

Comprehensive Statement

in Support of Promotion from Associate to Full Professor

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1 Preface

I was hired by the University of Guam, College of Natural and Applied Sciences on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as an extension entomologist with the academic rank of assistant professor. I received tenure and promotion to associate professor during spring term 2013. This comprehensive statement covers my activities from January 2013 through the present (Fall term 2018).

I retain my position as Guam's extension entomologist and I am also a member of the Environmental Science Program Graduate faculty and member of the Western Pacific Tropical Research Center.

I do not have a teaching appointment, but I have been tasked with being an instructor for undergraduate entomology courses. I have chosen to report on my teaching activities under the roll of *University and Community Service*.

I wish to be evaluated for promotion with proportional weight given to the following roles:

- 51% Extension and Community Activities
- 34% Creative / Scholarly Activity and Research
- 15% University and Community Service

1.1 What is Extension?

Not all readers will know what is meant by "extension". Here is concise definition:

"In the US, an extension agent is a university employee who develops and delivers educational programs to assist people in economic and community development, leadership, family issues, agriculture and environment. Another program area provided by extension agents is 4-H and youth activities. Many extension agents work for cooperative extension service programs at land-grant universities. They are sometimes referred to as county agents, or extension educators. Often confused with Extension agents, Extension specialists are subject matter experts usually employed as scientists and university professors in various departments in the land-grant university system. Subjects range from agriculture, life sciences, economics, engineering, food safety, pest management, veterinary medicine, and various other allied disciplines. These subject matter specialists work with agents

(usually in a statewide or regional team environment) to support programs within the cooperative extension system.”

Source: https://en.wikipedia.org/wiki/Agricultural_extension#Extension_terminology

1.2 Philosophy and Interests in Research and Extension

- In my opinion, an agricultural scientist should have one hand in the dirt and one hand reaching for the sky. I have a very strong interest in learning about, evaluating, developing, and adapting new technologies. However, I recognize the danger of spending all of one's efforts in developing new tools rather than using them to solve real-world problems. As an extension entomologist, I spend much of my time doing applied research aimed at finding practical solutions to real problems impacting Guam.
- I very much enjoy interacting with and learning from growers and other clients.
- The most fertile place to prospect for new knowledge and technical advancement is at interfaces between sciences. I enjoy working as a member of interdisciplinary teams and I try to keep up to date in several fields of science and technology
- I have a wide range of research interests in integrated pest management (IPM) including development of sustainable crop monitoring systems (simplified monitoring that can be done by the grower, or automated monitoring using instrumentation),, development of least-toxic or non-toxic pest control methods.and development of control methods based on control of insect behavior using chemical or physical attractants and repellents.
- I am interested in using information technology to facilitate access to extension and research information and I have experience in building and maintaining web sites and databases for this purpose. While working at Northern Marianas College during the 1990s, I was very appreciative of the vast amount of extension information becoming available on the World Wide Web and I was able to contribute to this global effort by creating web sites and providing content. After returning to Micronesia in 2003, I continued this effort by creating and maintaining several technical web sites.
- I am skilled in scientific programming. I started programming computers in the mid 1970s and I have used many languages and integrated development environments (FORTRAN, BASIC, APL, Pascal, Delphi, PHP, Drupal, R, and Python). I am also skilled at simulation modeling, database design, technical graphics, and geographical information systems. I enjoy using these tools for extension and applied research and I often share my skills with colleagues.

- I have first-hand experience of the effects of accidental introduction of pests on island ecosystems and economies. (I was the first to discover the silverleaf whitefly and scarlet gourd on Saipan.) Since returning to Guam in 2003, I have been the first detector for more than a dozen invasive species of insects. I spend much responding to problems caused by recently introduced insect pests and I work with colleagues within and outside UOG who are trying to improve Guam's biosecurity.

1.3 Philosophy and Interests in Teaching

- The instructor should act as a filter for the students. She or he should be very explicit in identifying essential core knowledge. I would rather have my students be rock-solid on fundamentals than knowing a lot of details.
- We should give students the tools to build on their foundation of knowledge by teaching them how to use modern information technology including research libraries, on-line databases, and other internet tools.
- Learning (*and teaching*) should be pleasurable.
- Students should be given ample opportunity to improve their skills in scientific communication, both oral and written, aimed at a wide variety of audiences.
- I promote a holistic, systems science approach to problem solving, requiring a high degree of critical thinking and creativity.
- I am a strong proponent of hands-on field work.

1.4 In a Nut Shell

My Activity between 2013 and 2018

Publications: authored or coauthored 8 articles published in peer-reviewed journals.
(Section 3.1)

Presentations: authored or coauthored 53 presentations for professional meetings.
(Section 3.2)

Technical Reports authored or coauthored 78 technical reports (Section 3.6)

Grants: served as principal investigator for 16 grants with a total budget of \$1,443,841.
(Section 3.5)

Instruction: taught AG109 *Insect World* during 2 semesters and AG/BI345 *General Entomology* during 4 semesters. (Section 4.1)

1.5 Note to Reader

I have tried to make this report easy to navigate by providing a table of contents and reference lists. Evidence of my work is included in appendices which are available in a digital copy of this report which is in portable document format (PDF). I have provided several memory sticks which contain a PDF of my comprehensive statement with appendices. This PDF contains active links to all sections listed in the table of contents and links to articles of evidence contained in the appendices.

The PDF can also be downloaded from:

<http://guaminsects.net/promotionPackage/comprehensive-statement.pdf>

2 Extension and Community Activities (51%)

2.1 Insect Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to diverse clients including commercial growers, gardeners, householders, GovGuam agencies, federal agencies, and UOG colleagues. Most client contacts are initiated by a phone call or a visit by the client to my office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem.

The number of extension calls requiring my assistance averages approximately three per day during the reporting period. Many of these are documented as postings to iNaturalist [1].

References

[1] My iNaturalist Postings, Jan. 1, 2013 - Nov. 28, 2018.

https://www.inaturalist.org/observations?created_d1=2013-01-01&created_d2=2018-11-28&place_id=any&subview=grid&user_id=aubreymoore&iconic_taxa=Arachnida, Insecta

2.2 Detection and Documentation of Invasive Species

Invasive insects are arriving on Guam at a very high rate (estimates range as high as one new species per day). Very few of these invasive species are detected and even fewer are identified because Guam suffers from [the taxonomic impediment](#). Even when reliable species determinations are made, new island records are only rarely documented in the scientific press. Thus, impacts of invasive insects on Guam and elsewhere in Micronesia are grossly underestimated. One of my professional goals is to work towards solving this problem by increasing the detection rate, getting specimens identified by qualified taxonomists, and publishing new island records in the scientific literature.

- 3 new invasive insects documented in iNaturalist posts, 1 new invasive species fact sheet, 1 peer-reviewed journal article.

- Pacific orange leafwing, *Doleschallia tongana* [1]
- Lobate lac scale, *Paratachardina pseudolobata* [2, 3]
- FIX THIS Mango fruit borer, *Citripestis eutraphera* (identification not yet confirmed) [4, 5]
- The International Union for Conservation of Nature (IUCN-ISSG) is building a Global Register of Introduced and Invasive Species. I have volunteered to coordinate building a check list for species on Guam.
- The Guam Invasive Species Council is required to maintain a list on invasive species on Guam. I have volunteered to be “registrar” for this list.

References

- [1] Manuel, Jake, W. John Tennent, Donald W. Buden, and Aubrey Moore 2018. First Record of *Doleschallia tongana* (Lepidoptera: Nymphalidae) for Guam Island. F1000Research 7: 366. <https://f1000research.com/articles/7-366/v1>, accessed April 17, 2018.
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- [5] Moore A. *Citripestis eutraphera* [Internet]. iNaturalist.org. 2018 [cited 2018 Aug 25]. Available from: <https://www.inaturalist.org/observations/13466275>

2.3 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I work with Dr. Ross Miller to curate and catalog this collection.

- I ported the digital catalog for the UOG Insect Collection from a CSIRO BioLink database to a more modern web-based Symbiota database which is now online [1].
- I established an internship to train entomology students how to curate an institutional insect collection [2].

- The Benita Laird-Hopkins collection includes more than 5,000 insect specimens reared from seeds of forest plants from Saipan and Guam as part of the Ecology of Bird Loss Project. This collection has been cataloged and accessioned into the UOG insect collection and a publication is being prepared [3].
- In June 2018 I attended the Second Annual Digital Data in Biodiversity Research Conference sponsored by iDigBio (Integrated Digital Biocollections) to attend a workshop entitled Sharing and Mobilization of Massive Specimen Image Databases from Collections of Tropical Island Biodiversity as an invited participant. I made a presentation on building a biodiversity inventory for Guam [2] and discussed ongoing collaboration with Dr. Alex Vandam, University of Puerto Rico, on writing an NSF proposal to support digitization of biological collections on American-affiliated islands [5].

References

- [1] Moore A. SCAN University of Guam Insect Collection Collection Profiles [Internet]. 2018 [cited 2018 Aug 23]. Available from: <http://scan-bugs.org/portal/collections/misc/collprofiles.php?collid=180>
- [2] Moore A. Internship: University of Guam Insect Collection Technician [Internet]. 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/internship.pdf>
- [3] Laird-Hopkins BC, Downey HF, Basset Y, Fricke E, Moore A, Quicke DLJ, et al. [IN PREPARATION] Fruit and seed-eating insect assemblages on island ecosystems. Biotropica.
- [4] Moore A. Building a Terrestrial Biodiversity Inventory for Guam [Internet]. Oral presentation presented at: Second Annual Digital Data in Biodiversity Research Conference; 2018 [cited 2018 May 30]; Berkeley, CA. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315
- [5] Moore A. Trip Report: Second Annual Digital Data in Biodiversity Research Conference, Berkeley, CA, June 2018 [Internet]. 2018 [cited 2018 Aug 25]. Available from: https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Berkeley_Trip_report.pdf

2.4 Guam Coconut Rhinoceros Beetle Project

This is currently my largest and most important project.

Please see CRB activities in the Creative/Research/Scholarly section

2.5 National Plant Diagnostic Network (NPDN)

I serve as the UOG Coordinator for the National Plant Diagnostic Network.

- Participated in monthly conference calls.
- Prepared an annual work plan and budget [1].
- Prepared annual report [2].
- Served on the NPDN IT Strategic Planning Committee.
- Trained and certified 14 First Detectors as part of my AL/BI 345 General Entomology course, Fall 2017.

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2.6 Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC)

- I am a founding member and regular participant in GISAC.
- President Underwood delegated me to represent UOG as a voting member of GISC.
- During 2018, I served on a GISC Import Data Harmonization Committee. This committee generated recommendations [1] resulting in a bill to amend the Guam Invasive Species Act [2].

References

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2.7 mosquito committee

2.8 Public Outreach (Guest lectures, presentations, interviews)

References

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3 Creative/Scholarly Activities or Research (34%)

3.1 Publications in Refereed Journals

I authored or coauthored 8 articles published in peer-reviewed journals between 2013 and 2018. Note that I have estimated my share of the effort contributed to each publication.

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Evidence: 5.4.1
My effort: 80%
- [2] Moore, A., Barahona, D. C., Lehman, K. A., Skabeikis, D. A., Iriarte, I. R., Jang, E. B., & Siderhurst, M. S. (2017). Judas beetles: Discovering cryptic breeding sites by radio-tracking coconut rhinoceros beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). Journal of Environmental Entomology, 46(1), 92-99. <https://doi.org/10.1093/ee/nvw152>
Evidence: 5.4.2
My effort: 80%
- [3] Marshall, S. D. G., Moore, A., Vaqalo, M., Noble, A., & Jackson, T. A. (2017). A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands. Journal of Invertebrate Pathology, 149, 127-134. <https://doi.org/10.1016/j.jip.2017.07.006>
Evidence: 5.4.3
My effort: 25%
- [4] Moore, A., Quitugua, R., Iriarte, I., Melzer, M., Watanabe, S., Cheng, Z., & Barnes, J. M. (2016). Movement of Packaged Soil Products as a Dispersal Pathway for Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) and Other Invasive Species. Proceedings of the Hawaiian Entomological Society, 48, 21-22. Retrieved from <http://scholarspace.manoa.hawaii.edu/handle/10125/42743>

Evidence: 5.4.4

My effort: 80%

- [5] Moore, A., Jackson, T., Quitugua, R., Bassler, P., & Campbell, R. (2015). Coconut rhinoceros beetles (Coleoptera: Scarabaeidae) develop in arboreal breeding sites in Guam. Florida Entomologist, 98(3), 1012-1014. Retrieved from <http://journals.fcla.edu/flaent/article/download/84794/84044>

Evidence: 5.4.5

My effort: 80%

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Evidence: 5.4.6

My effort: 80%

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Evidence: 5.4.7

My effort: 20%

- [8] Marler, T.E., Miller, R., and Moore A. (2013). Vertical stratification in predation of armored scale on *Cycas micronesica* seedlings. HortScience 48(1) 60-62. <http://hortsci.ashpublications.org/content/48/1/60.full>

Evidence: 5.4.8

My effort: 20%

3.1.1 Citations

According to Google Scholar, my journal articles have been cited 325 times since 2013.

References

- [1] Google Scholar Citations Web Site. Accessed 2018-11-29.
<https://scholar.google.com/citations?hl=en&user=LGb4OLwAAAAJ>
file:evidence/pubs/GoogleScholarCitations.png

3.2 Presentations

I authored or coauthored 53 presentations for professional meetings between 2013 and 2018:

References

- [1] Moore, Aubrey; Miller, Ross H.; Marler, Thomas E. 2013. Biological control of cycad scale, *Aulacaspis yasumatsui*, attacking Guams endemic cycad, *Cycas micronesica*. Entomological Society of America Annual Meeting. Austin, Texas. [BM8ZXEC9] <http://guaminsects.myspecies.info/sites/guaminsects.myspecies.info/files/CycadScaleBiocontrolAustin.pdf>
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- [5] Moore, Aubrey; Quitugua, Roland 2014. Overview of the Guam coconut rhinoceros beetle eradication project. Hawaii CRB Incident Command Meeting. Honolulu, Hawaii. [HE7PH8N9] <http://guaminsects.net/presentations/CRB-Hawaii-ICS-Jan-2014.pdf>
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- [9] Moore, Aubrey; Quitugua, Roland; Siderhurst, Matthew; Jang, Eric 2014. Improved traps for the coconut rhinoceros beetle, *Oryctes rhinoceros*. Entomological Society of America. Portland, OR. [NFTUN65F] http://guaminsects.net/anr/sites/default/files/Moore_1957_2.pdf
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Evidence: 5.5.35

3.3 Coconut Rhinoceros Beetle (CRB) Biocontrol

This is currently my largest and most important project. Funding for outreach and applied research is currently provided by three grants: USDA-APHIS FY17 Farm Bill, USDA-Farm FY18 Bill, and a grant from the Department of the Interior-Office of Island Affairs for FY18-19.

I have submitted a proposal for FY19 Farm Bill Fundings. The abstract from this proposal serves as a description of this ongoing project:

A newly discovered biotype of coconut rhinoceros beetle (CRB-G) is rapidly killing coconuts and other palms on Guam and on other Pacific islands. Following a failed eradication attempt on Guam, CRB-G proved hard to control because it is resistant to *Oryctes*

rhinoceros nudivirus (OrNV), which was previously used as the preferred biological control agent for control of CRB outbreaks on Pacific Islands and elsewhere. Previous to the discovery of CRB-G, all OrNV releases on Pacific Islands resulted in immediate and sustained suppression of CRB damage to low levels and prevented tree mortality.

Guam is currently experiencing an uncontrolled and unmonitored island-wide CRB-G outbreak which was triggered by abundant CRB-G breeding sites in the form of dead and dying vegetation left in the wake of Typhoon Dolphin which occurred in May 2015. of a recent typhoon. Most of these breeding sites are inaccessible to sanitation efforts, being either in the jungle or on military land (which covers one third of Guam). A positive feedback cycle has begun whereby large numbers of adult beetles are killing large numbers of palms which become breeding sites which generate even higher numbers of adults. Severe damage to Guam's palms prompted the Governor of Guam to declared a state of emergency in July 2017.

The main objective of this project is to stop the uncontrolled outbreak on Guam. Entomologists working on the CRB-G problem on several Pacific islands agree that the most feasible tactic to halt tree mortality and suppress damage to tolerable levels is establishment of biological control using an isolate of OrNV which is highly effective as a biological control agent for CRB-G. We are working with collaborators to identify populations of CRB-G throughout the Asia-Pacific region. We will sample these populations for biological control agent candidates which will be evaluated in laboratory bioassays performed at UOG. Promising candidates will be field released using autodissemination as per a USDA-APHIS import and release permit.

Concurrent with establishment of CRB-G biocontrol, success of the project will be monitored in a quarterly, island-wide tree health survey and incidence of OrNV infection will be monitored in a subsample of all field collected CRB-G.

If the Guam CRB-G infestation cannot be controlled, it is expected that most palms on the island will be killed and CRB-G will continue to spread to other islands and beyond. If CRB-G invades smaller islands and atolls where coconut is the tree of life, a human tragedy will ensue. On larger islands, coconut and oil palm industries will be severely impacted. Attempts to organize a regional project in response to CRB-G are underway.

Recent Activity in this Project

- Coauthored a peer-reviewed journal article documenting discovery of CRB-G [1].
- Wrote a magazine article for the Guam Invasive Species Awareness week. This was published by the Pacific Islands Times [2]. A similar article was archived in Zenodo [3].
- Recruited Dr. James Grasela, an insect pathologist, to work on the project for two years using funding from the US Department of Interior - Office of Island Affairs. Grasela's initial task will be to perform laboratory bioassays to evaluate OrNV isolates as candidates for biocontrol of CRB-G (Job announcement: [4]).

- Recruited Ian Iriarte as a research assistant using funds from Farm Bill grants. Ian is also my graduate student. He is working with me on development of an automated coconut rhinoceros beetle damage monitoring system using computer vision and deep learning. This project is likely to be the topic of his master's thesis.
- In August 2018, Moore, Grasela, Iriarte and Quitugua participated in the 51st Annual Meeting of the Society for Invertebrate Pathology and International Congress on Invertebrate Pathology and Microbial Control held at the Gold Coast, Australia. This conference provided a venue for was a symposium and a meeting to plan and promote collaboration among Pacific entomologists working on the CRB-G problem [5, 6].
- Created a private wiki site to facilitate sharing scientific/technical information among scientists working on the CRB-G problem [7].
- Laboratory bioassays of an OrNV isolate propagated from a virus-infected CRB-G adult we collected on Negros Island, Philippines in 2017 produced no response when applied to CRB-G adults [8, ?]

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3.4 Guam Biodiversity Inventory

I consider this to be my second most important project.

A biodiversity inventory is essentially a database containing a comprehensive check list of all taxa known occur within a defined area.

A terrestrial biodiversity inventory for Guam is needed to document rapid changes to Guam's ecosystems, to provide free and open access to information on Guam's flora and fauna, and to share Guam biodiversity information with the global scientific community, policy makers and the public.

The Guam Biodiversity Inventory will facilitate automatic generation and updates to lists such as: a list of all invasive species on Guam with year first recorded, a list of new species described from specimens collected on Guam, a list of observations for Guam's endangered species, a list of Guam's native plants with associated herbivores and pathogens, and a list of crops grown on Guam and pests and pathogens which attack them.

Recent Activity in This Project

- I made a couple of presentations on my plans for the Guam Biodiversity Inventory [[1](#), [2](#)].
- I designed data model for the Guam Biodiversity Inventory and created a prototype web site.

- I requested the Bishop Museum to publish primary entomological literature for Guam on-line and sponsored this using grant funding. Both volumes of *Insects of Guam* are now available for free download as PDFs from <http://hbs.bishopmuseum.org/pubs-online/pdf/bull172.pdf> and <http://hbs.bishopmuseum.org/pubs-online/pdf/bull189.pdf>.

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3.5 Grants

Between 2013 and 2018 I was the Principal Investigator for 16 grants with a total budget of \$1,443,841.

Title	Funding source	Start date	End date	Budget
CRB FS 2011	USDA Forest Service	2011-05-23	2013-12-31	\$200,000
CRB Biocontrol 2012	USDA-APHIS	2012-06-01	2014-05-31	\$40,000
WPDN 2013	NIFA via UC Davis	2013-04-11	2014-06-30	\$7,550
Management of the CRB	USDA Forest Service	2013-07-01	2015-06-30	\$150,000
CRB Biocontrol 2013 [42]	USDA-APHIS	2013-09-01	2015-08-31	\$40,000
Octocula	USFWS via GDOA	2014-05-13	2016-09-30	\$21,212
WPDN 2014-15	NIFA via UC Davis	2014-07-01	2015-06-30	\$10,672
Guam Forest Insect Survey	NIFA - McIntire-Stennis	2014-11-01	2018-09-30	\$12,302
WPDN 2016-17	NIFA via UC Davis	2016-09-01	2017-08-31	\$9,754
WPDN 2018	NIFA via UC Davis	2016-09-01	2018-08-31	\$9,754
Biological Control of Coconut Rhinoceros Beetle Guam Biotype in Micronesia	DOI Office of Island Affairs	2017-07-21	2019-09-30	\$176,553
Biological Control of Coconut Rhinoceros Beetle, Guam Biotype FB17	USDA APHIS; Farm Bill FY2017	2017-08-01	2019-07-31	\$200,000
Extension Core Funding FY2018	Extension Core Funds	2017-10-01	2018-09-30	\$4,000
Biological Control of Coconut Rhinoceros Beetle, Guam Biotype FB18	USDA APHIS; Farm Bill FY2018	2018-08-01	2019-07-31	\$200,000
Guam Forest Biodiversity Inventory	NIFA - McIntire-Stennis	2018-10-01	2023-10-30	\$80,000
Biological Control of Coconut Rhinoceros Beetle, Guam Biotype FB19 [PENDING]	USDA APHIS; Farm Bill FY2019	2019-08-01	2020-07-31	\$282,044

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Evidence: ??

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Evidence: 5.6.74

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Evidence: 5.6.81

4 University and Community Service (15%)

4.1 Undergraduate Instruction

Course	Semester	Student evaluations
AG109 Insect World	Spring 2013	5.3.1
AG345 General Entomology	Fall 2013	5.3.2
AG109 Insect World	Fall 2014	5.3.3
AGBI345 General Entomology	Fall 2014	5.3.4
AGBI345 General Entomology	Fall 2015	5.3.6
AGBI345 General Entomology	Fall 2017	5.3.7, 5.3.8, 5.3.9, 5.3.10

During the reporting period I taught AG 109 *Insect World* and AL/BI 345 *General Entomology*. Both courses consist of 2 one-and-a-half hour lectures per week and one three hour laboratory session per week. I prefer to teach both the lecture and lab sections because I think this results in delivery of a more integrated science instruction package. The syllabi for AG 109 [11] and AL/BI 345 [12] are provided in the appendices.

For each course I teach, I build and maintain a web site which I populate with online resources and tools. I have used several frameworks for building these sites including Drupal, Moodle [13] and Pelican. The current AL/BI 345 web site [14] was built using Nikola. Nikola uses a python script to generate static HTML pages hosted on GitHub pages.

References

- [1] Student evaluations for AG-109 (Lecture and lab sections) Spring 2013
Evidence: [5.3.1](#)
- [2] Student evaluations for AG/BI-345 (Lecture and lab sections) Fall 2013
Evidence: [5.3.2](#)

- [3] Student evaluations for AG-109 (Lecture and lab sections) Fall 2014
Evidence: 5.3.3
- [4] Student evaluations for AG-345 (Lecture and lab sections) Fall 2014
Evidence: 5.3.4
- [5] Student evaluations for BI-345 (Lecture and lab sections) Fall 2015
Evidence: 5.3.5
- [6] Student evaluations for AG-345 (Lecture and lab sections) Fall 2015
Evidence: 5.3.6
- [7] Student evaluations for AL-345 (Lecture section) Fall 2017
Evidence: 5.3.7
- [8] Student evaluations for AL-345L (Lab section) Fall 2017
Evidence: 5.3.8
- [9] Student evaluations for BI-345 (Lecture section) Fall 2017
Evidence: 5.3.9
- [10] Student evaluations for BI-345L (Lab section) Fall 2017
Evidence: 5.3.10
- [11] Syllabus for AG 109, *Insect World*, last updated Fall 2014.
Evidence: 5.7.4
- [12] Syllabus for Al/BI 345, *General Entomology*, last updated Fall 2017.
Evidence: 5.7.5
- [13] Moodle site for AG109, Fall 2014.
Screenshot of home page: **Evidence:** 5.7.1
- [14] AL/BI 345 Fall 2017 static web site built with Nikola. <https://aubreymoore.github.io/ALBI345F17>
Screenshot of home page: **Evidence:** 5.7.2
Screenshot of resources page: **Evidence:** 5.7.3

4.2 Graduate Instruction

- I am the major faculty advisor for Mr. Ian Iriarte who is pursuing a Master's degree in Environmental Science.
- Although I am not the instructor of record for any EV courses, I am often invited to give guest lectures.
- I served on thesis committee for Trent Hamada's EV masters degree.

4.3 Faculty Committees

4.3.1 Undergraduate Curriculum Review Committee

I was elected to serve on this committee in April 2013 and served for 2 years.

4.3.2 University Technical Advisory Committee

I was appointed by the Dean to serve on this committee and did so until it was disbanded.

4.3.3 Faculty Building Facilities Committee

This committee was formed by the Agriculture and Life Sciences Division to provide advice to the Dean on facilities problems within the Agriculture and Life Sciences Building. During the reporting period, I was re-elected as chair of this committee and I am joined by Dr. Jim McConnell and Dr. LaJoy Spears as the other members.

- Plans for improvements to the ALS124 teaching lab have been only partially achieved. For the past three years, faculty have asked for a dedicated computer and modern audiovisual equipment to facilitate science teaching. During the reporting period, lab tables were equipped with power sockets to replace those removed during a previous renovation.
- We continue to struggle with finding solutions to chronic air conditioning problems.

4.3.4 Search Committee: Extension Animal Scientist

I chaired this committee and was joined by Mari Marutani, LaJoy Spears, Bob Schlub, and Tom Poole, Guam's Territorial Veterinarian.

- Position announcement written [?] and advertisement placed on the web site of the American Association of Animal Scientists [?].

4.3.5 Search Committee: Extension Agricultural Economist

I am a member of this committee and I am joined by Bob Barber (chair), LaJoy Spears, and John Brown.

4.3.6 Search Committee: Research Associate II (CRB Project)

I chaired this committee and was joined by Jim Grasela, Roland Quitugua, and Jesse Bamba.

4.3.7 Continuing Employment Committee: Austin Shelton

I chair this committee and I am joined by Ross Miller and Hui Gong.

4.3.8 Continuing Employment Committee: Andrea Blas

I served on this committee with Ross Miller and Frank Camacho.

4.3.9 Extension Publications Committee

I served as a member of this committee.

5 Appendices

5.1 Curriculum Vitae

Please see next page.

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Aubrey Moore

Education

- 1988 Ph.D., Entomology, University of Hawaii, Honolulu, Hawaii
1984 M.S., Entomology, Michigan State University, East Lansing, Michigan
1979 B.Sc., Integrated Science Studies, Carleton University, Ottawa, Ontario

Professional Experience

- 2008-Pres. Extension Entomologist, Cooperative Extension Service, University of Guam, Guam
2003-2008 Research Associate, College of Natural & Applied Sciences, University of Guam, Guam
1999-2003 Pesticide Evaluator, Pest Management Regulatory Agency, Health Canada, Ottawa, ON
1998-1999 Entomologist, School of Agriculture & Life Sciences, Northern Marianas College, Saipan
1992-1997 Research Director, School of Agriculture & Life Sciences, Northern Marianas College, Saipan
1991-1992 Entomologist, Northern Mariana Islands Department of Natural Resources, Saipan
1990-1991 Entomologist, USDA Agricultural Development in the American Pacific Project, Guam & Maui
1989-1990 Research Associate, University of Hawaii Agricultural Experiment Station, Maui, Hawaii
1988 Post-doctoral Fellow, Hawaiian Evolutionary Biology Program, Honolulu, Hawaii
1985-1988 Graduate Assistant, Department of Entomology, University of Hawaii, Honolulu, Hawaii

1985-1986	Programmer/consultant, University of Hawaii Computing Centre, Honolulu, Hawaii
1984	Research Associate, Department of Entomology, Michigan State University, East Lansing, MI
1984	Entomologist, Insect and Rodent Control Section, Michigan Dept. of Public Health, Lansing, MI
1981-1984	Graduate Assistant, Department of Entomology, Michigan State University, East Lansing, MI
1979-1981	Research Tech., Forest Pest Management Institute, Environment Canada, Sault Ste. Marie, ON
1975-1979	Research Technician, Chemical Control Research Institute, Environment Canada, Ottawa, ON

Professional Memberships

Entomological Society of America
 Hawaiian Entomological Society
 Florida Entomological Society
 Pacific Science Association
 Sigma Xi Research Fraternity

Publications

Book Chapters

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5.2 Comprehensive Faculty Evaluation System Reports, Plans and Evaluations

Please see next page.

Comprehensive Faculty Evaluation System Reports, Plans, and Evaluations

Aubrey Moore

October 16, 2018

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1 2012-2013

1.1 Report

Please see next page.

CFES 2013

Aubrey Moore, Ph.D.

Assistant Professor / Extension Entomologist

PLANNED ACTIVITIES AND REPORT OF ACCOMPLISHMENTS

Evaluation Period: June 26, 2012 through June 25, 2013

I was hired by the University of Guam on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as extension entomologist with the academic rank of Assistant Professor. I am a faculty member of the Environmental Science Graduate Program and a member of the Western Pacific Tropical Research Center. At the end of the 2012 fall term I applied for tenure and promotion and received both.

This report documents my activities from June 2012 through the present. My current faculty role allocation is as follows:

- 51% Extension and Community Activities
- 34% Creative/Scholarly Activity or Research
- 15% University and Community Service

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1 Extension and Community Activities

1.1 Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam and federal agency personnel. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

1.2 Wilfred Leon Guerrero Insect Museum

The recently founded Wilfred Leon Guerrero Insect Museum houses the UOG insect collection, which is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the Museum and I work with Dr. Ross Miller to curate and catalog this collection. One of my grants employed Laura Barnhart as a part-time insect collection technician and also allowed us to sponsor visits by skilled taxonomists who are willing to help us identify specimens.

To increase my knowledge of collection management, I attend the annual meetings of the Entomological Collections Network, which are typically held in conjunction with annual meetings for the Entomological Society of America.

I have a personal goal of building an online website to share all available information on Micronesian insects. This will include specimen level information for the collection complete with digital images and literature references. I built a digital catalog for the collection using the BioLink Biodiversity Information Management System from CSIRO, Australia. The catalog currently contains 29,200 specimen records. BioLink is currently being redeveloped as an open source project (<http://code.google.com/p/biolink/>). I am an active collaborator in this project. In July 2012 I published an article entitled *Hosting a Biolink Database in the Amazon Web Services Cloud (EC2)* on the project's wiki (<http://code.google.com/p/biolink/wiki/BioLinkEC2>).

I have built and evaluated two websites for serving information on Micronesian insect biodiversity, including specimen level data from the collection. One is a Drupal content management system template called LifeDesk provided the Encyclopedia of Life Project

1 Extension and Community Activities

and the other is a similar template called ScratchPads provided by the Museum of Natural History in London. I am honored to have been selected as an advocate for ScratchPads as part of the project's Ambassadors program (<http://scratchpads.eu/locate-scratchpad-ambassadors>). Further information on my websites is provided in the Creative/Scholarly Activities section (2.3). I made a presentation entitled *Evaluation of a Scratchpad Template as an Online Database for the University of Guam Insect Collection* at the Entomological Collections Network annual meeting in Reno, December 2011. I participated in an online training workshop for advanced ScratchPad users on May 25, 2013.

1.3 Guam Coconut Rhinoceros Beetle Eradication Project

This is currently my largest and most time consuming project.

The coconut rhinoceros beetle (CRB) was first detected on Guam in the Tumon Beach hotel area on September 11, 2007. CRB is a very serious pest of coconut palms. Adult beetles may kill coconuts and other palms when they bore into the crowns to feed on sap. When CRB invaded Palau during the Second World War, it killed about half of all coconuts through the islands and totally exterminated the coconut palm from some of them. A delimitation survey indicated that the Guam infestation was limited to Tumon Bay and the adjacent Faifai Beach. In consultation with the Guam Department of Agriculture (GDOA), USDA-APHIS, and USDA-Forest Survey, it was decided to launch an eradication project.

I wrote the original eradication plan (available on-line at http://guaminsects.net/uogces/kbwiki/index.php?title=Coconut_Rhinoceros_Beetle_Eradication_Plan) and this was funded by USDA and local funds. USDA provided funds under the condition that the project was to be run under an Incident Command System with the USDA-APHIS Guam Port Director as the federal commander, and the GDOA Director, or designee, as the local commander.

My original role was to provide scientific/technical support for the project, with the Guam Department of Agriculture (GDOA) providing project management with assistance from USDA-APHIS and USDA-Forest Service. However, it soon became apparent that GDOA had serious bureaucratic impediments which prevented hiring staff and procuring supplies and equipment within a reasonable time frame. The eradication project directors, with the consent of the Dean, agreed to run project staffing, procurement, and fiscal management through the University. As a result, my role was expanded to include much of the project management. I am currently managing five grants which fund the project and supervise about 15 temporary employees. Report writing on current grants and proposal writing to keep the project in business occupies much of my time.

A recent progress report (Evidence 1.3.4; available on-line at <http://guaminsects.net/anr/sites/default/files/CRB%20Progress%20Report%2020090510.pdf>) provides a good overview of the current status of the project.

1 Extension and Community Activities

1.3.1 Activities:

1. **Biweekly Planning Meetings.** This project is run as an incident command system. I attend biweekly planning meetings as a program manager.
2. **Conference Calls.** These teleconferences are with stakeholders and advisers in USDA APHIS and USDA Forest Service. These agencies are funding the project. Until recently calls were biweekly. They are now monthly.
3. **Grant Writing.** During the past 2 years, the Guam CRB Eradication Project has been almost entirely funded from 12 grants for which I wrote proposals and act as principal investigator. These grants are listed in the Creative / Scholarly Activity section.
4. **Report Writing.** All grants supporting the Guam CRB Eradication Project require regular reporting. As an example, I provide a copy of my most recent semiannual report. (Evidence 1.3.4; available on-line at <http://guaminsects.net/anr/sites/default/files/CRB%20Progress%20Report%2020090510.pdf>)
5. **Project Websites.** I have endeavored to share and archive data and information associated with the Guam CRB Eradication Project on-line. Prior to May 2009, I used a wiki site at http://www.guaminsects.net/uogces/kbwiki/index.php?title=Oryctes_rhinoceros. Afterwards, I used a Drupal site at <http://www.guaminsects.net/anr/category/miscellaneous/coconut-rhinoceros-beetle>.
6. **Project Database.** Trapping data from a network of about 1200 traps, detections of CRB grubs or adults, and observations of CRB defoliation and bore holes are entered daily into a web-based georeferenced MySQL database which I designed. Data from this database is publicly accessible from a web page at <http://www.guaminsects.net/anr/content/public-access-data-collected-guam-coconut-rhinoceros>. Links on this page enable the user to view trap catch data as a spatiotemporal display using a Google Earth animation or a chart of monthly totals. I use this system to produce monthly surveillance reports. (Evidence 1.3.6; available on-line at <http://www.guaminsects.net/anr/sites/default/files/april2010.pdf>).
7. **Scientific/technical Support.** I do applied research in support of the Guam CRB Eradication Project. Results of this research is provided in a series of technical reports. I provide a report of the results of a CRB damage survey at the Padific Islands Club Resort as an example (Evidence 1.3.7; available on-line at <http://guaminsects.net/doc/tech/PIC%20CRB%20Damage%20Survey.pdf>). A second example is an identification aid I created for CRB grubs (Evidence 1.3.7A; available online at http://guaminsects.net/anr/sites/default/files/CRB%20larvae%20with%20guage_1.pdf)

1 Extension and Community Activities

1.4 Western Plant Diagnostics Network

I am the UOG coordinator for WPDN. This organization provides financial support for ANR's Plant Diagnostic Laboratory, offers First Detector Training workshops, and organizes identification workshops for important pest groups. As coordinator, I am required to organize First Detector Training workshops, attend monthly conference calls, attend annual meetings, and provide reports.

1.4.1 Activities:

1. The two most recent First Detector Training (FDT) workshops were run on October 24, 2009 (Evidence 1.4.1) and June 4, 2011. During spring term 2013, I provided FDT for my AG109 'Insect World' class. Twenty-four students were certified as First Detecters and there contact information has been added to the national registry of first detectors.
2. In March 2009, I participated in the Adult Lepidoptera Identification Workshop at UC Davis, CA (Evidence 1.4.2). USDA-APHIS funded my participation in this workshop to increase my knowledge as an approved insect identifier for the Guam Plant Inspection Facility.
3. I served as associate editor of Pacific Pest Detector News, a Quarterly Newsletter for First Detectors until September 2010. (Evidence 1.4.3; available online at <http://tinyurl.com/PPDN1>)
4. I participate in monthly calls as WPDN coordinator for Guam.

1.5 Guam Invasive Species Advisory Committee (GISAC)

I am an active, founding member of this informal group of Guam's biologists which meets irregularly about 6 times per year to discuss invasive species and what can be done to keep them out and mitigate the effects of those that do invade the island. I worked with Dr. Russell Campbell and Diane Vice to develop an emergency response plan for invasive species detected on Guam.

A wiki site which I built for GISAC was quickly adopted by the Western Micronesia Regional Invasive Species Council at http://guaminsects.net/gisac/index.php?title=Main_Page. (Evidence 1.6)

1 Extension and Community Activities

1.6 Insect Identification Service for USDA-APHIS / Guam Customs and Quarantine Agency

I am often called upon to identify insect specimens intercepted the Guam Customs and Quarantine Agency. USDA-APHIS has certified me for this service and has provided a very official looking badge to impress people with. (However, it is not quite as impressive as Dr. Millers bright red badge for getting onto the airport runways.)

1.7 Forensic Entomology Service for Guam Police Department (GPD)

I have been requested, along with Dr. Ross Miller and Dr. Russell Campbell, to assist the GPD in future homicide investigations by providing forensic entomology expertise. I attended and assisted with and a forensic science symposium during which Dr. Lee Goff, a forensic entomologist from Hawaii, held a workshop on forensic entomology. (Evidence 1.8)

In September 2010, I assisted UOG student Joey Lopez and Professor Ross Miller with pig decomposition studies. In June 2011, I assisted with and gave a lecture for the CSI Guam Forensic Science Summer Program.

1.8 Public Outreach (Guest lectures, presentations, interviews)

During the reporting period I was interviewed numerous times by newspaper reporters, radio talk show hosts, and television news reporters (Table 1.1). Most, but not all involved questions about the Guam coconut rhinoceros beetle eradication project. I produced several fact sheets and articles for public print media during my two years as extension entomologist year and also published a lot of content on various websites. I have evaluated several current technologies for building a web presence for the Agriculture and Natural Resources Unit and the Drupal content management system seems to be a good fit. This allows us to publish information for public access while keeping some documents private for internal use only. My print and online output are discussed in more detail in the Creative/Scholarly Activity section.

1.9 Public Outreach (Internet)

I maintain a website for the the UOG Cooperative Extension Service's Agriculture and Natural Resources Program at <http://guaminsects.net/ANR>. I frequently post blog articles of public interest to this site (Table 1.2). I also maintain a website at <http://guaminsects.myspecies.info> which is intended to facilitate sharing information on

1 Extension and Community Activities

insects in Micronesia. I frequently submit blog articles to this website which are of interest to entomologists (Table 1.3).

1.10 Regional Collaboration

1.10.1 Regional Invasive Species Council Website

I maintain a website for the Western Micronesia Regional Invasive Species Council (RISC) at <http://www.guaminsects.net/gisac/>. I attend RISC meetings whenever they are held on Guam and I make presentations at these meetings.
(Evidence 2.5.5)

1.10.2 Pacific Plant Protection Organization

I was honored to be nominated by the Government of Guam to represent the island along with Dr. Russell Campbell, Guam's territorial entomologist, at the past three Pacific Plant Protection Meetings (Fiji, 2006; Papua New Guinea, 2009; Fiji, 2012). These meetings were organized by the Secretariat of the Pacific Community to promote sharing of information on plant protection within the Pacific and to promote harmonization among plant pest quarantine regulations within the region. I presented a country report on emerging pest situations at each meeting.

1.10.3 Insect Diagnostics for Micronesia

I am often contacted with requests for help with identifying pests from throughout Micronesia and suggesting solutions to the problems they cause. I expect this workload to increase because the number of practicing PhD level entomologists in Micronesia has dropped from 9 to 3 within the last decade.

I Extension and Community Activities

Table 1.1: Public outreach activities since 2012-06-01.

N	Date	Title	Venue	Evidence
1	2013-07-08	presentation on CRB for CARIPAC scholars	Guam Plant Inspection Facility, Tiyan	
2	2013-07-06	CRB Workshop for General Public	UOG	
3	2013-06-28	talk on forest insect pests for Pacific island foresters	Dept. of Ag., Guam	
4	2013-06-27	CRB Workshop for Professionals	Tamuning, Guam	
5	2013-06-25	CRB Workshop for Professionals	Tamuning, Guam	
6	2013-06-07	Invasive species talk for Indigenous Fellows Institute	Guam Community College	
7	2013-05-15	PACSTEM/EPSCOR meeting	Hyatt Hotel, Guam	
8	2013-04-12	judge for science fair	Thomas Aquinas School	
9	2013-04-10	lecture for John Brown's EV class	UOG	
10	2013-03-21	school visit	MV Lujan School, Guam	
11	2012-11-14	Trent Hamada Thesis Defense	UOG	
12	2012-11-09	Marianas Variety Newspaper Article, 9 NOV 2012: Professor Warns Rhino Beetle Still Spreading	UOG	
13	2012-11-08	Presented an update on the rhino beetle infestation for the Rotary Club		
14	2012-11-08	Rhino Beetle talk for Rotary Club	Hilton Hotel, Guam	
15	2012-08-23	Lecture for EV class	UOG	

Continued on next page

1 Extension and Community Activities

Table 1.1: (Continued)

N	Date	Title	Venue	Evidence
16	2012-08-21	Lecture for EV class	UOG	
17	2012-07-18	Radio interview on rhino beetle with Steve Rice, Radio Australia, Pacific Beat Program		
18	2012-06-25	Presented island re- port for Guam at the Pacific Plant Protec- tion Meeting	Suva, Fiji	

Table 1.2: Blog posts to GuamInsects.net/ANR since 2012-06-01.

N	Date	Title
1	2013-08-11	testqwqwqwqwqwqw
2	2013-08-09	plain from
3	2013-08-06	Little Fire Ant PSAs
4	2013-07-08	Visualization of Coconut Rhinoceros Bee- tle Trap Catch Data
5	2013-07-07	KUAM TV News: Rhino Beetle Workshop Held at UOG
6	2013-07-05	Radio New Zealand Report on Guam Little Fire Ant Situation
7	2013-07-05	Trifold Flyer: Coconut Rhinoceros Beetle Control Tips
8	2013-07-05	Canned Edible Rhino Beetle Larvae
9	2013-07-05	CRB Life Cycle Diagram
10	2013-07-05	PDN Newspaper Article: Little fire ants are a nuisance, being tracked on island
11	2013-07-02	Guam Agricultural Resource Listing Flyer
12	2013-06-30	On-line References for Forest Insect Pests on Guam
13	2013-06-19	Weather Station on the Roof of the Agri- culture and Life Sciences Building at the University of Guam

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I Extension and Community Activities

Table 1.2: (Continued)

N	Date	Title
14	2013-06-17	Weather Station at the University of Guam Agricultural Experiment in Yigo is Back On Line
15	2013-06-13	PDN Newspaper Article: Steps to Prevent Invasive Species
16	2013-05-23	Experimental Trap Results
17	2013-05-22	Guam is Featured in Several Articles on the National Assoc of Conservation Districts Web Site
18	2013-05-07	UOG Researchers Search for Rare Butterflies and Snails
19	2013-04-27	Hawaii on the Lookout for Rhino Beetles
20	2013-04-14	Intel Uses Rhino Beetle in Advertisement
21	2012-12-15	Solar Energy Data from a Weather Station at the University of Guam's Yigo Agricultural Experiment Station
22	2012-10-10	test2
23	2012-10-10	test
24	2012-10-08	News Article: Giant Spider Traps Brown Treesnake in Web
25	2012-10-07	KUAM News Story; Nick Delgado interviews Aubrey Moore: "Guam has more spiders than all of CNMI"
26	2012-09-28	Mystery Vine
27	2012-09-27	Antmimicking Spider
28	2012-09-22	Marianas Variety Newspaper Article: Spiders Pose No Threat to Agriculture
29	2012-08-28	Unidentified spider
30	2012-07-25	What critter is making these sounds?
31	2012-07-21	Radio Australia Interview with Aubrey Moore: Guam's Palms Threatened by Rhino Beetle
32	2012-06-28	PDN Newspaper Article: Guam Infested by Invasive Species
33	2012-06-15	Internet Resources for the University of Guam 4H Entomology Course
34	2012-06-14	PDN Newspaper Article by Frank Ishizaki: We must help eradicate rhino beetle

Continued on next page

1 Extension and Community Activities

Table 1.2: (Continued)

N	Date	Title
35	2012-06-04	National Geographic Python Hunters TV Show features brown treesnake, monitor lizard, and rhino beetle on Guam
36	2012-06-04	PDN Newspaper Article: "UPDATE: Guam gets USDA funds to fight coconut rhinoceros beetle, biosecurity initiative"
37	2012-06-04	PDN Newspaper Article: "Check your compost for rhino beetles you may be shocked" by Helen Middlebrook
38	2012-06-04	PDN Newspaper Article: Rhino Beetle Needs to Be Stopped by Frank Ishizaki

Table 1.3: Content posted to s2.GuamInsects.MySpecies.info during the past year.

N	Date	Title	
1	2013-07-18	Biblio	Population size and distribution of <i>Latrodectus geometricus</i> (brown widow) with information on other common spiders of Guam
2	2013-07-17	Location	flame tree nr ALS building
3	2013-07-17	Media gallery	spiders
4	2013-07-17	Specimen/Observation	UOG - ESUG - AM20130716.001
5	2013-07-12	Media gallery	coconut termite
6	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.031
7	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.030
8	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.029
9	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.028
10	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.027
11	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.026
12	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.025
13	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.024
14	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.023
15	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.022
16	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.021
17	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.020
18	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.019
19	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.018

Continued on next page

I Extension and Community Activities

Table 1.3: (Continued)

N	Date	Title	
20	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.017
21	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.016
22	2013-07-01	Specimen/Observation	UOG - ESUG - 20130702.015
23	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.014
24	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.013
25	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.012
26	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.011
27	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.010
28	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.009
29	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.008
30	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.007
31	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.006
32	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.005
33	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.004
34	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.003
35	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.002
36	2013-07-01	Specimen/Observation	UOG - ESUG - 20130701.001
37	2013-07-01	Location	CBB trap
38	2013-06-13	Specimen/Observation	UOG - ESUG - AG109SP13-51b920f08801d
39	2013-06-13	Specimen/Observation	UOG - ESUG - AG109SP13-51b9216f648c0
40	2013-06-13	Location	Mangilao, Guam
41	2013-06-13	Blog entry	Liorhyssus hyalinus, hyaline grass bug, attacking lettuce
42	2013-06-13	Biblio	Heteroptera as Vectors of Plant Pathogens
43	2013-06-12	Biblio	INSECTS OF MICRONESIA Coreidae, Neididae, and Nabidae
44	2013-06-12	Biblio	Guam Coconut Rhinoceros Beetle Biological Control Project: Semiannual Report for USDA APHIS Grant Performance Period : June - December, 2012
45	2013-06-12	Biblio	Biological Control of Cycad Scale, Aulacaspis yasumatsui, Attacking Guam 's Endemic Cycad , Cycas micronesica
46	2013-03-12	Blog entry	Gregarious Caterpillar feeding on Pipturus argenteus
47	2013-03-08	Blog entry	Eurema caterpillars defoliating a Serianthes sapling

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1 Extension and Community Activities

Table 1.3: (Continued)

N	Date	Title	
48	2013-02-14	Media gallery	Mystery Spider
49	2013-02-14	Specimen/Observation	UOG - ESUG - AM20130214.001
50	2013-01-15	Specimen/Observation	UOG - ESUG - AM20130115.002
51	2013-01-15	Location	LFA2, Nimitz Hill, Piti, 13.4612, 144.7080
52	2013-01-15	Specimen/Observation	UOG - ESUG - AM20130115.001
53	2013-01-15	Location	LFA1 Primo Hard Fill, Yigo
54	2012-12-15	Blog entry	test
55	2012-12-13	Blog entry	Four Most Damaging Termites in the Philippines
56	2012-12-13	Biblio	Toxicity of thiamethoxam against Philippine subterranean termites.
57	2012-11-30	Media gallery	tangan tangan seed insects
58	2012-11-30	Media gallery	swami
59	2012-11-30	Media gallery	Polyrachis
60	2012-11-30	Media gallery	point and shoot camera plus microscope
61	2012-11-30	Media gallery	point and shoot camera
62	2012-11-30	Media gallery	Library
63	2012-11-30	Media gallery	Korean spiders
64	2012-11-30	Media gallery	Formicidae
65	2012-11-30	Media gallery	Eurema blanda
66	2012-11-30	Media gallery	digital SLR camera
67	2012-11-30	Media gallery	digital microscope
68	2012-11-30	Media gallery	cell phone phone camera plus microscope
69	2012-11-30	Media gallery	cell phone camera
70	2012-11-30	Media gallery	boonie bee
71	2012-11-30	Media gallery	AG109
72	2012-11-23	Specimen/Observation	UOG ESUG AM20121123.001
73	2012-11-23	Specimen/Observation	UOG ESUG AM20121123.004
74	2012-11-23	Specimen/Observation	UOG ESUG AM20121123.003
75	2012-11-23	Location	AAFB, Black Construction, Manny Concepcion 646-4861
76	2012-11-23	Specimen/Observation	UOG ESUG AM20121123.002
77	2012-11-22	Blog entry	Thrips Damaging Onion Leaves
78	2012-10-22	Blog entry	According to Holloway, the species of Eumelea on Guam is ludovicata
79	2012-10-18	Blog entry	Hypolimnas octocula on a Vanuatu Postage Stamp
80	2012-10-15	Specimen/Observation	UGUAM ESUG 507b78c288944

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1 Extension and Community Activities

Table 1.3: (Continued)

N	Date	Title
81	2012-10-15	Location Dededo 15-JUL-2012
82	2012-10-15	Specimen/Observation UGUAM ESUG 507b78c27dd69
83	2012-10-15	Blog entry Tobacco Flea Beetle, <i>Epitrix hirtipennis</i> , on hot pepper, <i>Capsicum annum</i>
84	2012-10-05	Blog entry Sphinx moth trapped while feeding from ginger lily flower
85	2012-08-25	Blog entry Utility for Adding Images to Blog Postings on guaminsects.myspecies.info
86	2012-08-24	Blog entry Caterpillar defoliating <i>Serianthes nelsonii</i> saplings

2 Creative/Scholarly Activities or Research

2.1 Publications and Presentations

My recent publications and presentations are listed in table 2.1 and table 2.2.

2.2 Patent

In 2012, I submitted a patent application entitled *Devices and Methods for Detecting and Trapping Pests* with co-inventor Darek Czokajlo, AlphaScents Inc. The application describes a simple insect trap consisting of an LED light source and a sticky card which can be used to detect insects in shipping containers during transit.

2.3 Web Sites Designed and Maintained by Me

For the past five years, I have been searching for the “right” technology for providing on-line extension information. The features I want include:

- Ease of use, including immediate, on-line editing, so that colleagues and collaborators can create content
- Ability to display digital images at several resolutions
- Full text search
- Methods for handling on-line and offline references
- Fine grained security which protects client confidentiality and allows for both protected, internal and public information sharing

My current technology of choice is Drupal, a free, open source contents management system. I am developing two Drupal websites, one for Agriculture and Natural Resources [1] and one for the Leon Guerrero Museum of Entomology [2]. I also use a Moodle website for my AG109 Insect World class [3].

2 Creative/Scholarly Activities or Research

2.3.1 ANR Web Site.

Home page: <http://guaminsects.net/anr>

This Drupal site is intended to facilitate sharing both internal and external information generated by the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. This site is currently being used heavily by the Guam CRB Eradication Project. I also use this site for documenting my diagnostics work. I provide a recent example web page documenting discovery of thrips in anthurium flowers. (Evidence 2.5.1; available on-line at <http://guaminsects.net/anr/content/thrips-damaging-anth>)

2.3.2 Insects of Guam Web Site

Home page: <http://guaminsects.myspecies.info>

This Drupal site is being evaluated for sharing information on Micronesian insects. Information will include specimen level information from the UOG insect collection complete with digital images and literature references. It was built using a template developed by the Scratchpad project <http://scratchpads.eu/> is sponsored by the European Institute of Distributed Taxonomy (EDIT) and the Natural History Museum in London. The ScratchPad project is celebrating the International Year of Biodiversity by highlighting a different Scratchpad taxon every week. I was honored to have one of my pages, describing the indigenous bug, *Leptocoris vicinus*, highlighted during the week of April 18 to 24, 2010.

(Evidence 2.5.2)

2.3.3 Micronesia Biosecurity Plan Review Web Site

Home page: MBP.GuamInsects.net

This is a secure, private Drupal site developed to facilitate sharing information among those reviewing the Micronesia Biosecurity Plan.

2.3.4 Moodle Site for my AG 109 Insect World Course

Home page: <http://campus.uogdistance.com/course/view.php?id=286>

This site was my first experience with Moodle, a content management system designed for teachers. I originally built it to provide on-line resources for my students, but later decided to open a few wikis to promote collaboration on laboratory exercises. I also kept track of grades using Moodle. Examples from this site include the course resource page (Evidence 2.5.3a; available on-line at <http://campus.uogdistance.com/mod/resource/view.php?id=7349>) and a small PHP program I wrote to facilitate printing pinned insect specimen labels (Evidence 2.5.3b; available on-line at <http://tinyurl.com/insect-labels>).

2 Creative/Scholarly Activities or Research

2.3.5 Knowledgebase Wiki for the UOG Cooperative Extension

Home page: <http://www.guaminsects.net/uogces/kbwiki/index.php>

This was my first attempt at building an extension website to facilitate collaborative content creation. Digital copies of all of ANR's pest fact sheets can be found on this site. There is also a list of insect pests found on all major crops grown in Micronesia. I stopped maintaining this site in May, 2009 because the ANR site built with Drupal has more of the features I need.

(Evidence 2.5.4)

2.3.6 Western Micronesia Regional Invasive Species Council Wiki

Home page: <http://www.guaminsects.net/gisac/index.php>

Originally built for the Guam Invasive Species Advisory Council, this site was quickly adopted for sharing regional information on invasive species by the Western Micronesia Regional Invasive Species Council.

(Evidence 2.5.5)

2.3.7 Guam Insects Blog Site

Home page: <http://blog.guaminsects.net/>

I ran into recurring technical problems with this site which uses the WordPress content management system and have more or less abandoned development and maintenance.

2.3.8 Life Desk Site for Micronesian Insects

Home page: <http://micronesianinsects.lifedesks.org/>

This site uses a Drupal template being developed by the Encyclopedia of Life Project. I evaluated it for sharing information on Micronesian insects, but decided that the Scratchpad template (number 2, above) had a better feature set for what I wanted to do.

2.4 Grants

My active and pending grants are listed in table 2.3) and table 2.4. Fourteen support staff positions have been supported, partially or fully, from my grants during 2012 and 2013 (Table 2.6). Two of my recent grant proposals were not funded (Table 2.5).

2 Creative/Scholarly Activities or Research

Table 2.1: Publications 2012 - 2013

N°	Citation	Type
1	Marler, TE, Moore A, Miller RH. 2013. Vertical stratification in predation of armored scales on <i>Cycas micronesica</i> seedlings. <i>HortScience</i> . 48(1):60-62.	Journal Article
2	Cave, RD, J-T C, Kumashiro B, Marler TE, Miles J, Moore A, Muniappan R, Watson GW. 2013. Status and biological control of Cycad Aulacaspis scale. <i>Biocontrol News and Information</i> . 34(1):1-4.	Journal Article
3	Marler, TE, Wiecko G, Moore A. 2012. Application of game theory to the interface between militarization and environmental stewardship in the Mariana Islands. <i>Commun Integr Biol</i> . 5:193-195. URL: http://dx.doi.org/10.4161/cib.18889 <i>NOTE: Evidence 134</i>	Journal Article
4	Yudin, LS, Moore A. 2012. Our Island Without Coconut Trees, Could It Happen: Coconut Rhinoceros Beetle. <i>Inspire Local Magazine</i> . URL:	Magazine Article

2 Creative/Scholarly Activities or Research

Table 2.2: Presentations at professional meetings 2012 - 2013

N	Citation	Type
1	Moore, A, Marler T, Miller RH, Yudin LS. 2013. Biological Control of Cycad Scale, <i>Aulacaspis yasumatsui</i> , Attacking Guam 's Endemic Cycad , <i>Cycas micronesica</i> . 4th International Symposium on Biological Control. :1-4.	Conference Paper
2	Moore, A. 2012. Guam as a source of new insects for Hawaii. Pacific Entomology Conference.	Conference Paper (oral presentation)
3	Moore, A. 2012. CRB is the BTS of the 21st Century. Brown Treesnake Technical Working Group Meeting. URL:	Conference Paper (oral presentation)
4	Moore, A. 2012. Insect pests of ironwoods. Ironwood Decline Conference. URL:	Conference Paper (oral presentation)
5	Moore, A. 2012. Insect pests of trees on Guam. Ironwood Decline Conference. URL:	Conference Paper (oral presentation)
6	Moore, A. 2012. Update on the Guam coconut rhinoceros beetle eradication project. Western Micronesia Invasive Species Committee Annual Meeting. URL:	Conference Paper (oral presentation)
7	Moore, A. 2012. Update on the Guam coconut rhinoceros beetle eradication project. Guam Invasive Species Council. URL:	Conference Paper (oral presentation)

2 Creative/Scholarly Activities or Research

Table 2.4: Active and pending grants other than those supporting the Guam Coconut Rhinoceros Beetle Eradication Project.

Title	Source	Grant No. or UOG Account	Amount
National Plant Diagnostic Network (NPDN)	via UC Davis	201223902-09	\$7,550
National Plant Diagnostic Network (NPDN) [PENDING]	via UC Davis		\$7,550
Octocula conservation [PENDING]	USFWS via DAWR		\$20,000

2 Creative/Scholarly Activities or Research

Table 2.5: Recent unfunded grant proposals.

Title	Source	Notes	Amount
Guam Insect Biodiversity	USFWS via DAWR		\$20,000
Octocula conservation	USFWS		\$18,000

2 Creative/Scholarly Activities or Research

Table 2.6: Staff support by my grants.

- | | |
|----|--------------------------------|
| 1 | Bob Bourgeois |
| 2 | Roger Brown (partially) |
| 3 | Roland Quitugua
(partially) |
| 4 | Ian Iriarte |
| 5 | Vincent Benavente |
| 6 | John Diego |
| 7 | Ken Leon Guerrero |
| 8 | Roland Cabrera |
| 9 | Derrick Diego |
| 10 | Marty Hara |
| 11 | Ken San Nicolas |
| 12 | Jessica Gross |
| 13 | Cris Crisostimo |
| 14 | Raymondo San Miquel |

2 Creative/Scholarly Activities or Research

Table 2.3: Active and pending grants in support of the Guam Coconut Rhinoceros Beetle Eradication Project.

Title	Source	Grant No.or UOG Account	Amount
Support for the Guam Coconut Rhinoceros Project	USDA Forest Service	11-DG-11052012- 101	\$227,000
Biological Control of the Coconut Rhinoceros Beetle	USDA APHIS	12-8515-1555- CA	\$40,000
Support for the Guam Coconut Rhinoceros Project	USDA Forest Service	11-DG-11052012- 101	\$150,000
Biological Control of the Coconut Rhinoceros Beetle [PENDING]	USDA APHIS		\$40,000

3 University and Community Service

3.1 Teaching

3.1.1 AG109 Insect World

I taught this course four times. My score on the student evaluations are consistently above average (Table 3.1).

Table 3.1: Student evaluation for AG109, *Insect World*.

Term	My Evaluation	College Average	University Average
Fall 2009	3.659	3.565	3.552
Spring 2011	3.986	3.519	3.617
Spring 2012	3.863	3.570	3.612
Spring 2013	na	na	na

3.1.2 Special Projects

McEllen Alfred and Sarah Taitano, both senior students, conducted a special project for credit under my guidance. They earned eubrey Moore to study hesperiids on Guam. They earned 1 course credit by doing research on Guam's hesperiids.

3.1.3 Annual Workshop for Micronesian Plant Quarantine Officers

I participated as an instructor in the annual one-week workshop sponsored by the Secretariat of the Pacific Community (SPC) and USDA-APHIS. This workshop trains plant protection quarantine officers from throughout Micronesia.

3.1.4 Environmental Science Thesis Committee: Trent Hamada

I served on an Environmental Science masters degree committee for Trent Hamada. Mr. Hamada did research on the effect of windspeed and direction on pollination of Guam's endemic cycad, *Cycas micronesica*, under the guidance of Dr. Thomas Marler.

3.2 Music

As an amateur horn player I play regularly, and often very badly, with the Guam Symphony Orchestra and occasionally with the Guam Territorial Band. I have played for UOG graduations and for concerts arranged by the UOG music department. In spring 2013, I played in a horn quartet in a UOG Music Department recital.

3.3 Conference on Island Sustainability

I served on the planning committee for the Island Conference on Island Sustainability in 2010, 2011 and 2012.

3.4 Micronesia Biosecurity Plan

I have been involved with the Micronesia Biosecurity Plan (MBP) since its inception. The MBP is being developed to mitigate an expected increase in invasive species associated with the Guam military buildup. A first draft of the MBP was written by federal agencies supported by a \$2.7M grant from the Department of Defense (DoD). In January, 2010, I made 2 presentations at an MBP organizational meeting: *Biological Invasion of Guam* and *Invasive Insects on Guam*. In 2011, I assisted the UOG Center for Island Sustainability in securing a DoD cooperative agreement (CA) which provides \$1.1M to UOG to provide a peer review of the MBP and to develop an implementation plan. I was named as CoPI with Dr. Frank Camacho on the original CA, but I have resigned from this position to concentrate more on my entomological interests. However, I am still involved as a reviewer and I recently helped out by building a private, secure website to facilitate sharing information among those working on the MBP.

3.5 Collaboration on CESU Rare Butterfly and Snails Survey Grant

I am collaborating with Dan Lindstrom, John Benedict, Frank Camacho, and Curt Fiedler (UOG Biology), Alex Kerr (UOG Marine Lab), Brent Holland and Dan Rubinoff (UH Manoa) on a DOD funded survey of rare butterflies and snails. My contribution is a literature review of *Hypolimnas octocula marianensis* for publication in Micronesica, design and maintenance of project website and development of butterfly camera traps.

3.6 Collaboration on Biocontrol of Cycad Aulacaspis Scale

I am working with Tom Marler on introduction of parasitoids for biocontrol of the *Aulacaspis yasumatsui*.

3 University and Community Service

3.7 Collaboration on EPSCOR Proposal

I have submitted many ideas to be incorporated into the EPSCOR proposal. I spent one and a half days at the PACSTEM meeting at the Hyatt discussing some of these ideas with colleagues.

3.8 University Technical Advisory Committee

I serve on UTAC as the representative for the College of Natural and Applied Sciences.

3.9 Undergraduate Curriculum Review Committee (UCRC)

In the April 2013 Faculty Elections, I was elected to serve on the UCRC.

1.2 Plan for Following Year

Aubrey Moore

Extension Entomologist and Associate Professor

Annual Evaluation Plan

June 26, 2013 through June 25, 2014

51% Extension and Community Activities

34% Creative/Scholarly Activity or Research

15% University and Community Service

Extension and Community Activities (51%)

Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, UoG colleagues, GovGuam employees and federal agency personnel. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

Planned Activities

- I will continue to develop and maintain an online presence for the UOG Cooperative Extension Service Agriculture and Natural Resources Program at <http://guaminsects.net/anr>. This site will document each call with client information, problem definition, specimen data, images, recommendations, and follow-up results. Privacy of confidential client information will be protected.
- I will continue to update the “Online Guide to Insect Pests in Micronesia” at http://guaminsects.net/uogces/kbwiki/index.php?title=List_of_Insects_and_Mites_Attacking_Crops_in_Micronesia. This resource lists all pests known to attack each crop on each Micronesian island.
- I will publish a field guide to common insects of Guam. This guide will include high quality images, and it will be available in print and on-line. The current version of this book is available at: <http://guaminsects.net/bookWriter/book.pdf>

Detection, Identification, and Control of Invasive Insect Species

Guam, being a small tropical island is susceptible to major economic and ecological damage from invasive insects. New pests arrive every year via transportation and trade links with North America, Asia and other Pacific Islands. If unmanaged, newly arrived pests typically undergo a population explosion during which they cause much damage. If new introductions are detected early enough, eradication may be possible. Otherwise, applied research needs to be done without delay to develop management strategies, often involving introduction of biocontrol agents. Arrival of invasive insect species and the severity of damage they may cause on Guam is unpredictable. During the past five years, I have detected over a dozen new insect pests on Guam. Dealing with the two most important pests, the cycad scale, *Aulacaspis yasumatsui*, detected in 2003 and the coconut rhinoceros beetle, *Oryctes rhinoceros*, detected in 2007 currently consumes over 50% of my time and effort.

Planned Activities

- Perform pest surveys aimed at early detection of invasive species. I am currently writing a proposal to support a survey of termites on Guam. At the request of the USDA Forest Service, I will be assisting the Guam Department of Agriculture to develop forest pest surveys as part of their Forest Health Program.
- Work with federal and local agencies to improve plant pest quarantine on Guam and within Micronesia.
- Participate in networks which facilitate sharing information on spread of pests among Pacific Islands (Pacific Islands Distant Diagnostics and Recommendation System (PIDDRS), Western Plant Diagnostics Network (WPDN), PestNet, etc.)
- Offer at least one "First Detector Training Workshop" each year. These workshops, designed by WPDN, teach participants about invasive species, how to detect them, and how to report them.
- Initiate rapid response when new invasive insect species are detected. This usually involves definitive identification, often requiring sending specimens to a taxonomic expert, a delimiting survey and damage assessment.
- Perform applied research leading to development of a management strategy specific to Guam for each new major pest that arrives. This management strategy may include development and implementation of an eradication plan, use of pesticides, and introduction of biocontrol agents. I am currently working with Dr. Marler and collaborators in Hawaii to introduce a parasitoid for biocontrol of the cycad scale. I am working with Dr. Jackson, a colleague in New Zealand, on biocontrol of the coconut rhinoceros beetle by auto-dissemination of an insect virus. I am working with Dr. Ambrose Alfiler (Philippines Coconut Authority) on an entomopathogenic fungus, *Metarhyzium anisopliae*, as a biocontrol agent for the rhino beetle. I am working with Dr. Reddy and Dr. Eric Jang (USDA-ARS-PBARC) on development of semiochemicals for the coconut rhinoceros beetle. I am working with ISCA Technologies on development of an attracticide for the rhino beetle based on their RB-SPLAT product.

Public Outreach

Planned Activities

- I will respond promptly to any requests from the media for information on insect pests and invasive species.
- I will be available for school visits and invitations to present information to civic groups, government agencies, etc.
- I plan further development of a website for ANR with the following features:
 - All ANR faculty and staff will be able to add content, including images
 - Internal information and confidential client information may be saved, but may only be viewed by ANR users
 - The site will contain print-on-demand copies of all fact sheets published by CNAS and CALS
 - The site will allow ANR to log all “extension calls”
- Fact sheets and pamphlets will be produced and updated as needed.

Regional Collaboration

Planned Activities

- I plan continued collaboration with colleagues in Hawaii, American Samoa, Marshall Islands, Federated States of Micronesia, Palau, and the Northern Marianas
- I plan to expand collaboration with colleagues in the Philippines.

Coordination of Extension Projects

Activities

Guam Coconut Rhinoceros Beetle Eradication Project.

- I will continue to act as PI for several federal and local grants which fund the project. About 20 part-time UOG employees are currently supported with these funds.
- I will continue to lead the efforts in applied research aimed at finding improved tactics with which to eradicate the rhino beetle from Guam.
- I will develop extension recommendations and control strategies for the rhino beetle should eradication prove impossible. Focus during this year will be on developing and testing a CRB integrated pest management plan for Guam.
- I will provide technical expertise and service in areas of database management, geographical information systems, and web site development and maintenance.

Western Plant Diagnostics Network (WPDN)

- As Guam coordinator for WPDN, I will attend annual meetings and attend monthly conference calls.
- I will organize at least one “First Detector Training” each year.

Annual Quarantine Workshop

- Each year, during Spring Break, I participate as an instructor in a one-week workshop sponsored by SPC and APHIS. This workshop trains plant protection quarantine officers from throughout Micronesia.

Creative/Scholarly Activity or Research (34%)

Applied Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project

Here are the objectives taken *verbatim* from the the plans of work for my CRB grants:

USFS FY13 grant; \$150,000

- Development of an Integrated Pest Management Program for Coconut Rhinoceros Beetle in Guam

APHIS FY12 Biocontrol Grant; \$40,000

- Obtain and Disseminate Virus Strains Which Kill Guam's Rhino Beetles (APHIS FY12 Biocontrol grant; \$40,000)
- Determine Why Previously Tested Viral Strains Failed to Kill Guam's Rhino Beetles (APHIS FY12 Biocontrol grant; \$40,000)

APHIS FY13 Biocontrol Grant; \$40,000; pending

- Estimate the Proportion of Guam's CRB Population Killed by the Biocontrol Agent, *Metarhizium majus*
- Develop DNA Profiles for CRB Populations in Asia and the Pacific with Respect to Virus Susceptibility

Development of Automated Digital Image Analysis for Insect Biodiversity Studies

I plan to complete this project, which was funded by a CNAS Research and Creative Activity Seed Grant. Working with project collaborators, I have analyzed three extensive series of insect samples provided by Tom Marler from his Rota biodiversity studies: pan traps, sticky traps, and Berlese funnel samples. Results have been prepared in the form of extensive lab reports. My goal is to prepare a methods paper for submission to the *American Entomologist*.

Planned Research Articles

I am preparing the following research articles and plan to submit them to peer reviewed journals within the next year:

Moore A. Mariana Eight Spot Butterfly, *Hypolimnas octocula marianensis*. [Draft targeting Micronesica. I am doing this review as part of my involvement in the CESU rare butterflies and snails project; about 30% complete]

Moore, A. and D. Bright. Three New Island Records for Bark Beetles (Curculionidae: Scolytinae) on Guam from a Single Coffee Berry Borer Trap. [Draft targeting Proceedings of the Hawaiian Entomological Society; 90% complete]

Moore, A. and R. H. Miller. The cycad blue butterfly, *Chilades panadava* (Lepidoptera: Lycaenidae), a new pest of cycads on Guam.[Draft targeting Proceedings of the Hawaiian Entomological Society; 90% complete]

Moore A. and J. Lopez, Jr. Increasing incidence of bed bugs on Guam.

Moore, A., F. Lee, R. Zack, T. Marler and A. Guerrero. Development of automated digital image analysis for insect biodiversity studies.

Moore A., T. Jackson, R. Quitugua and P. Bassler. Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), grubs develop in live coconut palm crowns on Guam.[Draft targeting the Florida Entomologist; 99% complete]

Moore A. Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), trap catch is maximum at full moon.

A. Moore, C. Apperson, J. McLaughlin and P Kirsch. Automated classification of female *Culex pipiens* (Diptera: Culicidae) and *Cx. quinquefasciatus* from optically-sensed wingbeat waveforms.[80% complete]

Curation of the Wilfred Leon Guerrero Insect Museum

The UOG insect collection, recently renamed as the Wilfred Leon Guerrero Insect Museum, is a valuable scientific resource for Guam and is often used as reference collection by the extension entomologist. I co-curate the collection with Dr. Ross Miller. The collection now has been moved into its own room. We are currently on building a digital catalog for the collection and catching up on a large backlog of unidentified specimens. One of my grants pays for a part-time collection technician and allows us to sponsor site visits by skilled taxonomists who are willing to help us identify specimens.

Planned Activities

- Complete a digital catalog of the collection. (Currently 90% complete; liquid collection yet to be organized)
- Migrate the catalog database from BioLink to a web-based application. The ScratchPad Drupal template developed by the Natural History Museum, London, England will probably be used. See <http://guaminsects.myspecies.info/>.
- Host visiting taxonomists who are willing to help clear up a backlog of unidentified specimens

Website Designed and Maintenance

Planned Activities

- I will continue to develop and maintain websites to facilitate storing and sharing extension and scientific information relevant to ANR's mission. I currently maintain:
 - <http://guaminsects.net/anr>
 - <http://guaminsects.myspecies.info>
 - <http://www.guaminsects.net/uogces/kbwiki/index.php>

- <http://www.guaminsects.net/gisac/index.php>
- <http://blog.guaminsects.net/>
- <http://micronesianinsects.lifedesks.org/>

Participation in Scientific Meetings

- I plan to attend the Entomological Collections Network meetings and Entomological Society of America meetings in Austin, Texas, November 10-13, 2013. I will be making an oral presentation entitled " Biological Control of Cycad Scale, Aulacaspis yasumatsui, Attacking Guam's Endemic Cycad, *Cycas micronesica*"
- I plan to attend the Bark and Ambrosia Beetle Academy at the University of Florida in May 2014. My application has been accepted.

University and Community Service (15%)

Community Involvement

I will continue to make myself available for formal and informal discussions of environmental issues on Guam. I have recently been involved in discussions regarding new Guam Pesticide Regulations, impacts of the military buildup on endangered butterflies, and the Micronesia Biosecurity Plan.

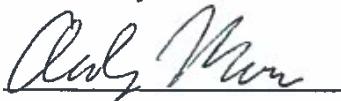
Guam Invasive Species Advisory Committee (GISAC)

I am a founding member and active participant in GISAC. Members of this committee are mostly biologists from the University, GovGuam and federal agencies. GISAC meets irregularly, about five times per year, to discuss invasive species activities on Guam and to provide advice to the Guam Invasive Species Council. I maintain a website for GISAC. This website is also used by the Western Micronesia Regional Invasive Species Committee (WM-RISC). The website can be found at <http://guaminsects.net/gisac/>.

Instruction

I will teach AG/BI 345, Introduction to Entomology, during the Fall 2013 term.

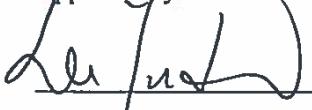
Submitted by:



Aubrey Moore, Ph.D.

Extension Entomologist/Assistant Professor

Approved by:



Lee S. Yudin, Ph.D.

Dean/Director, CNAS

Please see next page.

1.3 Evaluation

Please see next page.

University of Guam
Mangilao, Guam

FACULTY SALARY INCREMENT

AUBREY MOORE, Ph.D.
Faculty Member

ASSISTANT PROFESSOR
Rank or Title

06/26/2012 – 06/25/2013
Employment Period

III/14
Present Level/Step

CNAS/CES
College/Unit

ANR
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be
APPROVED/DISAPPROVED.


Victor T. Artero
Name

8/12/2013
Date

Interim Associate Director, CES
Position Title

Rationale:

Dr. Aubrey Moore continues to be among the most productive faculty members of CES, CNAS, as evidenced in his documented exemplary accomplishments of faculty roles. Below are examples of these accomplishments.

On Aubrey's primary role of Extension and Community Activities, addressing the needs of our clientele by providing diagnostic service, detection, identification, and control of invasive species continue to be the focal point of this endeavor. In addition, his public outreach efforts have been expanding and his regional involvement in addressing pest related issues ensures that Guam's concerns become a part of the cause for solution. Aubrey's role in Creative/Scholarly Activities or Research saw him securing additional grant funds to further applied research to provide workable solutions to dealing with Guam's deleterious Coconut Rhinoceros Beetle.

And for his accomplishments, Dr. Moore was recently informed that his application for promotion to Associate Professor was approved which take in effect this Fall 2013. Kudos to Dr. Moore for his achievements.

RECOMMENDATION OF DEAN/DIRECTOR

- I concur with the above recommendation.
 I do not concur with the above recommendation (see below).

Lee S. Yudin, Ph.D.
Dean/Director

11/14/13
Date

Rationale:

Concur w/ the evaluation. Dr. Moore
has shown sufficient evidence to be
allowed to receive his annual increment
for the period under review. Ld

The above recommendations have been discussed with me and my responses, if any, are as follows:

Aubrey Moore
Aubrey Moore, Ph.D.
Faculty Member

Oct. 15, 2013
Date

CERTIFICATION OF FUNDS

- Funds Available Funds Not Available

Nancy Cueto
Certifying Officer

Date

2 2013-2014

2.1 Report

Please see next page.

CFES 2014

Aubrey Moore, Ph.D.
Associate Professor / Extension Entomologist

July 27, 2014

CNAS/ANR
RECEIVED
7/28/14

I was hired by the University of Guam on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as extension entomologist with the academic rank of Assistant Professor. I am a faculty member of the Environmental Science Graduate Program and a member of the Western Pacific Tropical Research Center. At the end of the 2012 fall term I applied for tenure and promotion and received both.

This report documents my activities from June 2013 through the present. My current faculty role allocation is as follows:

- 51% Extension and Community Activities
- 34% Creative/Scholarly Activity or Research
- 15% University and Community Service

Note to Reader:

This report is available as an electronic document in PDF format on my website at <http://guaminsects.net/anr/content/cfes-2014-report-aubrey-moore>. Because this is not a public document, you will need to identify yourself to gain access by entering user name: **UogAdministrator** and password: **let_me_in**.

If you are reading the PDF version of the report, you will be able to follow hypertext links to documents I have referenced.

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Chapter 1

Extension and Community Activities

1.1 Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam and federal agency personnel. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

1.1.1 Insect Identification Service for USDA-APHIS / Guam Customs and Quarantine Agency

I am often called upon to identify insect specimens intercepted the Guam Customs and Quarantine Agency. USDA-APHIS has certified me for this service and has provided a very official looking badge to impress people with. (However, it is not quite as impressive as Dr. Millers bright red badge for getting onto the airport runways.)

1.1.2 Detection and Documentation of Invasive Species

I identify insects which are new invasive species at a rate of approximately one per month. The rate of detection of invasive species on Guam and other Micronesian

Islands is poorly documented and grossly under-reported. Few detections get published in the scientific literature.

My goal over the past year has been to establish a two step protocol for documenting new invasive species detections: in-house publication in a series of Guam New Invasive Species Alerts 2.4 followed by publication in a peer reviewed scientific journal. I have four new invasive species alerts but have only had time to publish one of these in the scientific literature.

1.1.3 Impediments

- My taxonomic skills are limited. I am attempting to educate myself by attending insect identification workshops. In May, 2014, the US Forest Service supported my attendance at the Bark Beetle and Ambrosia Beetle Academy at the University of Florida.
- Limited laboratory facilities. I do not have a laboratory equipped for insect diagnostics. I am very appreciative of the lab space (part of Dr. Wiecko's lab) assigned to me. Unfortunately, much of the equipment and supplies left behind by Dr. Muniappan and Dr. Reddy were removed from this lab. I am very much in need of a professional stereoscope with camera for identification of small insects. Both of the student microscopes (Lieca EZ4HD's) I purchased with my grants have been returned for repairs. I have asked USDA-APHIS to help by providing me with a used professional microscope with camera in return for the diagnostic services I provide to the Guam Plant Inspection Station. In addition to a microscope I need equipment for slide mounting small insects (scale insects, mealybugs, etc.) to enable identification. This requires a working fume hood and some specialized slide-making equipment.

1.2 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the collection and I work with Dr. Ross Miller to curate and catalog this collection.

To increase my knowledge of collection management, I attend the annual meetings of the Entomological Collections Network, which are typically held in conjunction with annual meetings for the Entomological Society of America.

I have a professional goal of building an online website to share all available information on Micronesian insects. This will include specimen level information for

the collection complete with digital images and literature references. I built a digital catalog for the collection using the BioLink Biodiversity Information Management System from CSIRO, Australia. The catalog currently contains 29,200 specimen records. BioLink is currently being redeveloped as an open source project (<http://code.google.com/p/biolink/>). I am an active collaborator in this project. In July 2012 I published an article entitled *Hosting a Biolink Database in the Amazon Web Services Cloud (EC2)* on the project's wiki (<http://code.google.com/p/biolink/wiki/BioLinkEC2>).

I have built and evaluated two websites for serving information on Micronesian insect biodiversity, including specimen level data from the collection. One is a Drupal content management system template called LifeDesk provided by the Encyclopedia of Life Project and the other is a similar template called ScratchPads provided by the Museum of Natural History in London. I am honored to have been selected as an advocate for ScratchPads as part of the project's Ambassadors program (<http://scratchpads.eu/locate-scratchpad-ambassadors>). Further information on my websites is provided in the Creative/Scholarly Activities section (2.6).

In March 2014 I travelled to Honolulu to attend the Biodiversity Collections Digitization in the Pacific workshop sponsored by the Integrated Digitized Biocollections (IDigBio). I made an oral presentation entitled Evaluation of a Scratchpad Template as an Online Database for the University of Guam Insect Collection at this workshop.

In May 2014 I met with Dr. Bob Foottit at the Canadian National Insect Collection in Ottawa to discuss progress and future directions for the UOG collection. Dr. Foottit is a member of the board of directors for the UOG Insect collection.

1.3 Guam Coconut Rhinoceros Beetle Eradication Project

The coconut rhinoceros beetle (CRB) was first detected on Guam in the Tumon Beach hotel area on September 11, 2007. CRB is a very serious pest of coconut palms. Adult beetles may kill coconuts and other palms when they bore into the crowns to feed on sap. When CRB invaded Palau during the Second World War, it killed about half of all coconuts through the islands and totally exterminated the coconut palm from some of them. A delimitation survey indicated that the Guam infestation was limited to Tumon Bay and the adjacent Faifai Beach. In consultation with the Guam Department of Agriculture (GDOA), USDA-APHIS, and USDA-Forest Survey, it was decided to launch an eradication project.

I wrote the original eradication plan (available on-line at <http://guaminsects>.

net/uogces/kbwiki/index.php?title=Coconut_Rhinoceros_Beetle_Eradication_Plan) and this was funded by USDA and local funds. USDA provided funds under the condition that the project was to be run under an Incident Command System with the USDA-APHIS Guam Port Director as the federal commander, and the GDOA Director, or designee, as the local commander.

My original role was to provide scientific/technical support for the project, with the Guam Department of Agriculture (GDOA) providing project management with assistance from USDA-APHIS and USDA-Forest Service. However, it soon became apparent that GDOA had serious bureaucratic impediments which prevented hiring staff and procuring supplies and equipment within a reasonable time frame. The eradication project directors, with the consent of the Dean, agreed to run project staffing, procurement, and fiscal management through the University. As a result, my role was expanded to include much of the project management. I am currently managing two grants which fund the project and supervise about 15 temporary employees. Report writing on current grants and proposal writing to keep the project in business occupies much of my time.

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command team set up by APHIS. Later, we were both added to a national technical working group for CRB. My activities in support of the Hawaii CRB Eradication project are detailed in the Regional Collaboration section 1.8.3.

1.3.1 Activities:

1. **Biweekly Planning Meetings.** This project is run as an incident command system. I attend biweekly planning meetings as a program manager.
2. **Conference Calls.** These teleconferences are with stakeholders and advisers in USDA APHIS and USDA Forest Service. These agencies are funding the project. Until recently calls were biweekly. They are now monthly.
3. **Grant Writing.** During the past 2 years, the Guam CRB Eradication Project has been almost entirely funded from 12 grants for which I wrote proposals and act as principal investigator. These grants are listed in the Creative / Scholarly Activity section.
4. **Report Writing.** All grants supporting the Guam CRB Eradication Project require regular reporting.

5. **Project Websites.** I have endeavored to share and archive data and information associated with the Guam CRB Eradication Project on-line. Prior to May 2009, I used a wiki site at http://www.guaminsects.net/uogces/kbwiki/index.php?title=Oryctes_rhinoceros. Afterwards, I used a Drupal site at <http://www.guaminsects.net/anr/category/miscellaneous/coconut-rhinoceros-beetle>
6. **Project Database.** Trapping data from a network of about 1200 traps, detections of CRB grubs or adults, and observations of CRB defoliation and bore holes are entered daily into a web-based georeferenced MySQL database which I designed. Data from this database is publicly accessible from a web page at <http://www.guaminsects.net/anr/content/public-access-data-collected-guam-cocon>. Links on this page enable the user to view trap catch data as a spatiotemporal display using a Google Earth animation or a chart of monthly totals. I use this system to produce monthly surveillance reports.
7. **Scientific/technical Support.** I do applied research in support of the Guam CRB Eradication Project. Results of this research is provided in a series of technical reports.
8. **Collaboration.** I have formed two collaborative research groups to do applied research aimed at controlling CRB damage. Dr. Sean Marshall and Dr. Trevor Jackson at AgResearch New Zealand collaborate with me on biological control using *oryctes nudivirus* (OrNV) and CRB population genetics. Dr. Matthew Siderhurst and Dr. Eric Jang of USDA-ARS-PBARC collaborate with me on CRB trap improvement.

1.3.2 Impediments

- I am sitting on a mountain of applied research results from the CRB program. Some of this needs to get into the scientific literature. My heavy workload, especially during terms when I am teaching, does not permit enough time to prepare research results for publication in scientific journals.

1.4 Western Plant Diagnostics Network

I am the UOG coordinator for WPDN. This organization provides financial support for ANR's Plant Diagnostic Laboratory, offers First Detector Training workshops, and organizes identification workshops for important pest groups. As coordinator,

Table 1.1: Contributions to the Pacific Pest Detector Newsletter

December 2013	coconut termite
March 2014	spotted cucumber beetle
March 2014	brown marmorated stink bug
June 2014	castor hairy caterpillar

I am required to organize First Detector Training workshops, attend monthly conference calls, attend annual meetings, and provide reports. During the past year, I have made four contributions to the Pacific Pest Detector Newsletter.

1.5 Guam Invasive Species Advisory Committee (GISAC)

I am an active, founding member of this informal group of Guam's biologists which meets irregularly about 6 times per year to discuss invasive species and what can be done to keep them out and mitigate the effects of those that do invade the island. I worked with Dr. Russell Campbell and Diane Vice to develop an emergency response plan for invasive species detected on Guam.

A wiki site which I built for GISAC was quickly adopted by the Western Micronesia Regional Invasive Species Council at http://guaminsects.net/gisac/index.php?title=Main_Page.

1.6 Public Outreach (Guest lectures, presentations, interviews)

During the reporting period I was interviewed numerous times by newspaper reporters, radio talk show hosts, and television news reporters (Table 1.2). Most, but not all involved questions about the Guam coconut rhinoceros beetle eradication project. I produced several fact sheets and articles for public print media during my two years as extension entomologist year and also published a lot of content on various websites. I have evaluated several current technologies for building a web presence for the Agriculture and Natural Resources Unit and the Drupal content management system seems to be a good fit. This allows us to publish information for public access while keeping some documents private for internal use only. My print and online output are discussed in more detail in the Creative/Scholarly Activity section.

1.7 Public Outreach (Internet)

I maintain a website for the the UOG Cooperative Extension Service's Agriculture and Natural Resources Program at <http://guaminsects.net/ANR>. I frequently post blog articles of public interest to this site (Table 1.3). I also maintain a website at <http://guaminsects.myspecies.info> which is intended to facilitate sharing information on insects in Micronesia. I frequently submit blog articles to this website which are of interest to entomologists (Table 1.4).

1.8 Regional Collaboration

1.8.1 Regional Invasive Species Council Website

I maintain a website for the Western Micronesia Regional Invasive Species Council (RISC) at <http://www.guaminsects.net/gisac/>. I attend RISC meetings whenever they are held on Guam and I make presentations at these meetings.

1.8.2 Insect Diagnostics for Micronesia

I am often contacted with requests for help with identifying pests from throughout Micronesia and suggesting solutions to the problems they cause. I expect this workload to increase because the number of practicing PhD level entomologists in Micronesia has dropped from 9 to 3 within the last decade. During thwe past year, I assisted in documenting the discovery of the coconut termite on Kosraie and the castor hairy caterpillar on Saipan.

1.8.3 Support for the Hawaii Coconut Rhinoceros Beetle Eradication Project

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command system (ICS) team set up by APHIS. Later, we were both added to a national technical working group (TWG) for CRB. I built and maintain an online, full-text bibliographic for use by the TWG at http://guaminsects.myspecies.info/CRB_biblio.

Frequent requests for scientific/technical information from the ICS, TWG and Hawaii Department of Agriculture (several queries per week) has significantly increased my workload over the past several months.

Table 1.2: Public outreach activities since 2013-06-01.

N	Date	Title	Venue
1	2014-06-11	Knowledge@Guam Initiative Data Modernization Conference	Pacific Star Hotel, Guam
2	2014-04-21	Presentation on coconut rhinoceros beetle for Guam EPA pesticide training workshop	Palm Ridge Hotel, Guam
3	2014-04-14	UOG Pacific STEM Coalition Meeting	Hyatt Hotel, Guam
4	2013-07-08	presentation on CRB for CARIPAC scholars	Guam Plant Inspection Facility, Tiyan
5	2013-07-06	CRB Workshop for General Public	UOG
6	2013-06-28	talk on forest insect pests for Pacific island foresters	Dept. of Ag., Guam
7	2013-06-27	CRB Workshop for Professionals	Tamuning, Guam
8	2013-06-25	CRB Workshop for Professionals	Tamuning, Guam
9	2013-06-07	Invasive species talk for Indigenous Fellows Institute	Guam Community College

Table 1.3: Blog posts to guaminsects.net/anr since 2013-06-01.

N	Date	Title
1	2014-06-12	PNC News Story: DoAG and UOG Team Up to Get Rid of the Little Fire Ant
2	2014-06-11	Visualization of Coconut Rhinoceros Beetle Trap Data
3	2014-03-31	Public opinion on invasive species issues
4	2014-03-26	iDigBio presentation - Honolulu, March 2014

Continued on next page

Table 1.3: (Continued)

N	Date	Title
5	2014-02-20	PNC News Story: Guam is Running Out of Options to Stop the Spread of Rhino Beetles and Save Guam's Coconut Trees
6	2014-02-10	CNN Article by Matt Smith: Meet the beetles: Hawaii mobilizes to fight bug invasion
7	2014-02-09	Pacific Daily News Nespaper Article: Mayors voice concerns over rhino beetle
8	2014-02-05	Pacific News Center Story: University of Guam Experts Help Hawaii with Rhino Beetles
9	2014-01-22	KITV4 Hawaii TV Story: Experts Brought to Hawaii to Battle the Rhino Beetle
10	2014-01-10	iNaturalist: Guam CRB Citizen Science
11	2014-01-10	KUAM News Story: Invasive species threaten local crops
12	2014-01-10	Coconut Rhinoceros Beetle Infestation Discovered at Hickam Air Force Base, Oahu, Hawaii
13	2014-01-10	Video: Little Fire Ant in Hawaii
14	2014-01-09	Relative Attractiveness of White and Ultraviolet Light Emmitting Diodes for Rhino Beetles
15	2014-01-09	Arnold Hara's Rhino Beetle Images Taken During his Trip to Guam
16	2014-01-04	No Rhino Pamphlet
17	2014-01-01	Pacific News Center Includes Invasive Species Issues in Top 10 Stories of 2013
18	2013-11-18	Guamanians Advised to Minimize Mosquito Bite Risk After Chikungunya Virus Reported on Yap
19	2013-11-18	Veto override will limit pesticide use, GMO crops on Hawaiian island
20	2013-09-16	Online Resources for Mosquito Surveillance on Guam

Continued on next page

Table 1.3: (Continued)

N	Date	Title
21	2013-08-28	Pacific News Center Video and Story by Clynt Ridgell: Idyllic Coconut Treeline at Ypao Beach Gone Because of Rhino Beetle
22	2013-08-20	Pacific News Article by Josh Tyquiengco: Little Fire Ant Infestation Found in 12-14 Sites on Guam; No Funding To Eradicate
23	2013-08-13	CRB Meeting
24	2013-08-13	CRB Meeting
25	2013-08-13	CRB MEETING 10
26	2013-08-13	default format
27	2013-08-13	plain text
28	2013-08-13	utf8 - html
29	2013-08-13	test monday 11:30
30	2013-08-12	PNC News: Hotel Rhino: A new trap that lets rhino beetles check in, but not check out
31	2013-08-06	Little Fire Ant PSAs
32	2013-07-07	KUAM TV News: Rhino Beetle Workshop Held at UOG
33	2013-07-05	Radio New Zealand Report on Guam Little Fire Ant Situation
34	2013-07-05	Trifold Flyer: Coconut Rhinoceros Beetle Control Tips
35	2013-07-05	Canned Edible Rhino Beetle Larvae
36	2013-07-05	CRB Life Cycle Diagram
37	2013-07-05	PDN Newspaper Article: Little fire ants are a nuisance, being tracked on island
38	2013-07-02	Guam Agricultural Resource Listing Flyer
39	2013-06-30	On-line References for Forest Insect Pests on Guam
40	2013-06-19	Weather Station on the Roof of the Agriculture and Life Sciences Building at the University of Guam

Continued on next page

Table 1.3: (Continued)

N	Date	Title
41	2013-06-17	Weather Station at the University of Guam Agricultural Experiment in Yigo is Back On Line
42	2013-06-13	PDN Newspaper Article: Steps to Prevent Invasive Species

Table 1.4: Blog posts to guaminsects.myspecies.info since 2013-06-01.

N	Date	Title
1	2014-06-09 10:09	Possible Observation of the Caterpillar of a Rare Appias sp. Butterfly
2	2013-12-16 00:28	Guam New Invasive Species Alert No. 2013-01: Eggplant Mealybug
3	2013-12-14 13:09	Notes for Coccidohystrix insolita
4	2013-12-13 20:39	Preprinting Specimen Labels
5	2013-12-10 19:28	Dipteran Leafminer on Noni (Morinda) Leaves
6	2013-11-11 03:54	Cycad Biocontrol Presentation - Austin, Texas
7	2013-10-13 17:07	Insect Label Generator
8	2013-10-10 07:48	Harvest of Fear
9	2013-10-07 16:17	Interesting article on crab lice
10	2013-10-07 10:52	Interesting article on a very rare stick insect
11	2013-09-16 07:35	Online Resources for Mosquito Surveillance on Guam
12	2013-06-13 08:05	Liorhyssus hyalinus, hyaline grass bug, attacking lettuce

Chapter 2

Creative/Scholarly Activities or Research

2.1 Refereed Scientific Journal Articles

Table 2.1: Refereed journal articles 2013 - 2014.

N	Citation	Type
1	Fisher, N, Moore A, Brown B, Purcell M, Taylor G, Salle J. 2014. Two new species of <i>Selitrichodes</i> (Hymenoptera: Eulophidae: Tetrastichinae) inducing galls on <i>Casuarina</i> (Casuarinaceae). <i>Zootaxa</i> . 3790:534-542. URL: http://biotaxa.org/ Zootaxa/article/view/zootaxa.3790.4.2/7933	Journal Article

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Table 2.1: (Continued)

N	Citation	Type
2	Moore, A, Watson G, Bamba J. 2014. First record of eggplant mealybug, <i>Coccidohystrix insolita</i> (Hemiptera: Pseudococcidae), on Guam: Potentially a major pest. Biodiversity Data Journal. 2 URL: http://biodiversitydatajournal.com/articles.php?id=1042	Journal Article
3	Marler, TE, Moore A, Miller RH. 2013. Vertical stratification in predation of armored scales on <i>Cycas micronesica</i> seedlings. HortScience. 48(1):60-62. URL: http://www.wptrc.org/userfiles/file/Marler/stratification%20reprint.pdf	Journal Article
4	Cave, RD, J-T C, Kumashiro B, Marler TE, Miles J, Moore A, Muniappan R, Watson GW. 2013. Status and biological control of Cycad Aulacaspis scale. Biocontrol News and Information. 34(1):1-4. URL: https://www.researchgate.net/publication/235793052_Status_and_biological_control_of_Cycad_Aulacaspis_scale/file/d912f5138f398cf49e.pdf	Journal Article

2.2 Presentations at Professional Meetings

Table 2.2: Presentations 2013 - 2014.

N	Citation	Type
1	Moore, A. 2014. Insects Attacking <i>Serianthes nelsonii</i>. 2014 Island Sustainability Conference.	Conference Paper (oral presentation)
2	Moore, A, Quitugua R. 2014. Overview of the Guam coconut rhinoceros beetle eradication project. Hawaii CRB Incident Command Meeting. URL: http://guaminsects.net/presentations/CRB-Hawaii-ICS-Jan-2014.pdf	Conference Paper (oral presentation)
3	Moore, A. 2014. Biological invasion of forests on Guam and other islands of Micronesia. 65th Western Forest Insect Work Conference . <i>NOTE: oral presentation</i>	Conference Paper
4	Moore, A. 2014. Evaluation of a Scratchpad template as an online database for the University of Guam insect collection. iDigBio Biodiversity Collections Digitization in the Pacific Workshop. <i>NOTE: oral presentation</i>	Conference Paper

Continued on next page

Table 2.2: (Continued)

N	Citation	Type
5	<p>Moore, A, Marler T, Miller RH, Yudin LS. 2013. Biological Control of Cycad Scale, <i>Aulacaspis yasumatsui</i>, Attacking Guam's Endemic Cycad, <i>Cycas micronesica</i>. 4th International Symposium on Biological Control. URL: http://guaminsects.net/anr/sites/default/files/Mooreetal.-2013-BiologicalControlofCycadScale,Aulacaspisyasumatsui,AttackingGuam'sEndemicCycad,Cycasmicronesica.pdf</p>	Conference Paper (abstract)
6	<p>Moore, A, Miller RH, Marler TE. 2013. Biological control of cycad scale, <i>Aulacaspis yasumatsui</i>, attacking Guam's endemic cycad, <i>Cycas micronesica</i>. Entomological Society of America Annual Meeting.</p>	Conference Paper (oral presentation)
7	<p>Moore, A, Miller RH, Marler TE. 2013. A coalition of invasive species attacks Guam's native cycads. Entomological Society of America Annual Meeting.</p>	Conference Paper (poster presentation)

2.3 Technical Reports Documenting Applied Research in Support of the Guam Coconut Rhinoceros Beetle Project

Table 2.3: CRB Technical Reports 2013 - 2014.

N	Citation	Type
1	Moore, A. 2014. CRB Sanitation at the University of Guam Yigo Agricultural Experiment Station. URL: http://guaminsects.net/anr/sites/default/files/2014-06-26-YigoSanitation.pdf	Report (CRB Technical Report)
2	Marshall, S, Moore A. 2014. Hawaii beetle dissections. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-17A.pdf	Report (CRB Technical Report)
3	Marshall, S, Moore A. 2014. DNA analysis of Hawaii CRB. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-02-12.pdf	Report (CRB Technical Report)
4	Moore, A. 2014. Cypermethrin applied to coconut palm crowns as a prophylactic treatment for prevention of CRB damage. URL: http://guaminsects.net/anr/sites/default/files/crownSpray.pdf	Report (CRB Technical Report)
5	Moore, A. 2014. Relative attractiveness of white and ultraviolet light emitting diodes plus oryzcalure. URL: http://guaminsects.net/anr/sites/default/files/LEDcolor_0.pdf	Report (CRB Technical Report)

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Table 2.3: (Continued)

N	Citation	Type
6	Moore, A. 2014. Chicken wire escape test. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-12A_0.pdf	Report (CRB Technical Report)
7	Moore, A. 2014. Plastic top catch test. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-12B.pdf	Report (CRB Technical Report)
8	Moore, A. 2014. Chicken wire vs plastic top. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-15.pdf	Report (CRB Technical Report)
9	Moore, A. 2014. Minibucket test. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-16.pdf	Report (CRB Technical Report)
10	Moore, A. 2014. Minibucket escape test. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-17.pdf	Report (CRB Technical Report)

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Table 2.3: (Continued)

N	Citation	Type
11	Moore, A. 2014. CRB mitigation for conservation of rare snails and butterflies at Haputo Beach. URL: http://guaminsects.net/anr/sites/default/files/2014-02-17Haputo.pdf	Report (CRB Technical Report)
12	Moore, A. 2014. CRB heat tolerance. URL: http://guaminsects.net/anr/content/2014-02-19-crb-heat-tolerance	Report (CRB Technical Report)
13	Moore, A. 2014. CRB dispersal by flight. URL: http://guaminsects.net/anr/content/2014-02-19a-crb-dispersal-flight	Report (CRB Technical Report)
14	Moore, A. 2014. CRB rearing. URL: http://guaminsects.net/anr/sites/default/files/CRBRearing_0.pdf	Report (CRB Technical Report)
15	Moore, A. 2014. APHIS biocontrol semiannual report. URL: http://guaminsects.net/anr/sites/default/files/CRB2014-05-04_0.pdf	Report (CRB Technical Report)

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Table 2.3: (Continued)

N	Citation	Type
16	Moore, A, Quitugua R. 2014. Bird net escape test. URL: http://guaminsects.net/anr/sites/default/files/BirdNet.pdf	Report (CRB Technical Report)
17	Moore, A, Quitugua R. 2014. Yigo barrel traps: trap catch comparison between pan and minibucket traps. URL: http://nbviewer.ipython.org/github/aubreymoore/YigoBarrels/blob/master/YigoBarrels.ipynb	Report (CRB Technical Report)
18	Moore, A. 2013. Development of barrel traps. URL: http://guaminsects.net/anr/sites/default/files/barrelTraps.pdf	Report (CRB Technical Report)
19	Moore, A. 2013. Improved pheromone traps for coconut rhinoceros beetle. URL: http://guaminsects.net/anr/sites/default/files/improvedPheromoneTraps.pdf	Report (CRB Technical Report)

2.4 Guam New Invasive Species Alert

Table 2.4: Guam New Invasive Species Alerts 2013 - 2014.

N	Citation	Type
1	Moore, A. 2014. Brown Marmorated Stink Bug <i>Halyomorpha halys</i> (Stal 1855) (Hemiptera: Pentatomidae). URL: http://guaminsects.net/anr/sites/default/files/brownMarmoratedStinkBug.pdf	Report (Guam New Invasive Species Alert)
2	Moore, A, Bamba J. 2014. Spotted Cucumber Beetle (Southern Corn Rootworm) <i>Diabrotica undecimpunctata</i> (Coleoptera: Chrysomelidae). URL: http://guaminsects.net/anr/sites/default/files/spottedcucumberbeetle.pdf	Report (Guam New Invasive Species Alert)
3	Route, A, Moore A. 2014. Castor Hairy Caterpillar <i>Olepa ricini</i> (Fabricius, 1775) (Lepidoptera: Arctiidae). URL: http://guaminsects.net/anr/sites/default/files/castorhairycaterpillar_0.pdf	Report (Guam New Invasive Species Alert)
4	Moore, A, Bamba J. 2013. Eggplant Mealybug <i>Coccidohystrix insolita</i> (Green 1908) (Hemiptera: Pseudococcidae). URL: http://guaminsects.net/anr/sites/default/files/eggplantMealybug.pdf	Report (Guam New Invasive Species Alert)

Table 2.5: Miscellaneous reports.

Citation	Type
1 Moore, A. 2014. The Mariana Eight Spot Butterfly, <i>Hypolimnas octocula marianensis</i> , and the Mariana Wandering Butterfly, <i>Vagrans egistina</i> of the Mariana Islands, Micronesia. Federal Candidate Species on Guam.	Report

2.5 Miscellaneous Reports

2.6 Web Sites Designed and Maintained by Me

For the past five years, I have been searching for the “right” technology for providing on-line extension information. The features I want include:

- Ease of use, including immediate, on-line editing, so that colleagues and collaborators can create content
- Ability to display digital images at several resolutions
- Full text search
- Methods for handling on-line and offline references
- Fine grained security which protects client confidentiality and allows for both protected, internal and public information sharing

My current technology of choice is Drupal, a free, open source contents management system.

2.6.1 ANR Web Site.

Home page: <http://guaminsects.net/anr>

This Drupal site is intended to facilitate sharing both internal and external information generated by the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. This site is currently being used heavily by the Guam CRB Eradication Project.

2.6.2 Insects of Guam Web Site

Home page: <http://guaminsects.myspecies.info>

This Drupal site is being evaluated for sharing information on Micronesian insects. Information will include specimen level information from the UOG insect collection complete with digital images and literature references. It was built using a template developed by the Scratchpad project <http://scratchpads.eu/> is sponsored by the European Institute of Distributed Taxonomy (EDIT) and the Natural History Museum in London .

2.6.3 Micronesia Biosecurity Plan Review Web Site

Home page: MBP.GuamInsects.net

This is a secure, private Drupal site developed to facilitate sharing information among those reviewing the Micronesia Biosecurity Plan.

2.6.4 Knowledgebase Wiki for the UOG Cooperative Extension

Home page: <http://www.guaminsects.net/uogces/kbwiki/index.php>

This was my first attempt at building an extension website to facilitate collaborative content creation. Digital copies of all of ANR's pest fact sheets can be found on this site. There is also a list of insect pests found on all major crops grown in Micronesia. I stopped maintaining this site in May, 2009 because the ANR site built with Drupal has more of the features I need.

2.6.5 Western Micronesia Regional Invasive Species Council Wiki

Home page: <http://www.guaminsects.net/gisac/index.php>

Originally built for the Guam Invasive Species Advisory Council, this site was quickly adopted for sharing regional information on invasive species by the Western Micronesia Regional Invasive Species Council.

2.6.6 Guam Insects Blog Site

Home page:<http://blog.guaminsects.net/>

I ran into recurring technical problems with this site which uses the WordPress

content management system and have more or less abandoned development and maintenance.

2.6.7 Life Desk Site for Micronesian Insects

Home page: <http://micronesianinsects.lifedesks.org/>

This site uses a Drupal template being developed by the Encyclopedia of Life Project. I evaluate it for sharing information on Micronesian insects, but decided that the Scratchpad template had a better feature set for what I wanted to do.

2.7 Grants

I managed six grants during the reporting period (Table ??). A Farm Bill suggestion to support CRB trap development submitted with my chemical ecology colleagues, Eric Jang (USDA-ARS-PBARC) and Matt Siderhurst (Eastern Mennonite University), was not funded.

My grants supported 14 staff positions (Table 2.7).

Table 2.6: Grants with an end date later than June 1, 2013.

	Title	grantID	startDate	endDate	Budget
1	CRB FS 2011	11-DG-11052012-101	2011-05-23	2013-12-31	\$200000
2	CRB Biocontrol 2012	12-8515-1555-CA	2012-06-01	2014-05-31	\$40000
3	WPDN 2013	201303063-09	2013-04-11	2014-06-30	\$7550
4	Management of the CRB	11-DG-11052021-101	2013-07-01	2015-06-30	\$150000
5	CRB Biocontrol 2013	13-8515-1555-CA	2013-09-01	2015-08-31	\$40000
6	Octocula		2014-05-13	2014-09-30	\$21000

2.7.1 US Forest Survey Program Review

During May 2014, officials from the US Forest Survey paid a visit to Guam to review performance on grants they have given to UoG over the past five years. A summary of my activities in support of these grants is available online at <http://guaminsects.net/anr/content/materials-forest-service-review-team>

Table 2.7: Staff supported by my grants.

1	Bob Bourgeois
2	Roger Brown (partially)
3	Roland Quitugua (partially)
4	Ian Iriarte
5	Vincent Benavente
6	John Diego
7	Ken Leon Guerrero
8	Roland Cabrera
9	Derrick Diego
10	Marty Hara
11	Ken San Nicolas
12	Jessica Gross
13	Cris Crisostimo
14	Raymondo San Miquel

2.2 Plan for Following Year

Please see next page.

Chapter 3

University and Community Service

3.1 Participation in the Good to Great Initiative

I participated in preparation of Good to Great reports for three entities:

- the Cooperative Extension Service
- the Agriculture and Life Sciences faculty
- the Environmental Sciences graduate faculty

3.2 Teaching

3.2.1 AG109 Insect World

I taught this course four times. My score on the student evaluations are consistently above average (Table 3.1).

Table 3.1: Student evaluation for AG109, *Insect World*.

Term	My Evaluation	College Average	University Average
Fall 2009	3.659	3.565	3.552
Spring 2011	3.986	3.519	3.617
Spring 2012	3.863	3.570	3.612
Spring 2013	3.659	3.554	3.627

3.2.2 AG/BIO 345 General Entomology

Teaching this course was not part of my plan of work. AG/BIO 345 was part of Dr. Reddy's teaching workload prior to his resignation. The course was assigned to Dr. ????, but she left the University unexpectedly for health reasons. Teaching this course for the first time required a lot of preparation in addition to presentation of two lectures and a three hour laboratory session each week. This unexpected overload impeded my progress on planned activities.

Table 3.2: Student evaluation for AG/BI-345, *General Entomology*.

Term	My Evaluation	College Average	University Average
Fall 2014	3.875	3.522	3.586

3.3 Music

As an amateur horn player I play regularly, and often very badly, with the Guam Symphony Orchestra and occasionally with the Guam Territorial Band. I have played for UOG graduations and for concerts arranged by the UOG music department. In spring 2013, I played in a horn quartet in a UOG Music Department recital.

3.4 Micronesia Biosecurity Plan

I have been involved with the Micronesia Biosecurity Plan (MBP) since its inception. The MBP is being developed to mitigate an expected increase in invasive species associated with the Guam military buildup. A first draft of the MBP was written by federal agencies supported by a \$2.7M grant from the Department of Defense (DoD). In January, 2010, I made 2 presentations at an MBP organizational meeting: *Biological Invasion of Guam* and *Invasive Insects on Guam*. In 2011, I assisted the UOG Center for Island Sustainability in securing a DoD cooperative agreement (CA) which provides \$1.1M to UOG to provide a peer review of the MBP and to develop an implementation plan. I was named as CoPI with Dr. Frank Camacho on the original CA, but I have resigned from this position to concentrate more on my entomological interests. However, I am still involved as a reviewer and I recently helped out by building a private, secure website to facilitate sharing information among the working on the MBP. I also served as a peer reviewer for the MBP.

3.5 Collaboration on CESU Rare Butterfly and Snails Survey Grant

I am collaborating with Dan Lindstrom, John Benedict, Frank Camacho, and Curt Fiedler (UOG Biology), Alex Kerr (UOG Marine Lab), Brent Holland and Dan Rubinoff (UH Manoa) on a DOD funded survey of rare butterflies and snails. My contribution is a literature review of *Hypolimnas octocula marianensis* for publication in Micronesica, design and maintenance of project website and development of butterfly camera traps.

3.6 Collaboration on Biocontrol of Cycad Aulacaspis Scale

I am working with Tom Marler on introduction of parasitoids for biocontrol of the *Aulacaspis yasumatsui*.

3.7 Collaboration on EPSCOR Proposal

I have submitted many ideas to be Incorporated into the EPSCOR proposal. I attended several meetings to discuss some of these ideas with colleagues.

3.8 University Technical Advisory Committee

I serve on UTAC as the representative for the College of Natural and Applied Sciences.

3.9 Undergraduate Curriculum Review Committee (UCRC)

In the April 2013 Faculty Elections, I was elected to serve on the UCRC. I attend monthly meetings as a member of this committee.

Aubrey Moore

Extension Entomologist and Associate Professor

Annual Work Plan

June 1, 2014 through May 31, 2015

51% Extension and Community Activities

34% Creative/Scholarly Activity or Research

15% University and Community Service

Extension and Community Activities (51%)

Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, UoG colleagues, GovGuam employees and federal agency personnel. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

Planned Activities

- I will continue to develop and maintain an online presence for the UOG Cooperative Extension Service Agriculture and Natural Resources Program at <http://guaminsects.net/anr>. This site will document each call with client information, problem definition, specimen data, images, recommendations, and follow-up results. Privacy of confidential client information will be protected.
- I will continue to update the “Online Guide to Insect Pests in Micronesia” at http://guaminsects.net/uogces/kbwiki/index.php?title=List_of_Insects_and_Mites_Attacking_Crops_in_Micronesia. This resource lists all pests known to attack each crop on each Micronesian island.
- I will publish a field guide to common insects of Guam. This guide will include high quality images, and it will be available in print and on-line. The current version of this book is available at: <http://guaminsects.net/bookWriter/book.pdf>

Detection, Identification, and Control of Invasive Insect Species

Guam, being a small tropical island is susceptible to major economic and ecological damage from invasive insects. New pests arrive every year via transportation and trade links with North America, Asia and other Pacific Islands. If unmanaged, newly arrived pests typically undergo a population explosion during which they cause much damage. If new introductions are detected early enough, eradication may be possible. Otherwise, applied research needs to be done without delay to develop management strategies, often involving introduction of biocontrol agents. Arrival of invasive insect species and the severity of damage they may cause on Guam is unpredictable. During the past five years, I have detected over a dozen new insect pests on Guam. Dealing with the two most important pests, the cycad scale, *Aulacaspis yasumatsui*, detected in 2003 and the coconut rhinoceros beetle, *Oryctes rhinoceros*, detected in 2007 currently consumes over 50% of my time and effort.

Planned Activities

- I will continue to document new island records for insects on Guam. I will continue to add to the Guam New Invasive Species Alerts series and strive to get new records into the scientific literature via refereed journals.
- Work with federal and local agencies to improve plant pest quarantine on Guam and within Micronesia.
- Participate in networks which facilitate sharing information on spread of pests among Pacific Islands (Pacific Islands Distant Diagnostics and Recommendation System (PIDDRS), Western Plant Diagnostics Network (WPDN), PestNet, etc.)
- Offer at least one “First Detector Training Workshop” each year. These workshops, designed by WPDN, teach participants about invasive species, how to detect them, and how to report them.
- Initiate rapid response when new invasive insect species are detected. This usually involves definitive identification, often requiring sending specimens to a taxonomic expert, a delimiting survey and damage assessment.
- Perform applied research leading to development of a management strategy specific to Guam for each new major pest that arrives. This management strategy may include development and implementation of an eradication plan, use of pesticides, and introduction of biocontrol agents. I am currently working with Dr. Marler and collaborators in Hawaii to introduce a parasitoid for biocontrol of the cycad scale. I am working with Dr. Jackson, a colleague in New Zealand, on biocontrol of the coconut rhinoceros beetle by auto-dissemination of an insect virus. I am working with Dr. Ambrose Alfiler (Philippines Coconut Authority) on an entomopathogenic fungus, *Metarhyzium majus*, as a biocontrol agent for the rhino beetle. I am working with Dr. Reddy and Dr. Eric Jang (USDA-ARS-PBARC) on development of semiochemicals for the coconut rhinoceros beetle. I am working with ISCA Technologies on development of an attracticide for the rhino beetle based on their RB-SPLAT product.

Public Outreach

Planned Activities

- I will respond promptly to any requests from the media for information on insect pests and invasive species.
- I will be available for school visits and invitations to present information to civic groups, government agencies, etc.
- I plan further development of a website for ANR with the following features:
 - All ANR faculty and staff will be able to add content, including images
 - Internal information and confidential client information may be saved, but may only be viewed by ANR users
 - The site will contain print-on-demand copies of all fact sheets published by CNAS and CALS
 - The site will allow ANR to log all “extension calls”
- Fact sheets and pamphlets will be produced and updated as needed.

Regional Collaboration

Planned Activities

- I plan continued collaboration with colleagues in Hawaii, American Samoa, Marshall Islands, Federated States of Micronesia, Palau, and the Northern Marianas
- I plan to expand collaboration with colleagues in the Philippines. I plan to attend an IPM and Invasive Species meeting in the Philippines organized by Dr. Muniappan during August, 2014 where I will make a presentation on the Guam Coconut Rhinoceros Beetle Project.

Coordination of Extension Projects

Activities

Guam Coconut Rhinoceros Beetle Eradication Project.

- I will continue to act as PI for several federal and local grants which fund the project. About 20 part-time UOG employees are currently supported with these funds.
- I will continue to lead the efforts in applied research aimed at finding improved tactics with which to eradicate the rhino beetle from Guam.
- I will develop extension recommendations and control strategies for the rhino beetle should eradication prove impossible. Focus during this year will be on developing and testing a CRB integrated pest management (IPM) plan for Guam.
- I will provide technical expertise and service in areas of database management, geographical information systems, and web site development and maintenance.

Western Plant Diagnostics Network (WPDN)

- As Guam coordinator for WPDN, I will attend annual meetings and attend monthly conference calls.
- I will organize at least one “First Detector Training” each year.

Annual Quarantine Workshop

- Each year, during Spring Break, I participate as an instructor in a one-week workshop sponsored by SPC and APHIS. This workshop trains plant protection quarantine officers from throughout Micronesia.

Creative/Scholarly Activity or Research (34%)

Applied Research in Support of the Guam Coconut Rhinoceros Beetle Eradication Project

Here are the objectives taken *verbatim* from the the plans of work for my CRB grants:

USFS FY13 grant; \$150,000

- Development of an Integrated Pest Management Program for Coconut Rhinoceros Beetle in Guam

APHIS FY13 Biocontrol Grant; \$40,000

- Estimate the Proportion of Guam's CRB Population Killed by the Biocontrol Agent, *Metarhizium majus*
- Develop DNA Proles for CRB Populations in Asia and the Pacific with Respect to Virus Susceptibility

Development of Automated Digital Image Analysis for Insect Biodiversity Studies

I plan to complete this project, which was funded by a CNAS Research and Creative Activity Seed Grant. Working with project collaborators, I have analyzed three extensive series of insect samples provided by Tom Marler from his Rota biodiversity studies: pan traps, sticky traps, and Berlese funnel samples. Results have been prepared in the form of extensive lab reports. My goal is to prepare a methods paper for submission to the *American Entomologist*.

Planned Research Articles

I am preparing the following research articles and plan to submit them to peer reviewed journals within the next year:

Moore A. Mariana Eight Spot Butterfly, *Hypolimnas octocula marianensis*. [Draft targeting Micronesica. I am doing this review as part of my involvement in the CESU rare butterflies and snails project; about 90% complete]

Moore, A. and D. Bright. Three New Island Records for Bark Beetles (Curculionidae: Scolytinae) on Guam from a Single Coffee Berry Borer Trap. [Draft targeting Proceedings of the Hawaiian Entomological Society; 90% complete]

Moore, A. and R. H. Miller. The cycad blue butterfly, *Chilades panadava* (Lepidoptera: Lycaenidae), a new pest of cycads on Guam.[Draft targeting Proceedings of the Hawaiian Entomological Society; 90% complete]

Moore A. and J. Lopez, Jr. Increasing incidence of bed bugs on Guam. *My be a note!*

Moore, A., F. Lee, R. Zack, T. Marler and A. Guerrero. Development of automated digital image analysis for insect biodiversity studies.

Moore A., T. Jackson, R. Quitugua and P. Bassler. Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), grubs develop in live coconut palm crowns on Guam.[Draft targeting the Florida Entomologist; 99% complete]

Moore A. Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), trap catch is maximum at full moon.

A. Moore, C. Apperson, J. McLaughlin and P Kirsch. Automated classification of female *Culex pipiens* (Diptera: Culicidae) and *Cx. quinquefasciatus* from optically-sensed wingbeat waveforms.[80% complete]

Curation of the Wilfred Leon Guerrero Insect Museum

The UOG insect collection, recently renamed as the Wilfred Leon Guerrero Insect Museum, is a valuable scientific resource for Guam and is often used as reference collection by the extension entomologist. I co-curate the collection with Dr. Ross Miller. The collection now has been moved into its own room. We are currently on building a digital catalog for the collection and catching up on a large backlog of unidentified specimens. One of my grants pays for a part-time collection technician and allows us to sponsor site visits by skilled taxonomists who are willing to help us identify specimens.

Planned Activities

- Complete a digital catalog of the collection. (Currently 90% complete; liquid collection yet to be organized)
- Migrate the catalog database from BioLink to a web-based application. The ScratchPad Drupal template developed by the Natural History Museum, London, England will probably be used. See <http://guaminsects.myspecies.info/>.
- Host visiting taxonomists who are willing to help clear up a backlog of unidentified specimens

Website Designed and Maintenance

Planned Activities

- I will continue to develop and maintain websites to facilitate storing and sharing extension and scientific information relevant to ANR's mission. I currently maintain:
 - <http://guaminsects.net/anr>
 - <http://guaminsects.myspecies.info>
 - <http://www.guaminsects.net/uogces/kbwiki/index.php>
 - <http://www.guaminsects.net/gisac/index.php>
 - <http://blog.guaminsects.net/>
 - <http://micronesianinsects.lifedesks.org/>

Participation in Scientific Meetings

- I plan to attend the Entomological Collections Network meetings and Entomological Society of America meetings in Portland, Oregon, November 10-13, 2013. I will be making an oral presentation entitled " Improved Trapping for Coconut Rhinoceros Beetle"
-

University and Community Service (15%)

Community Involvement

I will continue to make myself available for formal and informal discussions of environmental issues on Guam. I have recently been involved in discussions regarding new Guam Pesticide Regulations, impacts of the military buildup on endangered butterflies, and the Micronesia Biosecurity Plan.

Guam Invasive Species Advisory Committee (GISAC)

I am a founding member and active participant in GISAC. Members of this committee are mostly biologists from the University, GovGuam and federal agencies. GISAC meets irregularly, about five times per year, to discuss invasive species activities on Guam and to provide advice to the Guam Invasive Species Council. I maintain a website for GISAC. This website is also used by the Western Micronesia Regional Invasive Species Committee (WM-RISC). The website can be found at <http://guaminsects.net/gisac/>.

Instruction

I will teach AG-109, 'Insect World' , during the Fall 2014 term.

Submitted by:

Aubrey Moore Jul. 28/2014

Aubrey Moore, Ph.D.
Extension Entomologist/Assistant Professor

Approved by:

Lee S. Yudin

Lee S. Yudin, Ph.D.
Dean/Director, CNAS

2.3 Evaluation

Please see next page.

University of Guam
Mangilao, Guam

FACULTY SALARY INCREMENT

AUBREY MOORE, Ph.D.
Faculty Member

06/26/2013 – 06/25/2014
Employment Period

CNAS/CES
College/Unit

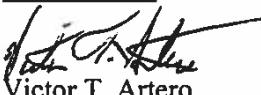
ASSOCIATE PROFESSOR
Rank or Title

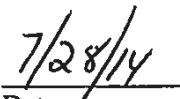
IV/14
Present Level/Step

ANR
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be
APPROVED.


Victor T. Artero
Name


7/28/14
Date

Interim Associate Director, CES
Position Title

Rationale:

As in past evaluations, Dr. Aubrey Moore ranks among the most productive faculty members of CES, CNAS. Below is a brief overview of his past year's performance.

In his primary CFES faculty role of Extension and Community Activities, Dr. Moore readily addresses the needs of our clientele by providing diagnostic service, detection, identification, and control of invasive species, the focal points of this endeavor. In fact, his public outreach efforts continue to expand as well as his regional involvement in addressing pest related issues ensures that Guam's concerns become a part of the cause for solution. I find that his professional involvement with the Guam Coconut Rhinoceros Beetle Eradication Project and the continued expansion of the UOG Insect Collection all help to fortify our entomological capacity as a educational/research entity.

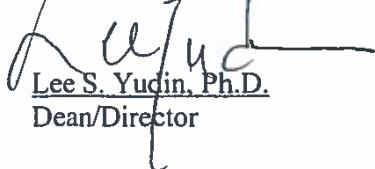
Dr. Moore's role in Creative/Scholarly Activities or Research saw him securing additional grant funds to further his and his colleagues applied research endeavors to provide workable solutions in dealing with Guam's deleterious insect problems. He has co-authored several referred journal articles and made several professional presentations at entomological conferences. Aubrey also created (designed) and maintains websites as sources of online extension clientele education information.

In University and Community Service, Aubrey has taught RI courses for the college with positive student reviews, participated and gave input to the University President's Good to Great endeavor, and last but not least, shared his musical talent by volunteering to play with the Guam Symphony Orchestra and the Guam Territorial Band.

RECOMMENDATION OF DEAN/DIRECTOR

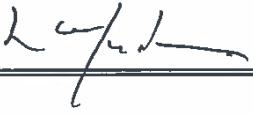
[] I concur with the above recommendation.

[] I do not concur with the above recommendation (see below).


Lee S. Yudin, Ph.D.
Dean/Director

07/28/14
Date

Rationale:

I concur with the evaluation. Dr. Moore continues to work extremely hard and continues to take on more of from her own shoulders. However, he strives for excellence.
Excellent cred. 

The above recommendations have been discussed with me and my responses, if any, are as follows:


Aubrey Moore, Ph.D.
Faculty Member

7/31/2014
Date

CERTIFICATION OF FUNDS

[] Funds Available

[] Funds Not Available

Nancy Cueto
Certifying Officer

Date

3 2014-2015

3.1 Report

Please see next page.

Comprehensive Faculty Evaluation System Report 2015

Aubrey Moore, Ph.D.
Associate Professor / Extension Entomologist

June 28, 2015

I was hired by the University of Guam on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as extension entomologist (100% extension) with the academic rank of Assistant Professor. I work in the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. I am also a faculty member of the Environmental Science Graduate Program and a member of the Western Pacific Tropical Research Center. At the end of the 2012 fall term I applied for tenure and promotion and received both.

My current faculty role allocation is as follows:

- 51% Extension and Community Activities
- 34% Creative/Scholarly Activity or Research
- 15% University and Community Service

Note to Reader:

This report is available as an electronic document, in PDF format, which can be downloaded from <http://guaminsects.net/doc/MooreCFES2015.pdf>. If you are reading the PDF version of the report, you will be able to follow hypertext links to documents I have referenced.

The L^AT_EX script used to generate this document is available at <https://github.com/aubreymoore/CFES2015>.

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1 Extension and Community Activities

1.1 Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam and federal agency personnel, and University of Guam colleagues. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

I am not well trained as an insect taxonomist. To improve my skills, I attend insect identification workshops whenever an opportunity occurs. In May, 2014, the US Forest Service sponsored my attendance at the Ambrosia and Bark Beetle Academy at the University of Florida.

1.1.1 Detection and Documentation of Invasive Species

As with any other tropical island, Guam is extremely susceptible to environmental and economic damage by invasive species. Despite this fact, Guam's biosecurity is very weak and invasive species, many of them insects, are arriving at unprecedented rates. Bioinvasions are grossly under-reported for several reasons:

1. Professional capacity is lacking. Twenty years ago, there were 9 PhD level entomologists practising in Micronesia. Only 3 remain (Moore, Miller, Campbell), despite an increased workload largely due to arrival of the cycad scale, coconut rhinoceros beetle and little fire ant and other invasive species of insects. UOG typically has 4 entomologists. We now have 2.
2. We suffer from the *taxonomic impediment*. The three remaining PhD level entomologists are generalists without the skills and resources for species determination. Timely and accurate species determination is a necessary first step in response to a new pest invasion.
3. There is no ongoing biological survey of Guam with the goal of establishing a baseline biodiversity inventory and detecting newly arrived invasive species. Un-

1 Extension and Community Activities

fortunately, CAPS surveys are usually focused on demonstrating absence of specific agricultural pests rather than detecting new invasions.

4. Even when invasive species are detected and properly identified, first island records are not documented and the information is not published in the scientific press.

In an attempt to improve this situation, I have set myself up as a *registrar* for new insect species arriving on Guam with the intent of properly documenting the ongoing bioinvasion of Guam. The procedure I am trying to establish is:

1. First detector sends me a digital image and/or specimen
2. Specimens are prepared and accessioned into the UOG insect collection
3. A fact sheet is prepared using a template for Guam New Invasive Species Alerts
4. The fact sheet is distributed to a list of stakeholders
5. Taxonomic assistance is obtained for an authoritative species determination.
6. A journal article is prepared and published in a refereed scientific journal. At this point the new geographical distribution data become available to the scientific community via the Global Biodiversity Facility (GBIF).

Although I have been able to generate about a dozen invasive species alerts over the past year (Section 2.4), only one new island record has made it into a peer reviewed journal (Moore, G. Watson, and Bamba 2014).

1.1.2 Insect Identification Service for USDA-APHIS / Guam Customs and Quarantine Agency

I am often called upon to identify insect specimens intercepted the Guam Customs and Quarantine Agency. USDA-APHIS has certified me for this service and has provided a very official looking badge to impress people with. (However, it is not quite as impressive as Dr. Millers bright red badge for getting onto the airport runways.)

USDA-APHIS has recently rewarded me for this service. In response to my 2015 Farm Bill suggestion, the agency kindly equipped me with two professional quality microscopes which will facilitate identification of smaller insects and slide-mounted specimens.

1.2 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the collection and I work with Dr. Ross Miller to curate and catalog this collection.

To increase my knowledge of collection management, I attend the annual meetings of the Entomological Collections Network, which are typically held in conjunction with annual meetings for the Entomological Society of America.

1 Extension and Community Activities

I have a professional goal of building an online website to share all available information on Micronesian insects. This will include specimen level information for the collection complete with digital images and literature references. I built a digital catalog for the collection using the BioLink Biodiversity Information Management System from CSIRO, Australia. The catalog currently contains 29,200 specimen records. BioLink is currently being redeveloped as an open source project (<http://code.google.com/p/biolink/>). I am an active collaborator in this project. In July 2012 I published an article entitled *Hosting a Biolink Database in the Amazon Web Services Cloud (EC2)* on the project's wiki (<http://code.google.com/p/biolink/wiki/BioLinkEC2>).

I have built and evaluated two websites for serving information on Micronesian insect biodiversity, including specimen level data from the collection. One is a Drupal content management system template called LifeDesk provided by the Encyclopedia of Life Project and the other is a similar template called ScratchPads provided by the Museum of Natural History in London. I am honored to have been selected as an advocate for ScratchPads as part of the project's Ambassadors program (<http://scratchpads.eu/locate-scratchpad-ambassadors>). Further information on my websites is provided in the Creative/Scholarly Activities section (2.5).

In March 2014 I travelled to Honolulu to attend the Biodiversity Collections Digitization in the Pacific workshop sponsored by the Integrated Digitized Biocollections (IDigBio). I made an oral presentation entitled [Evaluation of a Scratchpad Template as an Online Database for the University of Guam Insect Collection](#) at this workshop.

In May 2014 I met with Dr. Bob Foottit at the Canadian National Insect Collection in Ottawa to discuss progress and future directions for the UOG collection. Dr. Foottit is a member of the board of directors for the UOG Insect collection.

1.3 Guam Coconut Rhinoceros Beetle Eradication Project

This is currently my largest and most time consuming project.

The coconut rhinoceros beetle (CRB) was first detected on Guam in the Tumon Beach hotel area on September 11, 2007. CRB is a very serious pest of coconut palms. Adult beetles may kill coconuts and other palms when they bore into the crowns to feed on sap. When CRB invaded Palau during the Second World War, it killed about half of all coconuts through the islands and totally exterminated the coconut palm from some of them. A delimitation survey indicated that the Guam infestation was limited to Tumon Bay and the adjacent Faifai Beach. In consultation with the Guam Department of Agriculture (GDOA), USDA-APHIS, and USDA-Forest Survey, it was decided to launch an eradication project.

I wrote the original eradication plan (available on-line at http://guaminsects.net/uogces/kbwiki/index.php?title=Coconut_Rhinoceros_Beetle_Eradication_Plan) and this was funded by USDA and local funds. USDA provided funds under the condition that the project was to be run under an Incident Command System with the

1 Extension and Community Activities

USDA-APHIS Guam Port Director as the federal commander, and the GDOA Director, or designee, as the local commander.

My original role was to provide scientific/technical support for the project, with the Guam Department of Agriculture (GDOA) providing project management with assistance from USDA-APHIS and USDA-Forest Service. However, it soon became apparent that GDOA had serious bureaucratic impediments which prevented hiring staff and procuring supplies and equipment within a reasonable time frame. The eradication project directors, with the consent of the Dean, agreed to run project staffing, procurement, and fiscal management through the University. As a result, my role was expanded to include much of the project management. I am currently managing two grants which fund the project and supervise about 15 temporary employees. Report writing on current grants and proposal writing to keep the project in business occupies much of my time.

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command team set up by APHIS. Later, we were both added to a national technical working group for CRB. My activities in support of the Hawaii CRB Eradication project are detailed in the Regional Collaboration section 1.3.3.

1.3.1 Activities:

1. **Monthly Conference Calls.** These teleconferences are with stakeholders, collaborators, and advisers in USDA APHIS and USDA Forest Service.
2. **Project Websites.** I have endeavored to share and archive data and information associated with the Guam CRB Eradication Project on-line. Prior to May 2009, I used a wiki site at http://www.guaminsects.net/uogces/kbwiki/index.php?title=Oryctes_rhinoceros. Afterwards, I used a Drupal site at <http://www.guaminsects.net/anr/category/miscellaneous/coconut-rhinoceros-beetle>. I maintain a bibliographic database of CRB-related journal articles at http://guaminsects.myspecies.info/crb_biblio and research results are made available as on-line technical reports at http://guaminsects.net/anr/crb_tech_reports.
3. **Project Database.** Trapping data from a network of about 1200 traps, detections of CRB grubs or adults, and observations of CRB defoliation and bore holes are entered daily into a web-based georeferenced MySQL database which I designed. Data from this database is publicly accessible from a web page at <http://www.guaminsects.net/anr/content/public-access-data-collected-guam-coconut-rhinoceros>. Links on this page enable the user to view trap catch data as a spatiotemporal display using a Google Earth animation or a chart of monthly totals. I use this system to produce monthly surveillance reports.
4. **Collaboration.** I have formed two collaborative research groups to do applied research aimed at controlling CRB damage. Dr. Sean Marshall and Dr. Trevor

1 Extension and Community Activities

Jackson at AgResearch New Zealand collaborate with me on biological control using oryctes nudivirus (OrNV) and CRB population genetics. Dr. Matthew Siderhurst and Dr. Eric Jang of USDA-ARS-PBARC collaborate with me on CRB trap improvement and CRB behavior.

1.3.2 Impediment

- My heavy workload does not permit enough time to prepare research results for publication in scientific journals.

1.3.3 Support for the Hawaii Coconut Rhinoceros Beetle Eradication Project

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command system (ICS) team set up by APHIS. Later, we were both added to a national technical working group (TWG) for CRB. I built and maintain an online, full-text bibliographic for use by the TWG at http://guaminsects.myspecies.info/CRB_biblio.

Frequent requests for scientific/technical information from the ICS, TWG and Hawaii Department of Agriculture (several queries per week) has significantly increased my workload over the past several months.

Early in 2015, the directors of the Western IPM Center at UC Davis asked me to help organize a meeting to prioritize applied research needs for development of CRB IPM. I co-authored an agenda and attendance list with Arnold Hara and Roland Quitugua. The meeting took place at the Hawaii Department of Agriculture on April 3, 2015 and was chaired by WIPM Center Director Kassim Al-Khatib.

1.4 Western Plant Diagnostics Network

I am the UOG coordinator for WPDN. This organization provides financial support for ANR's Plant Diagnostic Laboratory, offers First Detector Training workshops, and organizes identification workshops for important pest groups. As coordinator, I am required to organize First Detector Training workshops, attend monthly conference calls, attend annual meetings, and provide reports. WPDN publishes newsletters for First Detectors, including the [Pacific Pest Detector](#) to which I occasionally contribute (Table 1.1).

Table 1.1: Contributions to the Pacific Pest Detector Newsletter

December 2013	coconut termite
March 2014	spotted cucumber beetle
March 2014	brown marmorated stink bug
June 2014	castor hairy caterpillar
<i>In press</i>	Pacific Pests and Pathogens Apps

1.5 Guam Invasive Species Advisory Committee (GISAC)

I am an active, founding member of this informal group of Guam's biologists which meets irregularly about 6 times per year to discuss invasive species and what can be done to keep them out and mitigate the effects of those that do invade the island. I worked with Dr. Russell Campbell and Diane Vice to develop an emergency response plan for invasive species detected on Guam.

A wiki site which I built for GISAC was quickly adopted by the Western Micronesia Regional Invasive Species Council at http://guaminsects.net/gisac/index.php?title>Main_Page.

1.6 Public Outreach (Guest lectures, presentations, interviews)

During the reporting period I was interviewed numerous times by newspaper reporters, radio talk show hosts, and television news reporters. Most, but not all involved questions about the Guam coconut rhinoceros beetle eradication project. I helped to produce several fact sheets and articles for public print media.

1.7 Public Outreach (Internet)

During the past decade I published a lot of content on various websites. I have evaluated several current technologies for building a web presence for the Agriculture and Natural Resources Unit and the Drupal content management system seems to be a good fit. This allows us to publish information for public access while keeping some documents private for internal use only. My print and online output are discussed in more detail in the Creative/Scholarly Activity section.

I maintain a website for the UOG Cooperative Extension Service's Agriculture and Natural Resources Program at <http://guaminsects.net/ANR>. I frequently post blog articles of public interest to this site (Table 1.2). I also maintain a website at <http://guaminsects.myspecies.info> which is intended to facilitate sharing information on insects in Micronesia. I submit blog articles to this website which are more technical

1 Extension and Community Activities

and are of interest to biologists. To see a list of my blog post on this site, visit <http://guaminsects.myspecies.info/blogs/aubrey-moore>.

Note that these blogs also contain posts containing information which is not intended for the public. These posts are shared with selected groups of clients and colleagues using a password authentication system.

Table 1.2: Public blog posts on *guaminsects.net/anr* posted 2014-15

Date	Title
2015 Jun 16 - 10:20am	Pacific Pests and Pathogens App for Cell Phones and Tablets
2015 May 30 - 12:20pm	Australian Northern Territory Agricultural Field Guides for Vegetables and Mangoes
2015 Apr 14 - 6:50am	Trap for In-transit Detection of Invasive Species
2015 Apr 10 - 5:55am	Attempts at Keeping Track of Invasive Species in the Marianas
2015 Mar 29 - 8:20am	KUAM News Story by Isa Baza: Funding to combat rhino beetle is lopsided
2015 Mar 28 - 8:21pm	Pacific Daily News Story: LeoPalace nets resort's rhino beetles
2015 Mar 28 - 7:27am	Marianas Variety Newspaper Article: Leo Palace uses nets to capture rhino beetles
2015 Mar 18 - 8:55pm	Pacific Daily News Story: UOG battles rhino beetles
2015 Mar 11 - 12:48pm	K57 Radio Interview: Roland Quitugua and Ray Gibson discuss rhino beetles and little fire ants
2015 Mar 11 - 12:44pm	Marianas Variety Newspaper Article: Rhino Beetle Nets Now on Sale
2015 Mar 11 - 6:55am	Facebook response to sale of tekken by Guam Home Improvement Center
2015 Mar 11 - 6:51am	K57 Radio Interview: Roland Quitugua and Patti Arroyo discuss tekken trap for coconut rhinoceros beetles
2015 Mar 1 - 6:55am	PhysOrg Article: Research to the rescue: Fishing for rhinos with tekken
2015 Feb 26 - 8:54pm	PNC Video: UOG Research to the Rescue
2015 Feb 26 - 4:54am	Hawaii News Now Article: Guam eyes nets to battle rhinoceros beetle
2015 Feb 22 - 10:25am	PNC Video: Family in Yigo finds coconut rhino beetle grubs in store-bought potting soil
2015 Feb 19 - 6:55am	Marianas Variety Newspaper Article: Rhino Beetle Traps Available next Month

Continued on next page

1 Extension and Community Activities

Table 1.2 – *Continued from previous page*

Date	Title
2015 Feb 18 - 5:54pm	PNC News Article by Clynt Ridgell: UOG Unveils New Tekken Trap For Coconut Rhino Beetle
2015 Feb 4 - 12:56pm	Marianas Variety Newspaper Article: Community-based rhino beetle program holds Guam workshop
2015 Jan 22 - 4:35am	Check List of Micronesian Insects
2014 Sep 28 - 12:37pm	Pacific Daily News Opinion: Fully implement the law to better combat invasive species
2014 Jun 12 - 1:20pm	PNC News Story: DoAG and UOG Team Up to Get Rid of the Little Fire Ant
2014 Jun 11 - 3:28pm	Visualization of Coconut Rhinoceros Beetle Trap Data
2014 Mar 31 - 3:39am	Public opinion on invasive species issues
2014 Mar 26 - 5:13am	iDigBio presentation - Honolulu, March 2014
2014 Feb 20 - 7:18pm	PNC News Story: Guam is Running Out of Options to Stop the Spread of Rhino Beetles and Save Guam's Coconut Trees
2014 Feb 10 - 11:47am	CNN Article by Matt Smith: Meet the beetles: Hawaii mobilizes to fight bug invasion
2014 Feb 9 - 7:17pm	Pacific Daily News Nespaper Article: Mayors voice concerns over rhino beetle
2014 Feb 5 - 1:18pm	Pacific News Center Story: University of Guam Experts Help Hawaii with Rhino Beetles
2014 Jan 22 - 5:48pm	KITV4 Hawaii TV Story: Experts Brought to Hawaii to Battle the Rhino Beetle
2014 Jan 10 - 9:33pm	iNaturalist: Guam CRB Citizen Science
2014 Jan 10 - 7:56pm	KUAM News Story: Invasive species threaten local crops
2014 Jan 10 - 12:52pm	Coconut Rhinoceros Beetle Infestation Discovered at Hickam Air Force Base, Oahu, Hawaii
2014 Jan 10 - 7:25am	Video: Little Fire Ant in Hawaii
2014 Jan 9 - 8:47am	Relative Attractiveness of White and Ultraviolet Light Emmitting Diodes for Rhino Beetles
2014 Jan 9 - 6:05am	Arnold Hara's Rhino Beetle Images Taken During his Trip to Guam
2014 Jan 4 - 5:47am	No Rhino Pamphlet

Continued on next page

Table 1.2 – *Continued from previous page*

Date	Title
2014 Jan 1 - 7:15pm	Pacific News Center Includes Invasive Species Issues in Top 10 Stories of 2013

1.8 Regional Collaboration

1.8.1 Regional Invasive Species Council Website

I maintain a website for the Western Micronesia Regional Invasive Species Council (RISC) at <http://www.guaminsects.net/gisac/>. I attend RISC meetings whenever they are held on Guam and I make presentations at these meetings.

1.8.2 Insect Diagnostics for Micronesia

I am often contacted with requests for help with identifying pests from throughout Micronesia and suggesting solutions to the problems they cause. This workload has increased because the number of practicing PhD-level entomologists in Micronesia has dropped from 9 to 3 within the last two decades.

2 Creative/Scholarly Activities or Research

2.1 Refereed Scientific Journal Articles

1. Fisher, Nicole, Aubrey Moore, Bradley Brown, Matthew Purcell, Gary Taylor, and John Salle (2014). “Two new species of Selitrichodes (Hymenoptera: Eulophidae: Tetrastichinae) inducing galls on Casuarina (Casuarinaceae)”. In: *Zootaxa* 3790.4, 534–542. ISSN: 1175-5334. URL: <http://biotaxa.org/Zootaxa/article/view/zootaxa.3790.4.2/7933>.
2. Moore, Aubrey, Chas Apperson, John McLaughlin, and Philipp Kirsch (In Preparation). “Automated classification of female *Culex pipiens* (Diptera: Culicidae) and *Cx. quinquefasciatus* from optically sensed wingbeat waveforms”. In: *Journal of Medical Entomology*. in preparation.
3. Moore, Aubrey and Donald Bright (In Preparation). “Three new island records for bark beetles (Curculionidae: Scolitinae) on Guam from a single coffee berry borer trap”. In: in preparation.
4. Moore, Aubrey, Trevor Jackson, Roland Quitugua, and Paul Bassler (In Press). “Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), grubs develop in live coconut palms on Guam”. In: *Florida Entomologist*. in preparation.
5. Moore, Aubrey, N-Y Su, and Leonard Sigrah (In Preparation). “First record of the coconut termite, *Neotermes rainbowi* (Isoptera: Kalotermitidae) from Micronesia”. In: in preparation.
6. Moore, Aubrey, Gillian Watson, and Jesse Bamba (2014). “First record of eggplant mealybug, *Coccidohystrix insolita* (Hemiptera: Pseudococcidae), on Guam: Potentially a major pest”. In: *Biodiversity Data Journal* 2. DOI: [10.3897/BDJ.1.e1042](https://doi.org/10.3897/BDJ.1.e1042). URL: <http://biodiversitydatajournal.com/articles.php?id=1042>.

2.2 Presentations at Professional Meetings

1. Ares, M.A., N. Meneses, A. Smith, Aubrey Moore, and R. Benford (2015). “Molecular Identification of a Lepidopteran Herbivore on a Critically Endangered Tree”. In: Northern Arizona Undergraduate Symposium 2015.

2 Creative/Scholarly Activities or Research

2. Moore, Aubrey (2014b). “Biological invasion of forests on Guam and other islands of Micronesia”. In: *65th Western Forest Insect Work Conference*. oral presentation. Sacramento, California.
3. Moore, Aubrey (2014n). “Evaluation of a Scratchpad template as an online database for the University of Guam insect collection”. In: *iDigBio Biodiversity Collections Digitization in the Pacific Workshop*. oral presentation. Honolulu, Hawaii.
4. Moore, Aubrey (2014p). “Insects Attacking *Serianthes nelsonii*”. In: *2014 Island Sustainability Conference*. Guam.
5. Moore, Aubrey (2015d). “Pacific Entomology Conference 2015 Oral Presentation: Failure Analysis of the Guam Coconut Rhinoceros Beetle Eradication Project”. In: URL: [http://guaminsects.net/anr/sites/default/files/pec2015-crb-failure\(10\).pdf](http://guaminsects.net/anr/sites/default/files/pec2015-crb-failure(10).pdf).
6. Moore, Aubrey and Roland Quitugua (2014d). “Overview of the Guam coconut rhinoceros beetle eradication project”. In: *Hawaii CRB Incident Command Meeting*. Honolulu, Hawaii. URL: <http://guaminsects.net/presentations/CRB-Hawaii-ICS-Jan-2014.pdf>.
7. Moore, Aubrey and Roland Quitugua (2014e). *Rhino Beetle Presentation for Hawaii ICS - January, 2014*. CRB Technical Report. URL: <http://guaminsects.net/presentations/CRB-Hawaii-ICS-Jan-2014.pdf>.
8. Moore, Aubrey and Roland Quitugua (2015c). “Pacific Entomology Conference 2015 Oral Presentation: Coconut Rhinoceros Beetle Trap Improvements”. In: URL: <http://guaminsects.net/anr/sites/default/files/pec2015-improved-traps.pdf>.
9. Moore, Aubrey, Roland Quitugua, Mattew Siderhurst, and Eric Jang (2014). “Improved traps for the coconut rhinoceros beetle, *Oryctes rhinoceros*”. In: *Entomological Society of America*. Portland, OR. URL: http://guaminsects.net/anr/sites/default/files/Moore_1957_2.pdf.

2.3 Technical Reports Documenting Applied Research in Support of the Guam Coconut Rhinoceros Beetle Project

1. Iriarte, Ian, Roland Quitugua, Olympia Terral, Aubrey Moore, and Mariana Sanders (2015a). *Fact Sheet: Coconut Rhinoceros Beetle Behavior and Biology*. CRB Technical Report. URL: <http://guaminsects.net/anr/sites/default/files/Behavior%20and%20Biology%20Ian.pdf>.

2 Creative/Scholarly Activities or Research

2. Iriarte, Ian, Roland Quitugua, Olympia Terral, Aubrey Moore, and Mariana Sanders (2015b). *Fact Sheet: Coconut Rhinoceros Beetle trapping Methods*. CRB Technical Report. URL: <http://guaminsects.net/anr/sites/default/files/Trapping%20Final.pdf>.
3. Marshall, Sean and Aubrey Moore (2014a). “DNA analysis of Hawaii CRB”. In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-02-12.pdf>.
4. Marshall, Sean and Aubrey Moore (2014b). “Hawaii beetle dissections”. In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-01-17A.pdf>.
5. Moore, Aubrey (2014a). “APHIS biocontrol semiannual report”. In: URL: http://guaminsects.net/anr/sites/default/files/CRB2014-05-04_0.pdf.
6. Moore, Aubrey (2014f). “Chicken wire escape test”. In: URL: http://guaminsects.net/anr/sites/default/files/CRB2014-01-12A_0.pdf.
7. Moore, Aubrey (2014g). “Chicken wire vs plastic top”. In: URL: <http://guaminsектs.net/anr/sites/default/files/CRB2014-01-15.pdf>.
8. Moore, Aubrey (2014h). “CRB dispersal by flight”. In: URL: <http://guaminsects.net/anr/content/2014-02-19a-crb-dispersal-flight>.
9. Moore, Aubrey (2014i). “CRB heat tolerance”. In: URL: <http://guaminsects.net/anr/content/2014-02-19-crb-heat-tolerance>.
10. Moore, Aubrey (2014j). “CRB mitigation for conservation of rear snails and butterflies at Haputo Beach”. In: URL: <http://guaminsects.net/anr/sites/default/files/2014-02-17%20Haputo.pdf>.
11. Moore, Aubrey (2014k). “CRB rearing”. In: URL: http://guaminsects.net/anr/sites/default/files/CRB%20Rearing_0.pdf.
12. Moore, Aubrey (2014l). “CRB Sanitation at the University of Guam Yigo Agricultural Experiment Station”. In: URL: <http://guaminsects.net/anr/sites/default/files/2014-06-26-YigoSanitation.pdf>.
13. Moore, Aubrey (2014m). “Cypermethrin applied to coconut palm crowns as a prophylactic treatment for prevention of CRB damage”. In: URL: <http://guaminsects.net/anr/sites/default/files/crownSpray.pdf>.
14. Moore, Aubrey (2014o). “Guam CRB project payroll simulation”. In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/CRB%20Payroll.ipynb>.
15. Moore, Aubrey (2014s). “Minibucket escape test”. In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-01-17.pdf>.

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16. Moore, Aubrey (2014t). "Minibucket test". In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-01-16.pdf>.
17. Moore, Aubrey (2014u). "Plastic top catch test". In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-01-12B.pdf>.
18. Moore, Aubrey (2014v). "Progress Report: Development of Integrated Pest Management for Coconut Rhinoceros Beetle on Guam". In: URL: <http://guaminsects.net/anr/sites/default/files/FS-CRB-Report-Sep-2014.pdf>.
19. Moore, Aubrey (2014w). "Relative attractiveness of white and ultraviolet light emitting diodes plus oryctalure". In: URL: http://guaminsects.net/anr/sites/default/files/LEDcolor_0.pdf.
20. Moore, Aubrey (2015a). "Best Way to Access Data in the Guam Coconut Rhinoceros Project Database". In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/bestWaySQL.ipynb>.
21. Moore, Aubrey (2015b). "Generating a Trap Map Animation". In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/trapMapViz.ipynb>.
22. Moore, Aubrey (2015c). "Harvesting data from the EpiCollect crb-yigo-barrel-epicollect Project". URL: http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/crb_yigo_barrel_epicollect.ipynb.
23. Moore, Aubrey (2015d). "Pacific Entomology Conference 2015 Oral Presentation: Failure Analysis of the Guam Coconut Rhinoceros Beetle Eradication Project". In: URL: [http://guaminsects.net/anr/sites/default/files/pec2015-crb-failure\(10\).pdf](http://guaminsects.net/anr/sites/default/files/pec2015-crb-failure(10).pdf).
24. Moore, Aubrey (2015e). "Standard CRB Pheromone Traps Catch More Females Than Males". In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/CRB-sex-ratio.ipynb>.
25. Moore, Aubrey (2015f). "Trap Thinning". URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Trap%20Thining.ipynb>.
26. Moore, Aubrey (2015g). *Yigo Palm Image Album 2015-01-04*. CRB Technical Report. URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Yigo%20Palm%20Image%20Album%202015-01-04.ipynb>.
27. Moore, Aubrey and Sean Marshall (2014). "Final Report for APHIS Biocontrol Grant: Entomopathogenic Virus for Biological Control of Coconut Rhinoceros Beetle on Guam". In: 20140709. URL: http://guaminsects.net/anr/sites/default/files/final_July14-CRB%20APHIS%20Biocontrol.pdf.

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28. Moore, Aubrey and Sean Marshall (2015). “Efficacy of Entomopathogenic Fungus for Biological Control of Coconut Rhinoceros Beetle (CRB) on Guam and DNA Profiling of Asia/Pacific CRB Populations with Respect to Virus Susceptibility”. URL: <http://guaminsects.net/anr/sites/default/files/semiannual-report-April2015.pdf>.
29. Moore, Aubrey and Roland Quitugua (2014a). “Adding CRB Breeding Site Material to Barrel Traps Does Not Increase Trap Catch”. In: URL: <http://guaminsects.net/anr/sites/default/files/barrelSubstrate.pdf>.
30. Moore, Aubrey and Roland Quitugua (2014b). “Bird net escape test”. In: ISSN: CRB-2014-02-23. URL: <http://guaminsects.net/anr/sites/default/files/BirdNet.pdf>.
31. Moore, Aubrey and Roland Quitugua (2014c). “Funnels Added to Pan Traps Increase Catch”. In: ISSN: CRB-2014-07-29. URL: <http://guaminsects.net/anr/sites/default/files/FunnelTest.pdf>.
32. Moore, Aubrey and Roland Quitugua (2014e). *Rhino Beetle Presentation for Hawaii ICS - January, 2014*. CRB Technical Report. URL: <http://guaminsects.net/presentations/CRB-Hawaii-ICS-Jan-2014.pdf>.
33. Moore, Aubrey and Roland Quitugua (2014f). “Test of Baffles to Prevent Escape from Pan Traps”. In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Baffle%20Escape%20Test.ipynb>.
34. Moore, Aubrey and Roland Quitugua (2014g). “Test of Netting as a Physical Barrier for CRB Adults”. In: URL: <http://guaminsects.net/anr/sites/default/files/FishNetTest.pdf>.
35. Moore, Aubrey and Roland Quitugua (2014h). “Yigo barrel traps: trap catch comparison between pan and minibucket traps”. In: URL: <http://nbviewer.ipython.org/github/aubreymoore/YigoBarrels/blob/master/YigoBarrels.ipynb>.
36. Moore, Aubrey and Roland Quitugua (2015a). “DeFence Traps: Using Fish Netting as Novel CRB Pheromone Trap Deployed on Fence Lines”. URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/DeFence%20Traps.ipynb>.
37. Moore, Aubrey and Roland Quitugua (2015b). “Harvesting data from the Epi-Collect CRB-TALAYA Project”. URL: http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/crb_talaya.ipynb.
38. Moore, Aubrey and Roland Quitugua (2015c). “Pacific Entomology Conference 2015 Oral Presentation: Coconut Rhinoceros Beetle Trap Improvements”. In: URL: <http://guaminsects.net/anr/sites/default/files/pec2015-improved-traps.pdf>.

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39. Moore, Aubrey and Roland Quitugua (2015d). "Protecting Coconut Palms from CRB Damage Using Fish Gill Netting". URL: http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Netted%20Coconut%20Palms_0.ipynb.
40. Moore, Aubrey and Roland Quitugua (2015e). "Taiwanese Gill Net Escape Test". In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Taiwanese%20Gill%20Net%20Escape%20Test.ipynb>.
41. Moore, Aubrey, Roland Quitugua, and Ian Iriarte (2015). "Netted Panel Traps to Test if CRB are Deflected". URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Netted%20Panel%20Traps%20Experiment%20to%20See%20if%20CRB%20are%20Deflected.ipynb>.
42. Moore, Aubrey, Roland Quitugua, Mattew Siderhurst, and Eric Jang (2014). "Improved traps for the coconut rhinoceros beetle, *Oryctes rhinoceros*". In: *Entomological Society of America*. Portland, OR. URL: http://guaminsects.net/anr/sites/default/files/Moore_1957_2.pdf.
43. Moore, Aubrey and Mattew Siderhurst (2015). "Oryctalure synergist candidates field trial". In: URL: <http://nbviewer.ipython.org/url/guaminsects.net/anr/sites/default/files/Oryctalure%20synergists%20field%20trial.ipynb>.
44. Quitugua, Roland, Mariana Sanders, Olympia Terral, and Aubrey Moore (2015). "Trifold Pamphlet: Coconut Rhinoceros Beetle Trapping". URL: <http://guaminsects.net/anr/sites/default/files/crb-trapping-trifold.pdf>.
45. Sanders, Mariana, Roland Quitugua, Olympia Terral, and Aubrey Moore (2014). *Coconut Rhinoceros Beetle Behavior and Biology*. Extension Pamphlet. URL: <http://guaminsects.net/anr/sites/default/files/crb-behavior-biology.pdf>.
46. Sanders, Mariana, Roland Quitugua, Olympia Terral, and Aubrey Moore (2015). "Trifold Pamphlet: Coconut Rhinoceros Beetle Behavior and Biology". URL: [http://guaminsects.net/anr/sites/default/files/crb-behavior-biology\(12\).pdf](http://guaminsects.net/anr/sites/default/files/crb-behavior-biology(12).pdf).

2.4 Guam New Invasive Species Alerts

1. McConnell, James and Aubrey Moore (2015). "Crambid moth, *Cydalima lati-costalis*". In: 2015-1. URL: [http://guaminsects.net/anr/sites/default/files/cydalima-laticostalis\(1\)_0.pdf](http://guaminsects.net/anr/sites/default/files/cydalima-laticostalis(1)_0.pdf).
2. Moore, Aubrey (2014c). "Brown marmorated stink bug, *Halymorpha halys*". In: 2014-1. URL: <http://guaminsects.net/anr/sites/default/files/brownMarmoratedStinkBug.pdf>.

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3. Moore, Aubrey (2014d). “Brown Marmorated Stink Bug *Halyomorpha halys* (Stål 1855) (Hemiptera: Pentatomidae)”. In: URL: <http://guaminsects.net/anr/sites/default/files/brownMarmoratedStinkBug.pdf>.
4. Moore, Aubrey (2014q). “Ixora leaf-mining weevil”. In: 2014-6. URL: <http://guaminsects.net/anr/sites/default/files/ixora-leafmining-weevil.pdf>.
5. Moore, Aubrey (2014r). “Leaf beetle, *Calligrapha californica*”. In: 2014-8. URL: [http://guaminsects.net/anr/sites/default/files/calligrapha-californica\(2\).pdf](http://guaminsects.net/anr/sites/default/files/calligrapha-californica(2).pdf).
6. Moore, Aubrey (2014x). “Spotted cucumber beetle (southern corn rootworm), *Diabrotica undecimpunctata*”. In: 2014-2. URL: <http://guaminsects.net/anr/sites/default/files/spotted%20cucumber%20beetle.pdf>.
7. Moore, Aubrey (2014z). “Unidentified roach”. In: 2014-07. URL: <http://guaminsектs.net/anr/sites/default/files/newpestroach.pdf>.
8. Moore, Aubrey and Jesse Bamba (2014). “Spotted Cucumber Beetle (Southern Corn Rootworm) *Diabrotica undecimpunctata* (Coleoptera: Chrysomelidae)”. In: URL: <http://guaminsects.net/anr/sites/default/files/spotted%20cucumber%20beetle.pdf>.
9. Moore, Aubrey, James McConnell, and Gillian Watson (2014). “Camphor scale, *Pseudaonidia duplex*”. In: 2014-5. URL: <http://guaminsects.net/anr/sites/default/files/camphorscale2.pdf>.
10. Rosario, Christopher A., Ross H. Miller, and Aubrey Moore (2014). “Varroa mite, Varroa destructor”. In: 2014-04. URL: <http://guaminsects.net/anr/sites/default/files/varroa%20mite.pdf>.
11. Route, Arnold and Aubrey Moore (2014a). “Castor hairy caterpillar, *Olepa ricini*”. In: 2014-3. URL: http://guaminsects.net/anr/sites/default/files/castor%20hairy%20caterpillar_0.pdf.
12. Route, Arnold and Aubrey Moore (2014b). “Castor Hairy Caterpillar *Olepa ricini* (Fabricius, 1775) (Lepidoptera: Arctiidae)”. In: URL: http://guaminsects.net/anr/sites/default/files/castor%20hairy%20caterpillar_0.pdf.

2.5 Web Sites Designed and Maintained by Me

For the past several years, I have been searching for the “right” technology for providing on-line extension information. The features I want include:

- Ease of use, including immediate, on-line editing, so that colleagues and collaborators can create content

2 Creative/Scholarly Activities or Research

- Ability to display digital images at several resolutions
- Full text search
- Methods for handling on-line and offline references
- Fine grained security which protects client confidentiality and allows for both protected, internal and public information sharing

My current technology of choice is Drupal, a free, open source contents management system.

2.5.1 ANR Web Site.

Home page: <http://guaminsects.net/anr>

This Drupal site is intended to facilitate sharing both internal and external information generated by the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. This site is currently being used heavily by the Guam CRB Eradication Project. I also use this site for documenting my diagnostics work. I provide a recent example web page documenting discovery of thrips in anthurium flowers. (Evidence 2.5.1; available on-line at <http://guaminsects.net/anr/content/thrips-damaging-anth>)

2.5.2 Insects of Guam Web Site

Home page: <http://guaminsects.myspecies.info>

This Drupal site is being evaluated for sharing information on Micronesian insects. Information will include specimen level information from the UOG insect collection complete with digital images and literature references. It was built using a template developed by the Scratchpad project <http://scratchpads.eu/> is sponsored by the European Institute of Distributed Taxonomy (EDIT) and the Natural History Museum in London . The ScratchPad project is celebrating the International Year of Biodiversity by highlighting a different Scratchpad taxon every week. I was honored to have one of my pages, describing the indigenous bug, *Leptocoris vicinus*, highlighted during the week of April 18 to 24, 2010.

(Evidence 2.5.2)

2.5.3 Micronesia Biosecurity Plan Review Web Site

Home page: MBP.GuamInsects.net

This is a secure, private Drupal site developed to facilitate sharing information among those reviewing the Micronesia Biosecurity Plan.

2.5.4 Moodle Site for my AG 109 Insect World Course

Home page: <http://campus.uogdistance.com/course/view.php?id=286>

This site was my first experience with Moodle, a content management system designed for teachers. I originally built it to provide on-line resources for my students, but later decided to open a few wikis to promote collaboration on laboratory exercises. I also kept track of grades using Moodle. Examples from this site include the course resource page (Evidence 2.5.3a; available on-line at <http://campus.uogdistance.com/mod/resource/view.php?id=7349>) and a small PHP program I wrote to facilitate printing pinned insect specimen labels (Evidence 2.5.3b; available on-line at <http://tinyurl.com/insect-labels>).

2.5.5 Knowledgebase Wiki for the UOG Cooperative Extension

Home page: <http://www.guaminsects.net/uogces/kbwiki/index.php>

This was my first attempt at building an extension website to facilitate collaborative content creation. Digital copies of all of ANR's pest fact sheets can be found on this site. There is also a list of insect pests found on all major crops grown in Micronesia. I stopped maintaining this site in May, 2009 because the ANR site built with Drupal has more of the features I need.

(Evidence 2.5.4)

2.5.6 Western Micronesia Regional Invasive Species Council Wiki

Home page: <http://www.guaminsects.net/gisac/index.php>

Originally built for the Guam Invasive Species Advisory Council, this site was quickly adopted for sharing regional information on invasive species by the Western Micronesia Regional Invasive Species Council.

(Evidence 2.5.5)

2.5.7 Guam Insects Blog Site

Home page: <http://blog.guaminsects.net/>

I ran into recurring technical problems with this site which uses the WordPress content management system and have more or less abandoned development and maintenance.

2.5.8 Life Desk Site for Micronesian Insects

Home page: <http://micronesianinsects.lifedesks.org/>

This site uses a Drupal template being developed by the Encyclopedia of Life Project. I evaluated it for sharing information on Micronesian insects, but decided that the Scratchpad template (number 2, above) had a better feature set for what I wanted to do.

2.6 Grants

During 2014 and 2015, I managed 8 grants totalling \$345,040 (listed below). These grants partially or fully supported 14 staff positions (Table 2.1).

2.6.1 Support for the Guam Coconut Rhinoceros Beetle Eradication Project

Funding Source US Forest Service

Amount \$150,000

End Date 2015 Jun 30

Description The objective of this project its to develop an integrated pest management (IPM) program for coconut rhinoceros beetle on Guam.

Project Documents <http://guaminsects.net/anr/content/crb-biocontrol-2013>

2.6.2 Efficacy of Entomopathogenic Fungus for Biological Control of Coconut Rhinoceros Beetle (CRB) on Guam and DNA Profiling of Asia/Pacific CRB Populations with Respect to Virus Susceptibility

Funding Source USDA-APHIS

Amount \$40,000

End Date 2015 Aug 31

Description This project has two objectives:

1. To measure the impact of *Metarhizium majus*, green muscardine fungus (GMF), as a biological control agent for the Guam CRB population
2. To survey and map the extent of the Guam CRB genotype. This work is done in collaboration with Sean Marshall at AgResearch New Zealand.

Project Documents <http://guaminsects.net/anr/content/crb-biocontrol-2013>

2.6.3 Microscopes for UOG Extension Entomology Lab and Guam Customs and Quarantine Agency

Funding Source USDA-APHIS

Amount \$80,000

Description Proposal submitted as a 2015 Farm Bill suggestion. However, APHIS decided to fund this equipment grant from AQI funds. Professional grade equipment including a Nikon stereozoom microscope, a Nikon compound microscope, a digital microscope camera, and camera control were delivered to UOG in June 2015. The grant also provided a stereozoom for the Guam Customs and Quarantine Agency.

2.6.4 Establishment of Captive and Managed Populations of the Mariana Eight-spot Butterfly, *Hypolimnas octocula marianensis*

Funding Source USFWS via an MOU with GDOA-DAWR

Amount \$21,212

End Date 2015 Sep 30 (1 year no cost extension requested)

Description This project will investigate the feasibility of rearing and breeding *H. o. marianensis* and also in field sites where ungulates are excluded.

Project Documents <http://guaminsects.net/anr/content/octocula>

2.6.5 Western Plant Diagnostic Network FY2015

Funding Source UC Davis

Amount \$10,672

End Date 2015 Jun 30

Description WPDN provides first detector training and support of diagnosis of plant pests and pathogens.

Project Documents <http://guaminsects.net/anr/content/wpdn-2014-15>

2.6.6 Western Plant Diagnostic Network FY2016

Funding Source UC Davis

Amount \$10,854

End Date 2016 Jun 30

Description WPDN provides first detector training and support of diagnosis of plant pests and pathogens.

2.6.7 Guam Forest Insect Survey

Funding Source NIFA-McIntire-Stennis

Amount \$12,302 per year

End Date 2018 Jun 30

Description The objective of the survey is to build a knowledge-base on insects associated with plants in Guam's forests. The survey will result in a reference collection of Guam's forest insects and a publicly available online database to facilitate sharing of specimen data, images and ecological associations among plants and insects. The knowledge base will be useful to natural resource managers responsible for maintaining the health of Guam's forests and to biologists trying to understand Guam's terrestrial ecosystems in the wake of major biological invasions.

Project Documents <http://guaminsects.net/anr/content/guam-forest-insect-survey>

2.6.8 Detector Beetles: Radio-tracking Coconut Rhinoceros Beetles to Discover Breeding Sites

Funding Source US Forest Service

Amount \$20,000 (additional \$20,000 pending)

End Date 2016 Apr 30

Description This project is a feasibility study to see if CRB adults equipped with glue-on miniature radio transmitters can be tracked to cryptic breeding sites.

Project Documents <http://guaminsects.net/anr/content/detector-beetles>

Table 2.1: Staff support by my grants in 2014-2015.

1	Bob Bourgeois
2	Roger Brown (partially)
3	Roland Quitugua (partially)
4	Ian Iriarte
5	Vincent Benavente
6	John Diego
7	Ken Leon Guerrero
8	Roland Cabrera
9	Derrick Diego
10	Marty Hara
11	Ken San Nicolas
12	Jessica Gross
13	Cris Crisostimo
14	Raymondo San Miquel

3 University and Community Service

3.1 Teaching

In addition to my job as an extension entomologist, I am required to teach a four credit course every year. My student evaluations are consistently above average (Tables 3.1 and 3.2).

3.1.1 AG-109 Insect World

Table 3.1: Student evaluation for AG109, *Insect World*.

Term	My Evaluation	College Average	University Average
Fall 2009	3.659	3.565	3.552
Spring 2011	3.986	3.519	3.617
Spring 2012	3.863	3.570	3.612
Spring 2013	3.659	3.552	3.627
Fall 2014	3.645	3.471	3.553

3.1.2 AG/BIO-345 General Entomology

Table 3.2: Student evaluation for AG/BIO-345, *General Entomology*.

Term	My Evaluation	College Average	University Average
Fall 2013	3.875	3.522	3.586

3.2 Service as a Reviewer

- I served as an external examiner and reviewed Maclean Vaqalo's PhD dissertation entitled *Biology and ecology of Nisota basselae on Abelmoschus manihot Medicus in Solomon Islands* for the University of Queensland.

3 University and Community Service

- I reviewed the manual *New Pest Response Guidelines for Coconut Rhinoceros Beetle* for USDA-APHIS.
- I reviewed a scientific note for publication in the Proceedings of the Hawaiian Entomological Society.

3.3 Music

As an amateur horn player I play regularly, and often very badly, with the Guam Symphony Orchestra and occasionally with the Guam Territorial Band. I have played for UOG graduations and for concerts arranged by the UOG music department.

3.4 Collaboration on CESU Rare Butterfly and Snails Survey Grant

I am collaborating with Dan Lindstrom, John Benedict, Frank Camacho, and Curt Fiedler (UOG Biology), Alex Kerr (UOG Marine Lab), Brent Holland and Dan Rubinoff (UH Manoa) on a DOD funded survey of rare butterflies and snails. My contribution is a literature review of *Hypolimnas octocula mariannensis* for publication in Micronesica, design and maintenance of project website and development of butterfly camera traps.

3.5 Collaboration on Biocontrol of Cycad Aulacaspis Scale

I am working with Tom Marler on introduction of parasitoids for biocontrol of the *Aulacaspis yasumatsui*.

3.6 University Technical Advisory Committee

I serve on UTAC as the representative for the College of Natural and Applied Sciences.

3.7 Undergraduate Curriculum Review Committee (UCRC)

In the April 2013 Faculty Elections, I was elected to serve on the UCRC. I served for two years on this committee, 2013-14, and 2014-15.

3.2 Plan for Following Year

Please see next page.

Work Plan 2015

Aubrey Moore, Ph.D.
Associate Professor / Extension Entomologist

June 29, 2015

I was hired by the University of Guam on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as extension entomologist (100% extension) with the academic rank of Assistant Professor. I work in the Agriculture and Naturals Resources Unit of the University of Guam Cooperative Extension Service. I am also a faculty member of the Environmental Science Graduate Program and a member of the Western Pacific Tropical Research Center. At the end of the 2012 fall term I applied for tenure and promotion and received both.

My current faculty role allocation is as follows:

- 51% Extension and Community Activities
- 34% Creative/Scholarly Activity or Research
- 15% University and Community Service

Tasks in this work plan for June 2015 through May 2016 are organized under these roles.

Note to Reader:

This report is available as an electronic document, in PDF format, which can be downloaded from <http://guainsects.net/doc/MooreWorkPlan2015.pdf>. If you are reading the PDF version of the report, you will be able to follow hypertext links to documents I have referenced.

The L^AT_EX script used to generate this document is available at <https://github.com/aubreymoore/CFES2015>.

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1 Extension and Community Activities

1.1 Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam and federal agency personnel, and University of Guam colleagues. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem. The number of extension calls requiring my assistance averages approximately three per day.

I am not well trained as an insect taxonomist. To improve my skills, I attend insect identification workshops whenever an opportunity occurs. In May, 2014, the US Forest Service sponsored my attendance at the Ambrosia and Bark Beetle Academy at the University of Florida.

1.1.1 Detection and Documentation of Invasive Species

As with any other tropical island, Guam is extremely susceptible to environmental and economic damage by invasive species. Despite this fact, Guam's biosecurity is very weak and invasive species, many of them insects, are arriving at unprecedented rates. Bioinvasions are grossly under-reported for several reasons:

1. Professional capacity is lacking. Twenty years ago, there were 9 PhD level entomologists practising in Micronesia. Only 3 remain (Moore, Miller, Campbell), despite an increased workload largely due to arrival of the cycad scale, coconut rhinoceros beetle and little fire ant and other invasive species of insects. UOG typically has 4 entomologists. We now have 2.
2. We suffer from the *taxonomic impediment*. The three remaining PhD level entomologists are generalists without the skills and resources for species determination. Timely and accurate species determination is a necessary first step in response to a new pest invasion.
3. There is no ongoing biological survey of Guam with the goal of establishing a baseline biodiversity inventory and detecting newly arrived invasive species. Un-

1 Extension and Community Activities

fortunately, CAPS surveys are usually focused on demonstrating absence of specific agricultural pests rather than detecting new invasions.

4. Even when invasive species are detected and properly identified, first island records are not documented and the information is not published in the scientific press.

In an attempt to improve this situation, I have set myself up as a *registrar* for new insect species arriving on Guam with the intent of properly documenting the ongoing bioinvasion of Guam. The procedure I am trying to establish is:

1. First detector sends me a digital image and/or specimen
2. Specimens are prepared and accessioned into the UOG insect collection
3. A fact sheet is prepared using a template for Guam New Invasive Species Alerts
4. The fact sheet is distributed to a list of stakeholders
5. Taxonomic assistance is obtained for an authoritative species determination.
6. A journal article is prepared and published in a refereed scientific journal. At this point the new geographical distribution data become available to the scientific community via the Global Biodiversity Facility (GBIF).

Although I have been able to generate about a dozen invasive species alerts over the past year (Section 2.4), only one new island record has made it into a peer reviewed journal (Moore, G. Watson, and Bamba 2014).

1.1.2 Insect Identification Service for USDA-APHIS / Guam Customs and Quarantine Agency

I am often called upon to identify insect specimens intercepted the Guam Customs and Quarantine Agency. USDA-APHIS has certified me for this service and has provided a very official looking badge to impress people with. (However, it is not quite as impressive as Dr. Millers bright red badge for getting onto the airport runways.)

USDA-APHIS has recently rewarded me for this service. In response to my 2015 Farm Bill suggestion, the agency kindly equipped me with two professional quality microscopes which will facilitate identification of smaller insects and slide-mounted specimens.

1.2 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the collection and I work with Dr. Ross Miller to curate and catalog this collection.

To increase my knowledge of collection management, I attend the annual meetings of the Entomological Collections Network, which are typically held in conjunction with annual meetings for the Entomological Society of America.

1 Extension and Community Activities

I have a professional goal of building an online website to share all available information on Micronesian insects. This will include specimen level information for the collection complete with digital images and literature references. I built a digital catalog for the collection using the BioLink Biodiversity Information Management System from CSIRO, Australia. The catalog currently contains 29,200 specimen records. BioLink is currently being redeveloped as an open source project (<http://code.google.com/p/biolink/>). I am an active collaborator in this project. In July 2012 I published an article entitled *Hosting a Biolink Database in the Amazon Web Services Cloud (EC2)* on the project's wiki (<http://code.google.com/p/biolink/wiki/BioLinkEC2>).

I have built and evaluated two websites for serving information on Micronesian insect biodiversity, including specimen level data from the collection. One is a Drupal content management system template called LifeDesk provided by the Encyclopedia of Life Project and the other is a similar template called ScratchPads provided by the Museum of Natural History in London. I am honored to have been selected as an advocate for ScratchPads as part of the project's Ambassadors program (<http://scratchpads.eu/locate-scratchpad-ambassadors>). Further information on my websites is provided in the Creative/Scholarly Activities section (2.5).

In March 2014 I travelled to Honolulu to attend the Biodiversity Collections Digitization in the Pacific workshop sponsored by the Integrated Digitized Biocollections (IDigBio). I made an oral presentation entitled [Evaluation of a Scratchpad Template as an Online Database for the University of Guam Insect Collection](#) at this workshop.

In May 2014 I met with Dr. Bob Foottit at the Canadian National Insect Collection in Ottawa to discuss progress and future directions for the UOG collection. Dr. Foottit is a member of the board of directors for the UOG Insect collection.

1.3 Guam Coconut Rhinoceros Beetle Eradication Project

This is currently my largest and most time consuming project.

The coconut rhinoceros beetle (CRB) was first detected on Guam in the Tumon Beach hotel area on September 11, 2007. CRB is a very serious pest of coconut palms. Adult beetles may kill coconuts and other palms when they bore into the crowns to feed on sap. When CRB invaded Palau during the Second World War, it killed about half of all coconuts through the islands and totally exterminated the coconut palm from some of them. A delimitation survey indicated that the Guam infestation was limited to Tumon Bay and the adjacent Faifai Beach. In consultation with the Guam Department of Agriculture (GDOA), USDA-APHIS, and USDA-Forest Survey, it was decided to launch an eradication project.

I wrote the original eradication plan (available on-line at http://guaminsects.net/uogces/kbwiki/index.php?title=Coconut_Rhinoceros_Beetle_Eradication_Plan) and this was funded by USDA and local funds. USDA provided funds under the condition that the project was to be run under an Incident Command System with the

1 Extension and Community Activities

USDA-APHIS Guam Port Director as the federal commander, and the GDOA Director, or designee, as the local commander.

My original role was to provide scientific/technical support for the project, with the Guam Department of Agriculture (GDOA) providing project management with assistance from USDA-APHIS and USDA-Forest Service. However, it soon became apparent that GDOA had serious bureaucratic impediments which prevented hiring staff and procuring supplies and equipment within a reasonable time frame. The eradication project directors, with the consent of the Dean, agreed to run project staffing, procurement, and fiscal management through the University. As a result, my role was expanded to include much of the project management. I am currently managing two grants which fund the project and supervise about 15 temporary employees. Report writing on current grants and proposal writing to keep the project in business occupies much of my time.

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command team set up by APHIS. Later, we were both added to a national technical working group for CRB. My activities in support of the Hawaii CRB Eradication project are detailed in the Regional Collaboration section 1.3.3.

1.3.1 Activities:

1. **Monthly Conference Calls.** These teleconferences are with stakeholders, collaborators, and advisers in USDA APHIS and USDA Forest Service.
2. **Project Websites.** I have endeavored to share and archive data and information associated with the Guam CRB Eradication Project on-line. Prior to May 2009, I used a wiki site at http://www.guaminsects.net/uogces/kbwiki/index.php?title=Oryctes_rhinoceros. Afterwards, I used a Drupal site at <http://www.guaminsects.net/anr/category/miscellaneous/coconut-rhinoceros-beetle>. I maintain a bibliographic database of CRB-related journal articles at http://guaminsects.myspecies.info/crb_biblio and research results are made available as on-line technical reports at http://guaminsects.net/anr/crb_tech_reports.
3. **Project Database.** Trapping data from a network of about 1200 traps, detections of CRB grubs or adults, and observations of CRB defoliation and bore holes are entered daily into a web-based georeferenced MySQL database which I designed. Data from this database is publicly accessible from a web page at <http://www.guaminsects.net/anr/content/public-access-data-collected-guam-coconut-rhinoceros>. Links on this page enable the user to view trap catch data as a spatiotemporal display using a Google Earth animation or a chart of monthly totals. I use this system to produce monthly surveillance reports.
4. **Collaboration.** I have formed two collaborative research groups to do applied research aimed at controlling CRB damage. Dr. Sean Marshall and Dr. Trevor

1 Extension and Community Activities

Jackson at AgResearch New Zealand collaborate with me on biological control using oryctes nudivirus (OrNV) and CRB population genetics. Dr. Matthew Siderhurst and Dr. Eric Jang of USDA-ARS-PBARC collaborate with me on CRB trap improvement and CRB behavior.

1.3.2 Impediment

- My heavy workload does not permit enough time to prepare research results for publication in scientific journals.

1.3.3 Support for the Hawaii Coconut Rhinoceros Beetle Eradication Project

In December 2013, an infestation of CRB was detected on Hickam Air Force Base on Oahu. Roland Quitugua and myself were recruited as subject matter experts and spent a week in Honolulu advising an incident command system (ICS) team set up by APHIS. Later, we were both added to a national technical working group (TWG) for CRB. I built and maintain an online, full-text bibliographic for use by the TWG at http://guaminsects.myspecies.info/CRB_biblio.

Frequent requests for scientific/technical information from the ICS, TWG and Hawaii Department of Agriculture (several queries per week) has significantly increased my workload over the past several months.

Early in 2015, the directors of the Western IPM Center at UC Davis asked me to help organize a meeting to prioritize applied research needs for development of CRB IPM. I co-authored an agenda and attendance list with Arnold Hara and Roland Quitugua. The meeting took place at the Hawaii Department of Agriculture on April 3, 2015 and was chaired by WIPM Center Director Kassim Al-Khatib.

1.4 Western Plant Diagnostics Network

I am the UOG coordinator for WPDN. This organization provides financial support for ANR's Plant Diagnostic Laboratory, offers First Detector Training workshops, and organizes identification workshops for important pest groups. As coordinator, I am required to organize First Detector Training workshops, attend monthly conference calls, attend annual meetings, and provide reports. WPDN publishes newsletters for First Detectors, including the [Pacific Pest Detector](#) to which I occasionally contribute (Table 1.1).

Table 1.1: Contributions to the Pacific Pest Detector Newsletter

December 2013	coconut termite
March 2014	spotted cucumber beetle
March 2014	brown marmorated stink bug
June 2014	castor hairy caterpillar
<i>In press</i>	Pacific Pests and Pathogens Apps

1.5 Guam Invasive Species Advisory Committee (GISAC)

I am an active, founding member of this informal group of Guam's biologists which meets irregularly about 6 times per year to discuss invasive species and what can be done to keep them out and mitigate the effects of those that do invade the island. I worked with Dr. Russell Campbell and Diane Vice to develop an emergency response plan for invasive species detected on Guam.

A wiki site which I built for GISAC was quickly adopted by the Western Micronesia Regional Invasive Species Council at http://guaminsects.net/gisac/index.php?title>Main_Page.

1.6 Public Outreach (Guest lectures, presentations, interviews)

During the reporting period I was interviewed numerous times by newspaper reporters, radio talk show hosts, and television news reporters. Most, but not all involved questions about the Guam coconut rhinoceros beetle eradication project. I helped to produce several fact sheets and articles for public print media.

1.7 Public Outreach (Internet)

During the past decade I published a lot of content on various websites. I have evaluated several current technologies for building a web presence for the Agriculture and Natural Resources Unit and the Drupal content management system seems to be a good fit. This allows us to publish information for public access while keeping some documents private for internal use only. My print and online output are discussed in more detail in the Creative/Scholarly Activity section.

I maintain a website for the UOG Cooperative Extension Service's Agriculture and Natural Resources Program at <http://guaminsects.net/ANR>. I frequently post blog articles of public interest to this site (Table 1.2). I also maintain a website at <http://guaminsects.myspecies.info> which is intended to facilitate sharing information on insects in Micronesia. I submit blog articles to this website which are more technical

1 Extension and Community Activities

and are of interest to biologists. To see a list of my blog post on this site, visit <http://guaminsects.myspecies.info/blogs/aubrey-moore>.

Note that these blogs also contain posts containing information which is not intended for the public. These posts are shared with selected groups of clients and colleagues using a password authentication system.

Table 1.2: Public blog posts on *guaminsects.net/anr* posted 2014-15

Date	Title
2015 Jun 16 - 10:20am	Pacific Pests and Pathogens App for Cell Phones and Tablets
2015 May 30 - 12:20pm	Australian Northern Territory Agricultural Field Guides for Vegetables and Mangoes
2015 Apr 14 - 6:50am	Trap for In-transit Detection of Invasive Species
2015 Apr 10 - 5:55am	Attempts at Keeping Track of Invasive Species in the Marianas
2015 Mar 29 - 8:20am	KUAM News Story by Isa Baza: Funding to combat rhino beetle is lopsided
2015 Mar 28 - 8:21pm	Pacific Daily News Story: LeoPalace nets resort's rhino beetles
2015 Mar 28 - 7:27am	Marianas Variety Newspaper Article: Leo Palace uses nets to capture rhino beetles
2015 Mar 18 - 8:55pm	Pacific Daily News Story: UOG battles rhino beetles
2015 Mar 11 - 12:48pm	K57 Radio Interview: Roland Quitugua and Ray Gibson discuss rhino beetles and little fire ants
2015 Mar 11 - 12:44pm	Marianas Variety Newspaper Article: Rhino Beetle Nets Now on Sale
2015 Mar 11 - 6:55am	Facebook response to sale of tekken by Guam Home Improvement Center
2015 Mar 11 - 6:51am	K57 Radio Interview: Roland Quitugua and Patti Arroyo discuss tekken trap for coconut rhinoceros beetles
2015 Mar 1 - 6:55am	PhysOrg Article: Research to the rescue: Fishing for rhinos with tekken
2015 Feb 26 - 8:54pm	PNC Video: UOG Research to the Rescue
2015 Feb 26 - 4:54am	Hawaii News Now Article: Guam eyes nets to battle rhinoceros beetle
2015 Feb 22 - 10:25am	PNC Video: Family in Yigo finds coconut rhino beetle grubs in store-bought potting soil
2015 Feb 19 - 6:55am	Marianas Variety Newspaper Article: Rhino Beetle Traps Available next Month

Continued on next page

1 Extension and Community Activities

Table 1.2 – *Continued from previous page*

Date	Title
2015 Feb 18 - 5:54pm	PNC News Article by Clynt Ridgell: UOG Unveiles New Tekken Trap For Coconut Rhino Beetle
2015 Feb 4 - 12:56pm	Marianas Variety Newspaper Article: Community-based rhino beetle program holds Guam workshop
2015 Jan 22 - 4:35am	Check List of Micronesian Insects
2014 Sep 28 - 12:37pm	Pacific Daily News Opinion: Fully implement the law to better combat invasive species
2014 Jun 12 - 1:20pm	PNC News Story: DoAG and UOG Team Up to Get Rid of the Little Fire Ant
2014 Jun 11 - 3:28pm	Visualization of Coconut Rhinoceros Beetle Trap Data
2014 Mar 31 - 3:39am	Public opinion on invasive species issues
2014 Mar 26 - 5:13am	iDigBio presentation - Honolulu, March 2014
2014 Feb 20 - 7:18pm	PNC News Story: Guam is Running Out of Options to Stop the Spread of Rhino Beetles and Save Guam's Coconut Trees
2014 Feb 10 - 11:47am	CNN Article by Matt Smith: Meet the beetles: Hawaii mobilizes to fight bug invasion
2014 Feb 9 - 7:17pm	Pacific Daily News Nespaper Article: Mayors voice concerns over rhino beetle
2014 Feb 5 - 1:18pm	Pacific News Center Story: University of Guam Experts Help Hawaii with Rhino Beetles
2014 Jan 22 - 5:48pm	KITV4 Hawaii TV Story: Experts Brought to Hawaii to Battle the Rhino Beetle
2014 Jan 10 - 9:33pm	iNaturalist: Guam CRB Citizen Science
2014 Jan 10 - 7:56pm	KUAM News Story: Invasive species threaten local crops
2014 Jan 10 - 12:52pm	Coconut Rhinoceros Beetle Infestation Discovered at Hickam Air Force Base, Oahu, Hawaii
2014 Jan 10 - 7:25am	Video: Little Fire Ant in Hawaii
2014 Jan 9 - 8:47am	Relative Attractiveness of White and Ultraviolet Light Emmitting Diodes for Rhino Beetles
2014 Jan 9 - 6:05am	Arnold Hara's Rhino Beetle Images Taken During his Trip to Guam
2014 Jan 4 - 5:47am	No Rhino Pamphlet

Continued on next page

Table 1.2 – *Continued from previous page*

Date	Title
2014 Jan 1 - 7:15pm	Pacific News Center Includes Invasive Species Issues in Top 10 Stories of 2013

1.8 Regional Collaboration

1.8.1 Regional Invasive Species Council Website

I maintain a website for the Western Micronesia Regional Invasive Species Council (RISC) at <http://www.guaminsects.net/gisac/>. I attend RISC meetings whenever they are held on Guam and I make presentations at these meetings.

1.8.2 Insect Diagnostics for Micronesia

I am often contacted with requests for help with identifying pests from throughout Micronesia and suggesting solutions to the problems they cause. This workload has increased because the number of practicing PhD-level entomologists in Micronesia has dropped from 9 to 3 within the last two decades.

2 Creative/Scholarly Activities or Research

2.1 Refereed Scientific Journal Articles

1. Fisher, Nicole, Aubrey Moore, Bradley Brown, Matthew Purcell, Gary Taylor, and John Salle (2014). “Two new species of Selitrichodes (Hymenoptera: Eulophidae: Tetrastichinae) inducing galls on Casuarina (Casuarinaceae)”. In: *Zootaxa* 3790.4, 534–542. ISSN: 1175-5334. URL: <http://biotaxa.org/Zootaxa/article/view/zootaxa.3790.4.2/7933>.
2. Moore, Aubrey, Chas Apperson, John McLaughlin, and Philipp Kirsch (In Preparation). “Automated classification of female *Culex pipiens* (Diptera: Culicidae) and *Cx. quinquefasciatus* from optically sensed wingbeat waveforms”. In: *Journal of Medical Entomology*. in preparation.
3. Moore, Aubrey and Donald Bright (In Preparation). “Three new island records for bark beetles (Curculionidae: Scolitinae) on Guam from a single coffee berry borer trap”. In: in preparation.
4. Moore, Aubrey, Trevor Jackson, Roland Quitugua, and Paul Bassler (In Press). “Coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), grubs develop in live coconut palms on Guam”. In: *Florida Entomologist*. in preparation.
5. Moore, Aubrey, N-Y Su, and Leonard Sigrah (In Preparation). “First record of the coconut termite, *Neotermes rainbowi* (Isoptera: Kalotermitidae) from Micronesia”. In: in preparation.
6. Moore, Aubrey, Gillian Watson, and Jesse Bamba (2014). “First record of eggplant mealybug, *Coccidohystrix insolita* (Hemiptera: Pseudococcidae), on Guam: Potentially a major pest”. In: *Biodiversity Data Journal* 2. DOI: [10.3897/BDJ.1.e1042](https://doi.org/10.3897/BDJ.1.e1042). URL: <http://biodiversitydatajournal.com/articles.php?id=1042>.

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1. Ares, M.A., N. Meneses, A. Smith, Aubrey Moore, and R. Benford (2015). “Molecular Identification of a Lepidopteran Herbivore on a Critically Endangered Tree”. In: Northern Arizona Undergraduate Symposium 2015.

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3. Moore, Aubrey (2014n). “Evaluation of a Scratchpad template as an online database for the University of Guam insect collection”. In: *iDigBio Biodiversity Collections Digitization in the Pacific Workshop*. oral presentation. Honolulu, Hawaii.
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4. Marshall, Sean and Aubrey Moore (2014b). “Hawaii beetle dissections”. In: URL: <http://guaminsects.net/anr/sites/default/files/CRB2014-01-17A.pdf>.
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2. Moore, Aubrey (2014c). "Brown marmorated stink bug, *Halymorpha halys*". In: 2014-1. URL: <http://guaminsects.net/anr/sites/default/files/brownMarmoratedStinkBug.pdf>.

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2.5 Web Sites Designed and Maintained by Me

For the past several years, I have been searching for the “right” technology for providing on-line extension information. The features I want include:

- Ease of use, including immediate, on-line editing, so that colleagues and collaborators can create content

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- Ability to display digital images at several resolutions
- Full text search
- Methods for handling on-line and offline references
- Fine grained security which protects client confidentiality and allows for both protected, internal and public information sharing

My current technology of choice is Drupal, a free, open source contents management system.

2.5.1 ANR Web Site.

Home page: <http://guaminsects.net/anr>

This Drupal site is intended to facilitate sharing both internal and external information generated by the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. This site is currently being used heavily by the Guam CRB Eradication Project. I also use this site for documenting my diagnostics work. I provide a recent example web page documenting discovery of thrips in anthurium flowers. (Evidence 2.5.1; available on-line at <http://guaminsects.net/anr/content/thrips-damaging-anth>)

2.5.2 Insects of Guam Web Site

Home page: <http://guaminsects.myspecies.info>

This Drupal site is being evaluated for sharing information on Micronesian insects. Information will include specimen level information from the UOG insect collection complete with digital images and literature references. It was built using a template developed by the Scratchpad project <http://scratchpads.eu/> is sponsored by the European Institute of Distributed Taxonomy (EDIT) and the Natural History Museum in London . The ScratchPad project is celebrating the International Year of Biodiversity by highlighting a different Scratchpad taxon every week. I was honored to have one of my pages, describing the indigenous bug, *Leptocoris vicinus*, highlighted during the week of April 18 to 24, 2010.

(Evidence 2.5.2)

2.5.3 Micronesia Biosecurity Plan Review Web Site

Home page: MBP.GuamInsects.net

This is a secure, private Drupal site developed to facilitate sharing information among those reviewing the Micronesia Biosecurity Plan.

2.5.4 Moodle Site for my AG 109 Insect World Course

Home page: <http://campus.uogdistance.com/course/view.php?id=286>

This site was my first experience with Moodle, a content management system designed for teachers. I originally built it to provide on-line resources for my students, but later decided to open a few wikis to promote collaboration on laboratory exercises. I also kept track of grades using Moodle. Examples from this site include the course resource page (Evidence 2.5.3a; available on-line at <http://campus.uogdistance.com/mod/resource/view.php?id=7349>) and a small PHP program I wrote to facilitate printing pinned insect specimen labels (Evidence 2.5.3b; available on-line at <http://tinyurl.com/insect-labels>).

2.5.5 Knowledgebase Wiki for the UOG Cooperative Extension

Home page: <http://www.guaminsects.net/uogces/kbwiki/index.php>

This was my first attempt at building an extension website to facilitate collaborative content creation. Digital copies of all of ANR's pest fact sheets can be found on this site. There is also a list of insect pests found on all major crops grown in Micronesia. I stopped maintaining this site in May, 2009 because the ANR site built with Drupal has more of the features I need.

(Evidence 2.5.4)

2.5.6 Western Micronesia Regional Invasive Species Council Wiki

Home page: <http://www.guaminsects.net/gisac/index.php>

Originally built for the Guam Invasive Species Advisory Council, this site was quickly adopted for sharing regional information on invasive species by the Western Micronesia Regional Invasive Species Council.

(Evidence 2.5.5)

2.5.7 Guam Insects Blog Site

Home page: <http://blog.guaminsects.net/>

I ran into recurring technical problems with this site which uses the WordPress content management system and have more or less abandoned development and maintenance.

2.5.8 Life Desk Site for Micronesian Insects

Home page: <http://micronesianinsects.lifedesks.org/>

This site uses a Drupal template being developed by the Encyclopedia of Life Project. I evaluated it for sharing information on Micronesian insects, but decided that the Scratchpad template (number 2, above) had a better feature set for what I wanted to do.

2.6 Grants

During 2014 and 2015, I managed 8 grants totalling \$345,040 (listed below). These grants partially or fully supported 14 staff positions (Table 2.1).

2.6.1 Support for the Guam Coconut Rhinoceros Beetle Eradication Project

Funding Source US Forest Service

Amount \$150,000

End Date 2015 Jun 30

Description The objective of this project its to develop an integrated pest management (IPM) program for coconut rhinoceros beetle on Guam.

Project Documents <http://guaminsects.net/anr/content/crb-biocontrol-2013>

2.6.2 Efficacy of Entomopathogenic Fungus for Biological Control of Coconut Rhinoceros Beetle (CRB) on Guam and DNA Profiling of Asia/Pacific CRB Populations with Respect to Virus Susceptibility

Funding Source USDA-APHIS

Amount \$40,000

End Date 2015 Aug 31

Description This project has two objectives:

1. To measure the impact of *Metarhizium majus*, green muscardine fungus (GMF), as a biological control agent for the Guam CRB population
2. To survey and map the extent of the Guam CRB genotype. This work is done in collaboration with Sean Marshall at AgResearch New Zealand.

Project Documents <http://guaminsects.net/anr/content/crb-biocontrol-2013>

2.6.3 Microscopes for UOG Extension Entomology Lab and Guam Customs and Quarantine Agency

Funding Source USDA-APHIS

Amount \$80,000

Description Proposal submitted as a 2015 Farm Bill suggestion. However, APHIS decided to fund this equipment grant from AQI funds. Professional grade equipment including a Nikon stereozoom microscope, a Nikon compound microscope, a digital microscope camera, and camera control were delivered to UOG in June 2015. The grant also provided a stereozoom for the Guam Customs and Quarantine Agency.

2.6.4 Establishment of Captive and Managed Populations of the Mariana Eight-spot Butterfly, *Hypolimnas octocula marianensis*

Funding Source USFWS via an MOU with GDOA-DAWR

Amount \$21,212

End Date 2015 Sep 30 (1 year no cost extension requested)

Description This project will investigate the feasibility of rearing and breeding *H. o. marianensis* and also in field sites where ungulates are excluded.

Project Documents <http://guaminsects.net/anr/content/octocula>

2.6.5 Western Plant Diagnostic Network FY2015

Funding Source UC Davis

Amount \$10,672

End Date 2015 Jun 30

Description WPDN provides first detector training and support of diagnosis of plant pests and pathogens.

Project Documents <http://guaminsects.net/anr/content/wpdn-2014-15>

2.6.6 Western Plant Diagnostic Network FY2016

Funding Source UC Davis

Amount \$10,854

End Date 2016 Jun 30

Description WPDN provides first detector training and support of diagnosis of plant pests and pathogens.

2.6.7 Guam Forest Insect Survey

Funding Source NIFA-McIntire-Stennis

Amount \$12,302 per year

End Date 2018 Jun 30

Description The objective of the survey is to build a knowledge-base on insects associated with plants in Guam's forests. The survey will result in a reference collection of Guam's forest insects and a publicly available online database to facilitate sharing of specimen data, images and ecological associations among plants and insects. The knowledge base will be useful to natural resource managers responsible for maintaining the health of Guam's forests and to biologists trying to understand Guam's terrestrial ecosystems in the wake of major biological invasions.

Project Documents <http://guaminsects.net/anr/content/guam-forest-insect-survey>

2.6.8 Detector Beetles: Radio-tracking Coconut Rhinoceros Beetles to Discover Breeding Sites

Funding Source US Forest Service

Amount \$20,000 (additional \$20,000 pending)

End Date 2016 Apr 30

Description This project is a feasibility study to see if CRB adults equipped with glue-on miniature radio transmitters can be tracked to cryptic breeding sites.

Project Documents <http://guaminsects.net/anr/content/detector-beetles>

Table 2.1: Staff support by my grants in 2014-2015.

1	Bob Bourgeois
2	Roger Brown (partially)
3	Roland Quitugua (partially)
4	Ian Iriarte
5	Vincent Benavente
6	John Diego
7	Ken Leon Guerrero
8	Roland Cabrera
9	Derrick Diego
10	Marty Hara
11	Ken San Nicolas
12	Jessica Gross
13	Cris Crisostimo
14	Raymondo San Miquel

3 University and Community Service

3.1 Teaching

In addition to my job as an extension entomologist, I am required to teach a four credit course every year. My student evaluations are consistently above average (Tables 3.1 and 3.2).

3.1.1 AG-109 Insect World

Table 3.1: Student evaluation for AG109, *Insect World*.

Term	My Evaluation	College Average	University Average
Fall 2009	3.659	3.565	3.552
Spring 2011	3.986	3.519	3.617
Spring 2012	3.863	3.570	3.612
Spring 2013	3.659	3.552	3.627
Fall 2014	3.645	3.471	3.553

3.1.2 AG/BIO-345 General Entomology

Table 3.2: Student evaluation for AG/BIO-345, *General Entomology*.

Term	My Evaluation	College Average	University Average
Fall 2013	3.875	3.522	3.586

3.2 Service as a Reviewer

- I served as an external examiner and reviewed Maclean Vaqalo's PhD dissertation entitled *Biology and ecology of Nisota basselae on Abelmoschus manihot Medicus in Solomon Islands* for the University of Queensland.

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- I reviewed the manual *New Pest Response Guidelines for Coconut Rhinoceros Beetle* for USDA-APHIS.
- I reviewed a scientific note for publication in the Proceedings of the Hawaiian Entomological Society.

3.3 Music

As an amateur horn player I play regularly, and often very badly, with the Guam Symphony Orchestra and occasionally with the Guam Territorial Band. I have played for UOG graduations and for concerts arranged by the UOG music department.

3.4 Collaboration on CESU Rare Butterfly and Snails Survey Grant

I am collaborating with Dan Lindstrom, John Benedict, Frank Camacho, and Curt Fiedler (UOG Biology), Alex Kerr (UOG Marine Lab), Brent Holland and Dan Rubinoff (UH Manoa) on a DOD funded survey of rare butterflies and snails. My contribution is a literature review of *Hypolimnas octocula mariannensis* for publication in Micronesica, design and maintenance of project website and development of butterfly camera traps.

3.5 Collaboration on Biocontrol of Cycad Aulacaspis Scale

I am working with Tom Marler on introduction of parasitoids for biocontrol of the *Aulacaspis yasumatsui*.

3.6 University Technical Advisory Committee

I serve on UTAC as the representative for the College of Natural and Applied Sciences.

3.7 Undergraduate Curriculum Review Committee (UCRC)

In the April 2013 Faculty Elections, I was elected to serve on the UCRC. I served for two years on this committee, 2013-14, and 2014-15.

3.3 Evaluation

Please see next page.



College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

FACULTY SALARY INCREMENT

Aubrey Moore
Faculty Member

Extension Agent IV
Rank or Title

May 27, 2014 - June 26, 2015
Employment Period Under Review

IV - 15
Present Level/Step

College of Natural and Applied Sciences
College/Unit

Cooperative Extension Service
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be
 APPROVED DISAPPROVED

James R. Hollyer
Associate Director, Dean

Dec 28, 2015
Date

Rationale:

My apologies for finding this on my desk today when we did the review in late December. We did the review back in late December and Aubrey has done good work. He has taught classes, wrote and won grants, performed successful research, and in some cases published. He has provided clients and Govt. Guam agencies with insect identification services. He is passionate about eliminating the coconut rhinoceros beetle. He asked not to teach any more for an entomology tech, and for more money to support his extension work, and those requests were forwarded to the Dean.

Approved
unfilled
04/18/16



College of Natural & Applied Sciences
COLLEGE OF NATURAL & APPLIED SCIENCES

RECOMMENDATION OF DEAN/DIRECTOR

- I concur with the above recommendation
 I do not concur with the above recommendation (see below)

[Signature]
Lee S. Ydoin
Dean/Director

[Signature]
Date

Rationale

RESPONSES OF EMPLOYEE TO THIS EVALUATION

The above Salary increment recommendations have been discussed with me and my responses, if any, are as follows:

[Signature]
Audrey Moore
Faculty Member

Date

CERTIFICATION OF FUNDS

- Funds Available Funds Not Available

Certifying Officer

Date

4 2015-2016

4.1 Report

Please see next page.

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

Reflective Form

Comprehensive Faculty Evaluation System – Part I

Your name: Aubrey Moore

Your current Rank and Step: Extension Entomologist / Associate Professor

This CFES evaluation period: June 15, 2015 – June 14, 2016

Role Assignments	Percent of Time
Extension & Outreach	51% (primary focus must be a minimum of 50%)
Creative/Research/Scholarly	34%
Instruction	0%
University Service	15%
TOTAL	100%

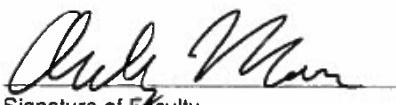
Please list any outside consulting activities for this performance period:

None.

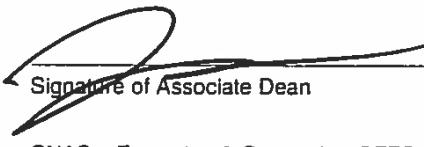
The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

As called for by the University Comprehensive Faculty Evaluation System, I hereby acknowledge that I have notified my unit Chair and unit colleagues of my preferences for role assignments.

Further, I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating.


Signature of Faculty

8/10/16
Date:


Signature of Associate Dean

Date: 8/10/16

Alycia

Signature of Dean/Director

Date 09/27/16

Comprehensive Faculty Evaluation System – Part II

Directions: This document serves as a Plan of Work for the upcoming period and then as the Annual Report, a year later, relative to your accomplishments in the Plan of Work. Please note any deviations from your original plan – activities that changed and the ones that got added for some reason – in the second table. DO NOT ALTER THE ORIGINAL TOP TABLE. For any papers, presentations, workshops, attach hard copy evidence at the end of this document.

Role Assignment: Extension & Outreach 51%

Planned Activities for this CFES year: June 15, 2015 – June 14, 2016

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
1. Insect Diagnostic Services Identify insects and make control recommendations when requested.	Records of insect identifications and control recommendations, some in the form of iNaturalist observation postings.	Jim Hollyer
2. Detection and Documentation of Invasive Species Continue adding to and maintaining the Guam Invasive Species Alerts fact sheet series.	Publish Guam Invasive Species Alerts fact sheets	Jim Hollyer
3. University of Guam Insect Collection Continue curation and databasing of the UOG Insect Collection.	none	Jim Hollyer
4. Guam Coconut Rhinoceros Beetle Project	presentations, technical reports, journal articles	Jim Hollyer
5. National Plant Diagnostic Network (NPDN) Participate in monthly conference calls. Train and certify First Detectors. Attend the NPDN National Conference in Washington, D.C., March 8-12, 2016.	none	Jim Hollyer
6. Guam Invasive Species Advisory Committee (GISAC)	GISAC meeting minutes	Jim Hollyer

Participate in GISAC meetings.		
7. Public Outreach (Guest lectures, presentations, interviews) Provide accurate scientific and technical information to the public as required.	Radio, TV, and newspaper articles	Jim Hollyer
8. Public Outreach(Internet) Assist in migrating the CNAS-RE WordPress test site on DreamHost to a more permanent home. Phase out use of the ANR Drupal site and move content to the new CNAS-RE WordPress Site.	None.	Jim Hollyer

Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2015 – June 14, 2016

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
1. Insect Diagnostic Services The number of extension calls requiring my assistance during the reporting year averaged approximately three per day. During this reporting year, my USDA-APHIS cooperator workload was very high because the Guam Territorial Entomologist retired and there was a campaign to intercept pests arriving with the Pacific Festival of the Arts.	Insect diagnostic cases documented as iNat observations: [1] Press stories on flies discovered in nipa leaves imported from the Philippines for FestPac: [2, 3, 4, 5, 6] On May 26, 2016 I wrote a press release with Olympia Terral, intended to highlight cooperation among GCQA, Guam Agriculture, USDA-APHIS, and UOG: [7] Press stories triggered by the above press release: [8][9]	Jim Hollyer
2. Detection and Documentation of Invasive Species Added a page to the CNAS-RE web site which links to the Guam Invasive Species Alerts fact sheets. [10] Prepared a fact sheet for <i>Vespa tropica</i> . [10]	References provided.	Jim Hollyer
University of Guam Insect Collection I have begun evaluating Specify as an online database for the UOG Insect Collection. iDigBiorecommends Specify as the online collection database of choice for small biological collections. Whenever, taxonomists visit Guam, I recruit their expert help to improve the collection. Dr. Mary-Liz Jamison and Dr. Josh Dunlap visited during January 14-16, 2016 and worked on the scarab beetles. Dr. Peter Maddison visited Guam June 23-29, 2016 and put together a	Sorted and identified specimens.	Jim Hollyer

<p>synoptic collection of common insects using specimens collected by students.</p> <p>4. Guam Coconut Rhinoceros Beetle Project</p> <p>Discovery of arboreal breeding of CRB on Guam:</p> <ul style="list-style-type: none"> • Refereed journal article published [12, hard copy provided] • Prepared press release and web post with Olympia Terral [13] • Press articles : [14, 15, 13] <p>Radio-tracking CRB to find cryptic breeding sites:</p> <ul style="list-style-type: none"> • Referred journal article submitted [16] • Press release and generated articles. [17][18] [19][20] <p>Discovery of the CRB-Guam biotype:</p> <ul style="list-style-type: none"> • Whitepaper prepared as requested by the Western IPM Center [21] • Discovery of the CRB-Guam biotype announced at a Society for Invertebrate Pathology meeting [22] • Fact sheet on CRB-G prepared for SPC [23] • Made a presentation on CRB-G and participated in discussions on a coordinated response to CRB-G at the Pacific Plant Protection Meeting in Fiji, September 2015. [24] • In June 2016, I attended a meeting on CRB Biocontrol in Fiji. I made a presentation on CRB-G and discussed a 	<p>References provided.</p>	<p>Jim Hollyer</p>
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<p>coordinated response to CRB-G with participants from SPC, PNG, Solomon Islands, and Samoa. I compiled an online repository of materials from this meeting on my Open Science Framework site. [25]</p> <p>Movement of packaged soil as a dispersal pathway for coconut rhinoceros beetles:</p> <ul style="list-style-type: none"> • Refereed journal article submitted and accepted [26] • Press release and web article prepared with Olympia Terral [27] • RPress articles generated: [28, 29, 30] 		
<p>5. National Plant Diagnostic Network (NPDN)</p> <p>Participated in monthly conference calls.</p> <p>Submitted an article to the Pacific Pest Detector Newsletter [31].</p> <p>Trained and certified students as First Detectors via a module in my Fall 2015 AG/BI 345 course.</p> <p>Attended the NPDN National Conference in Washington, D.C., March 8-12, 2016.</p>	<p>Conference call minutes, article in Pacific Pest Detector Newsletter, National Certified First Detector List</p>	<p>James Hollyer</p>
<p>6. Guam Invasive Species Advisory Committee (GISAC)</p> <p>Participated in meetings.</p>	<p>GISAC meeting minutes</p>	<p>Jim Hollyer</p>
<p>7. Public Outreach (Guest lectures, presentations, interviews)</p> <p>During the reporting period I was interviewed numerous times by newspaper reporters, radio talk show hosts, and television news reporters. Most, but not all involved</p>	<p>A Google news archive search for pages containing "Aubrey Moore" and "Guam" posted between June 15, 2015 and June 14, 2016 returned 23 results. [32]</p>	<p>Jim Hollyer</p>

questions about the Guam coconut rhinoceros beetle and other invasive species issues. I helped to produce several fact sheets and articles for public print media.		
<p>8. Public Outreach (Internet)</p> <p>The CNAS-RE test site http://guaminsects.net/wp was moved to its more permanent home https://cnas-re.uog.edu during August 2015.</p> <p>Some content, bibliographic information for instance, was imported into the CNAS-RE site from ANR site.</p>		Jim Hollyer

Role Assignment: Creative/Research/Scholarly 34%

Planned Activities for this CFES year: June 15, 2015 – June 14, 2016

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
<p>1. Coconut Rhinoceros Beetle (CRB) Biocontrol</p> <p>Complete bioassays to recheck pathogenicity of previously tested OrNV samples from AgResearch New Zealand. This task is already included in the work plan for 2 of my grants.</p> <p>As per an action item from the WIPM CRB IPM meeting in Honolulu, I will work with Sean Marshall (AgResearch NZ) and Maclean Vaqalo (SPC) on generating a white paper prioritizing applied research needs for CRB management.</p> <p>I plan to attend the Pacific Plant Protection Conference as a technical rep for Guam and will make a presentation based on the white paper.</p> <p>I will work to set up an international collaborative project with the goal of mapping the CRB-Guam biotype and finding a strain of OrNV which can be used as an effective biocontrol agent. Potential collaborators are AgResearch NZ, SPC, Philippine Coconut Authority, and USDA. This project will have a foreign exploration component which will collect CRB and virus samples throughout the Asian/Pacific region. Genotyping and virus detection will be done by AgResearch NZ. Bioassays in which CRB-Guam beetles will be</p>	None	Jim Hollyer

<p>challenged with virus candidates will be done in my laboratory at UOG.</p> <p>I will set up an insect pathology lab and recruit Ian Iriarte as a graduate assistant to run bioassays.</p> <p>I have already applied to US Forest Service for \$20K to fund this assistantship.</p>		
<p>2. Cycad Aulacaspis Scale Biocontrol</p> <p>Evaluate the impact of <i>Arrhenophagus</i> sp. on the Guam cycad population</p> <p>Write and submit a peer-reviewed scientific journal article entitled something like Fortuitous introduction of the parasitoid <i>Arrhenophagus</i> sp. to Guam and its impact on cycas aulacaspis scale, <i>Aulacaspis yasumatsui</i>, infesting endemic cycads, <i>Cycas micronesica</i>.</p> <p>If Ron Cave is willing to collect <i>Coccobius fulvus</i> again and if APHIS approves, attempt a direct field release of this parasitoid.</p>	none	Jim Hollyer
<p>3. Guam Forest Insect Survey</p> <p>The objective of the proposed survey is to build a knowledgebase on insects associated with plants in Guam's forests. The survey will result in a reference collection of Guam's forest insects and a publicly available online database to facilitate sharing of specimen data, images and ecological associations among plants and insects. The knowledgebase will be useful to natural resource managers</p>	none	Jim Hollyer

responsible for maintaining the health of Guam's forests and to biologists trying to understand Guam's terrestrial ecosystems in the wake of major biological invasions.		
<p>4. Eight Spot Butterfly Conservation</p> <p>Propagate and maintain at least 100 plants of each of the eight-spot's known host plants, <i>Procrispendunculata</i> and <i>Elatostema calcareum</i> in a plant nursery.</p> <p>Establish a self-sustaining, caged, breeding colony of eight-spot butterflies using 30 field-collected caterpillars reared on plants from the nursery.</p> <p>Propagate host plants throughout two 10 x 10 meter, wooded limestone areas at the University of Guam's Agricultural Experiment Station in Yigo.</p> <p>Release 60 cage-reared eight-spot butterflies and larvae on protected host plants.</p>	None	Jim Holler

Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2015 – June 14, 2016

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
<p>1. Coconut Rhinoceros Beetle (CRB) Biocontrol</p> <p>We have gone through 4 cycles of the witch's brew bioassays and the mortality increases for each iteration. Gut samples from beetles are being sent to AgResearch NZ to test for OrNV.</p>	technical reports, etc. References provided in the Actual Activities column	Jim Hollyer

	<p>White paper was written [37] and used as a source for the SPC fact sheet on CRB .</p>	
<p>Made a presentation on CRB-G and participated in discussions on a coordinated response to CRB-G at the Pacific Plant Protection Meeting in Fiji, September 2015. [24]</p>		
<p>I continue working to set up an international collaborative project with the goal of mapping the CRB-Guam biotype and finding a strain of OrNV which can be used as an effective biocontrol agent. Potential collaborators are AgResearch NZ, SPC, Philippine Coconut Authority, and USDA. This project will have a foreign exploration component which will collect CRB and virus samples throughout the Asian/Pacific region. Genotyping and virus detection will be done by AgResearch NZ. Bioassays in which CRB-Guam beetles will be challenged with virus candidates will be done in my laboratory at UOG.</p>		
<p>I recruited Ian Iriarte as a graduate assistant to run bioassays and have secured one year of support from my FY16 Farm Bill grant.</p>		
<p>2. Cycad Aulacaspis Scale Biocontrol</p> <p>Journal article not written due to lack of time.</p> <p>Made direct releases of <i>Coccobius fulvus</i> at Ritidian in September and November 2015. <i>C. fulvus</i> has not been reared from recent leaf collections, so there is no proof that</p>	<p>No evidence provided.</p>	<p>Jim Hollyer</p>

this parasitoid has established.		
3. Guam Forest Insect Survey	Please see McIntire Stennis FY2015 Annual Report [38, hard copy provided]	Jim Hollyer
4. Eight Spot Butterfly Conservation Twelve Procris plants were collected and propagated by Lauren Gutierrez. These plants were delivered to the Yigo Ag. Expt. Stn. and were immediately attacked by Cuban slugs. Prior to this observation, introduced slugs were not considered as serious competitors for 8-spot butterfly host plants. 2. A contract was written to support Lauren Gutierrez as a collaborator on the project. Gutierrez's role is to collect and propagate host plants. Due to beaurocratic delays, the contract has not yet been signed by UOG. 3. In November 2015, Hypolimnus octocula marianensis was list by the US Fish and Wildlife Service as an endangered species. A permit is now required to perform scientific work aimed at conserving this species. A permit application has been written [39].	Surviving Procris plants are growing in front of ALS105. Permit application pending.	Jim Hollyer

Role Assignment: Instruction 0%

Planned Activities for this CFES year: June 15, 2015 – June 14, 2016

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
1.		
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Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2015 – June 14, 2016

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
1.		
2.		
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Role Assignment: Community Service 15%

Planned Activities for this CFES year: June 15, 2015 – June 14, 2016

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
1. Instruction I will teach General Entomology AG/BIO-345 during the Fall 2015 term. This is a 4 credit course consisting of 2 lectures per week plus a 3 hour lab session. I plan to have Ian Iriarte as my first masters student in the EV program.	Student evaluation.	Jim Hollyer
2. Service as a Reviewer	None.	Jim Hollyer
3. University Technical Advisory Committee I will continue to serve on UTAC as the representative for the College of Natural and Applied Sciences.	UTAC meeting minutes	Jim Hollyer
4. Faculty Building Facilities Committee for ALS	None.	Jim Hollyer

Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2015 – June 14, 2016

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
1. Instruction I taught General Entomology AG/BIO-345 during the Fall 2015 term. I recruited Ian Iriarte as my first masters student in the EV program and secured support for his first year from my FY2016 Farm Bill grant (CRB-G Biocontrol).	Syllabus for General Entomology AG/BIO-345. [40] Web site for General Entomology AG/BIO-345 (static web site built using Pelican) [41]. Student evaluation for General Entomology AG/BIO-345. My score (3.63) was above the university average (3.55) and the CNAS average (3.48).	Jim Hollyer
2. Service as a Reviewer	References provided.	Jim Hollyer

<p>Acted as external examiner for master's student John Tuivavalagi, University of Queensland. I was an external examiner of his thesis entitled Investigating The Impacts of the natural enemy <i>Trichogramma chilonis</i> Ishii on populations of <i>Crocidolomia pavonana</i> in Samoa. [42]</p> <p>2. In September 2015: I acted as peer reviewer for Public Library of Science (PLoS) manuscript PONE-D-15-29086R1 Insect Biometrics: Optoacoustic signal processing and its applications to remote monitoring of McPhail type traps. submitted by Ilyas Potamitis.</p> <p>3. In July 2016: I acted as peer reviewer for Journal of Medical Entomology manuscript JME-2016-0177 2D Optoacoustic sensors embedded in mosquito insectary cages report species identity through wingbeats. submitted by Ilyas Potamitis et al. [43]</p>		
<p>3. University Technical Advisory Committee</p> <p>I continue to serve on UTAC as the representative for the College of Natural and Applied Sciences.</p>		Jim Hollyer
<p>4. Faculty Building Facilities Committee for ALS</p> <p>I became chair of this committee when Dr. Laura Biggs left during 2015.</p> <p>Documented air conditioning problems, especially excessively high humidity (>60% RH) and met with Dr. Rachel Leon Guerrero and Jesse Rosario to discuss possible solutions</p>	<p>Recommendations for improving the ALS 124 as a science teaching environment [44] and obtained a quote for installation of audiovisual equipment.</p>	Jim Hollyer

<p>Procured a large screen HDTV for the teaching lab (ALS 124) (Thanks to Jim Hollyer for help with this)</p> <p>Installed Internet cable to provide sufficient bandwidth for streaming video (Thanks to Rudy Magallanes for help with this)</p> <p>Organized clean up of the teaching lab following the Fall 2015 semester.</p> <p>Compiled recommendations for improving the ALS 124 as a science teaching environment.</p>		
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Comprehensive Faculty Evaluation System – Part III

Summary of Publications and Grant Activities

On this page, list specific outputs generated during the evaluation period so that they can be entered into the CNAS website databases.

Publications and other media produced during the review period

1. Peer Reviewed Journal Articles

Moore et al. 2015. Coconut rhinoceros beetles (Coleoptera : Scarabaeidae) develop in arboreal breeding sites in Guam. *Florida Entomologist* 98(3) 1012-1014. [12]

Moore et al. 2016. Movement of packaged soil products as a dispersal pathway for coconut rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera:Scarabaeidae) and other invasive species. *Proceedings of the Hawaiian Entomological Society* [In press]. [26]

Moore et al. 2016. Judas beetles: Discovering cryptic breeding sites by radio-tracking coconut rhinoceros beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Environmental Entomology* [Submitted] [16]

2. Fact Sheets

Moore et al. (2014-2016) Guam Invasive Species Alert Series. [10]

Vaqalo, M., Marshall, S., Jackson, T., & Moore, A. (2015). An emerging biotype of coconut rhinoceros beetle discovered in the Pacific (Pest Alert No. 51) (p. 2). Secretariat of the Pacific Community. [23]

Moore, A. (2015). The new Pacific pests and pathogens app. In *Pacific Pest Detector News* 23.[31]

3. Presentations

Ares, M. A., Meneses, N., Smith, A., Moore, A., & Benford, R. (2015). Molecular Identification of a Lepidopteran Herbivore on a Critically Endangered Tree. Northern Arizona Undergraduate Symposium 2015. [45]

Marshall, S. D. G., Vaqalo, M., Moore, A., Quitugua, R., & Jackson, T. A. (2015). A new invasive biotype of the coconut rhinoceros beetle (*Oryctes rhinoceros*) has escaped from biocontrol by *Oryctes rhinoceros* nudivirus. In International Congress on Invertebrate Pathology and Microbial Control and the 48th Annual Meeting of the Society for Invertebrate Pathology. Retrieved from [22]

Moore, A. (2015). A report on the Guam coconut rhinoceros beetle infestation for the 8th Pacific Plant Protection Organisation Board Meeting and 16th Regional Technical Meeting on Plant Protection. Nadi, Fiji. September 21-25, 2015.

Moore, A, (2015). Update on the Guam Coconut Rhinoceros Beetle for the Guam Invasive Species Council. Guam, November 20, 2015. [46]

Aubrey Moore. (2016, March). Guam Report. Presented at the National Plant Diagnostics Network Meeting, Washington, D.C. [47]

Aubrey Moore. (2016, April). New variant of rhinoceros beetle, Guam biotype, and implications for global control. Presented at the Annual Meeting of the Pacific Branch of the Entomological Society of America, Honolulu, Hawaii. [48]

Aubrey Moore. (2016, June). Discovery of the Coconut Rhinoceros Beetle Guam Biotype and Implications for Global Control. Presented at the Future proofing the palm industries: Limiting damage by existing (CRB-P) and invasive (CRB-G) coconut rhinoceros beetle (*Oryctes rhinoceros*) in the Pacific, Suva, Fiji. [49]

Grants applied for during the review period

USDA-Aphis Biocontrol Program: *Oryctes nudivirüs* for biocontrol of the Guam biotype of the coconut rhinoceros beetle; \$20,000 requested, Not funded, Proposal[50]

2015-16 USDA Farm Bill: *Oryctes nudivirüs* for biocontrol of the Guam biotype of the coconut rhinoceros beetle; \$120,000 requested; \$100,000 awarded; Work plan [51]

US Forest Service: Detector Beetles: Radio-Tracking Coconut Rhinoceros Beetles (CRB) to Discover Breeding Sites and CRB Biocontrol; \$40,000 requested, \$40,000 awarded, Proposal [52]

McIntire-Stennis, Guam Forest Pest Survey. \$5,000

Dean's 2016 High-impact Project Pool Competition: Coconut rhino beetle as a transmission vector for Tinangaja disease., \$39,911 requested; \$39,911 awarded, Proposal [53]

US Fish and Wildlife Service FY2016 (funds passed through GDOA-DAWR via an MOU): Establishment of Captive and Establishment of Captive and Managed Populations of Mariana Eight-spot Butterfly,\$18,000 requested; \$18,000 awarded; Work Plan [54]

Grants won during the review period

As indicated above, applied for 6 grants with a total request of \$242,911. I was funded on 5 grants totalling \$202,911.

References

- [1] Aubrey Moore. "iNaturalist.org · Observations by aubreymoore between June 15, 2015 and June 14, 2016." [Online]. Available: <http://www.inaturalist.org/observations/aubreymoore.html?dl=2015-06-15&d2=2016-06-14> 5
- [2] "Blue flies in FestPac shipment of nipa leaves." [Online]. Available: <http://www.guampdn.com/story/news/2016/04/02/blue-flies-festpac-shipment-nipa-leaves/82452640> 5
- [3] "FestPac nipa leaves fumigated | Local News | postguam.com." [Online]. Available: http://www.postguam.com/news/local/festpac-nipa-leaves-fumigated/article_e76a1ec6-f63c-11e5-82a8-7b0fcdf743ba.html 5
- [4] "Invasive Flies Found in Shipment of Nipa Leaves For FESTPAC Cement Huts - YouTube." [Online]. Available: <https://www.youtube.com/watch?v=DfwUcSE1-X4> 5
- [5] "OUR VIEW: Protect Guam from invasive species during FestPac - Pacific Daily News - Guam Info." [Online]. Available: <http://guam.localco.net/our-view-protect-guam-from-invasive-species-during-festpac-pacific-daily-news> 5
- [6] "Quarantine." [Online]. Available: <http://www.desmoinesregister.com/topic/9e8d95f2-1de4-4d3a-b376-acd6bf73fb97/quarantine> 5
- [7] "press release: GovGuam, UOG and USDA Cooperate to Prevent Arrival of Invasive Species Hitch-hiking on FestPac Materials - aubreymoore2013@gmail.com - Gmail." [Online]. Available: <https://mail.google.com/mail/u/0/#search/%22press%20release%22/154eb579c58231c> 5
- [8] "Invasive species found on coconut leaves for FestPac." [Online]. Available: <http://www.guampdn.com/story/news/2016/05/27/invasive-species-found-coconut-leaves-festpac/84957454> 5
- [9] "Guam prevents invasive species hitchhiking on FestPac materials - Saipan News, Headlines, Events, Ads | Saipan Tribune." [Online]. Available: <http://www.saipantribune.com/index.php/guam-prevents-invasive-species-hitchhiking-festpac-materials> 5
- [10] Aubrey Moore, "Guam New Invasive Species Alerts." [Online]. Available: <http://cnas-re.uog.edu/guam-new-invasive-species-alerts> 6
- [11] A. Moore, "Evaluation of a Scratchpad template as an online database for the University of Guam insect collection," in *iDigBio Biodiversity Collections Digitization in the Pacific Workshop*, Honolulu, Hawaii, Mar. 2014, oral presentation. [Online]. Available: https://www.idigbio.org/wiki/images/a/aa/Scratchpads_iDigBio-part1.pdf 8
- [12] A. Moore, T. Jackson, R. Quitugua, P. Bassler, and R. Campbell, "Coconut rhinoceros beetles (Coleoptera : Scarabaeidae) develop in arboreal breeding sites in Guam," *Florida Entomologist*, vol. 98, no. 3, pp. 1012-1014, 2015. [Online]. Available: <http://journals.fcla.edu/laent/article/download/84794/84044> 9, 27
- [13] Olympia Terral, "Breeding in the crowns of coconut palms: Unusual coconut rhinoceros beetle behavior in Guam." [Online]. Available: <http://cnas-re.uog.edu/breeding-in-the-crowns-of-coconut-palms-unusual-coconut-rhinoceros-beetle-behavior-in-guam> 9
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4.2 Plan for Following Year

Please see next page.

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

Future Plan Form

Comprehensive Faculty Evaluation System – Part I

Your name: Aubrey Moore

Your current Rank and Step: Extension Entomologist / Associate Professor

This CFES evaluation period: June 15, 2016 – June 14, 2017

Role Assignments	Percent of Time
Extension & Outreach	51% (primary focus must be a minimum of 50%)
Creative/Research/Scholarly	34%
Instruction	0%
University Service	15%
TOTAL	100%

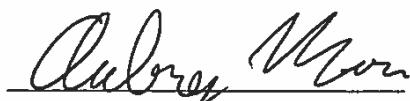
Please list any outside consulting activities for this performance period:

None.

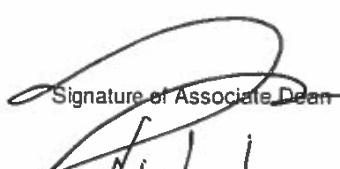
The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

As called for by the University Comprehensive Faculty Evaluation System, I hereby acknowledge that I have notified my unit Chair and unit colleagues of my preferences for role assignments.

Further, I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating.


Signature of Faculty

8/10/16
Date:


Signature of Associate Dean


Signature of Dean/Director

Date: 8/10/16

Date: 9/24/14

Comprehensive Faculty Evaluation System – Part II

Directions: This document serves as a Plan of Work for the upcoming period and then as the Annual Report, a year later, relative to your accomplishments in the Plan of Work. Please note any deviations from your original plan – activities that changed and the ones that got added for some reason – in the second table. DO NOT ALTER THE ORIGINAL TOP TABLE. For any papers, presentations, workshops, attach hard copy evidence at the end of this document.

Role Assignment: Extension & Outreach 51%

Planned Activities for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
1. Insect Diagnostic Services Identify insects and make control recommendations when requested.	iNaturalist posts documenting insect identifications	Jim Hollyer
2. Detection and Documentation of Invasive Species Continue adding to and maintaining the Guam Invasive Species Alerts fact sheet series.	Guam Invasive Species Alerts fact sheets	Jim Hollyer
3. University of Guam Insect Collection Continue curation and databasing of the UOG Insect Collection. Continue evaluation of Specify as an online database for the UOG Insect Collection.	Specimen records.	Jim Hollyer
4. Guam Coconut Rhinoceros Beetle Project Provide scientific/technical support to the Guam Coconut Rhinoceros Beetle Project. My focus will be on CRB-G biocontrol and monitoring health of coconut palms on Guam. For details, see the CRB Biocontrol section under Creative / Scholarly / Research for details .	Technical reports, refereed journal articles	Jim Hollyer
5. National Plant Diagnostic Network (NPDN)	Conference call minutes, NPDN First Detector Certifications, annual report	Jim Hollyer

Participate in monthly conference calls.		
Train and certify First Detectors.		
Prepare annual work plan and annual report.		
6. Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC)	meeting minutes	Jim Hollyer
Participate in meetings.		
7. Public Outreach (Guest lectures, presentations, interviews)	Newspaper articles, radio and television interviews	Jim Hollyer
Provide accurate scientific and technical information to the public as required.		
8. Public Outreach(Internet)	Blog posts, online database of crop pests	Jim Hollyer
Phase out use of the ANR Drupal site and move content to the new CNAS-RE WordPress Site.		
Provide an online database of insect crop pests in Micronesia with links to images and fact sheets. This activity overlaps with plans to create a Guam Biodiversity Inventory (see section in Create/Scholarly/Research)		
9.		
10.		

Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2016 – June 14, 2017

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
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Role Assignment: Creative/Research/Scholarly 34%

Planned Activities for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
<p>1. Coconut Rhinoceros Beetle (CRB) Biocontrol</p> <p>Complete bioassays to recheck pathogenicity of previously tested OrNV samples from AgResearch New Zealand.</p> <p>2. Participate in the International Congress of Entomology in Orlando, Florida in September 2016. I have been invited to give an oral presentation on CRB-G at a symposium on scarab beetles.</p> <p>3. I will work to set up an international collaborative project with the goal of mapping the CRB-Guam biotype and finding a strain of OrNV which can be used as an effective biocontrol agent. Potential collaborators are AgResearch NZ, SPC, Philippine Coconut Authority, and USDA. This project will have a foreign exploration component which will collect CRB and virus samples throughout the Asian/Pacific region. Genotyping and virus detection will be done by AgResearch NZ. Bioassays in which CRB-Guam beetles will be challenged with virus candidates will be done in my laboratory at UOG.</p> <p>As per my approved FY2016 Farm Bill grant, I plan to visit Palau and Negros Island, Philippines with Dr. Sean Marshall, AgResearch New Zealand and my graduate student, Ian Iriart in early 2017. CRB-G has</p>	Technical reports, scientific journal articles, presentations.	Jim Hollyer

<p>been detected at both of these locations.</p> <p>I plan to submit a FY2017 Farm Bill suggestion to continue my work on establish biocontrol of CRB-G to prevent further coconut palm mortality on Guam. This suggestion will also request support for establishment of a semiannual coconut palm health survey.</p>		
<p>2. Cycad Aulacaspis Scale Biocontrol</p> <p>Determine if the parasitoid <i>Coccobius fulvus</i> which was released twice at Ritidian at the end of 2016 has established.</p> <p>Evaluate the impact of <i>Arrhenophagus</i> sp. on the Guam cycad population</p> <p>Write and submit a peer-reviewed scientific journal article on CAS biocontrol.</p>	<p>Peer reviewed article on CAS biocontrol.</p>	<p>Jim Hollyer</p>
<p>3. Guam Forest Insect Survey</p> <p>A database of insect pests associated with Guam's forest plants will be built using information from the literature, specimens, and surveys.</p> <p>The database will be made available on-line.</p>	<p>Online database of insect pests associated with Guam's forest plants.</p>	<p>Jim Hollyer</p>
<p>4. Eight Spot Butterfly Conservation</p> <p>Propagate and maintain at least 100 plants of each of the eight-spot's known host plants, <i>Procris pendunculata</i> and <i>Elatostema calcaricum</i> in a plant nursery.</p> <p>Establish a self-sustaining, caged, breeding colony of eight-spot</p>	<p>Technical reports.</p>	<p>Jim Hollyer</p>

<p>butterflies using 30 field-collected caterpillars reared on plants from the nursery.</p> <p>Propagate host plants throughout two 10 x 10 meter, wooded limestone areas at the University of Guam's Agricultural Experiment Station in Yigo.</p> <p>Release 60 cage-reared eight-spot butterflies and larvae on protected host plants.</p>		
<p>5. Guam Biodiversity Inventory</p> <p>Design and build Check List Plus (CLP) an online database to store a "tree of life" for Guam using a reference taxonomy from the National Center for Biotechnology (NCBI). The database will contain synonyms, references (to the literature, observations and specimens), and ecological relationships (such as links between herbivores and host plants).</p> <p>Populate CLP with the flora and fauna of Guam from the scientific literature. Initial targets will include a list of all crops and important forest plants growing on Guam, insect pests that feed on these plants, and biocontrol agents controlling these insects.</p>	<p>An online database which can be queried to return useful information on Guam's organisms and interactions between them.</p> <p>Applications will query this database to return useful information such as: "return list of all caterpillars feeding on cabbage on Guam with links to images and fact sheets for these species."</p>	Jim Hollyer
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Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2016 – June 14, 2017

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
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Role Assignment: Instruction 0%

Planned Activities for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
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Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2016 – June 14, 2017

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
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Role Assignment: Community Service 15%

Planned Activities for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities	Planned Evidence of Accomplishment	Planned Evaluation By
1. Instruction I will serve as Ian Iriarte's major professor during his masters program in environmental science.	None	Jim Hollyer
2. University Technical Advisory Committee I will continue to serve on UTAC as the representative for the College of Natural and Applied Sciences.	meeting minutes	Jim Hollyer
3. Faculty Building Facilities Committee for the ALS I will continue to serve as chair of the Faculty Building Facilities Committee for the ALS	None	Jim Hollyer
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Activities that were planned above the year before and these are the Actual Activities that took place during the evaluation period: June 15, 2016 – June 14, 2017

Actual Activities	Actual Evidence of Accomplishment	Actual Evaluation By
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Comprehensive Faculty Evaluation System – Part III

Summary of Publications and Grant Activities

On this page, list specific outputs generated during the evaluation period so that they can be entered into the CNAS website databases.

Publications and other media produced during the review period

Grants applied for during the review period

Grants won during the review period

4.3 Evaluation

Please see next page.



College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

FACULTY SALARY INCREMENT

Aubrey Moore
Faculty Member

June 15 2016 – June 14, 2017
Employment Period Under Review

College of Natural and Applied Sciences
College/Unit

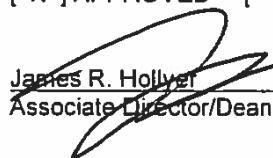
Associate Professor/Extension Agent IV
Rank or Title

IV - 16
Present Level/Step

Cooperative Extension Service
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be:
[X] APPROVED [] DISAPPROVED.


James R. Hollyer
Associate Director/Dean

8/10/16
Date

Rationale:

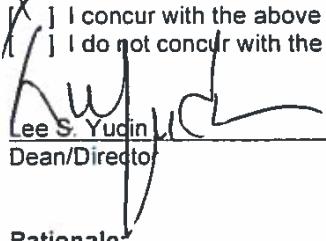
Dr Moore continues to be passionate about managing the Coconut Rhinoceros Beetle (CRB) and that is a good thing for Guam and the Pacific. In the fall of 2016 Dr. Moore has requested from the Dean time to write up his research findings and I encourage that work to get done on time. I am looking forward to the reconnaissance work that Dr Moore will be doing in early 2017 to find a virus that might manage CRB. I appreciate that Dr Moore wants to help Guam manage its invasive species and I encourage him to make sure that he continues to write for grant funding, teach classes, do University/Community Service, and do Extension & Outreach work. Finally, I acknowledge that Dr Moore received better than average student evaluations.



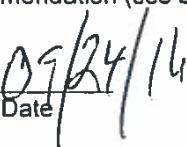
College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

RECOMMENDATION OF DEAN/DIRECTOR

- I concur with the above recommendation.
 I do not concur with the above recommendation (see below).


Lee S. Yudin

Dean/Director


Date

09/24/16

Rationale:

RESPONSES OF EMPLOYEE TO THIS EVALUATION

The above Salary Increment recommendations have been discussed with me and my responses, if any, are as follows:


Aubrey Moore

Faculty Member

Oct. 27, 2016

Date

CERTIFICATION OF FUNDS

Funds Available Funds Not Available


Certifying Officer

Date

5 2016-2017

5.1 Report

Please see next page.

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

Reflective Form

**Comprehensive Faculty Evaluation System (CFES) – Part I
or Plan of Work (POW)**

Your name: Aubrey Moore

Your current Rank and Step: Associate Professor

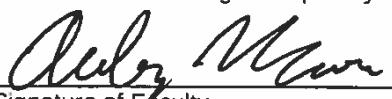
This CFES/POW evaluation period: June 15, 2016 – June 14, 2017

Role Assignments	Percent of Time
Extension & Outreach	51% (primary focus must be a minimum of 50%)
Creative/Research/Scholarly	34%
Instruction	0%
University Service	15%
TOTAL	100%

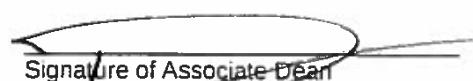
Please list any outside consulting or paid board activities for this performance period:
None.

The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating.


Signature of Faculty

9/15/2017
Date:


Signature of Associate Dean

9/15/17
Date:


Signature of Dean/Director

9/25/17
Date:

Comprehensive Faculty Evaluation System/POW – Part II

Directions: This document serves as a reflection on your Plan of Work for the last 12 months and then as the Take the Planned Activities and the Planned Evidence of Accomplishment from your last year's Future Form and put it here in the right section. Then add content into the Actual Activities and Actual Evidence of Accomplishment fields. Please include all your original Planned Activities and if there was a deviation, explain the problem with accomplishing it and then list what you did instead. If you added an additional activity, just add it to your original list of activities and give evidence of accomplishment. For any papers, presentations, workshops, and grant applications you accomplished, note them on the last page and please attach hard copy evidence.

Role Assignment: Extension & Outreach 51%

Planned and Actual Activities for this CFES year: June 15, 2016 – June 14, 2017

NOTE: for workshops taught, put the number of attendees in [brackets] when reporting Actual Activities, and attach any course evaluation summaries.

*** indicates reference has been selected as an example and has been made available in hard copy

Planned Activities (in last years' Future form)	Planned Evidence of Accomplishment (in last years' Future form)	Actual Activities (as they relate to Planned). Indicate any modifications and add new activities at the end of the list.	Actual Evidence of Accomplishment
1. Insect Diagnostic Services Identify insects and make control recommendations when requested.	iNaturalist posts documenting insect identifications	<u>30 insect observations</u> and identifications were posted to iNaturalist during the reporting period. performed 2 major investigations this year: 1. Ifit stem borer 2. Scaevola dieback	https://www.inaturalist.org/observations/aubreymoore?d1=2016-06-15&d2=2017-06-14 Ifit stem borer News media: N9 N10*** Scaevola dieback Web site: GP2*** News media: N2*** N8 N14
2. Detection and Documentation of Invasive Species Continue adding to and maintaining the Guam Invasive Species Alerts fact sheet series.	Guam Invasive Species Alerts fact sheets	<u>4 new island records</u> documented as iNaturalist posts. No new invasive species fact sheets. iNat 5296088 palm infesting whitefly, <i>Aleurotrachelus atratus</i> iNat 4525105 armored scale insect <i>Parlatoria ziziphi</i> iNat 4000498 a clerid beetle <i>Tilloidea notata</i> iNat 3564434 a cockroach <i>Arenivaga sp.</i>	New Island Records https://www.inaturalist.org/observations/5296088 *** https://www.inaturalist.org/observations/4525105 *** https://www.inaturalist.org/observations/4000498 *** https://www.inaturalist.org/observations/3564434 ***

<p>3. University of Guam Insect Collection Continue curation and databasing of the UOG Insect Collection.</p> <p>Continue evaluation of Specify as an online database for the UOG Insect Collection.</p>	Specimen records.	Made little progress on databasing the UOG insect collection. Made contact with Specify developers who are willing to help. Met with Dr. Chris Lobban who uses Specify for the UOG diatom collection. Working on a grant proposal with Dr. Alex Vandam at the University of Puerto Rico.	<p>Checklist of terrestrial Micronesian arthropods: http://guaminsects.net/mad/tree2.php?id=Reduviidae ***</p> <p>Insect pin label printer: http://guaminsects.net/insect_label_printer/insect_label_printer2.php ***</p>
<p>4. Guam Coconut Rhinoceros Beetle Project Provide scientific/technical support to the Guam Coconut Rhinoceros Beetle Project. My focus will be on CRB-G biocontrol and monitoring health of coconut palms on Guam. For details, see the CRB Biocontrol section under Creative / Scholarly / Research for details .</p>	Technical reports, refereed journal articles	Please see CRB activities in Creative/Research/Scholarly section	Please see CRB outputs in Creative/Research/Scholarly section
<p>5. National Plant Diagnostic Network (NPDN) Participate in monthly conference calls.</p> <p>Train and certify First Detectors.</p> <p>Prepare annual work plan and annual report.</p>	Conference call minutes, NPDN First Detector Certifications, annual report.		<p>Annual report:</p> <p>Work plan and budget:</p>
<p>6. Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC) Participate in meetings.</p>	meeting minutes	President Underwood delegated me to represent UOG as the official, voting member of the Guam Invasive Species Council.	

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<p>7. Public Outreach (Guest lectures, presentations, interviews) Provide accurate scientific and technical information to the public as required.</p>			Please see list in the Part III
<p>8. Public Outreach(Internet) Phase out use of the ANR Drupal site and move content to the new CNAS-RE WordPress Site.</p> <p>Provide an online database of insect crop pests in Micronesia with links to images and fact sheets. This activity overlaps with plans to create a Guam Biodiversity Inventory (see section in Create/Scholarly/Research)</p>	Blog posts, online database of crop pests		Please see list in Part III.
<p>9. Unplanned: Meeting to Discuss Response to CRB-G at the International Congress of Entomology, Orlando, Florida, September 2017.</p>		I helped to organize this USDA-APHIS sponsored at the ICE.	I used the Open Science Framework to organize material associated with this meeting. Web site https://osf.io/67g2m/wiki/home/ ***
<p>10. Unplanned: Forest Entomology Workshop</p>		Dr. Sheri Lee Smith, US Forest Service requested me to conduct a 4 hour session on Forest Entomology at the US Forest Service Forest Health Protection Workshop, Guam, April, 2017.	I used the Open Science Framework to organize material associated with this meeting. Web site https://osf.io/a6t7v/wiki/home/ ***

Additional information about this Role Assignment.

Role Assignment: Creative/Research/Scholarly 34%

Planned Activities and Actual for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities (in last years' Future form)	Planned Evidence of Accomplishment (in last years' Future form)	Actual Activities (as they relate to Planned). Indicate any modifications and add new activities at the end of the list.	Actual Evidence of Accomplishment
<p>1. Coconut Rhinoceros Beetle (CRB) Biocontrol</p> <p>1. Complete bioassays to recheck pathogenicity of previously tested OrNV samples from AgResearch New Zealand.</p> <p>2. Participate in the International Congress of Entomology in Orlando, Florida in September 2016. I have been invited to give an oral presentation on CRB-G at a symposium on scarab beetles.</p> <p>3. I will work to set up an international collaborative project with the goal of mapping the CRB-Guam biotype and finding a strain of OrNV which can be used as an effective biocontrol agent. Potential collaborators are AgResearch NZ, SPC, Philippine Coconut Authority, and USDA. This project will have a foreign exploration component which will collect CRB and virus samples throughout the Asian/Pacific region. Genotyping and virus detection will be done by AgResearch NZ. Bioassays in which CRB-Guam beetles will be challenged with virus candidates will be done in my laboratory at UOG.</p> <p>4. As per my approved FY2016 Farm Bill grant, I plan to visit Palau and Negros Island, Philippines with Dr. Sean Marshall, AgResearch New Zealand and my graduate student, Ian Irniart in early 2017. CRB-G has been detected at both of these locations.</p> <p>5. I plan to submit a FY2017 Farm Bill suggestion to continue my work on establishing biocontrol of CRB-G to prevent further coconut palm mortality on Guam. This suggestion will also request support for establishment of a semiannual coconut palm health survey.</p>	<p>Technical reports, scientific journal articles, presentations.</p>	<p>Two refereed journal articles published, one more submitted.</p>	<p>Refereed Journal Articles J1*** J2***</p> <p>Presentations P1 P2 P3 P4 P5 P10</p> <p>GitHub Repositories GR2 GR3 GR4 GR6 GR7 GR8 GR9 GR10</p> <p>News Media N1 N13 N15</p> <p>Philippine Expedition Trip report *** Technical report***</p> <p>Farm Bill FY2016 Semiannual Report*** FY2017 Work Plan*** FY2017 Financial Plan***</p>

<p>2. Cycad Aulacaspis Scale Biocontrol</p> <p>Determine if the parasitoid <i>Coccobius fulvus</i> which was released twice at Ritidian at the end of 2016 has established.</p> <p>Evaluate the impact of <i>Arrhenophagus</i> sp. on the Guam cycad population</p> <p>Write and submit a peer-reviewed scientific journal article on CAS biocontrol.</p>	<p>Peer reviewed article on CAS biocontrol.</p>	<p>No progress</p>	
<p>3. Guam Forest Insect Survey</p> <p>A database of insect pests associated with Guam's forest plants will be built using information from the literature, specimens, and surveys.</p> <p>The database will be made available on-line.</p>	<p>Online database of insect pests associated with Guam's forest plants.</p>	<p>Database designed and prototyped.</p>	

<p>4. Eight Spot Butterfly Conservation</p> <p>Propagate and maintain at least 100 plants of each of the eight-spot's known host plants, <i>Procris pendunculata</i> and <i>Elatostema calcaratum</i> in a plant nursery.</p> <p>Establish a self-sustaining, caged, breeding colony of eight-spot butterflies using 30 field-collected caterpillars reared on plants from the nursery.</p> <p>Propagate host plants throughout two 10 x 10 meter, wooded limestone areas at the University of Guam's Agricultural Experiment Station in Yigo.</p> <p>Release 60 cage-reared eight-spot butterflies and larvae on protected host plants.</p>	<p>Technical reports.</p>	<p>The eight spot butterfly was listed as an endangered species. I submitted a permit application to work on this species and submitted it to USFWS.</p>	<p>Recovery permit application***</p>
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5. Guam Biodiversity Inventory	An online database which can be queried to return useful information on Guam's organisms and interactions between them. The database will contain synonyms, references (to the literature, observations and specimens), and ecological relationships (such as links between herbivores and host plants).	Database designed and prototyped	
Design and build Check List Plus (CLP) an online database to store a "tree of life" for Guam using a reference taxonomy from the National Center for Biotechnology (NCBI). The database will contain synonyms, references (to the literature, observations and specimens), and ecological relationships (such as links between herbivores and host plants).	Applications will query this database to return useful information such as: "return list of all caterpillars feeding on cabbage on Guam with links to images and fact sheets for these species."		
Populate CLP with the flora and fauna of Guam from the scientific literature. Initial targets will include a list of all crops and important forest plants growing on Guam, insect pests that feed on these plants, and biocontrol agents controlling these insects.			
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Additional information about this Role Assignment.

Role Assignment: Instruction 0%

Planned and Actual Activities for this CFES year: June 15, 2016 – June 14, 2017

NOTE: for courses taught, put the number of in brackets [] when reporting Actual Activities and attach evaluation summaries for each course.

Planned Activities (in last years' Future form)	Planned Evidence of Accomplishment (in last years' Future form)	Actual Activities (as they relate to Planned). Indicate any modifications and add new activities at the end of the list.	Actual Evidence of Accomplishment
1			
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Additional information about this Role Assignment.

Role Assignment: Community Service 15%

Planned Activities for this CFES year: June 15, 2016 – June 14, 2017

Planned Activities (in last years' Future form)	Planned Evidence of Accomplishment (in last years' Future form)	Actual Activities (as they relate to Planned). Indicate any modifications and add new activities at the end of the list.	Actual Evidence of Accomplishment
1. Instruction I will serve as Ian Iriarte's major professor during his masters program in environmental science.	None		
2. University Technical Advisory Committee I will continue to serve on UTAC as the representative for the College of Natural and Applied Science.	meeting minutes	Attended meetings	
3. Faculty Building Facilities Committee for the ALS I will continue to serve as chair of the Faculty Building Facilities Committee for the ALS	None	Organised clean up of ALS 124 teaching lab	
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Additional information about this Role Assignment.

Comprehensive Faculty Evaluation System/POW – Part III

Summary of Publications and Grant Activities

On this page, list specific outputs generated during the evaluation period so that they can be entered into the CNAS website databases.

Publications and other media produced during the review period

Refereed Journal Articles

J1 Moore, Aubrey, Roland Quitugua, Ian Iriarte, Michael Melzer, Shizu Watanabe, Zhiqiang Cheng, and Jathan Muna Barnes. 2016. "Movement of Packaged Soil Products as a Dispersal Pathway for Coconut Rhinoceros Beetle, *Oryctes Rhinoceros* (Coleoptera: Scarabaeidae) and Other Invasive Species." Proceedings of the Hawaiian Entomological Society 48 (December): 21–22. <http://scholarspace.manoa.hawaii.edu/handle/10125/42743>.

J2 Moore, Aubrey, Diego C. Barahona, Katherine A. Lehman, Dominick A. Skabeikis, Ian R. Iriarte, Eric B. Jang, and Matthew S. Siderhurst. 2017. "Judas Beetles: Discovering Cryptic Breeding Sites by Radio-Tracking Coconut Rhinoceros Beetles, *Oryctes Rhinoceros* (Coleoptera: Scarabaeidae)." Journal of Environmental Entomology 46 (1): 92–99. doi:<https://doi.org/10.1093/ee/nvw152>.

J3 Marshall, Sean D G, Aubrey Moore, Maclean Vaqalo, and Trevor A Jackson. 2017. "A New Haplotype of the Coconut Rhinoceros Beetle, *Oryctes Rhinoceros*, Has Escaped Biological Control by *Oryctes Rhinoceros Nudivirus* and Is Invading Pacific Islands [in Preparation]." Journal of Invertebrate Pathology.

Presentations at Professional Meetings

P1 Moore, Aubrey 2016c. "Discovery of the Coconut Rhinoceros Beetle Guam Biotype and Implications for Global Control." presented at the Future proofing the palm industries: Limiting damage by existing (CRB-P) and invasive (CRB-G) coconut rhinoceros beetle (*Oryctes rhinoceros*) in the Pacific, Suva, Fiji, June. http://guaminsects.net/GISC_NOV2015/GISC_NOV2015/Moore_ESA_PB_APR2016.html.

P2 Moore, Aubrey, Roland Quitugua, Trevor A Jackson, Sean David Goldie Marshall, and Mattew S. Siderhurst. 2016. "The Rhinoceros Beetle Invasion of Guam: An Unprecedented Disaster." presented at the XXV International Congress of Entomology, Orlando, FL, September 26. <https://aubreymoore.github.io/CRB-G-ICE2016/Paper94967.html>.

P3 Marshall, Sean David Goldie, Maclean Vaqalo, Aubrey Moore, Roland Quitugua, and Trevor A Jackson. 2016. "Detection of an Invasive Biotype of *Oryctes Rhinoceros* (L.) in the Pacific." presented at the XXV International Congress of Entomology, Orlando, FL, September 26. <https://aubreymoore.github.io/CRB-G-ICE2016/Paper95540.html>.

P4 Moore, Aubrey. 2017. "Coconut Rhinoceros Beetle." presented at the Extension and Outreach Monthly Meeting, University of Guam, April 7. https://aubreymoore.github.io/extalk-APR2017/EXTALK_APRApr2017.html.

P5 Moore, Aubrey, Roland Quitugua, Trevor Jackson, Sean Marshall, and Mattew Siderhurst. 2017. "Invasion of Guam by the Coconut Rhinoceros Beetle." presented at the 8th Regional Island Sustainability Conference, Tumon, Guam, April 20.

P6 Moore, Aubrey. 2017a. "Access to Information on Forest Insect Pests in Micronesia." presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4.

P7 Moore, Aubrey. 2017b. "Biological Control of Cycad Scale, *Aulacaspis Yasumatsui*, Attacking Guam's Endemic Cycad, *Cycas Micronesica*." presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4. <https://github.com/aubreymoore/Guam-Forestry-Workshop-Resources/raw/master/CycadScaleBiocontrolChile.pdf>.

P8 Moore, Aubrey. 2017c. "Biological Invasion of Forests on Guam and Other Islands in Micronesia." presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4. https://aubreymoore.github.io/PDF_to_Reveal/reveal.js/slides.html.

P9 Moore, Aubrey. 2017d. "Biological Invasion of Guam." presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4. https://aubreymoore.github.io/PDF_to_Reveal/reveal.js/slides.html.

P10 Moore, Aubrey. 2017e. "The Coconut Rhinoceros Beetle Invasion of Guam: An Unprecedented Disaster," presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4. The coconut rhinoceros beetle invasion of Guam: An unprecedented disaster.

P11 Moore, Aubrey. 2017f. "Using Free Cell Phone Apps for Forest Pest Surveys." presented at the 2017 Pacific Island Forestry Professionals Workshop, Tumon Bay, Guam, April 4.

GitHub Pages

GP1 Moore, Aubrey. 2017a. "List of Insects and Mites Attacking Crops in Micronesia." Accessed July 24. <https://aubreymoore.github.io/crop-pest-list/>.

GP2 Moore, Aubrey. 2017b. "Scaevola Dieback." Accessed July 24. <https://aubreymoore.github.io/Scaevola-dieback/>.

GP3 Moore, Aubrey. 2017c. "Technical Blog: A Static Site Published Using Nikola and GitHub Pages." Accessed July 24. <https://aubreymoore.github.io/blog/>.

GitHub Project Repositories

GR1 "Aubreymoore/Blog." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/blog>.

GR2 "Aubreymoore/Crbdist." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/crbdist>.

GR3 "Aubreymoore/CRB-G_article_review." 2017. GitHub. Accessed July 24. https://github.com/aubreymoore/CRB-G_article_review.

GR4 "Aubreymoore/CRB-Trap-Improvement." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/CRB-trap-improvement>.

GR5 "Aubreymoore/Crop-Pest-List." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/crop-pest-list>.

GR6 "Aubreymoore/Doi-Project-Proposal." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/doi-project-proposal>.

GR7 "Aubreymoore/EPA-GMF-OrNV." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/EPA-GMF-OrNV>.

GR8 "Aubreymoore/Extalk-APR2017." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/extalk-APR2017>.

GR9 "Aubreymoore/FY2018-Farm-Bill-Suggestion." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/FY2018-Farm-Bill-Suggestion>.

GR10 "Aubreymoore/GISC-NOV2015." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/GISC-NOV2015>.

GR11 "Aubreymoore/Guam-Forestry-Workshop-Resources." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/Guam-Forestry-Workshop-Resources>.

GR12 "Aubreymoore/PDF_to_Reveal." 2017. GitHub. Accessed July 24. https://github.com/aubreymoore/PDF_to_Reveal.

GR13 "Aubreymoore/Scaevola-Dieback." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/Scaevola-dieback>.

GR14 "Aubreymoore/SQLite-Database-for-Guam-Forest-Inventory." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/SQLite-database-for-Guam-Forest-Inventory>.

GR15 "Aubreymoore/Vespa-Tropica." 2017. GitHub. Accessed July 24. <https://github.com/aubreymoore/Vespa-tropica>.

Blog Entries Posted on My Technical Blog

- TB1 Moore, Aubrey. 2016a. "Configuring Nikola and Adding Full Text Search." Aubrey Moore. December 29.<https://aubreymoore.github.io/blog/posts/test-post/>.
- TB2 Moore, Aubrey. 2016b. "Taxonomic Notes." Aubrey Moore. December 29.<https://aubreymoore.github.io/blog/posts/taxonomic-notes/>.
- TB3 Moore, Aubrey. 2017a. "Failed Attempt to Run Wep2py on Dreamhost." Aubrey Moore. February 11.<https://aubreymoore.github.io/blog/posts/failed-attempt-to-run-wep2py-on-dreamhost/>.
- TB4 Moore, Aubrey. 2017b. "Publish Nikola Site on GitHub." Aubrey Moore. February 11.<https://aubreymoore.github.io/blog/posts/publish-nikola-site-on-github/>.
- TB5 Moore, Aubrey. 2017c. "Using Scrapy to Find a String in a Web Site." Aubrey Moore. February 11.<https://aubreymoore.github.io/blog/posts/using-scrapy-to-find-a-string-in-a-web-site/>.
- TB6 Moore, Aubrey. 2017d. "Tweaking Nikola CSS to Improve Table Display." Aubrey Moore. February 20.<https://aubreymoore.github.io/blog/posts/tweeking-nikola-css-to-improve-table-display/>.
- TB7 Moore, Aubrey. 2017e. "USDA Farm Bill Funding for Coconut Rhinoceros Beetle Projects in Guam." Aubrey Moore. February 20. <https://aubreymoore.github.io/blog/posts/usda-farm-bill-funding-for-coconut-rhinoceros-beetle-projects-in-guam-and-hawaii/>.
- TB8 Moore, Aubrey. 2017f. "Install Web2py in a Conda Virtual Environment." Aubrey Moore. February 21.<https://aubreymoore.github.io/blog/posts/play-pen/>.
- TB9 Moore, Aubrey. 2017g. "Setting Up an Online Weather Station." Aubrey Moore. April 9.<https://aubreymoore.github.io/blog/posts/setting-up-an-online-weather-station/>.
- TB10 Moore, Aubrey. 2017h. "Using the Species API to Mine the GBIF Backbone Taxonomy." Aubrey Moore. April 22.<https://aubreymoore.github.io/blog/posts/using-the-species-api-to-mine-the-gbif-backbone-taxonomy/>.
- TB11 Moore, Aubrey. 2017i. "Finding Lost Parents." Aubrey Moore. May 1.<https://aubreymoore.github.io/blog/posts/finding-lost-parents/>.
- TB12 Moore, Aubrey. 2017j. "Using Scrapy to Extract Scientific Names from PestNet Fact Sheets." Aubrey Moore. May 18. <https://aubreymoore.github.io/blog/posts/using-scrapy-to-extract-scientific-names-from-pestnet-fact-sheets/>.
- TB13 Moore, Aubrey. 2017k. "Calculate Geographical Coordinates for Equidistant Points at Vertices." Aubrey Moore. May 28. <https://aubreymoore.github.io/blog/posts/calculate-geographical-coordinates-for-equidistant-points-at-vertices-of-a-triangular-grid/>.
- TB14 Moore, Aubrey. 2017l. "Converting a Web2py Web Site to a Static Web Site." Aubrey Moore. May 28.<https://aubreymoore.github.io/blog/posts/web2py-to-static/>.
- TB15 Moore, Aubrey. 2017m. "Migrate a MySQL Database to Postgreql." Aubrey Moore. June 11.<https://aubreymoore.github.io/blog/posts/migrate-a-mysql-database-to-postgreql/>.

News Media

- N1 Entomology Today. 2016. "Judas Beetles: How Coconut Rhinoceros Beetles Are Betraying Each Other's Secrets." *Entomology Today*, December 5. <https://entomologytoday.org/2016/12/05/judas-beetles-how-coconut-rhinoceros-beetles-are-betraying-each-others-secrets/>.
- N2 Pacific Daily News. 2017a. "Bark Beetle Found on Saipan 70 Years Ago Resurfaces on Guam." Accessed July 26. <http://www.guampdn.com/story/news/2017/06/23/bark-beetle-found-saipan-70-years-ago-resurfaces-guam/418835001/>.
- N3 Pacific Daily News. 2017b. "GovGuam Develops Plan to Combat New Hornet." Accessed July 26. <http://www.guampdn.com/story/news/2016/09/14/govguam-develops-plan-combat-new-wasp/90333634/>.

N4 Pacific Daily News. 2017c. "Guam Invasive Species Council Establishes Committee for Managing Green Waste." Accessed July 26. <http://www.guampdn.com/story/news/2017/06/28/invasive-species-council-creates-committee-green-waste-management/434482001/>.

N5 Pacific Daily News. 2017d. "Opinion: How to Address Invasive Species Problems in Guam." Accessed July 26. <http://www.guampdn.com/story/opinion/readers/2016/08/20/opinion-how-address-invasive-species-problems-guam/89032032/>.

N6 Pacific Daily News. 2017e. "Our View: Manage Green Waste, Help Stop Spread of Invasive Species." Accessed July 26. <http://www.guampdn.com/story/opinion/editorials/2017/06/29/manage-green-waste-help-stop-spread-invasive-species/438310001/>.

N7 Pacific Daily News. 2017f. "University of Guam Scientists: Hornet Eradication Unlikely." Accessed July 26. <http://www.guampdn.com/story/news/2016/09/16/university-guam-scientists-hornet-eradication-unlikely/90440636/>.

N8 Post, Manny Cruz | The Guam Daily. 2017a. "Bark Beetle Identified." *The Guam Daily Post*. Accessed July 26. https://www.postguam.com/news/local/bark-beetle-identified/article_00127814-5721-11e7-bcdb-8b345ddcd581.html.

N9 Post, Manny Cruz. 2017b. "Possibly Invasive Species Hits Ifit Seedlings." *The Guam Daily Post*. Accessed July 26. https://www.postguam.com/news/local/possibly-invasive-species-hits-ifit-seedlings/article_3bb3a03a-2f00-11e7-9a8c-47b85f74c1c4.html.

N10 Post, Manny Cruz. 2017c. "Stemborer Ifit Damage Contained, Research Ongoing." *The Guam Daily Post*. Accessed July 26. https://www.postguam.com/news/local/stemborer-ifit-damage-contained-research-ongoing/article_e34bf450-4fd5-11e7-b7c9-5795c938917e.html.

N12 Guam Daily Post. 2017. "Greater Banded Hornet Finds Its Way to Guam." Accessed July 26. https://www.postguam.com/community/greater-banded-hornet-finds-its-way-to-guam/article_1f26214c-57b3-11e6-8bee-e3cc07facef6.html.

N13 Saipan Tribune. 2016. "New Coconut Rhino Beetle Invading Pacific Islands| Saipan Tribune," October 3. <http://www.saipantribune.com/index.php/new-coconut-rhino-beetle-invading-pacific-islands/>.

N14 Saipan Tribune. 2017. "Bark Beetle Attacking Guam's Nanåsu Plants Now Identified| Saipan Tribune," June 29. <http://www.saipantribune.com/index.php/bark-beetle-attacking-guams-nanasu-plants-now-identified/>.

N15 PhysOrg News. 2017. "The Dirt on Packaged Rhino Beetles." Accessed July 26. <https://phys.org/news/2016-12-dirt-packaged-rhino-beetles.html>.

Grants applied for during the review period

USDA-APHIS Farm Bill FY2017: Biological Control of Coconut Rhinoceros Beetle Biotype G Using Oryctes Nudivirüs

Grants won during the review period

USDA-APHIS Farm Bill FY2017: Biological Control of Coconut Rhinoceros Beetle Biotype G Using Oryctes Nudivirüs

Other output you want to talk about

5.2 Plan for Following Year

Please see next page.

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

Future Plan Form

**Comprehensive Faculty Evaluation System (CFES) – Part I
or Plan of Work (POW)**

Your name: Aubrey Moore

Your current Rank and Step: Associate Professor

This CFES/POW evaluation period: June 15, 2017 – June 14, 2018

Role Assignments	Percent of Time
Extension & Outreach	51% (primary focus must be a minimum of 50%)
Creative/Research/Scholarly	34%
Instruction	0%
University Service	15%
TOTAL	100%

Please list any outside consulting or paid board activities for this performance period:
None.

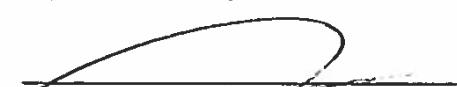
The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

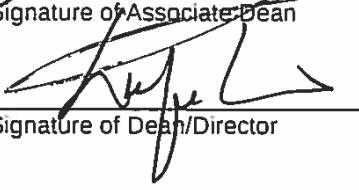
I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating.



Signature of Faculty

9/15/2017
Date:



Signature of Associate Dean


Signature of Dean/Director

9/15/17
Date:
9/26/17
Date:

Comprehensive Faculty Evaluation System/POW – Part II

Directions: This document serves as a Plan of Work for the *upcoming period*. Simply list your planned activities with as much detail as possible. For workshop presentations and papers, for example, give a draft or final name. For any papers, presentations, workshops, and grant applications you plan to accomplish, note them on the last page.

Role Assignment: Extension & Outreach 51%

Planned Activities for this CFES year: June 15, 2017 – June 14, 2018

Planned Activities	Planned Evidence of Accomplishment
1. Insect Diagnostic Services Identify insects and make control recommendations when requested.	iNaturalist posts documenting insect identifications
2. Detection and Documentation of Invasive Species Continue adding to and maintaining the Guam Invasive Species Alerts fact sheet series.	Guam Invasive Species Alerts fact sheets
3. University of Guam Insect Collection Continue curation and databasing of the UOG Insect Collection. Continue evaluation of Specify as an online database for the UOG Insect Collection.	Specimen records.
4. Guam Coconut Rhinoceros Beetle Project Provide scientific/technical support to the Guam Coconut Rhinoceros Beetle Project. My focus will be on CRB-G biocontrol and monitoring health of coconut palms on Guam. For details, see the CRB Biocontrol section under Creative / Scholarly / Research for details .	Grant proposals, grant project reports, technical reports, refereed journal articles
5. National Plant Diagnostic Network (NPDN) Participate in monthly conference calls. Train and certify First Detectors. Prepare annual work plan and annual report.	Conference call minutes, NPDN First Detector Certifications, annual report.
6. Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC) Participate in meetings.	meeting minutes
7. Public Outreach (Guest lectures, presentations, interviews) Provide accurate scientific and technical information to the public as required.	
8. Public Outreach(Internet) Phase out use of the ANR Drupal site and move	Blog posts, online database of crop pests

content to the new CNAS-RE WordPress Site.	
Provide an online database of insect crop pests in Micronesia with links to images and fact sheets. This activity overlaps with plans to create a Guam Biodiversity Inventory (see section in Create/Scholarly/Research).	
9	
10	

Additional information about this Role Assignment.

Role Assignment: Creative/Research/Scholarly 34%

Planned Activities for this CFES year: June 15, 2017 – June 14, 2018

Planned Activities	Planned Evidence of Accomplishment
1. Coconut Rhinoceros Beetle (CRB) Biocontrol Complete applied research on biological control of coconut rhinoceros beetle as per work plans in my FY2016 Farm Bill grant, my FY2017 Farm Bill grant, and Department of the Interior grant. Prepare and submit a Farm Bill suggestion for FY2018. Collaborate with colleagues in establishing a regional project in response to coconut rhinoceros beetle biotype G invasions.	Technical reports, scientific journal articles, presentations.
2. Cycad Aulacaspis Scale Biocontrol Determine if the parasitoid <i>Coccobius fulvus</i> which was released twice at Ritidian at the end of 2016 has established. Evaluate the impact of <i>Arrhenophagus</i> sp. on the Guam cycad population Write and submit a peer-reviewed scientific journal article on CAS biocontrol.	Peer reviewed article on CAS biocontrol.
3. Guam Forest Insect Survey A database of insect pests associated with Guam's forest plants will be built using information from the literature, specimens, and surveys. The database will be made available on-line.	Online database of insect pests associated with Guam's forest plants.
4. Eight Spot Butterfly Conservation Propagate and maintain at least 100 plants of each of the eight-spot's known host plants, <i>Procris pendunculata</i> and <i>Elatostema calcareum</i> in a plant nursery.	Technical reports.

<p>Establish a self-sustaining, caged, breeding colony of eight-spot butterflies using 30 field-collected caterpillars reared on plants from the nursery.</p> <p>Propagate host plants throughout two 10 x 10 meter, wooded limestone areas at the University of Guam's Agricultural Experiment Station in Yigo.</p> <p>Release 60 cage-reared eight-spot butterflies and larvae on protected host plants.</p>	
<p>5. Guam Biodiversity Inventory</p> <p>Design and build Check List Plus (CLP) an online database to store a "tree of life" for Guam using a reference taxonomy from the National Center for Biotechnology (NCBI). The database will contain synonyms, references (to the literature, observations and specimens), and ecological relationships (such as links between herbivores and host plants).</p> <p>Populate CLP with the flora and fauna of Guam from the scientific literature. Initial targets will include a list of all crops and important forest plants growing on Guam, insect pests that feed on these plants, and biocontrol agents controlling these insects.</p>	<p>An online database which can be queried to return useful information on Guam's organisms and interactions between them.</p> <p>Applications will query this database to return useful information such as: "return list of all caterpillars feeding on cabbage on Guam with links to images and fact sheets for these species."</p>
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Additional information about this Role Assignment.

Role Assignment: Instruction 0%

Planned Activities for this CFES year: June 15, 2017 – June 14, 2018

Planned Activities	Planned Evidence of Accomplishment
1	
2	
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Additional information about this Role Assignment.

Role Assignment: Community Service 15%

Planned Activities for this CFES year: June 15, 2017 – June 14, 2018

Planned Activities	Planned Evidence of Accomplishment
1. Instruction I will serve as Ian Iriarte's major professor during his masters program in environmental science. I will present guest lectures in my role as an Environmental Sciences graduate school faculty. I will teach General Entomology during Fall 2017.	student evaluation
2. University Technical Advisory Committee I will continue to serve on UTAC as the representative for the College of Natural and Applied Sciences.	
3. Faculty Building Facilities Committee for the ALS I will continue to serve as chair of the Faculty Building Facilities Committee for the ALS	
4. Faculty and Committee Meetings I will attend faculty meetings and committees as required.	
5	
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8	
9	
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Additional information about this Role Assignment.

Comprehensive Faculty Evaluation System/POW – Part III

Summary of Publications and Grant Activities

On this page, list specific outputs you plan to generate during the evaluation period.

Publications and other media you will produce during the review period

Grants you will apply for during the review period

Farm Bill FY2019

Other output you want to talk about

5.3 Evaluation

Please see next page.



UNIVERSITY OF
GUAM
UNIBETSEDAT GUAHAN

College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

FACULTY SALARY INCREMENT

Aubrey Moore
Faculty Member

June, 15 2016 – June 14, 2017
Employment Period Under Review

College of Natural and Applied Sciences
College/Unit

Associate Professor / Extension Agent IV
Rank or Title

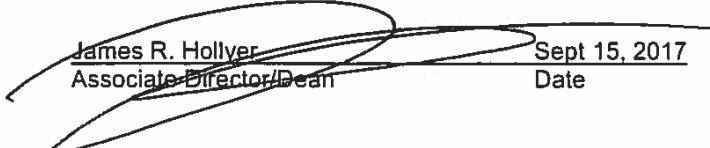
Step 16
Present Level/Step

Cooperative Extension Service
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be:
[X] APPROVED [] DISAPPROVED.

(NOTE: Dr Moore is frozen at Step 16 and will need to apply and attain a promotion for any further increments.)


James R. Hollyer
Associate Director/Dean

Sept 15, 2017
Date

Rationale:

Dr Moore has been fulfilling his role as an extension entomologist with researching and responding to invasive species and species already present on Guam. His documentation is very thorough and appreciated. Dr Moore has applied for and received outside funding and has published 3 refereed journal articles during the last year. During the review period, he was given a year-off from teaching, and CNAS hired an instructor to handle the course(s) he normally teaches. He is back teaching in the fall of 2017.

Dr Moore needs to hire someone who can help him manage and implement his grants so that timelines and deliverables are met without stress.



College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

RECOMMENDATION OF DEAN/DIRECTOR

- I concur with the above recommendation.
 I do not concur with the above recommendation (see below).

Lee S. Yudin
Dean/Director

09/26/17
Date

Rationale:

RESPONSES OF EMPLOYEE TO THIS EVALUATION

The above Salary Increment recommendations have been discussed with me and my responses, if any, are as follows:

30

Aubrey Moore
Faculty Member

Sept 26/2017
Date

CERTIFICATION OF FUNDS

- Funds Available Funds Not Available

Certifying Officer

Date

6 2017-2018

6.1 Report

Please see next page.

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

1

Reflective Form

**Comprehensive Faculty Evaluation System (CFES) – Part I
or Plan of Work (POW)**

Your name: Aubrey Moore

Your current Rank and Step: Associate Professor

This CFES/POW evaluation period: June 15, 2017 – June 14, 2018

Role Assignments	Percent of Time
Extension & Outreach	51% (primary focus must be a minimum of 50%)
Creative/Research/Scholarly	34%
Instruction	0%
University Service	15% 2.5
TOTAL	100%

*Sorry about this -
I have changed this -
changed 9/6/18*

Please list any outside consulting or paid board activities for this performance period:
None.

The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating.

Aubrey Moore
Signature of Faculty

Sept. 6, 2018
Date

John M. Gaskins
Signature of Associate Dean

9/6/18
Date

John M. Gaskins
Signature of Dean/Director

9/4/18
Date

Aubrey Moore
F 2017 BI/AL 345 General Entomology

Enrolled:

Respond: 15

Mean: 3.7707

CNAS: 3.4969

KOG: 3.56

→ "Learned a great deal" $\bar{x} = 3.93 / 4$

CFES Report 2018

Aubrey Moore, Ph.D.
Associate Professor / Extension Entomologist

September 3, 2018

I was hired by the University of Guam on October 1, 2003 under a limited-term, split appointment (50% extension and 50% research). On June 26, 2008, I started a tenure-track appointment as extension entomologist (100% extension) with the academic rank of Assistant Professor. At the end of the 2012 fall term I applied for tenure and promotion and received both in 2013. I intend to submit my application for promotion to full professor during Fall semester 2018.

I work within the Agriculture and Natural Resources Unit of the University of Guam Cooperative Extension Service. I am a faculty member of the Environmental Science Graduate Program and a member of the Western Pacific Tropical Research Center.

This report documents my activities from June 2017 through the present. My current faculty role allocation is as follows:

- 51% Extension and Community Activities
- 34% Creative/Scholarly Activity or Research
- 15% University and Community Service

Note to Reader:

This report is available as an electronic document in PDF format at <https://tinyurl.com/am-cfes-rept-2018>.

If you are reading the PDF version of the report on a device connected to the internet, you will be able to follow hypertext links to documents I have referenced.

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1 Extension and Community Activities

1.1 Insect Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam agencies, federal agencies, and UOG colleagues. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem.

Activity

- The number of extension calls requiring my assistance averages approximately three per day during the reporting period. Many of these are documented as postings to iNaturalist [1]

Reference(s)

- [1] Moore A. iNaturalist Observations of Arthropods from June 15, 2017 to June 14, 2018; 2018. Available from: https://www.inaturalist.org/observations/aubreymoore?d1=2017-06-15&d2=2018-06-14&filter_spam=true&page=1&taxon_name=Arthropoda&user_id=7547.

1.2 Detection and Documentation of Invasive Species

Invasive insects are arriving on Guam at a very high rate (estimates range as high as one new species per day). Very few of these are detected and even fewer are identified because Guam suffers from [the taxonomic impediment](#). Even when reliable species determinations are made, new island records are only rarely documented in the scientific press. Thus, impacts of invasive insects on Guam and elsewhere in Micronesia are grossly underestimated. One of my professional goals is to work towards solving this problem by increasing the detection rate, getting specimens identified by qualified taxonomists, and publishing new island records in the scientific literature.

Activity

- 3 new invasive insects documented in iNaturalist posts, 1 new invasive species fact sheet, 1 peer-reviewed journal article.
 - Pacific orange leafwing, *Doleschallia tongana* [2, 4]
 - Lobate lac scale, *Paratachardina pseudolobata* [7, 6, 3]
 - Mango fruit borer, *Citripestis eutraphera* (identification not yet confirmed) [5, 1]
- The International Union for Conservation of Nature (IUCN-ISSG) is building a Global Register of Introduced and Invasive Species. I have volunteered to coordinate building a check list for species on Guam.
- The Guam Invasive Species Council is required to maintain a list on invasive species on Guam. I have volunteered to be “registrar” for this list.

Reference(s)

- [1] Moore A. *Citripestis eutraphera*; 2018. Available from:
<https://www.inaturalist.org/observations/13466275>.
- [2] Manuel J. Pacific Orange Leafwing (*Doleschallia tongana*); 2017. Available from:
<https://www.inaturalist.org/observations/8515898>.
- [3] Leon Guerrero P. Interview with Phil Leon Guerrero, Talk Radio 57, about lobate lac scale; 2018.
- [4] Manuel J, Tennent WJ, Buden DW, Moore A. First record of *Doleschallia tongana* (Lepidoptera: Nymphalidae) for Guam Island. F1000Research. 2018 Mar;7:366. Available from: <https://f1000research.com/articles/7-366/v1>.
- [5] Moore A. *Citripestis eutraphera*; 2018. Available from:
<https://www.inaturalist.org/observations/15067449>.

1 Extension and Community Activities

- [6] Post MS|TGD. New tree pest found at UOG site in Yigo;,. Available from:
[https://www.postguam.com/news/local/
new-tree-pest-found-at-uog-site-in-yigo/article_9ae0a830-8fa0-11e8-8cb6-c3bd2a08c887.html](https://www.postguam.com/news/local/new-tree-pest-found-at-uog-site-in-yigo/article_9ae0a830-8fa0-11e8-8cb6-c3bd2a08c887.html).
- [7] Moore A. Lobate Lac Scale (*Paratachardina pseudolobata*); 2018. Available from:
<https://www.inaturalist.org/observations/12779405>.

1.3 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the collection and I work with Dr. Ross Miller to curate and catalog this collection.

Activity

- I ported the digital catalog for the UOG Insect Collection from a CSIRO BioLink database to a more modern web-based Symbiota database which is now online [3].
- I established an internship to train entomology students how to curate an institutional insect collection [4].
- The Benita Laird-Hopkins collection includes more than 5,000 insect specimens reared from seeds of forest plants from Saipan and Guam as part of the Ecology of Bird Loss Project. This collection has been cataloged and accessioned into the UOG insect collection and a publication is being prepared [5, 1].
- In June, I attended the Second Annual Digital Data in Biodiversity Research Conference sponsored by iDigBio (Integrated Digital Biocollections) to attend a workshop entitled Sharing and Mobilization of Massive Specimen Image Databases from Collections of Tropical Island Biodiversity as an invited participant. I made a presentation on building a biodiversity inventory for Guam [6] and discussed ongoing collaboration with Dr. Alex Vandam on writing an NSF proposal to support digitization of biological collections on American-affiliated islands [2].

Reference(s)

- [1] Moore A. Online Catalog for the Laird-Hopkins Collection of Insects Reared from Seeds of Forest Plants from Saipan and Guam; 2018. Available from: <http://scan-bugs.org/portal/collections/list.php?collector=Laird-Hopkins&db=all&page=1>.
- [2] Moore A. Trip Report: Second Annual Digital Data in Biodiversity Research Conference, Berkely, CA, June 2018; 2018. Original-date: 2018-08-22T09:25:09Z. Available from: https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Berkeley_Trip_report.pdf.
- [3] Moore A. SCAN University of Guam Insect Collection Collection Profiles; 2018. Available from: <http://scan-bugs.org/portal/collections/misc/collprofiles.php?collid=180>.
- [4] Moore A. Internship: University of Guam Insect Collection Technician; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/internship.pdf>.

1 Extension and Community Activities

- [5] Laird-Hopkins BC, Downey HF, Bassett Y, Fricke E, Moore A, Quicke DLJ, et al. [IN PREPARATION] Fruit and seed-eating insect assemblages on island ecosystems. *Biotropica*. 2018;.
- [6] Moore A. Building a Terrestrial Biodiversity Inventory for Guam [oral presentation]. Berkeley, CA; 2018. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315.

1.4 Guam Coconut Rhinoceros Beetle Project

This is my largest and most important project.

Activity

- Please see CRB activities in the Creative/Research/Scholarly section [2.1](#).

1.5 National Plant Diagnostic Network (NPDN)

I serve as the UOG Coordinator for the National Plant Diagnostic Network.

Activity

- Participated in monthly conference calls.
- Prepared an annual work plan and budget [2].
- Prepared annual report [1].
- Served on the NPDN IT Strategic Planning Committee.
- Trained and certified 14 First Detectors as part of my AL/BI 345 General Entomology course.

Reference(s)

- [1] Moore A. NPDN Accomplishments Survey for University of Guam, April 1, 2017 through April 1, 2018; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Guam%20WPDN-Accomplishments%20Summary%20Form%202018%20final.pdf>.
- [2] Moore A. University of Guam: WPDN Funded Budget September 1, 2017 through August 1, 2018; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Univ%20of%20Guam%20WPDN%20budget%202017-18-Final.pdf>.

1.6 Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC)

Activity

- I am a founding member and regular participant in GISAC.
- President Underwood delegated me to represent UOG as a voting member of GISC.
- During 2018, I served on a GISC Import Data Harmonization Committee. This committee generated recommendations [2] resulting in a bill to amend the Guam Invasive Species Act [1].

Reference(s)

- [1] Guerrero D, Santos J. Bill to Amend 5 GCA Chapter 70: Guam Invasive Species Act; 2018. Available from:
<https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/5%20GCA%20Chapter%2070%20Bill%20Draft%20D.%20Guerrero%20%26%20Joseph%20Santos%20V.5%202018%20no%20password.pdf>.
- [2] Guerrero D. Guam Invasive Species Council: Import Data Harmonization Committee Report; 2018. Available from:
<https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/GISC%20IDHC%20Report%20%26%20Recommendations.pdf>.

1.7 Public Outreach (Guest lectures, presentations, interviews)

Activity

- Presentations [30, 23, 29, 2, 7, 9, 13, 27, 5, 32, 22, 21, 25, 18, 24, 26, 1, 12, 4, 14]
- Workshops [16, 10, 3, 8]
- Press [6, 19, 31, 15, 28, 11, 20, 17]

Reference(s)

- [1] Moore A. Biological Control of Cycad Scale, *Aulacaspis yasumatsui*, Attacking Guam's Endemic Cycad, *Cycas micronesica*. Tumon Bay, Guam; 2017. Available from:
<https://github.com/aubreymoore/Guam-Forestry-Workshop-Resources/raw/master/CycadScaleBiocontrolChile.pdf>.
- [2] Moore A. Free Cell Phone Apps for Pest Surveys. UOG, Guam; 2018.
- [3] Moore A, Bamba J. CNAS Workshop Series: Bring Your Own Bug, April 7, 2018; 2018. Available from: https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/BYOB_03_07_18.pdf.
- [4] Moore A. Biological Invasion of Guam. Tumon Bay, Guam; 2017. Available from: https://aubreymoore.github.io/PDF_to_Reveal/reveal.js/slides.html.
- [5] Moore A, Marshall SDG, Quitugua R, Iriarte IR. Attempted microbial control of coconut rhinoceros beetle, *Oryctes rhinoceros*, biotype G on Guam using *Oryctes rhinoceros* nudivirus and *Metarhizium majus*. Gold Coast, Australia; 2018. Available from:
<https://www.zotero.org/aubreymoore/items/7VDF7QFR/file>.
- [6] Moore A. Special Report for Guam Invasive Species Awareness Week: Invasive Species are a Crisis for Guam and the Pacific, Right Now. Pacific Island Times. 2018 Feb; Available from:
<https://www.pacificislandtimes.com/single-post/2018/02/25/Special-Report-Invasive-species-are-a-crisis-for-Guam-and-the-Pacific-right-now>
- [7] Moore A. Coconut Rhinoceros Beetle Invasion of Guam. UOG, Guam; 2018.
- [8] Quitugua R, Moore A. 2018 Coconut Rhinoceros Beetle Training for CNMI, July 30 - August 3; 2018. Available from: <https://github.com/aubreymoore/Free-Cell-Phone-Apps-for-Pest-Surveys/raw/master/2018%20CRB%20workshop%20for%20CNMI.pdf>.

1 Extension and Community Activities

- [9] Moore A. Biological Invasion of Guam. UOG, Guam; 2018.
- [10] Moore A, Bamba J. Bring Your Own Bug: Insect ID Workshop, July 1, 2017; 2017. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/BYOB%20Flyer.png>.
- [11] Leon Guerrero P. Interview with Phil Leon Guerrero, Talk Radio 57, about lobate lac scale; 2018.
- [12] Moore A. The coconut rhinoceros beetle invasion of Guam: An unprecedented disaster. Tumon Bay, Guam; 2017. Available from: [ThecoconutrhinocerosbeetleinvasionofGuam:Anunprecedenteddisaster](#).
- [13] Moore A. Free Cell Phone Apps for Pest Surveys; 2018. Prepared for and presented at the Coconut Rhinoceros Beetle workshop for the CNMI. Available from: <https://github.com/aubreymoore/Free-Cell-Phone-Apps-for-Pest-Surveys/raw/master/iNatEpi.pdf>.
- [14] Moore A. Biological Invasion of Forests on Guam and Other Islands in Micronesia. Tumon Bay, Guam; 2017. Available from: https://aubreymoore.github.io/PDF_to_reveal/reveal.js/slides.html.
- [15] Viral control wanted for Coconut Rhinoceros Beetle; 2018. Available from: <https://www.radionz.co.nz/international/programmes/datelinepacific/audio/2018657196/viral-control-wanted-for-coconut-rhinoceros-beetle>.
- [16] Berringer D. Sixteenth Annual Quarantine Training Workshop in Guam, March 19th to 23rd, 2018; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/2018%20PPQ%20Agenda.pdf>.
- [17] Tracking some of the world's biggest killers, via cellphone. Public Radio International; 2017. Available from: <https://www.pri.org/stories/2017-12-25/tracking-some-worlds-biggest-killers-cellphone>.
- [18] Moore A. Coconut Rhinoceros Beetle. University of Guam; 2017. Available from: https://aubreymoore.github.io/extalk-APR2017/EXTALK_APR2017.html.
- [19] Variety M. UOG seeks virus that can kill rhino beetle on Guam and Rota;. Available from: <http://www.mvariety.com/cnmi/cnmi-news/local/106535-uog-seeks-virus-that-can-kill-rhino-beetle-on-guam-and-rota>.
- [20] G2G: Human Resources and Financial Sustainability: Greatness Through Grants: Research Corporation positions UOG for federal funding. University of Guam Newsletter. 2018 Jun;.

1 Extension and Community Activities

- [21] Moore A. Building a Terrestrial Biodiversity Inventory for Guam. Tumon Bay, Guam; 2018. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315.
- [22] Moore A. Biological Invasion of Guam. Guam; 2018. Available from: https://github.com/aubreymoore/Guam-Bioinvasion-July-2018/raw/master/compress_biological_invasion_of_guam_July_2018.pdf.
- [23] Blas AL, Quitugua R, Moore A. Protecting a cultural icon and food resource: Current research and status of Coconut palm in Guam and the Northern Marianas. Porthnd, Oregon; 2018. Available from: https://www.apsnet.org/members/divisions/pac/meetings/Documents/APS_PacificDivisionCSPP_2018_PROGRAM%20SCHEDULE.pdf.
- [24] Moore A. Using free Cell Phone Apps for Forest Pest Surveys. Tumon Bay, Guam; 2017.
- [25] Moore A. Invasion of Guam by the Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (Linnaeus 1758). Guam; 2017.
- [26] Moore A. Access to Information on Forest Insect Pests in Micronesia. Tumon Bay, Guam; 2017.
- [27] Marshall SDG, Moore A, Ero M, Fanai C, Vaqalo M, Jackson TA. Progress with control of a virus resistant coconut rhinoceros beetle. Gold Coast, Australia; 2018.
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1.8 Public Outreach(Internet)

Activity

- On-line output [4, 22, 6, 2, 15, 8, 31, 26, 3, 32, 9, 10, 20, 13, 5, 28, 19, 18, 25, 29, 14, 12, 24, 1, 7, 27, 23, 30, 11, 21, 17, 16]

Reference(s)

- [1] Moore A. Install web2py in a Conda Virtual Environment; 2017. Available from: <https://aubreymoore.github.io/blog/posts/play-pen/>.
- [2] Moore A. Lobate Lac Scale (*Paratachardina pseudolobata*); 2018. Available from: <https://www.inaturalist.org/observations/12779405>.
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- [6] Moore A. *Citripestis eutraphera*; 2018. Available from: <https://www.inaturalist.org/observations/13466275>.
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- [8] Moore A. Web Site for General Entomology AL/BI345 Fall 2017; 2017. Available from: <https://aubreymoore.github.io/ALBI345F17/>.
- [9] Manuel J. Pacific Orange Leafwing (*Doleschallia tongana*); 2017. Available from: <https://www.inaturalist.org/observations/8515898>.
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- [16] Moore A. Crop-pest-list by aubreymoore; 2018. Available from: <https://aubreymoore.github.io/crop-pest-list/>.
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2 Creative/Scholarly Activities or Research

2.1 Coconut Rhinoceros Beetle (CRB) Biocontrol

This is my largest and most important project. Funding for outreach and applied research is currently provided by three grants: USDA-APHIS FY17 Farm Bill, USDA-Farm FY18 Bill, and a grant from the Department of the Interior-Office of Island Affairs for FY18-19.

I have submitted a proposal for FY19 Farm Bill Fundings. The abstract from this proposal serves as a description of this ongoing project:

A newly discovered biotype of coconut rhinoceros beetle (CRB-G) is rapidly killing coconuts and other palms on Guam and on other Pacific islands. Following a failed eradication attempt on Guam, CRB-G proved hard to control because it is resistant to *Oryctes rhinoceros* nudivirus (OrNV), which was previously used as the preferred biological control agent for control of CRB outbreaks on Pacific Islands and elsewhere. Previous to the discovery of CRB-G, all OrNV releases on Pacific Islands resulted in immediate and sustained suppression of CRB damage to low levels and prevented tree mortality.

Guam is currently experiencing an uncontrolled and unmonitored island-wide CRB-G outbreak which was triggered by abundant CRB-G breeding sites in the form of dead and dying vegetation left in the wake of Typhoon Dolphin which occurred in May 2015. of a recent typhoon. Most of these breeding sites are inaccessible to sanitation efforts, being either in the jungle or on military land (which covers one third of Guam). A positive feedback cycle has begun whereby large numbers of adult beetles are killing large numbers of palms which become breeding sites which generate even higher numbers of adults. Severe damage to Guam's palms prompted the Governor of Guam to declared a state of emergency in July 2017.

The main objective of this project is to stop the uncontrolled outbreak on Guam. Entomologists working on the CRB-G problem on several Pacific islands agree that the most feasible tactic to halt tree mortality and suppress damage to tolerable levels is establishment of biological control using an isolate of OrNV which is highly effective as a biological control agent for CRB-G. We are working with collaborators to identify populations of CRB-G throughout the Asia-Pacific region. We will sample these populations for biological control agent candidates which will be evaluated in laboratory bioassays performed at UOG. Promising candidates will be field released using autodissemination as per a USDA-APHIS import and release permit.

2 Creative/Scholarly Activities or Research

Concurrent with establishment of CRB-G biocontrol, success of the project will be monitored in a quarterly, island-wide tree health survey and incidence of OrNV infection will be monitored in a subsample of all field collected CRB-G.

If the Guam CRB-G infestation cannot be controlled, it is expected that most palms on the island will be killed and CRB-G will continue to spread to other islands and beyond. If CRB-G invades smaller islands and atolls where coconut is the tree of life, a human tragedy will ensue. On larger islands, coconut and oil palm industries will be severely impacted. Attempts to organize a regional project in response to CRB-G are underway.

Activity

- Coauthored a peer-reviewed journal article documenting discovery of CRB-G [6].
- Wrote a magazine article for the Guam Invasive Species Awareness week. This was published by the Pacific Islands Times [2]. A similar article was archived in Zenodo [1].
- Recruited Dr. James Grasela, an insect pathologist, to work on the project for two years using funding from the US Department of Interior - Office of Island Affairs. Grasela's initial task will be to perform laboratory bioassays to evaluate OrNV isolates as candidates for biocontrol of CRB-G (Job announcement: [4]).
- Recruited Ian Iriarte as a research assistant using funds from Farm Bill grants. Ian is also my graduate student. He is working with me on development of an automated coconut rhinoceros beetle damage monitoring system using computer vision and deep learning. This project is likely to be the topic of his master's thesis.
- In August 2018, Moore, Grasela, Iriarte and Quitugua participated in the 51st Annual Meeting of the Society for Invertebrate Pathology and International Congress on Invertebrate Pathology and Microbial Control held at the Gold Coast, Australia. This conference provided a venue for a symposium and a meeting to plan and promote collaboration among Pacific entomologists working on the CRB-G problem [8, 7].
- Created a private wiki site to facilitate sharing scientific/technical information among scientists working on the CRB-G problem [3].
- Laboratory bioassays of an OrNV isolate propagated from a virus-infected CRB-G adult we collected on Negros Island, Philippines in 2017 produced no response when applied to CRB-G adults [5]

Reference(s)

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2 Creative/Scholarly Activities or Research

- [2] Moore A. Special Report for Guam Invasive Species Awareness Week: Invasive Species are a Crisis for Guam and the Pacific, Right Now. Pacific Island Times. 2018 Feb; Available from:
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- [3] Moore A. CRB-G Wiki - CRB-G Wiki; 2018. Available from:
http://guaminsects.net/CRBG/index.php?title=CRB-G_Wiki.
- [4] Moore A. Position Announcement: Post-Doctoral Researcher (Insect Pathologist); 2018. Available from: [https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/blob/master/JA-RC-18-06%20Post%20Doctoral%20Researcher%20\(Insect%20Pathology\).pdf](https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/blob/master/JA-RC-18-06%20Post%20Doctoral%20Researcher%20(Insect%20Pathology).pdf).
- [5] Moore A. Initial bioassay of Dumaguete isolate of *Oryctes rhinoceros* nudivirus. Zenodo. 2018 Jan; Available from: <https://zenodo.org/record/1134737>.
- [6] Marshall SDG, Moore A, Vaqalo M, Noble A, Jackson TA. A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands. Journal of Invertebrate Pathology. 2017 Oct;149:127–134. Available from:
<http://www.sciencedirect.com/science/article/pii/S0022201117300289>.
- [7] Marshall SDG, Moore A, Ero M, Fanai C, Vaqalo M, Jackson TA. Progress with control of a virus resistant coconut rhinoceros beetle. Gold Coast, Australia; 2018.
- [8] Moore A. Failed Attempts to Establish IPM for Asian Cycad Scale and Coconut Rhinoceros Beetle on Guam. Vancouver, BC, Canada; 2018.

2.2 Cycad Aulacaspis Scale (CAS) Biocontrol

A US Forest Service survey published in 2002 reported that the most abundant tree in Guam's forests (DBH > 5 inches) was Guam's endemic cycad, *Cycas micronesica*. In 2003, an invasive scale insect, *Aulacaspis yasumatsui*, was detected on ornamental cycads but it soon infested wild cycads and started killing them. Within a decade, 90% of Guam's endemic cycads have been killed by the scale and other invasive species. *Cycas micronesica* was placed on the US National Endangered Species List in 2015.

Mature plants are protected by a lady beetle I introduced, but no natural reproduction is occurring because seeds and seedlings are still being killed by the scale insect. A likely solution to this problem is establishment of a small biocontrol agent, such as a miniature parasitic wasp which will control scale insects infesting seeds and seedlings.

Activity

- Worked with Ben DeLoso, Tom Marler's grad student, to perform a CAS parasitoid survey [1].

Reference(s)

- [1] Deloso BE, Moore A, Marler TE. Parasitoid Surveys in Cycad Habitats on Guam. Washington, D.C.; 2018. Available from:
<https://ashs.confex.com/ashs/2018/meetingapp.cgi/Paper/28523>.

2.3 Guam Forest Insect Survey

The objective of this project is to compile a comprehensive check list of insects impacting Guam's forests. While it is notable that Guam's two most numerous forest trees, namely fadang, *Cycas micronesica*, and coconut palm, *Cocos nucifera*, are under simultaneous attack by invasive insects, there are many other forest plants under attack from invasive insects. This project is funded by McIntire-Stennis.

Activity

- I work closely with Jim McConnell's Guam Plant Extinction Prevention Program. Many of Guam's rare plants are being attacked by invasive insects. I routinely identify and document insect specimens collected from the GPEPP plant nursery and from field surveys.
 - Annual report [1].
 - Proposal [2].

Reference(s)

- [1] Moore A. McIntire-Stennis Project - REEIS Online Report: Guam Forest Insect Survey; 2018. Available from: <https://reeis.usda.gov/web/crisprojectpages/1005269-guam-forest-insect-survey.html>.
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2.4 Eight Spot Butterfly (ESB) Conservation

The Guam Department of Agriculture Division of Aquatic and Wildlife Resources (GDOA-DAWR) requested assistance with conservation of the rare Mariana eight-spot butterfly, *Hypolimnas octocula marianensis*. A grant proposal for this work was funded by US Fish and Wildlife and funds were made available to the Guam Department of Agriculture for this work. The project was halted shortly after it began because USFWS listed ESB on the National Endangered Species List. This required a permit to work with this species. I worked with GDOA-DAWR on a permit application. I am ready to restart this project, but GDOA-DAWR is unable to access grant funding from GovGuam.

Activity

- Progress on this project blocked by GovGuam bureaucracy. No progress to report.

2.5 Guam Biodiversity Inventory

I consider this to be my second most important project.

A biodiversity inventory is essentially a database containing a comprehensive check list of all taxa known occur within a defined area.

A terrestrial biodiversity inventory for Guam is needed to document rapid changes to Guam's ecosystems, to provide free and open access to information on Guam's flora and fauna, and to share Guam biodiversity information with the global scientific community, policy makers and the public.

The Guam Biodiversity Inventory will facilitate automatic generation and updates to lists such as: a list of all invasive species on Guam with year first recorded, a list of new species described from specimens collected on Guam, a list of observations for Guam's endangered species, a list of Guam's native plants with associated herbivores and pathogens, and a list of crops grown on Guam and pests and pathogens which attack them.

Activity

- I made a couple of presentations on my plans for the Guam Biodiversity Inventory [2, 1].
- I designed data model for the Guam Biodiversity Inventory and created a prototype web site.

Reference(s)

- [1] Moore A. Building a Terrestrial Biodiversity Inventory for Guam. Tumon Bay, Guam; 2018. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315.
- [2] Moore A. Building a Terrestrial Biodiversity Inventory for Guam [oral presentation]. Berkeley, CA; 2018. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315.

3 University and Community Service

3.1 Instruction

Activity

- According to the UOG Registrar, I taught 4 courses during the Fall semester: AL345, AL345L, BI345, and BI345L. In reality this was a single course, AL/BI 345 *General Entomology* consisting of two, one and a half hour lectures and one three hour lab per week.
 - I built and maintained a web site for this course [1]
 - In student evaluations for AL345, AL345L, BI345, and BI345L, my scores were consistently higher than the University and College average [2].
- I acted as the major faculty advisor for Mr. Ian Iriarte who is pursuing a Master's degree in Environmental Science.

Reference(s)

- [1] Moore A. Web Site for General Entomology AL/BI345 Fall 2017; 2017. Available from: <https://aubreymoore.github.io/ALBI345F17/>.
- [2] Moore A. Student Evaluations: AL/BI 345 General Entomology, Fall 2017; 2018. Available from: [title={2017-18},filter={presentations}](#).

3.2 Faculty Committees

3.2.1 Faculty Building Facilities Committee for the ALS

This committee was formed by the Agriculture and Life Sciences Division to provide advice to the Dean on facilities problems within the Agriculture and Life Sciences Building. During the reporting period, I was re-elected as chair of this committee and I am joined by Dr. Jim McConnell and Dr. LaJoy Spears as the other members.

Activity

- Plans for improvements to the ALS124 teaching lab have been only partially achieved. For the past three years, faculty have asked for a dedicated computer and modern audiovisual equipment to facilitate science teaching. During the reporting period, lab tables were equipped with power sockets to replace those removed during a previous renovation.
- We continue to struggle with finding solutions to chronic air conditioning problems.

3.2.2 Search Committee: Extension Animal Scientist

I chair this committee. I am joined by Mari Marutani, LaJoy Spears, Bob Schlub, and Tom Poole, Guam's Territorial Veterinarian.

Activity

- Position announcement written [1] and advertisement placed on the web site of the American Association of Animal Scientists [2].

3.2.3 Search Committee: Extension Agricultural Economist

I am a member of this committee and I am joined by Bob Barber (chair), LaJoy Spears, and John Brown.

3.2.4 Search Committee: Research Associate II (CRB Project)

I chaired this committee and was joined by Jim Grasela, Roland Quitugua, and Jesse Bamba.

3.2.5 Continuing Employment Committee: Austin Shelton

I chair this committee and I am joined by Ross Miller and Hui Gong.

3.2.6 Continuing Employment Committee: Andrea Blas

I served on this committee with Ross Miller and Frank Camacho.

3.2.7 Extension Publications Committee

I served as a member of this committee.

Reference(s)

- [1] Moore A. UOG Animal Scientist Announcement; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/UOG%20Animal%20Scientist%20Announcement.pdf>.
- [2] Moore A. Animal Scientist Announcement - American Society of Animal Science; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Animal%20Scientist%20Announcement%20-%20American%20Society%20of%20Animal%20Science.pdf>.

4 Appendix

4.1 Grants

Funding Source	Title	Years	Budget
Active			
Farm Bill 2017 [2]	Biological Control of Coconut Rhinoceros Beetle Biotype-G	1	\$200,000
Farm Bill 2018 [1]	Biological Control of Coconut Rhinoceros Beetle Biotype-G	1	\$200,000
Department of the Interior - Office of Insular Affairs [3]	Biological Control of Coconut Rhinoceros Beetle Biotype-G in Micronesia	2	\$176,553
McIntire-Stennis [6]	Guam Forest Insect Survey	4	\$40,000
National Plant Diagnostic Network 2017 [9] [8]		1	\$10,000
Pending			
Farm Bill 2019 [4, 5]	Biological Control of Coconut Rhinoceros Beetle Biotype-G	1	\$282,044
McIntire-Stennis [7]		5	\$80,000
National Plant Diagnostic Network		1	\$10,000

Reference(s)

- [1] Moore A. Farm Bill Work Plan - FY2018: Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/FY18-GU-CRB%20biocontrol%20workplan.pdf>.
- [2] Moore A. Farm Bill Work Plan - FY2017: Oryctes Nudivirus for Biocontrol of the Guam Biotype of the Coconut Rhinoceros Beetle; 2017. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/FY17-GU-FB-CRB%20biocontrol%20workplan.pdf>.
- [3] Moore A. DOI Proposal: Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia; 2017. Available from:

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https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/doi_proposal.pdf.

- [4] Moore A. FY19 Farm Bill Suggestion: Biocontrol of Coconut Rhinoceros Beetle Biotype G; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/MooreFB19.pdf>.
- [5] Moore A. FY19 Farm Bill Suggestion: Budget; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/MooreFB19.pdf>.
- [6] Moore A. McIntire-Stennis Project - REEIS Online Report: Guam Forest Insect Survey; 2018. Available from: <https://reeis.usda.gov/web/crisprojectpages/1005269-guam-forest-insect-survey.html>.
- [7] Moore A. McIntire-Stennis Proposal: Guam Forest Biodiversity Inventory; 2018. Available from: https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/ms_proposal_2018.pdf.
- [8] Moore A. NPDN Accomplishments Survey for University of Guam, April 1, 2017 through April 1, 2018; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Guam%20WPDN-Accomplishments%20Summary%20Form%202018%20final.pdf>.
- [9] Moore A. University of Guam: WPDN Funded Budget September 1, 2017 through August 1, 2018; 2018. Available from: <https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/Univ%20of%20Guam%20WPDN%20budget%202017-18-Final.pdf>.

4.2 Selected Examples of My Work

Peer-reviewed journal article Sean D. G. Marshall, Aubrey Moore, Maclean Vaqalo, Alasdair Noble, and Trevor A. Jackson, "A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands", Journal of Invertebrate Pathology 149 (2017), pp. 127–134.[7]

Peer-reviewed journal article Jake Manuel, W. John Tennent, Donald W. Buden, and Aubrey Moore, "First record of *Doleschallia tongana* (Lepidoptera: Nymphalidae) for Guam Island", F1000Research 7 (2018), pp. 366.[4]

Grant proposal Aubrey Moore, "DOI Proposal: Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia" (2017).[1]

Grant proposal Aubrey Moore, "McIntire-Stennis Proposal: Guam Forest Biodiversity Inventory" (2018).[6]

Magazine article Aubrey Moore, "Special Report for Guam Invasive Species Awareness Week: Invasive Species are a Crisis for Guam and the Pacific, Right Now", Pacific Island Times (2018).[2]

Oral presentation slide set Aubrey Moore, "Building a Terrestrial Biodiversity Inventory for Guam" (2018).[5]

Web site [3] Aubrey Moore, "CRB-G Wiki - CRB-G Wiki" (2018).

Reference(s)

- [1] Moore A. DOI Proposal: Biological Control of Coconut Rhinoceros Beetle Biotype G in Micronesia; 2017. Available from:
https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/doi_proposal.pdf.
- [2] Moore A. Special Report for Guam Invasive Species Awareness Week: Invasive Species are a Crisis for Guam and the Pacific, Right Now. Pacific Island Times. 2018 Feb;Available from:
<https://www.pacificislandtimes.com/single-post/2018/02/25/Special-Report-Invasive-species-are-a-crisis-for-Guam-and-the-Pacific-right-now>
- [3] Moore A. CRB-G Wiki - CRB-G Wiki; 2018. Available from:
http://guaminsects.net/CRBG/index.php?title=CRB-G_Wiki.
- [4] Manuel J, Tennent WJ, Buden DW, Moore A. First record of *Doleschallia tongana* (Lepidoptera: Nymphalidae) for Guam Island. F1000Research. 2018 Mar;7:366. Available from: <https://f1000research.com/articles/7-366/v1>.

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- [5] Moore A. Building a Terrestrial Biodiversity Inventory for Guam. Tumon Bay, Guam; 2018. Available from: https://figshare.com/articles/Building_a_Terrestrial_Biodiversity_Inventory_for_Guam/6188315.
- [6] Moore A. McIntire-Stennis Proposal: Guam Forest Biodiversity Inventory; 2018. Available from: https://github.com/aubreymoore/Miscellaneous-Docs-for-CFES2018/raw/master/ms_proposal_2018.pdf.
- [7] Marshall SDG, Moore A, Vaqalo M, Noble A, Jackson TA. A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands. Journal of Invertebrate Pathology. 2017 Oct;149:127–134. Available from: <http://www.sciencedirect.com/science/article/pii/S0022201117300289>.

6.2 Plan for Following Year

Please see next page.

1

University of Guam
College of Natural & Applied Sciences
Cooperative Extension & Outreach

Future Plan Form

**Comprehensive Faculty Evaluation System (CFES) – Part I
or Plan of Work (POW)**

Your name: Aubrey Moore

Your current Rank and Step: Associate Professor

This CFES/POW evaluation period: June 15, ~~2017~~ June 14, ~~2018~~
~~2018~~ 2019 *sas ✓*

Role Assignments

Extension & Outreach

Creative/Research/Scholarly

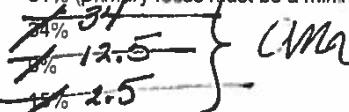
Instruction

University Service

TOTAL

Percent of Time

51% (primary focus must be a minimum of 50%)



34%
0%
15%

*SAS 9/6/18
BESD doc
attached memo*

Please list any outside consulting or paid board activities for this performance period:
None.

The components of: (1) Planned Activities, (2) Evidence of Accomplishment, and (3) Evaluated By for each of the Roles identified above are found in Part II.

I have met with my appropriate administrative supervisor and discussed my evaluation plan for the period above cited. I understand that amendments to my plan are possible and that said amendments, if any, are to be discussed with and agreed upon by my administrator prior to initiating

Aubrey Moore
Signature of Faculty

Sept. 6, 2018

Date:

9/6/18

Signature of Associate Dean

Date:

Signature of Dean/Director

9/18/18

Date:

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1 Extension and Community Activities

1.1 Insect Diagnostic Services

As an extension entomologist, a major part of my job is providing insect identification and pest control recommendations to a diverse clientele including commercial growers, gardeners, householders, GovGuam and federal agency personnel. Most client contacts are initiated by a phone call or a visit by the client to the ANR office. In many cases identification and pest control recommendations require a site visit by me and/or extension associates to collect samples and define the problem.

- I will continue to attend to insect related extension enquiries on a daily basis
- I will continue to document insect pest problems by posting information in the iNaturalist Insects of Micronesia Project

1.2 Detection and Documentation of Invasive Species

Invasive insects are arriving on Guam at a very high rate (estimates range as high as one new species per day). Very few of these are detected and even fewer are identified because Guam suffers from the taxonomic impediment. Even when reliable species determinations are made, new island records are only rarely documented in the scientific press. Thus, impacts of invasive insects on Guam and elsewhere in Micronesia are grossly underestimated.

One of my professional goals is to work towards solving this problem by increasing the detection rate, getting specimens identified by qualified taxonomists, and publishing new island records in the scientific literature.

- I will continue to send suspected new invasive species to taxonomists at the Systematic Entomology Laboratory or elsewhere for species determination.
- I will document new island records via fact sheets and peer-reviewed journal articles
- The International Union for Conservation of Nature (IUCN-ISSG) is building a Global Register of Introduced and Invasive Species. I have volunteered to coordinate building a check list for species on Guam.
- The Guam Invasive Species Council is required to maintain a list on invasive species on Guam. I have volunteered to be "registrar" for this list.

1 Extension and Community Activities

1.3 University of Guam Insect Collection

The UOG insect collection is a valuable reference collection for extension entomology, teaching and research. I am a member of the board of directors for the collection and I work with Dr. Ross Miller to curate and catalog this collection.

- I recently ported the digital catalog for the UOG Insect Collection from a CSIRO BioLink database to a more modern web-based Symbiota database which is now online at <http://scan-bugs.org/portal/collections/misc/collprofiles.php?collid=180>. I will continue to maintain this database.
- I recently established an internship to train entomology students how to curate an institutional insect collection. If my pending McIntire-Stennis grant proposal is funded, I will continue this internship.
- Digital images of specimens will be made for each taxon in the UOG insect collection and these will be assed to the online database.

1.4 Guam Coconut Rhinoceros Beetle Project

This is my largest and most important project. Please see the CRB section in the Creative/Research/Scholarly chapter 2.1.

1.5 National Plant Diagnostic Network (NPDN)

I serve as the UOG Coordinator for the National Plant Diagnostic Network.

- I will participate in monthly conference calls.
- I will prepared an annual work plan and budget.
- I will prepared an annual report.
- I will serve on the NPDN IT Strategic Planning Committee.
- I will continue to train and certified First Detectors.
- I will attend the National Plant Diagnostic Network meeting in Indianapolis during April 2019.

1.6 Guam Invasive Species Advisory Committee (GISAC) and Guam Invasive Species Council (GISC)

I am a founding member and regular participant in GISAC. President Underwood delegated me to represent UOG as the official, voting member of GISC.

1 Extension and Community Activities

- I will continue to participate in GISAC and GISC meetings and will serve on committees as needed.

1.7 Public Outreach (Guest lectures, presentations, interviews)

- I will provide guest lectures, presentations, and interviews when requested.

1.8 Public Outreach (Internet)

- I will continue to provide online information about Guam's insects.

2 Creative/Scholarly Activities or Research

2.1 Coconut Rhinoceros Beetle (CRB) Biocontrol

This is my largest and most important project. Funding for outreach and applied research is currently provided by three grants: USDA-APHIS FY17 Farm Bill, USDA-Farm FY18 Bill, and a grant from the Department of the Interior-Office of Island Affairs for FY18-19.

I have submitted a proposal for FY19 Farm Bill Fundings. The abstract from this proposal serves as a description of this ongoing project:

A newly discovered biotype of coconut rhinoceros beetle (CRB-G) is rapidly killing coconuts and other palms on Guam and on other Pacific islands. Following a failed eradication attempt on Guam, CRB-G proved hard to control because it is resistant to *Oryctes rhinoceros* nudivirus (OrNV), which was previously used as the preferred biological control agent for control of CRB outbreaks on Pacific Islands and elsewhere. Previous to the discovery of CRB-G, all OrNV releases on Pacific Islands resulted in immediate and sustained suppression of CRB damage to low levels and prevented tree mortality.

Guam is currently experiencing an uncontrolled and unmonitored island-wide CRB-G outbreak which was triggered by abundant CRB-G breeding sites in the form of dead and dying vegetation left in the wake of Typhoon Dolphin which occurred in May 2015, of a recent typhoon. Most of these breeding sites are inaccessible to sanitation efforts, being either in the jungle or on military land (which covers one third of Guam). A positive feedback cycle has begun whereby large numbers of adult beetles are killing large numbers of palms which become breeding sites which generate even higher numbers of adults. Severe damage to Guam's palms prompted the Governor of Guam to declared a state of emergency in July 2017.

The main objective of this project is to stop the uncontrolled outbreak on Guam. Entomologists working on the CRB-G problem on several Pacific islands agree that the most feasible tactic to halt tree mortality and suppress damage to tolerable levels is establishment of biological control using an isolate of OrNV which is highly effective as a biological control agent for CRB-G. We are working with collaborators to identify populations of CRB-G throughout the Asia-Pacific region. We will sample these populations for biological control agent candidates which will be evaluated in laboratory bioassays performed at UOG. Promising candidates will be field released using autodissemination as per a USDA-APHIS import and release permit.

2 Creative/Scholarly Activities or Research

Concurrent with establishment of CRB-G biocontrol, success of the project will be monitored in a quarterly, island-wide tree health survey and incidence of OrNV infection will be monitored in a subsample of all field collected CRB-G.

If the Guam CRB-G infestation cannot be controlled, it is expected that most palms on the island will be killed and CRB-G will continue to spread to other islands and beyond. If CRB-G invades smaller islands and atolls where coconut is the tree of life, a human tragedy will ensue. On larger islands, coconut and oil palm industries will be severely impacted. Attempts to organize a regional project in response to CRB-G are underway.

- I recently created a private wiki site (<http://guaminsects.net/CRBG>) to facilitate sharing scientific/technical information among scientists working on the CRB-G problem. I will continue to maintain this site.
- The immediate focus in this project is to find isolates of OrNV which are candidates for classical biological control of CRB-G.
 - I will develop a protocol for transport of frozen CRB-G tissue samples containing OrNV
 - I will work with my post-doc, Dr. Jim Grasela, to develop bioassay protocols to evaluate OrNV samples
 - I will lead an expedition to Taiwan where there is a CRB-G population with a an OrNV incidence of 80%. This trip is likely to occur at the end of September 2018.
 - I will arrange for OrNV samples to be sent to Guam from a Dr. Nur Ain's lab in Malaysia
 - Potential OrNV biocontrol candidates will be field tested using autodissemination
 - Samples of potential OrNV biocontrol candidates will be shared with collaborators throughout the Pacific
- A secondary objective of my Farm Bill grants is to establish an island-wide monitoring system to track temporal and spatial changes in the extent of CRB damage to Guam's coconut palms. Damage symptoms such as v-shaped cuts to fronds, bore holes, and dead standing coconut palm stems are readily observed during roadside surveys. Survey data will be collected using a 360 degree digital video camera mounted on a truck. Initially, video images of coconut palm damage by CRB-G will be detected, classified and tagged by a technician. When a large number of images have been tagged, these will be used to train a fully automated CRB damage detection and monitoring system. In addition to being used to evaluate response to pest control programs, this automated system may be useful as an early detection device for CRB. This research is expected to be the topic of Ian Iriarte's Master's thesis.

2 Creative/Scholarly Activities or Research

- I will deliver an invited presentation at the Annual Meeting of the Entomological Society of America meeting in Vancouver, BC during November 2018. The title of my presentation is *Failed Attempts to Establish IPM for Asian Cycad Scale and Coconut Rhinoceros Beetle on Guam*.

2.2 Cycad Aulacaspis Scale (CAS) Biocontrol

A US Forest Service survey published in 2002 reported that the most abundant tree in Guam's forests (DBH > 5 inches) was Guam's endemic cycad, *Cycas micronesica*. In 2003, an invasive scale insect, *Aulacaspis yasumatsui*, was detected on ornamental cycads but it soon infested wild cycads and started killing them. Within a decade, 90% of Guam's endemic cycads have been killed by the scale and other invasive species. *Cycas micronesica* was placed on the US National Endangered Species List in 2015.

Mature plants are protected by a lady beetle I introduced, but no natural reproduction is occurring because seeds and seedlings are still being killed by the scale insect. A likely solution to this problem is establishment of a small biocontrol agent, such as a miniature parasitic wasp which will control scale insects infesting seeds and seedlings.

- This project will be placed on hold while I work on the CRB-G problem.

2.3 Guam Forest Insect Survey

The objective of this project is to compile a comprehensive check list of insects impacting Guam's forests. While it is notable that Guam's two most numerous forest trees, namely fadang, *Cycas micronesica*, and coconut palm, *Cocos nucifera*, are under simultaneous attack by invasive insects, there are many other forest plants under attack from invasive insects. This project is funded by McIntire-Stennis.

- I will write a final report for the 4-year McIntire-Stennis project which ends August 31, 2018.
- This project will be continued if my pending 5-year McIntire-Stennis proposal is funded.
 - If funded, will continue to work closely with Jim McConnell's Guam Plant Extinction Prevention Program. Many of Guam's rare plants are being attacked by invasive insects. I routinely identify and document insect specimens collected from the GPEPP plant nursery and from field surveys.

2.4 Eight Spot Butterfly (ESB) Conservation

The Guam Department of Agriculture Division of Aquatic and Wildlife Resources (GDOA-DAWR) requested assistance with conservation of the rare Mariana eight-spot butterfly, *Hypolimnas octocula marianensis*. I grant proposal for this work was funded by US Fish

2 Creative/Scholarly Activities or Research

and Wildlife and funds were made available to the Guam Department of Agriculture for this work. The project was halted shortly after it began because USFWS listed ESB on the National Endangered Species List. This required a permit to work with this species. I worked with GDOA-DAWR on a permit application. I am ready to restart this project, but GDOA-DAWR is unable to access grant funding from GovGuam.

- This project is on hold pending progress on the CRB-G project and resolution of problems with GovGuam bureaucracy which are blocking funds.

2.5 Guam Biodiversity Inventory (GBI)

I consider this to be my second most important project.

A biodiversity inventory is essentially a database containing a comprehensive check list of all taxa known occur within a defined area.

A terrestrial biodiversity inventory for Guam is needed to document rapid changes to Guam's ecosystems, to provide free and open access to information on Guam's flora and fauna, and to share Guam biodiversity information with the global scientific community, policy makers and the public.

The Guam Biodiversity Inventory will facilitate automatic generation and updates to lists such as: a list of all invasive species on Guam with year first recorded, a list of new species described from specimens collected on Guam, a list of observations for Guam's endangered species, a list of Guam's native plants with associated herbivores and pathogens, and a list of crops grown on Guam and pests and pathogens which attack them.

- I will continue to evaluate my data model for the Guam Biodiversity Inventory and finish the prototype web site.
- The prototype GBI database will be used as infrastructure for some of the tasks listed in my *Detection and Documentation of Invasive Species* project 1.2.

3 University and Community Service

3.1 Instruction

- I am the major faculty advisor for Mr. Ian Iriarte who is pursuing a Master's degree in Environmental Science.
- As a member of the Environmental Science Graduate Faculty, I will provide guest lectures when requested.

~~Where is
Course
instruction...?~~ → Teach: Gen Entomology - AL/B1 345 J119

3.2 Faculty Committees

3.2.1 Faculty Building Facilities Committee for the ALS

This committee was formed by the Agriculture and Life Sciences Division to provide advice to the Dean on facilities problems within the Agriculture and Life Sciences Building. During the reporting period, I was re-elected as chair of this committee and I am joined by Dr. Jim McConnell and Dr. LaJoy Spears as the other members.

- Plans for improvements to the ALS124 teaching lab have been only partially achieved. For the past three years, faculty have asked for a dedicated computer and modern audiovisual equipment to facilitate science teaching. I will continue to push to properly equip the only science teaching lab in the Agriculture and Life Sciences Building.

3.2.2 Search Committee: Extension Animal Scientist

I chair this committee. I am joined by Mari Marutani, Dr. LaJoy Spears, Bob Schlub, plant pathologist, and Tom Poole, Guam's Territorial Veterinarian.

- I will continue to chair this committee until a candidate is selected.

3.2.3 Continuing Employment Committee: Austin Shelton

I chair this committee and I am joined by Ross Miller and Hui Gong.

- I will continue to chair this committee until Dr. Shelton's paper work is submitted.

Bio Blitz (Immediate -)

Pilot project: options \hookrightarrow Yigo ST.

\hookrightarrow small area

\hookrightarrow south side Pago Bay

\hookrightarrow students UOG

\hookrightarrow nature reserve / reservoir \swarrow Maso

\hookrightarrow Micronesian Challenge

\hookrightarrow needs taxonomist -

\hookrightarrow use iNaturalist -

\hookrightarrow social media skills -

\hookrightarrow engaged w/ community

\hookrightarrow Pilot project - Jan 2019

Yigo St. - Saturday -

\hookrightarrow training: leaders

\hookrightarrow training: participants

\hookrightarrow simultaneous planning
of a bigger proj.



\rightarrow new recorded observations

\rightarrow new species...

\rightarrow new audiences

\rightarrow ↑ science literacy

\rightarrow saturation of Extension + outreach + contribution
back to science/research base

"Raspberry Pi" → full operating system
→ coding →
→ Python →
→ teaching coding
→ girls
→ 4H →

→ talk w/ 4H — $\frac{1}{2}$ day / 5 days
→ integrate w/ real world
→ real world / real time data
→ sensors in the field

→ 15 kids (experiment)

→ After: acoustic monitoring
→ getting others engaged
from across university
→ Connect to real world !!.

→ Evaluation plan

Kits: \$80 — 15 — (20)
monitor for each —
Large monitor —

Summer 2019

15 youth
Supplies
Staffing
Mentors

6.3 Evaluation

Please see next page.

University of Guam
Mangilao, Guam

FACULTY SALARY INCREMENT

Aubrey Moore
Faculty Member

June 15, 2017 – June 14, 2018
Employment Period

College of Natural and Applied Sciences
College/Unit

Associate Professor
Rank or Title

IV - 16
Present Level/Step

Cooperative Extension Service
Department

RECOMMENDATION OF EVALUATOR

I recommend that a salary increment increase for the above named faculty member be
APPROVED/DISAPPROVED.


Sereana H. Dresbach 9/6/18
Date

Associate Director, Cooperative Extension Service
Position Title

Rationale:

Dr. Moore has reached the top step in his appointment for Associate Professor. Dr. Moore has two major goals for the next three months: submission of the promotion packet and the CRBG research. We outlined two inter-related programs specifically for Extension Programming for FY2019: Bio Blitz and youth coding 4H program. Both will begin planning in the last quarter of 2018 calendar year.



See attached memo

RECOMMENDATION OF DEAN/DIRECTOR

- I concur with the above recommendation.
 I do not concur with the above recommendation (see below).

Lee S. Yudin
Dean/Director

Date

09/7/17

Rationale:

The above Salary Increment recommendations have been discussed with me and my responses, if any are as follows:

Aubrey Moore
Aubrey Moore
Faculty Member

Sept. 26/2018
Date

CERTIFICATION OF FUNDS

[] Funds Available

[] Funds Not Available

Certifying Officer

Date



College of Natural & Applied Sciences
COOPERATIVE EXTENSION & OUTREACH

MEMORANDUM

September 18, 2018

TO: Dr. Tanisha Alfague, ALS Division Chair *PLA*

VIA: Dr. Lee Yudin *LJ*

FROM: Dr. Kate Moots, Associate Dean, Academics *KAM* *gad*
Dr. Sereana H. Driesbach, Associate Dean/ Director

RE: Teaching load for Aubrey Moore, PhD – AY 2018-2019/FY 2019

9/20 / 2018

9/10 / 2018

18/Sept / 2018

9/18 / 2018

Dr. Aubrey Moore taught AL/BI 351 (3 credits) and the AL/BI 351 Lab (3 credits) in the Fall semester 2017 (Academic Year 2017-2018).

This pair of courses was taught without overload compensation, under the premise that this load would eliminate teaching for the next academic year (2018-2019).

While this premise may have been practiced under other contracts, this is not the desired path for faculty within CNAS. Therefore, Dr. Moore's Plan of Work for federal FY 2019 will reflect that he will not be teaching in Spring 2018, but will be preparing to teach in Fall 2019. This pattern will not be repeated.

Beginning with AY 2019-2020, as per the expectations of the College of Natural and Applied Sciences, Dr. Aubrey Moore, like every other faculty member in Agriculture & Life Sciences will teach at least one course per year.

If the teaching course load exceeds the minimum amount per year, then overload compensation will be arranged. Alternative measures, such as a graduate student or other faculty teaching the lab class, are also acceptable, rather than incurring an overload. However, graduate student teaching is not a substitute for the faculty teaching expectation.

The primary requirement that each faculty member teach a class during the academic year must first be fulfilled. Special arrangements and exceptions are highly discouraged as the burden then falls to other faculty and ultimately, the students.

5.3 Student Evaluations

5.3.1 AG-109 (Lecture and lab sections) Spring 2013

According to my CFES report for June 2013 to June 2014, my eval score was 3.659 (CNAS mean = 3.554; UOG mean = 3.627)

Please see next page.

University of Guam
Faculty Evaluation
Course Section by Instructor

INSTR ID: 0030333
 INSTR LAST NAME: MOORE
 INSTR FIRST NAME: AUBREY
 INSTR MIDDLE INITIAL:
 COLLEGE: 40 CNAS-CALS
 TERM: 13/SP
 COURSE: AG*109*01

STMT	FREQUENCY-----						TOT	AVG SCORE	STD DEV
	4	3	2	1	0	-			
1.	7	5	0	0	0	0	12	3.5833	0.4930
2.	9	3	0	0	0	0	12	3.7500	0.4330
3.	10	2	0	0	0	0	12	3.8333	0.3727
4.	10	2	0	0	0	0	12	3.8333	0.3727
5.	9	3	0	0	0	0	12	3.7500	0.4330
6.	8	4	0	0	0	0	12	3.6667	0.4714
7.	6	6	0	0	0	0	12	3.5000	0.5000
8.	7	5	0	0	0	0	12	3.5833	0.4930
9.	6	6	0	0	0	0	12	3.5000	0.5000
10.	10	2	0	0	0	0	12	3.8333	0.3727
11.	7	5	0	0	0	0	12	3.5833	0.4930
13.	8	4	0	0	0	0	12	3.6667	0.4714
14.	8	4	0	0	0	0	12	3.6667	0.4714
15.	8	4	0	0	0	0	12	3.6667	0.4714
16.	8	4	0	0	0	0	12	3.6667	0.4714
17.	8	4	0	0	0	0	12	3.6667	0.4714
18.	3	8	0	0	0	1	12	3.2727	0.4454
19.	6	5	0	0	0	1	12	3.5455	0.4979
20.	8	3	0	0	0	1	12	3.7273	0.4454
21.	7	4	0	0	0	1	12	3.6364	0.4810
22.	7	4	0	0	0	1	12	3.6364	0.4810
23.	10	1	0	0	0	1	12	3.9091	0.2875
						OVERALL		3.6589	

UNIVERSITY AVG.: 3.6270

COLLEGE AVERAGE: 3.5536

OMITTED STATEMENTS FROM OVERALL CALCULATION:

12.	5	4	2	1	0	0	12	3.0833	0.9538
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UNIVERSITY OF GUAM
STUDENT EVALUATION OF FACULTY COMMENTS
Spring SEMESTER 2013

Dept. Code (*first 7 digits of Faculty ID & last 6 digits on Course Serial No.:*): 0030333 031569

Faculty Name: Aubrey Moore

Faculty ID Number (7 digits): 0030333 Course Enrollment No. 23
Year: 2013 Semester: Spring Course No.: AG-109 Section: 01

COMMENTS:

A. STRENGTHS OF THIS INSTRUCTOR:

1. Professor Moore really made AG-109 very interesting for me. He really knew his material and taught it extremely well enough for me to understand. I really liked how he was very understanding towards assignment dates which had deadlines.
2. Prof. Moore really showed an interest in this class. He really helped us achieve accomplishment of our bug collections.
3. The instructor did an excellent job of making his class interesting and is very enthusiastic about his material. Very nice and open minded.
4. His lectures and field instructions are very clear. He makes class very interesting and exciting. His enthusiasm is contagious and makes me want to learn more and excel in class. Awesome class!
5. Pro: Moore is truly strong in this subject and knows much about insects.
6. He allowed us, students, to use any materials that are available on our test. Such as book, notes, computer. He made it relatable to real life situations where all situations were all source are easy to get.
7. Instructor knew his work, answered my questions.
8. He knows what he's teaching.
9. The instructor made this class interesting for learning. He is always available for class lectures and always arrive on time => A Fair teacher.

B. AREAS IN WHICH INSTRUCTOR NEEDS IMPROVEMENT:

1. There were more movie nights than actual lecture nights. He should have more lecture nights and also make attendance part of the grades to ensure the student shows up.
2. NONE.
3. He needs to have more materials.
4. He doesn't encourage students to show up to class, or at least not enough.
5. Dr. Moore could be more prepared for class. When we watched a movie there was always an issue to be dealt with.

5.3.2 AG/BI-345 (Lecture and lab sections) Fall 2013

According to my CFES report for June 2013 to June 2014, my evaluation score was 3.875 (CNAS mean = 3.522; UOG mean = 3.586)

Unfortunately, I was unable to locate a copy of this student evaluation. It was not found in the files of CNAS, the Human Resources Office, or the Registrar's office. Please see next page for a memo confirming this.



COLLEGE OF NATURAL & APPLIED SCIENCES
Agricultural and Life Science Division

November 13, 2018

MEMORANDUM

To whom it may concern,

Dr. Aubrey Moore's student evaluations for Fall 2013 could not be located as we checked with the Human Resources office as well as the Register's office, both have stated that they do not have Dr. Moore's evaluations for that semester. Our past administrative assistant whom filed student evaluations did not process the student evaluations for that semester which is why we and the other offices do not have the documents for Fall 2013. Please see attached student evaluations that I have found for Spring 2013.

Thank You,

A handwritten signature in black ink, appearing to read "M. Hikichi".

Megumi Hikichi, Extension Assistant

T: +1 671.735.2021 F: +1 671.734.4600 W: cnas-re.uog.edu

Mailing Address: 303 University Drive UOG Station Mangilao, Guam 96913

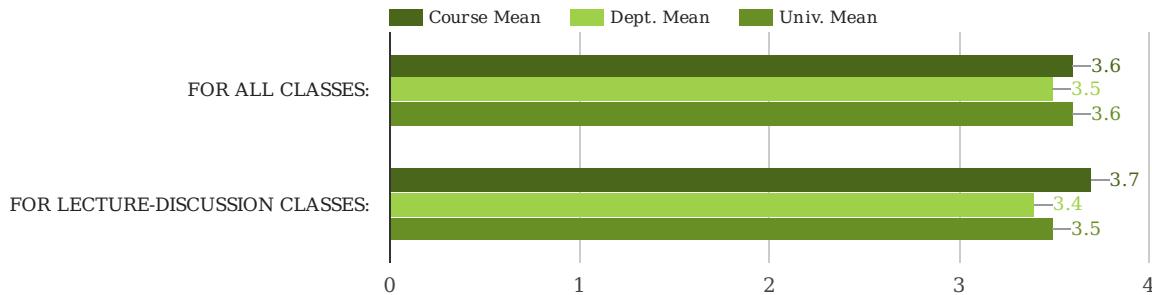
The University of Guam is a U.S. Land Grant Institution accredited by the Western Association of Schools and Colleges Senior College and University Commission and is an equal opportunity provider and employer.

5.3.3 AG-109 (Lecture and lab sections) Fall 2014

Please see next page.

Instructor: **MOORE, AUBREY**Section: **1**Course Title: **AG-109-01**Course ID: **035398**

Objectives:

Enrollment: **23**Responses Incl Declines: **7**Declines: **0****Category Summary**

Category	Number of Responses	Response Rate	Dept. Mean	Univ. Mean	Median	Dept. Median	Univ. Median	STDEV
FOR ALL CLASSES:	118	30.2%	3.6	3.5	3.6	4.0	4.0	0.5
FOR LECTURE-DISCUSSION CLASSES:	42	30.4%	3.7	3.4	3.5	4.0	4.0	0.5

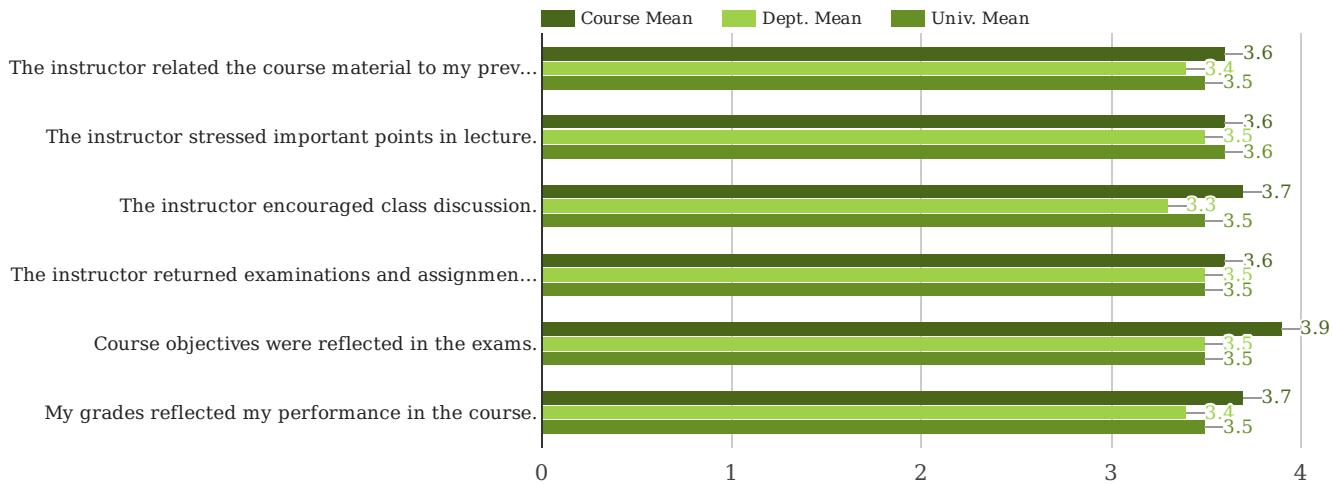
FOR ALL CLASSES:



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor made the subject matter interesting.	7	30%	3.7	3.3	3.5	4.0	3.0	4.0
The instructor's speaking style was clear enough to be understood.	7	30%	3.6	3.3	3.5	4.0	3.0	4.0
The instructor seemed interested in what he/she was teaching.	7	30%	3.9	3.6	3.7	4.0	4.0	4.0
The instructor knows the course material well.	7	30%	3.9	3.6	3.7	4.0	4.0	4.0
The instructor answered students' questions.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor was well prepared for each class.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor used class time well.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor encouraged students to do their best work.	7	30%	3.7	3.5	3.6	4.0	4.0	4.0
The instructor was fair to the students.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor made himself/herself available for help in and out of class.	7	30%	3.9	3.4	3.5	4.0	4.0	4.0
I spent two hours studying for this class for every hour of actual in-class time.	7	30%	3.3	3.3	3.2	3.0	3.0	3.0
I feel I've learned a great deal in this course.	7	30%	3.7	3.4	3.5	4.0	4.0	4.0
The instructor followed his/her syllabus.	7	30%	3.4	3.4	3.5	4.0	4.0	4.0
The instructor encouraged me to think.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor has done an effective job in this course.	6	26%	3.7	3.4	3.5	4.0	4.0	4.0
I would like to acknowledge this instructor for excellence.	7	30%	3.7	3.4	3.6	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR LECTURE-DISCUSSION CLASSES:



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor related the course material to my previous learning experiences.	7	30%	3.6	3.4	3.5	4.0	4.0	4.0
The instructor stressed important points in lecture.	7	30%	3.6	3.5	3.6	4.0	4.0	4.0
The instructor encouraged class discussion.	7	30%	3.7	3.3	3.5	4.0	4.0	4.0
The instructor returned examinations and assignments promptly.	7	30%	3.6	3.5	3.5	4.0	4.0	4.0
Course objectives were reflected in the exams.	7	30%	3.9	3.5	3.5	4.0	4.0	4.0
My grades reflected my performance in the course.	7	30%	3.7	3.4	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR ALL CLASSES:

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	7	30%	3.7	4.0
The instructor's speaking style was clear enough to be understood.	7	30%	3.6	4.0
The instructor seemed interested in what he/she was teaching.	7	30%	3.9	4.0
The instructor knows the course material well.	7	30%	3.9	4.0
The instructor answered students' questions.	7	30%	3.6	4.0
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	7	30%	3.6	4.0
The instructor was well prepared for each class.	7	30%	3.6	4.0
The instructor used class time well.	7	30%	3.6	4.0
The instructor encouraged students to do their best work.	7	30%	3.7	4.0
The instructor was fair to the students.	7	30%	3.6	4.0
The instructor made himself/herself available for help in and out of class.	7	30%	3.9	4.0
I spent two hours studying for this class for every hour of actual in-class time.	7	30%	3.3	3.0
I feel I've learned a great deal in this course.	7	30%	3.7	4.0
The instructor followed his/her syllabus.	7	30%	3.4	4.0
The instructor encouraged me to think.	7	30%	3.6	4.0
The instructor has done an effective job in this course.	6	26%	3.7	4.0
I would like to acknowledge this instructor for excellence.	7	30%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR LECTURE-DISCUSSION CLASSES:

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor related the course material to my previous learning experiences.	7	30%	3.6	4.0
The instructor stressed important points in lecture.	7	30%	3.6	4.0
The instructor encouraged class discussion.	7	30%	3.7	4.0
The instructor returned examinations and assignments promptly.	7	30%	3.6	4.0
Course objectives were reflected in the exams.	7	30%	3.9	4.0
My grades reflected my performance in the course.	7	30%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

COMMENTS: (2 comments)**Q: Strengths of this instructor:**

1 great instructor, easy to understand

2 this instructors passion for this class is great. I learned more than I hoped to. Thank you

5.3.4 AG-345 (Lecture and lab sections) Fall 2014

Instructor: **MOORE, AUBREY**Section: **70**Course Title: **AG-345-70**Course ID: **037387**

Objectives:

Enrollment: **1**Responses Incl Declines: **0**Declines: **0****Category Summary**

Category	Number of Responses	Response Rate	Dept. Mean	Univ. Mean	Median	Dept. Median	Univ. Median	STDEV
FOR ALL CLASSES:	0	0.0%	0.0	3.5	3.6	0.0	4.0	4.0
FOR LECTURE-DISCUSSION CLASSES:	0	0.0%	0.0	3.4	3.5	0.0	4.0	0.0

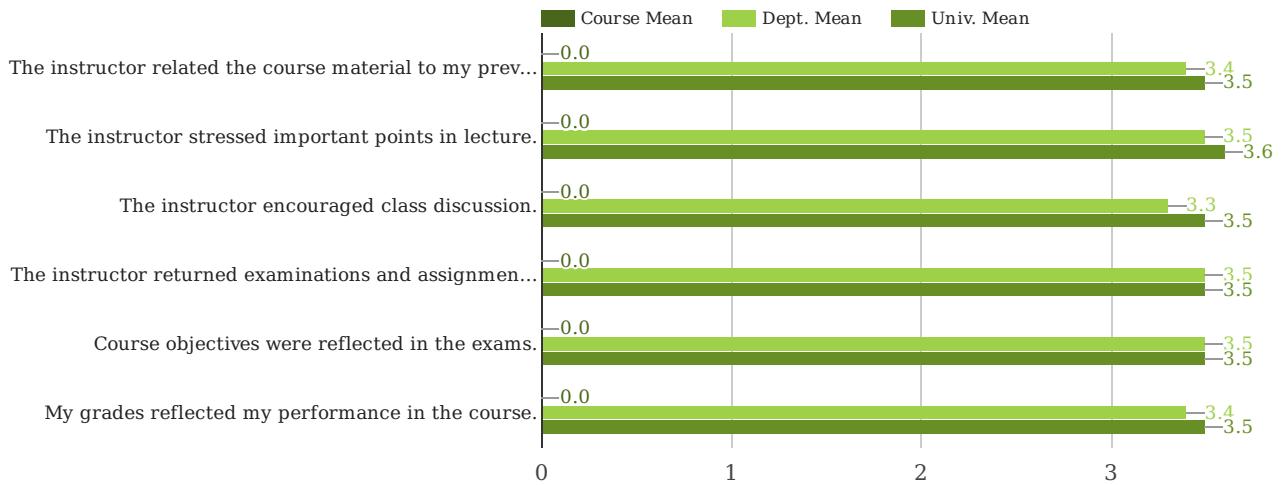
FOR ALL CLASSES:



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor made the subject matter interesting.	0	0%	0.0	3.3	3.5	0.0	3.0	4.0
The instructor's speaking style was clear enough to be understood.	0	0%	0.0	3.3	3.5	0.0	3.0	4.0
The instructor seemed interested in what he/she was teaching.	0	0%	0.0	3.6	3.7	0.0	4.0	4.0
The instructor knows the course material well.	0	0%	0.0	3.6	3.7	0.0	4.0	4.0
The instructor answered students' questions.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor was well prepared for each class.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor used class time well.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor encouraged students to do their best work.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor was fair to the students.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor made himself/herself available for help in and out of class.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0
I spent two hours studying for this class for every hour of actual in-class time.	0	0%	0.0	3.3	3.2	0.0	3.0	3.0
I feel I've learned a great deal in this course.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0
The instructor followed his/her syllabus.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0
The instructor encouraged me to think.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor has done an effective job in this course.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0
I would like to acknowledge this instructor for excellence.	0	0%	0.0	3.4	3.6	0.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR LECTURE-DISCUSSION CLASSES:



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor related the course material to my previous learning experiences.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0
The instructor stressed important points in lecture.	0	0%	0.0	3.5	3.6	0.0	4.0	4.0
The instructor encouraged class discussion.	0	0%	0.0	3.3	3.5	0.0	4.0	4.0
The instructor returned examinations and assignments promptly.	0	0%	0.0	3.5	3.5	0.0	4.0	4.0
Course objectives were reflected in the exams.	0	0%	0.0	3.5	3.5	0.0	4.0	4.0
My grades reflected my performance in the course.	0	0%	0.0	3.4	3.5	0.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR ALL CLASSES:

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	0	0%	0.0	0.0
The instructor's speaking style was clear enough to be understood.	0	0%	0.0	0.0
The instructor seemed interested in what he/she was teaching.	0	0%	0.0	0.0
The instructor knows the course material well.	0	0%	0.0	0.0
The instructor answered students' questions.	0	0%	0.0	0.0
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	0	0%	0.0	0.0
The instructor was well prepared for each class.	0	0%	0.0	0.0
The instructor used class time well.	0	0%	0.0	0.0
The instructor encouraged students to do their best work.	0	0%	0.0	0.0
The instructor was fair to the students.	0	0%	0.0	0.0
The instructor made himself/herself available for help in and out of class.	0	0%	0.0	0.0
I spent two hours studying for this class for every hour of actual in-class time.	0	0%	0.0	0.0
I feel I've learned a great deal in this course.	0	0%	0.0	0.0
The instructor followed his/her syllabus.	0	0%	0.0	0.0
The instructor encouraged me to think.	0	0%	0.0	0.0
The instructor has done an effective job in this course.	0	0%	0.0	0.0
I would like to acknowledge this instructor for excellence.	0	0%	0.0	0.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

FOR LECTURE-DISCUSSION CLASSES:

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor related the course material to my previous learning experiences.	0	0%	0.0	0.0
The instructor stressed important points in lecture.	0	0%	0.0	0.0
The instructor encouraged class discussion.	0	0%	0.0	0.0
The instructor returned examinations and assignments promptly.	0	0%	0.0	0.0
Course objectives were reflected in the exams.	0	0%	0.0	0.0
My grades reflected my performance in the course.	0	0%	0.0	0.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

5.3.5 BI-345 (Lecture and lab sections) Fall 2015

Please see next page.

Instructor: MOORE, AUBREY

Section: 01

Course Title: BI-345-01

Course ID: 038418

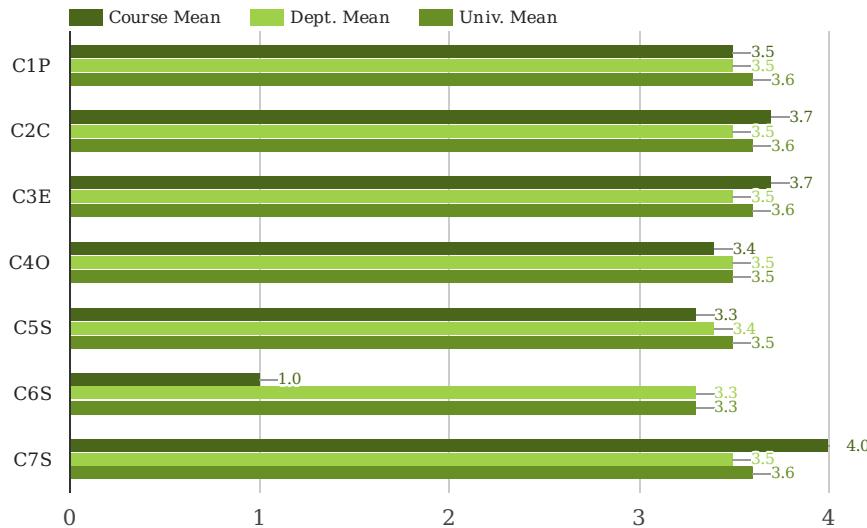
Objectives:

Enrollment: 2

Responses Incl Declines: 1

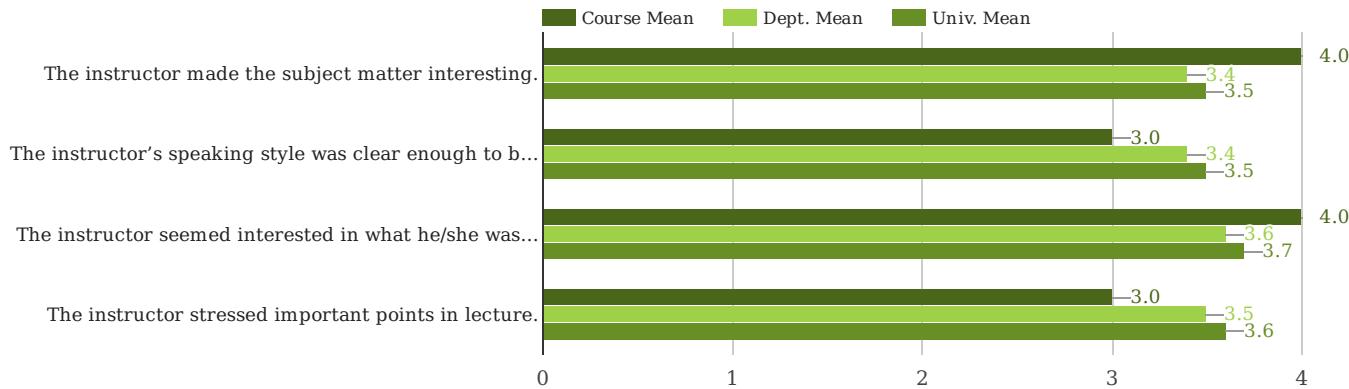
Declines: 0

Category Summary



Category	Number of Responses	Response Rate	Mean	Dept. Mean	Univ. Mean	Median	Dept. Median	Univ. Median	STDEV
C1P	4	50.0%	3.5	3.5	3.6	3.5	4.0	4.0	0.5
C2C	3	50.0%	3.7	3.5	3.6	4.0	4.0	4.0	0.5
C3E	6	50.0%	3.7	3.5	3.6	4.0	4.0	4.0	0.5
C4O	5	50.0%	3.4	3.5	3.5	3.0	4.0	4.0	0.5
C5S	3	50.0%	3.3	3.4	3.5	3.0	4.0	4.0	0.5
C6S	1	50.0%	1.0	3.3	3.3	1.0	3.0	3.0	0.0
C7S	1	50.0%	4.0	3.5	3.6	4.0	4.0	4.0	0.0

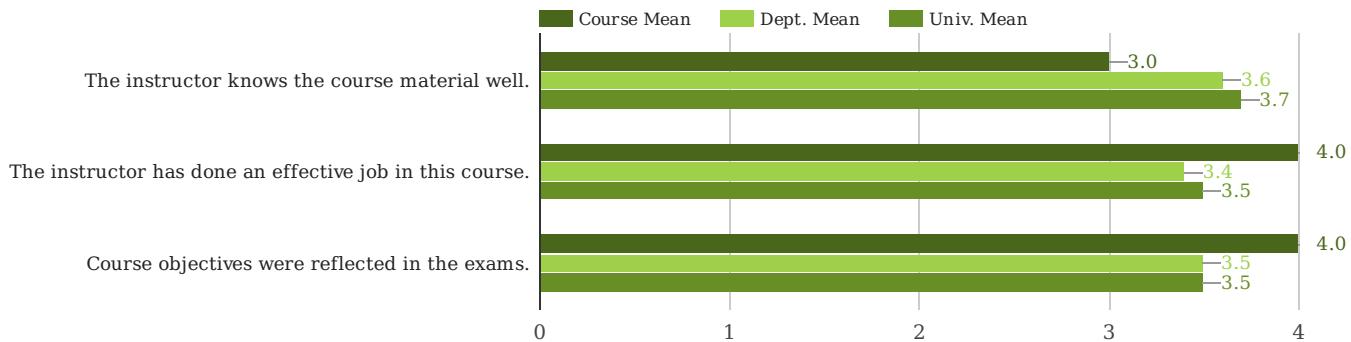
C1P



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor made the subject matter interesting.	1	50%	4.0	3.4	3.5	4.0	3.0	4.0
The instructor's speaking style was clear enough to be understood.	1	50%	3.0	3.4	3.5	3.0	3.0	4.0
The instructor seemed interested in what he/she was teaching.	1	50%	4.0	3.6	3.7	4.0	4.0	4.0
The instructor stressed important points in lecture.	1	50%	3.0	3.5	3.6	3.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

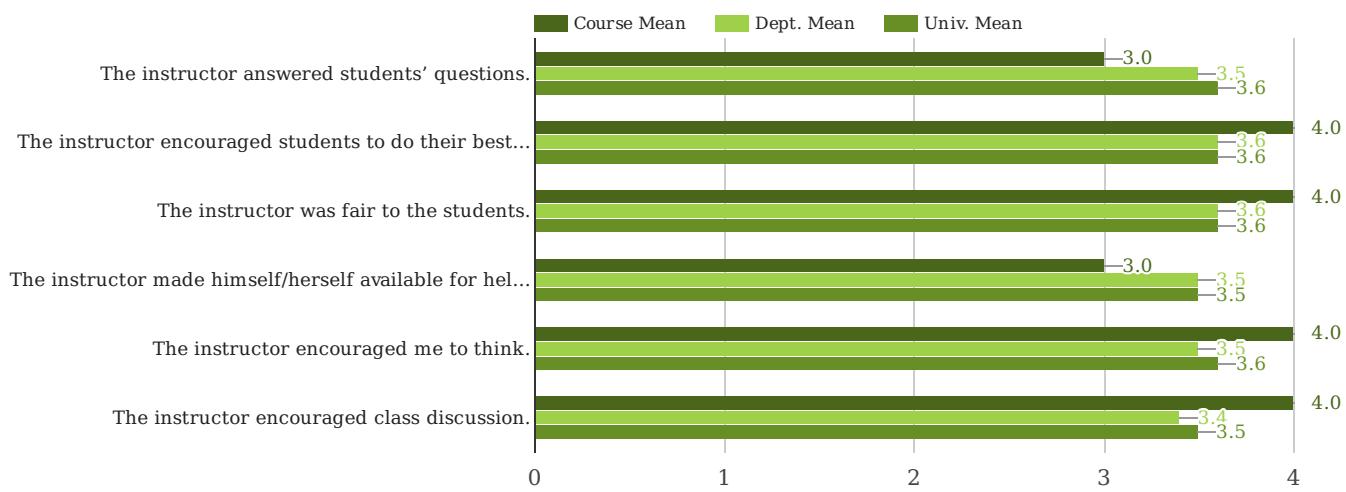
C2C



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor knows the course material well.	1	50%	3.0	3.6	3.7	3.0	4.0	4.0
The instructor has done an effective job in this course.	1	50%	4.0	3.4	3.5	4.0	4.0	4.0
Course objectives were reflected in the exams.	1	50%	4.0	3.5	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

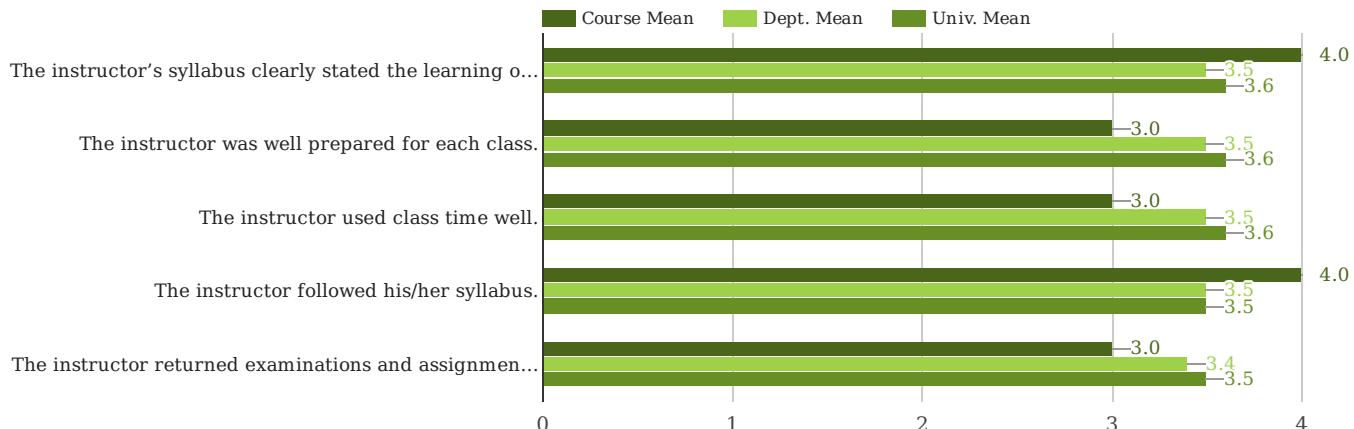
C3E



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor answered students' questions.	1	50%	3.0	3.5	3.6	3.0	4.0	4.0
The instructor encouraged students to do their best work.	1	50%	4.0	3.6	3.6	4.0	4.0	4.0
The instructor was fair to the students.	1	50%	4.0	3.6	3.6	4.0	4.0	4.0
The instructor made himself/herself available for help in and out of class.	1	50%	3.0	3.5	3.5	3.0	4.0	4.0
The instructor encouraged me to think.	1	50%	4.0	3.5	3.6	4.0	4.0	4.0
The instructor encouraged class discussion.	1	50%	4.0	3.4	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

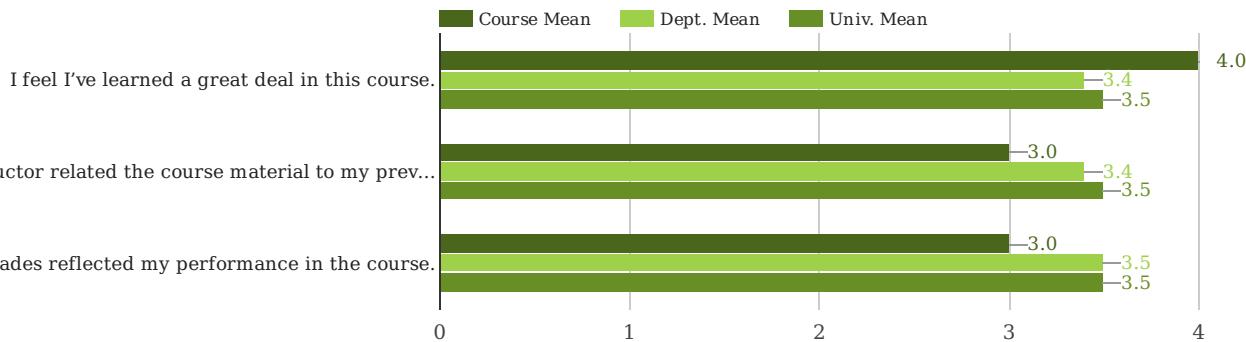
C4O



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	1	50%	4.0	3.5	3.6	4.0	4.0	4.0
The instructor was well prepared for each class.	1	50%	3.0	3.5	3.6	3.0	4.0	4.0
The instructor used class time well.	1	50%	3.0	3.5	3.6	3.0	4.0	4.0
The instructor followed his/her syllabus.	1	50%	4.0	3.5	3.5	4.0	4.0	4.0
The instructor returned examinations and assignments promptly.	1	50%	3.0	3.4	3.5	3.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

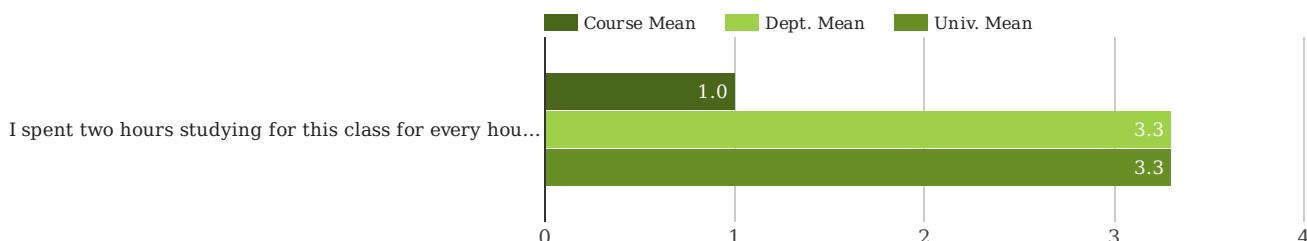
C5S



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I feel I've learned a great deal in this course.	1	50%	4.0	3.4	3.5	4.0	4.0	4.0
The instructor related the course material to my previous learning experiences.	1	50%	3.0	3.4	3.5	3.0	4.0	4.0
My grades reflected my performance in the course.	1	50%	3.0	3.5	3.5	3.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

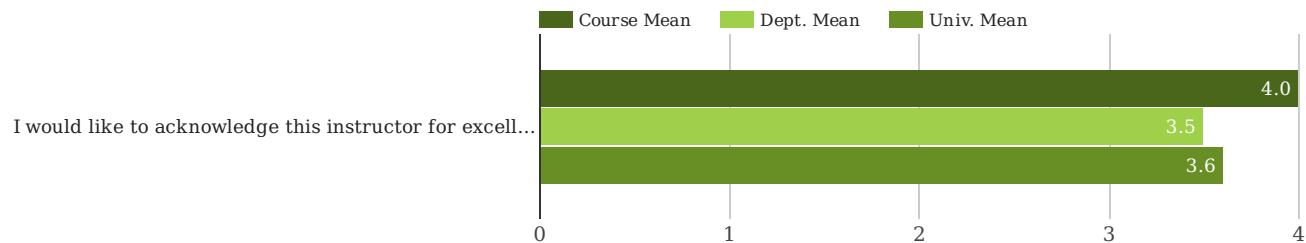
C6S



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I spent two hours studying for this class for every hour of actual in-class time.	1	50%	1.0	3.3	3.3	1.0	3.0	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I would like to acknowledge this instructor for excellence.	1	50%	4.0	3.5	3.6	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C1P

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	1	50%	4.0	4.0
The instructor's speaking style was clear enough to be understood.	1	50%	3.0	3.0
The instructor seemed interested in what he/she was teaching.	1	50%	4.0	4.0
The instructor stressed important points in lecture.	1	50%	3.0	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C2C

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor knows the course material well.	1	50%	3.0	3.0
The instructor has done an effective job in this course.	1	50%	4.0	4.0
Course objectives were reflected in the exams.	1	50%	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C3E

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor answered students' questions.	1	50%	3.0	3.0
The instructor encouraged students to do their best work.	1	50%	4.0	4.0
The instructor was fair to the students.	1	50%	4.0	4.0
The instructor made himself/herself available for help in and out of class.	1	50%	3.0	3.0
The instructor encouraged me to think.	1	50%	4.0	4.0
The instructor encouraged class discussion.	1	50%	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C4O

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	1	50%	4.0	4.0
The instructor was well prepared for each class.	1	50%	3.0	3.0
The instructor used class time well.	1	50%	3.0	3.0
The instructor followed his/her syllabus.	1	50%	4.0	4.0
The instructor returned examinations and assignments promptly.	1	50%	3.0	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C5S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I feel I've learned a great deal in this course.	1	50%	4.0	4.0
The instructor related the course material to my previous learning experiences.	1	50%	3.0	3.0
My grades reflected my performance in the course.	1	50%	3.0	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C6S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I spent two hours studying for this class for every hour of actual in-class time.	1	50%	1.0	1.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I would like to acknowledge this instructor for excellence.	1	50%	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

Comments (2 comments)

Q: Strengths of this instructor:

1 This instructor has very much helped me with my insectphobia. He gave me the knowledge to understand these creatures.

He made class very relate-able to real world experiences and identification.

He answered questions. Honestly, and if he did not know the answer he would ask another student who was also very knowledgeable.

Q: Areas in which this instructor needs improvement:

- 1 The only recommendation I have is that I would have liked to have had the course syllabus earlier in the year.

5.3.6 AG-345 (Lecture and lab sections) Fall 2015

Please see next page.

Instructor: **MOORE, AUBREY**Section: **01**Course Title: **AG-345-01**Course ID: **038417**

Objectives:

Enrollment: **7**Responses Incl Declines: **7**Declines: **0****Category Summary****C1P****C2C****C3E****C4O****C5S****C6S****C7S**

C1P

C2C

C3E

C4O

C5S

C6S

C7S

Comments (4 comments)**Q: Strengths of this instructor:**

- 1 very knowledgeable about insects and their biodiversity
- 2 Knows his materials well. He works well with improving weaknesses of students.

Q: Areas in which this instructor needs improvement:

- 1 Just keep teaching the course
- 2 Promptness in returning assignments

5.3.7 AL-345 (Lecture section) Fall 2017

Please see next page.

Instructor: **MOORE, AUBREY**Section: **01**Course Title: **AL-345-01**Course ID: **044456**

Objectives:

Enrollment: **9**Responses Incl Declines: **8**Declines: **0****Category Summary****C1P****C2C****C3E****C4O****C5S****C6S****C7S**

C1P

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	8	88%	3.9	4.0
The instructor's speaking style was clear enough to be understood.	8	88%	4.0	4.0
The instructor seemed interested in what he/she was teaching.	8	88%	4.0	4.0
The instructor stressed important points in lecture.	8	88%	3.8	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C2C

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor knows the course material well.	8	88%	4.0	4.0
The instructor has done an effective job in this course.	8	88%	3.9	4.0
Course objectives were reflected in the exams.	8	88%	3.8	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C3E

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor answered students' questions.	8	88%	3.9	4.0
The instructor encouraged students to do their best work.	8	88%	3.9	4.0
The instructor was fair to the students.	8	88%	4.0	4.0
The instructor made himself/herself available for help in and out of class.	8	88%	3.8	4.0
The instructor encouraged me to think.	8	88%	3.8	4.0
The instructor encouraged class discussion.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C4O

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	8	88%	3.9	4.0
The instructor was well prepared for each class.	8	88%	3.8	4.0
The instructor used class time well.	8	88%	3.8	4.0
The instructor followed his/her syllabus.	8	88%	3.8	4.0
The instructor returned examinations and assignments promptly.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C5S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I feel I've learned a great deal in this course.	8	88%	4.0	4.0
The instructor related the course material to my previous learning experiences.	8	88%	3.9	4.0
My grades reflected my performance in the course.	8	88%	3.8	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C6S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I spent two hours studying for this class for every hour of actual in-class time.	8	88%	3.5	3.5

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I would like to acknowledge this instructor for excellence.	8	88%	3.8	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

Comments (9 comments)

Q: Strengths of this instructor:

- 1 Knows the subject very, encouraged informative discussions, and made me view my surroundings in a whole new perspective.
- 2 Always willing to help identify insects or assist in finding the species.
- 3 Dr. Moore was very good at expressing the importance of insect knowledge and relating that to the current issues in the pacific. This was beneficial to me in broadening my area of interests in Agriculture and Biology. He is a wealth of knowledge and should be acknowledged as he is one of only a few Entomologists in Micronesia.

-
- 4 Great at what he does, as well making me appreciate the insect world.
 - 5 I truly appreciated the fact that Dr. Moore allowed each student to participate and perform a research project upon the student's choosing. It allowed the process to be very fun, informative and personal in regards to interest. Thank you Dr. Moore!
-

Q: Areas in which this instructor needs improvement:

- 1 Maybe take classes off campus to help encourage better collection for lab projects.
 - 2 Time to practice identifying insect orders in class so that we are prepared for exams.
 - 3 Nothing, awesome class!
 - 4 N/A.
-

5.3.8 AL-345L (Lab section) Fall 2017

Please see next page.

AL-345L-01 (17/FA)

Instructor: **MOORE, AUBREY**

Section: **01**

Course Title: **AL-345L-01**

Course ID: **044459**

Objectives:

Enrollment: **9**

Responses Incl Declines: **8**

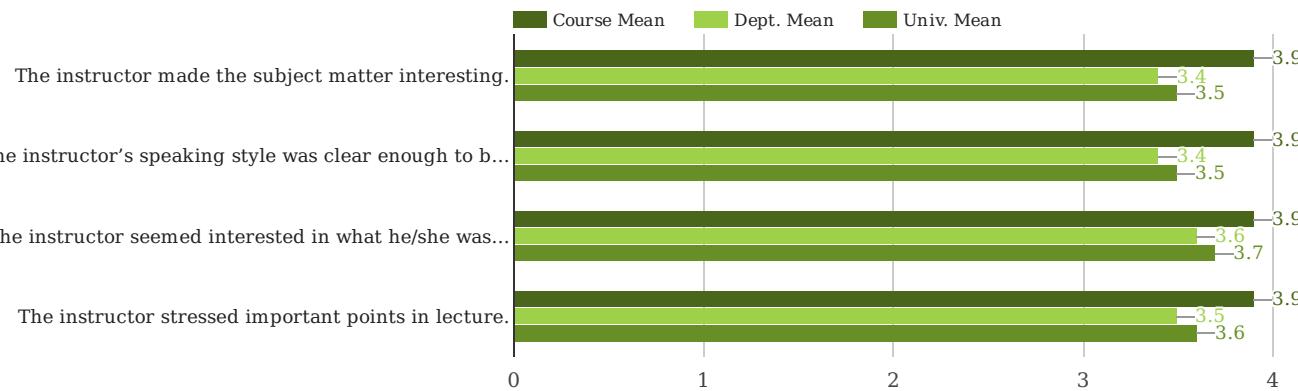
Declines: **0**

Category Summary



Category	Number of Responses	Response Rate	Mean	Dept. Mean	Univ. Mean	Median	Dept. Median	Univ. Median	STDEV
C1P	32	88.9%	3.9	3.5	3.6	4.0	4.0	4.0	0.3
C2C	24	88.9%	3.9	3.5	3.6	4.0	4.0	4.0	0.3
C3E	48	88.9%	3.8	3.5	3.6	4.0	4.0	4.0	0.4
C4O	40	88.9%	3.8	3.5	3.5	4.0	4.0	4.0	0.4
C5S	24	88.9%	3.8	3.4	3.5	4.0	4.0	4.0	0.4
C6S	8	88.9%	3.9	3.3	3.3	4.0	3.0	3.0	0.3
C7S	8	88.9%	3.9	3.5	3.6	4.0	4.0	4.0	0.3

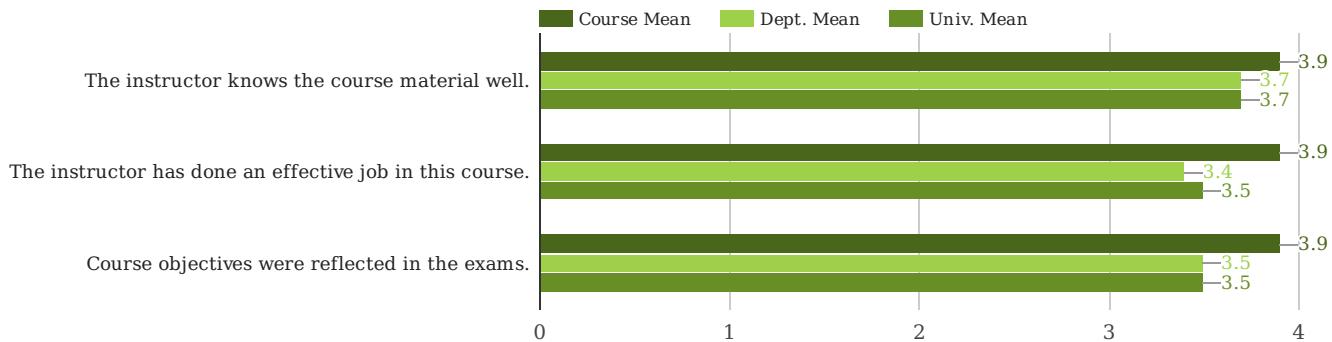
C1P



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor made the subject matter interesting.	8	88%	3.9	3.4	3.5	4.0	3.0	4.0
The instructor's speaking style was clear enough to be understood.	8	88%	3.9	3.4	3.5	4.0	3.0	4.0
The instructor seemed interested in what he/she was teaching.	8	88%	3.9	3.6	3.7	4.0	4.0	4.0
The instructor stressed important points in lecture.	8	88%	3.9	3.5	3.6	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

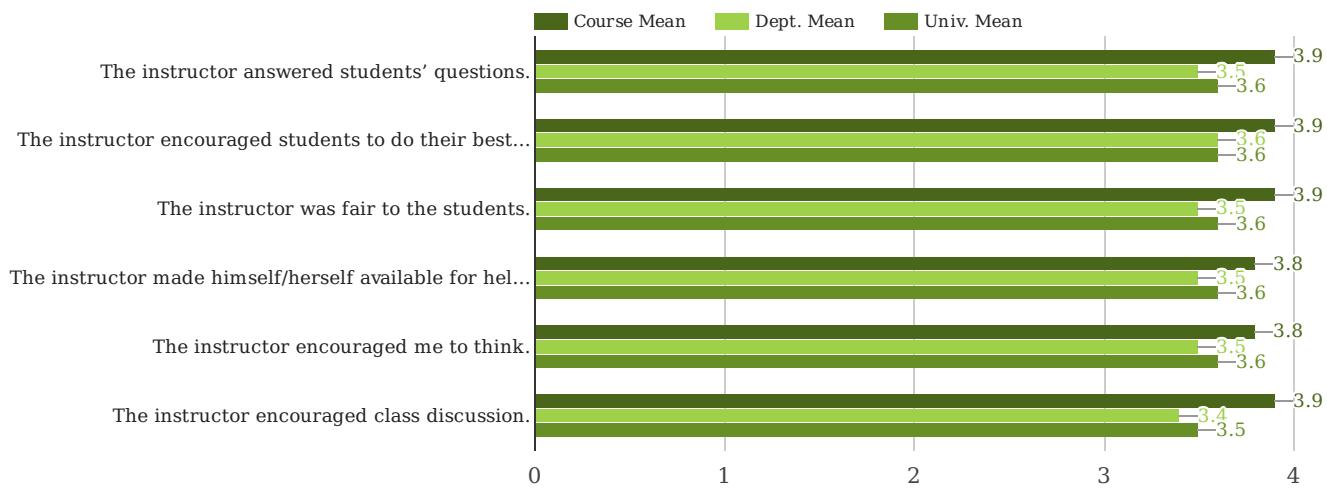
C2C



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor knows the course material well.	8	88%	3.9	3.7	3.7	4.0	4.0	4.0
The instructor has done an effective job in this course.	8	88%	3.9	3.4	3.5	4.0	4.0	4.0
Course objectives were reflected in the exams.	8	88%	3.9	3.5	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

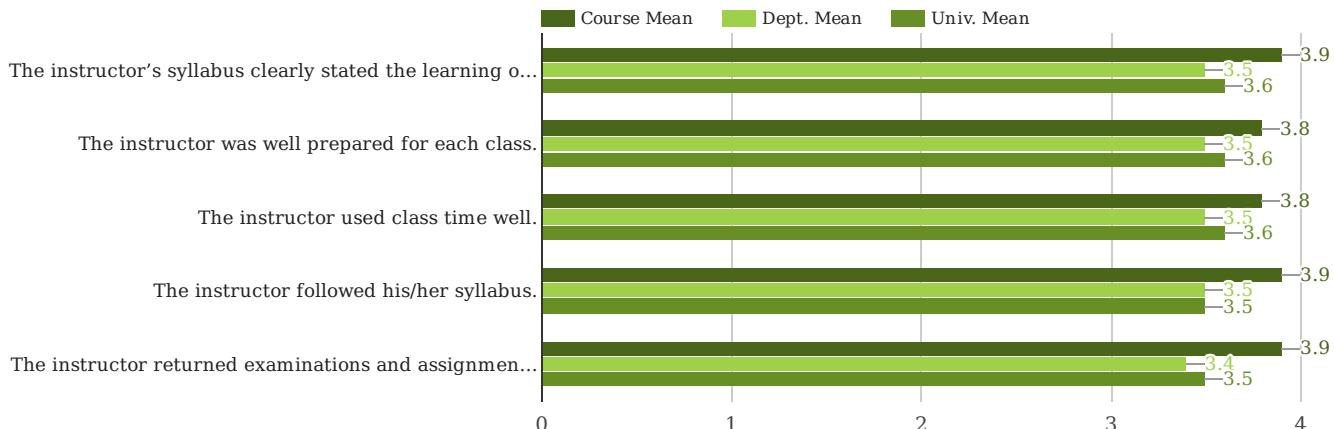
C3E



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor answered students' questions.	8	88%	3.9	3.5	3.6	4.0	4.0	4.0
The instructor encouraged students to do their best... work.	8	88%	3.9	3.6	3.6	4.0	4.0	4.0
The instructor was fair to the students.	8	88%	3.9	3.5	3.6	4.0	4.0	4.0
The instructor made himself/herself available for help in and out of class.	8	88%	3.8	3.5	3.6	4.0	4.0	4.0
The instructor encouraged me to think.	8	88%	3.8	3.5	3.6	4.0	4.0	4.0
The instructor encouraged class discussion.	8	88%	3.9	3.4	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

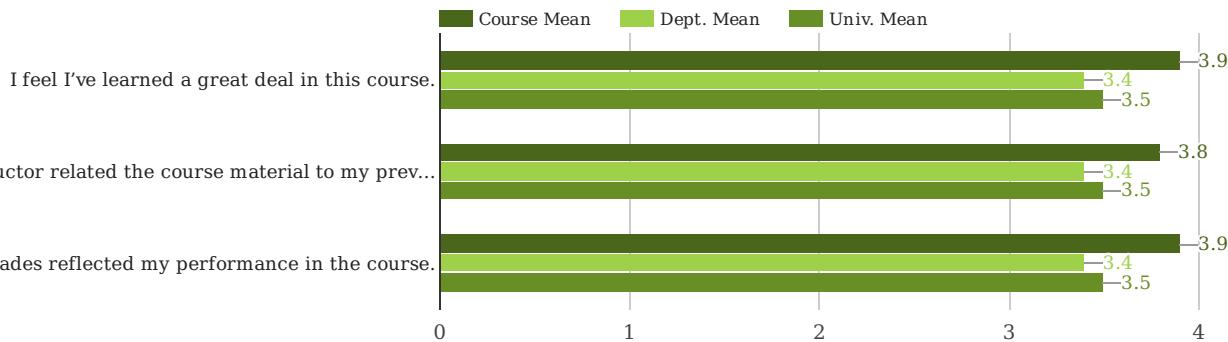
C4O



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	8	88%	3.9	3.5	3.6	4.0	4.0	4.0
The instructor was well prepared for each class.	8	88%	3.8	3.5	3.6	4.0	4.0	4.0
The instructor used class time well.	8	88%	3.8	3.5	3.6	4.0	4.0	4.0
The instructor followed his/her syllabus.	8	88%	3.9	3.5	3.5	4.0	4.0	4.0
The instructor returned examinations and assignments promptly.	8	88%	3.9	3.4	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

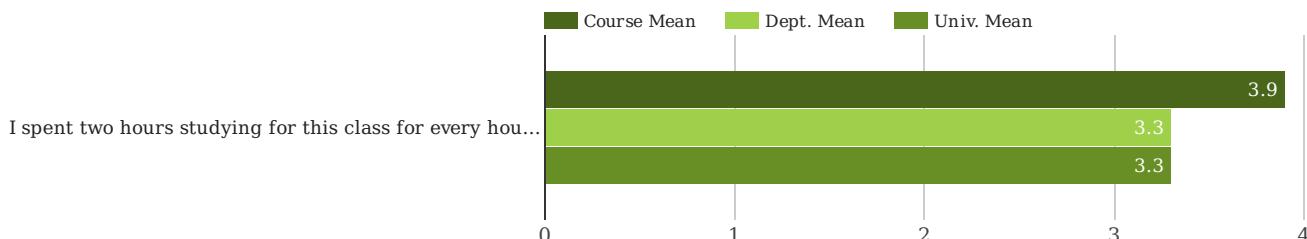
C5S



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I feel I've learned a great deal in this course.	8	88%	3.9	3.4	3.5	4.0	4.0	4.0
The instructor related the course material to my previous learning experiences.	8	88%	3.8	3.4	3.5	4.0	4.0	4.0
My grades reflected my performance in the course.	8	88%	3.9	3.4	3.5	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

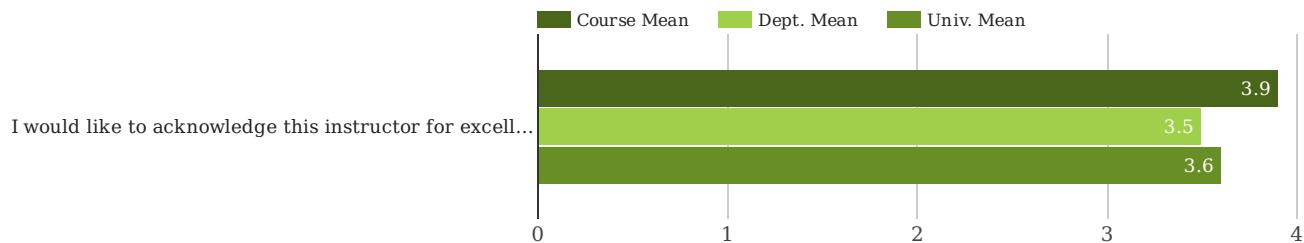
C6S



Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I spent two hours studying for this class for every hour of actual in-class time.	8	88%	3.9	3.3	3.3	4.0	3.0	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S



I would like to acknowledge this instructor for excell...

Question	Number of Responses	Response Rate	Course Mean	Dept. Mean	Univ. Mean	Course Median	Dept. Median	Univ. Median
I would like to acknowledge this instructor for excellence.	8	88%	3.9	3.5	3.6	4.0	4.0	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C1P

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	8	88%	3.9	4.0
The instructor's speaking style was clear enough to be understood.	8	88%	3.9	4.0
The instructor seemed interested in what he/she was teaching.	8	88%	3.9	4.0
The instructor stressed important points in lecture.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C2C

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor knows the course material well.	8	88%	3.9	4.0
The instructor has done an effective job in this course.	8	88%	3.9	4.0
Course objectives were reflected in the exams.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C3E

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor answered students' questions.	8	88%	3.9	4.0
The instructor encouraged students to do their best work.	8	88%	3.9	4.0
The instructor was fair to the students.	8	88%	3.9	4.0
The instructor made himself/herself available for help in and out of class.	8	88%	3.8	4.0
The instructor encouraged me to think.	8	88%	3.8	4.0
The instructor encouraged class discussion.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C4O

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	8	88%	3.9	4.0
The instructor was well prepared for each class.	8	88%	3.8	4.0
The instructor used class time well.	8	88%	3.8	4.0
The instructor followed his/her syllabus.	8	88%	3.9	4.0
The instructor returned examinations and assignments promptly.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C5S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I feel I've learned a great deal in this course.	8	88%	3.9	4.0
The instructor related the course material to my previous learning experiences.	8	88%	3.8	4.0
My grades reflected my performance in the course.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C6S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I spent two hours studying for this class for every hour of actual in-class time.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I would like to acknowledge this instructor for excellence.	8	88%	3.9	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

Comments (10 comments)

Q: Strengths of this instructor:

- 1 easygoing/calm and able to make course material engaging for students to learn and grasp
- 2 Wealth of knowledge on the class subject(s)
- 3 Allows us to use materials that we would have available in real life circumstances.
- 4 Dr. Moore really opened up the doors to the vast insect population we live among through assigning personal insect collections!

5 The instructor was very professional in delivering the class lecture. He helped students particularly in insect ID, explaining major concepts in general entomology. He is very keen to share current problems and his research in entomology in the region and I think that is a smart way to help the community aware of what's going on these days and how to basically how to apply concepts to deal with insect problems. He's been very motivational in encouraging his students to pursue graduate studies in entomology. All of the above I liked it when he showed the class about his email communication with an entomologist in Maldives about the migration of dragon flies, that tells us how the important technology is, communication/ collaboration and what its like to email a scientist. He also did professionally in telling the class the success story of invasive species on Guam. There is a lot more to cover in this class and I will like to take it again if time and money allows me to.

6 He made collecting bugs fun! haha...

Q: Areas in which this instructor needs improvement:

1 Take class out to other areas of the island for a more broad collection for class projects.

2 Assign the research project sooner.

3 N/A

4 Nothing! but maybe incorporate some field studies aaayyyy...

5.3.9 BI-345 (Lecture section) Fall 2017

Please see next page.

Instructor: **MOORE, AUBREY**Section: **01**Course Title: **BI-345-01**Course ID: **044458**

Objectives:

Enrollment: **8**Responses Incl Declines: **7**Declines: **0****Category Summary****C1P****C2C****C3E****C4O****C5S****C6S****C7S**

C1P

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	7	87%	3.6	4.0
The instructor's speaking style was clear enough to be understood.	7	87%	3.7	4.0
The instructor seemed interested in what he/she was teaching.	7	87%	3.7	4.0
The instructor stressed important points in lecture.	7	87%	3.4	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C2C

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor knows the course material well.	7	87%	3.7	4.0
The instructor has done an effective job in this course.	7	87%	3.6	4.0
Course objectives were reflected in the exams.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C3E

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor answered students' questions.	7	87%	3.7	4.0
The instructor encouraged students to do their best work.	7	87%	3.7	4.0
The instructor was fair to the students.	7	87%	3.7	4.0
The instructor made himself/herself available for help in and out of class.	7	87%	3.7	4.0
The instructor encouraged me to think.	7	87%	3.4	4.0
The instructor encouraged class discussion.	7	87%	3.6	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C4O

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	7	87%	3.7	4.0
The instructor was well prepared for each class.	7	87%	3.7	4.0
The instructor used class time well.	7	87%	3.7	4.0
The instructor followed his/her syllabus.	7	87%	3.7	4.0
The instructor returned examinations and assignments promptly.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C5S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I feel I've learned a great deal in this course.	7	87%	3.6	4.0
The instructor related the course material to my previous learning experiences.	7	87%	3.7	4.0
My grades reflected my performance in the course.	7	87%	3.6	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C6S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I spent two hours studying for this class for every hour of actual in-class time.	7	87%	3.3	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I would like to acknowledge this instructor for excellence.	7	87%	3.6	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

Comments (6 comments)

Q: Strengths of this instructor:

- 1 Well versed in the material and explained things thoroughly
- 2 Dr. Moore is a great professor. His lectures were very informative and he showed how passionate he was about the subject matter.
- 3 He's passionate about what he does
- 4 Instructor is extremely knowledgeable about subject matter and made the course interesting with his presentation style. I learned a

great deal in this course and enjoyed the whole semester. 5 out of 5 stars

Q: Areas in which this instructor needs improvement:

- 1 Nothing. This was the most fun class I have been apart.
- 2 Only feedback I can think of is I would have loved to learned more about crop pests but Dr. Moore has taught Entomology as a general course and provides students with resources to learn more information about any specific topic one may wish to learn.

5.3.10 BI-345L (Lab section) Fall 2017

Please see next page.

Instructor: **MOORE, AUBREY**Section: **01**Course Title: **BI-345L-01**Course ID: **044460**

Objectives:

Enrollment: **8**Responses Incl Declines: **7**Declines: **0****Category Summary****C1P****C2C****C3E****C4O****C5S****C6S****C7S**

C1P

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor made the subject matter interesting.	7	87%	3.7	4.0
The instructor's speaking style was clear enough to be understood.	7	87%	3.7	4.0
The instructor seemed interested in what he/she was teaching.	7	87%	3.7	4.0
The instructor stressed important points in lecture.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C2C

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor knows the course material well.	7	87%	3.7	4.0
The instructor has done an effective job in this course.	7	87%	3.7	4.0
Course objectives were reflected in the exams.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C3E

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor answered students' questions.	7	87%	3.7	4.0
The instructor encouraged students to do their best work.	7	87%	3.7	4.0
The instructor was fair to the students.	7	87%	3.7	4.0
The instructor made himself/herself available for help in and out of class.	7	87%	3.7	4.0
The instructor encouraged me to think.	7	87%	3.7	4.0
The instructor encouraged class discussion.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C4O

Question	Number of Responses	Response Rate	Course Mean	Course Median
The instructor's syllabus clearly stated the learning objectives, requirements, and grading procedures for the course.	7	87%	3.7	4.0
The instructor was well prepared for each class.	7	87%	3.7	4.0
The instructor used class time well.	7	87%	3.7	4.0
The instructor followed his/her syllabus.	7	87%	3.7	4.0
The instructor returned examinations and assignments promptly.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C5S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I feel I've learned a great deal in this course.	7	87%	3.7	4.0
The instructor related the course material to my previous learning experiences.	7	87%	3.7	4.0
My grades reflected my performance in the course.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C6S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I spent two hours studying for this class for every hour of actual in-class time.	7	87%	3.3	3.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

C7S

Question	Number of Responses	Response Rate	Course Mean	Course Median
I would like to acknowledge this instructor for excellence.	7	87%	3.7	4.0

Note: 4:Strongly Agree; 3:Agree; 2:Disagree; 1:Strongly Disagree; -1:Not applicable or no response;

Comments (3 comments)

Q: Strengths of this instructor:

- 1 He's passionate about what he does
- 2 Labs were really fun considering we were tasked to catch insects the whole time.

Q: Areas in which this instructor needs improvement:

1 Nothing. I have had labs before but I never had this much fun while doing work.

5.4 Evidence: Publications in Refereed Journals

5.4.1 First record of *Doleschalia tongana* for Guam Island

Please see next page.



RESEARCH NOTE

First record of *Doleschallia tongana* (Lepidoptera: Nymphalidae) for Guam Island [version 1; referees: 2 approved]

Jake Manuel¹, W. John Tennent², Donald W. Buden³, Aubrey Moore  1

¹College of Natural and Applied Sciences, University of Guam, Mangilao, Guam, 96923, USA

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³Division of Natural Sciences and Mathematics, College of Micronesia-FSM, Kolonia, Pohnpei, 96941, Federated States of Micronesia

V1 First published: 23 Mar 2018, 7:366 (doi: [10.12688/f1000research.14316.1](https://doi.org/10.12688/f1000research.14316.1))

Latest published: 23 Mar 2018, 7:366 (doi: [10.12688/f1000research.14316.1](https://doi.org/10.12688/f1000research.14316.1))

Abstract

A single specimen of the butterfly, *Doleschallia tongana* Hopkins 1927, was collected on Guam Island on October 23, 2017 (13.430478°N, 144.800419°E). This is a new species record for Guam and Micronesia, indicating a geographical range expansion for *D. tongana*.

Keywords

Doleschallia tongana, Pacific orange leafwing, Guam, Micronesia, range expansion, invasive species, new country record

Open Peer Review

Referee Status:  

Invited Referees

1 2

version 1  
published  report  report

1 Richard S. Zack, Washington State University, USA

2 Niklas Wahlberg , Lund University, Sweden

Discuss this article

Comments (0)

Corresponding author: Aubrey Moore (aubreymoore@guam.net)

Author roles: **Manuel J:** Writing – Review & Editing; **Tennent WJ:** Writing – Review & Editing; **Buden DW:** Writing – Review & Editing; **Moore A:** Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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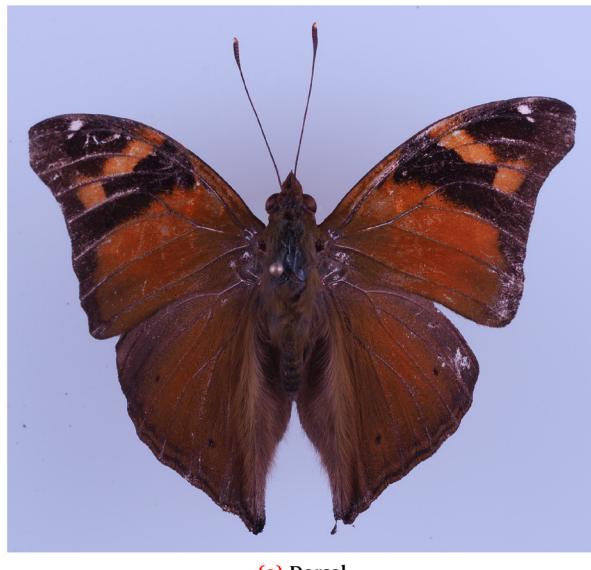
First published: 23 Mar 2018, 7:366 (doi: [10.12688/f1000research.14316.1](https://doi.org/10.12688/f1000research.14316.1))

Introduction

On October 23, 2017, a butterfly was taken from the underside of a leaf of soursop, *Annona muricata*, by a student (JM) assembling an insect collection as a requirement for the General Entomology course at the University of Guam. The collection site was the University of Guam campus in Mangilao, Guam (13.430478° N, 144.800419° E).

The specimen was pinned, images were made (Figure 1), documented in iNaturalist¹ and deposited in the University of Guam insect collection (Accession code: iNat8515898).

This specimen does not match any of the descriptions in *Butterflies of Micronesia*², the standard reference for Guam's butterflies.



(a) Dorsal



(b) Ventral

Figure 1. First specimen of *Doleschallia tongana* collected on Guam.

Identification

Digital images of the specimen were sent to DB and JT for identification. On 7 November 2017, DB tentatively identified the specimen as a species in the genus *Doleschallia*, and indicated it possibly belonging to the *bisaltide* complex. On 24 February, 2018 JT determined the butterfly as *Doleschallia tongana* Hopkins, 1927, based on images and comparison with the extensive collections of the Natural History Museum, London.

In common with other species in the “*bisaltide* species-group, *D. tongana* is individually variable.

The convex outer margin of the forewing; the general appearance of the specimen; and geography all suggest *D. tongana* (*tongana* Hopkins, 1927, is a name to replace *drusias* Fabricius, 1781, the type locality for which is Tonga). Some minor ‘unusual’ features include the fact that *tongana* usually has a sub-apical ‘half-moon’ series of 4–5 spots on the forewing, lacking in this specimen, which only has two, but this lies within the wide individual variation of the species. Considering a distribution of Papua New Guinea (including the Bismarcks), the Solomon Islands, Fiji, Samoa, Tonga and New Caledonia, we are confident of associating this specimen with *D. tongana*. No doubt further material will confirm this association in due course. The species-group is in need of some revision³. The GBIF Backbone Taxonomy lists the accepted name for this taxon as *Doleschallia bisaltide* subsp. *tongana* Hopkins, 1927⁴. However, the taxon record is tagged as a “name parent mismatch” issue.

D. tongana is listed in the iNaturalist database⁵ and has been assigned the vernacular name ‘Pacific orange leafwing’.

Geographical distribution

D. tongana, as it is currently understood, occurs throughout much of New Guinea, including the island groups in the east (see above).

Occurrence of *D. tongana* in Samoa is a relatively recently recorded range expansion. It was first detected on Tutuila Island in American Samoa in 1997⁶. Cook and Vargo 2000⁶ state that “The inclusion of Samoa in this species’ range by Parsons, 1998⁷ appears to be based on a misreading of Hopkins (1927).”

Description of caterpillar

Cook and Vargo 2000⁶ provide a description of a last instar *D. tongana* caterpillar:

“Just prior to pupation, the caterpillar measured ca. 50 mm in length. It possessed a black ground color with light speckling dorsally and prominent cream colored stripes running longitudinally, located dorso-laterally and ventro-laterally. Each body segment had seven prominent black spines, with numerous smaller secondary spines. The base of each primary spine was pale metallic blue. From a distance, the most prominent features of the caterpillar are the black ground color with metallic blue spots, and the pair of light parallel stripes running longitudinally on each side.”

Only a few larval host plants have been recorded for *D. tongana* (Table 1).

Table 1. Larval host plants of *Doleschallia tongana*.

Larval host plant	Reference(s)
Acanthaceae	
<i>Graptophyllum</i>	
<i>Graptophyllum insularum</i>	8
<i>Graptophyllum pictum</i>	6,7
Pseuderanthemum	
<i>Pseuderanthemum carruther</i>	6
<i>Pseuderanthemum laxiflorum</i>	8
<i>Pseuderanthemum</i> sp.	9
Moraceae	
<i>Artocarpus</i>	
<i>Artocarpus altilis</i>	8
Fabaceae	
<i>Erythrina</i>	
<i>Erythrina</i> sp.	8

Discussion

An informal survey has been initiated on Guam to search for more specimens of *D. tongana* and to record host plants.

This insect has the potential to do economic damage because it has been reported to feed on breadfruit, *Artocarpus altilis*⁸.

Data availability

All data underlying the results are available as part of the article and no additional source data are required.

Competing interests

No competing interests were disclosed.

Grant information

The author(s) declared that no grants were involved in supporting this work.

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Referee Report 23 May 2018

doi:[10.5256/f1000research.15578.r33973](https://doi.org/10.5256/f1000research.15578.r33973)

 Niklas Wahlberg 

Department of Biology, Lund University, Lund, Sweden

This is a simple report on a range extension of a butterfly species. What makes it interesting is that the range extension is to the island of Guam, which is relatively isolated. It seems this species is on a rampage through the South Pacific, colonizing new isolated islands! The only reference I missed was the relatively recent book by Patrick and Patrick (2012) "Butterflies of the South Pacific". I unfortunately do not have access to my copy at the moment to see what it says about the species. Perhaps good to look at that?

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 28 March 2018

doi:[10.5256/f1000research.15578.r32407](https://doi.org/10.5256/f1000research.15578.r32407)

**Richard S. Zack**

Department of Entomology, Washington State University, Pullman, WA, USA

A well-written report and discussion of a new species of butterfly to Guam. The record is a significant range extension for the species. Introductions to islands such as Guam are of concern because of potential economic and environmental effects and reports such as this are important to document. It will be interesting to see if the species has established or if this was a one-time occurrence.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Not applicable

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.**I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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**5.4.2 Judas beetles: Discovering cryptic breeding sites by
radio-tracking coconut rhinoceros beetles, *Oryctes rhinoceros*
(Coleoptera: Scarabaeidae)**

Please see next page.

Sampling

Judas Beetles: Discovering Cryptic Breeding Sites by Radio-Tracking Coconut Rhinoceros Beetles, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae)

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Subject Editor: Francis Reay-Jones

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Abstract

The coconut rhinoceros beetle, *Oryctes rhinoceros* L., is a serious pest of coconut and other palms throughout Southeast Asia and on several Pacific Islands. Adults damage and sometimes kill palms when they bore into the crown to feed. In contrast, larvae feed only on dead plant material at breeding sites. Typically, coconut rhinoceros beetle populations are controlled with a combination of biocontrol, pheromone traps, and breeding site removal. A field trial was performed at two locations on Guam to test the feasibility of using the Judas technique, releasing radio-tagged adults to discover cryptic breeding sites, for potential coconut rhinoceros beetle control. Of 33 radio-tagged beetles that were released, 19 were successfully tracked to landing sites, 11 of which were considered to be active or potential breeding sites, in five different microhabitats. The remaining 14 beetles were lost when they flew beyond the range of receivers. Only one of the radio-tagged beetles was caught in the numerous pheromone traps present at the release sites. Percent emergence weight (%EW, ratio of current/emergence weight) varied significantly by the microhabitat to which coconut rhinoceros beetles were tracked. When microhabitats were further grouped, the difference in mean %EW between the arboreal ($74 \pm 2\%$) and the soil-associated ($82 \pm 3\%$) groups were found to be highly significant. The %EW for coconut rhinoceros beetles that were successfully located ($78 \pm 2\%$) and those that were lost ($72 \pm 2\%$) also differed significantly. Radio-tracking coconut rhinoceros beetles shows promise as a method to identify cryptic breeding sites, which could then be treated, removed, or destroyed.

Key words: forest entomology, pest management, GIS, ecology & behavior, detection

The coconut rhinoceros beetle, *Oryctes rhinoceros* L. (Coleoptera: Scarabaeidae, Dynastinae), is a serious pest of coconut trees, *Cocos nucifera* L., and other palms throughout the Pacific and Southeast Asia. Adult beetles damage and sometimes kill palms when they bore into crowns of palms to feed on sap. Palms die when boring and feeding activities kill the apical meristem. Although coconut rhinoceros beetle damage does not always result in coconut tree mortality, the characteristic V-cut damage to palm fronds can adversely affect the nut production and the aesthetic value of ornamental trees (Hinckley 1973, Zelazny 1979, Bedford 2013).

Coconut rhinoceros beetle damage to coconut palms is caused only by adult coconut rhinoceros beetles feeding in crowns. In contrast, larvae cause no economic damage, as they feed on decaying vegetation at breeding sites, which include dead standing coconut

palms, fallen coconut logs, rotting coconut stumps, and decaying wood of many tree species (Bedford 1976, 2013). Breeding sites are also found in piles of compost, sawdust, and manure where these materials are available. When coconut rhinoceros beetle breeding sites are abundant following damage from typhoons, war, or large-scale agricultural operations, a self-sustaining positive feedback loop may be initiated in which large numbers of coconut rhinoceros beetle adults kill large numbers of palms, creating new breeding sites that generate even more coconut rhinoceros beetles. This worst case scenario was observed in the Palau Islands when coconut rhinoceros beetles arrived near the end of World War II. A coconut rhinoceros beetle population outbreak was fueled by the availability of abundant breeding sites in the form of trees killed by military activities. Fifty percent of coconut palms were killed by coconut rhinoceros

beetles throughout the Parlay Islands, and some of the smaller islands lost all of their coconut palms (Gressit 1953).

After feeding in the crowns of palms, adults of both sexes return to breeding sites, where they aggregate and mate, and females oviposit (Bedford 1980). Coconut rhinoceros beetle aggregation at breeding sites is facilitated by an aggregation pheromone produced by adult males (Hallett et al. 1995). The feeding-aggregation/mating-oviposition cycle generally repeats multiple times throughout the lifetime of adult beetles (Gressit 1953). Vander Meer (1987) developed a body mass index, percent emergence weight (%EW), which is strongly correlated with the physiological and behavioral status of coconut rhinoceros beetle adults.

Coconut rhinoceros beetle was first detected in Guam in the Tumon Bay tourist hotel area in September, 2007. A delimiting survey indicated that the infestation was restricted to only a small region of the island (<500 ha) and an eradication project was launched (Smith and Moore 2008). The project relied on mass trapping using pheromone traps to capture adults and sanitation to remove rotting vegetation used as breeding sites. In addition, four detector dogs were trained to assist in finding breeding sites on the ground by sniffing out coconut rhinoceros beetle grubs.

Despite these efforts, coconut rhinoceros beetles damage in central Tumon Bay remained high and the infestation spread to all parts of Guam by 2010, making eradication impractical at that time. Attempts at population suppression using *Oryctes nuditivirus* (OrNV), the preferred biocontrol agent for coconut rhinoceros beetles (Bedford 1986), also failed. It has recently been determined that the Guam coconut rhinoceros beetle population is genetically different from other populations in Asia and the Pacific, and it is considered to be a new invasive biotype of coconut rhinoceros beetles that is not subject to biocontrol by OrNV (Marshall et al. 2015). In addition to being resistant to all currently available isolates of OrNV, it appears that the coconut rhinoceros beetle-Guam biotype behaves differently. Coconut rhinoceros beetle breeding sites are commonly found in coconut palm crowns on Guam (Moore et al. 2015), but arboreal breeding sites are found only occasionally in other areas where the beetle occurs.

Eradication of coconut rhinoceros beetles from an island is difficult once this pest has become established. On two islands in Fiji, mass trapping using the now superseded synthetic attractant ethyl chrysanthemate coupled with sanitation from 1971 through 1974 failed to eradicate coconut rhinoceros beetles (Bedford 1980). The only proven tactic for eradication is a vigorous sanitation program that discovers and destroys all active and potential breeding sites. The single successful coconut rhinoceros beetle eradication to date was accomplished during the 1920s on the tiny (36 km²) Niuatoputapu Island (also known as Keppel Island), which lies between Samoa and Tonga, using sanitation alone (Catley 1969, Bedford 1976). Given the importance of finding and destroying breeding sites in coconut rhinoceros beetle eradication and the inherent difficulty of locating breeding sites, which are often cryptic and are found in a wide range of locations (Hinckley 1973, Bedford 2013), there is a pressing need to develop detection methods to reliably find these sites.

Potential agents for detecting pest species include predators, parasitoids, and conspecifics of pest animals, which have often evolved behaviors and superior sensory systems that aid in finding either prey or mates in a complex natural environment. An example is the use of the predatory wasp, *Cerceris fumipennis* Say, a natural predator of different beetles in the Buprestidae, to monitor the emerald ash borer (Swink et al. 2013). While *C. fumipennis* specializes on Buprestids, they are not a species-specific predator, capturing 52 different species in 11 different genera (Swink et al. 2013). A more species-specific monitoring technique is the use of “Judas goats,”

referencing the Biblical character Judas Iscariot, in the eradication of feral goat populations, particularly on islands (Taylor and Katahira 1988, Campbell and Donlan 2005). This technique involves fitting a Judas goat with a radio transmitter and releasing it into the wild to seek out other goats, which are then tracked and shot (Taylor and Katahira 1988). The Judas technique has been most commonly employed to control mammals (Spencer et al. 2015), but has also been used against fish (Bajer et al. 2011) and birds (Woolnough et al. 2006). Until recently, using the Judas technique with insects would have been impractical due to the relatively high mass of available transmitters. However, the recent development of lightweight, miniaturized radio-tracking transmitters now allows application of this technique to larger species of insect pests that aggregate in the wild.

Radio telemetry to monitor flying insects was first employed by Hedin and Ranius (2002) to study the dispersal range of the Russian leather beetle, *Osmodesma eremita* (Scopoli), and since then, several studies have adopted this technique to study the behavior of beetles. Large beetles in the Carabidae (Negro et al. 2008), Lucanidae (Rink and Sinsch 2007), and Scarabaeidae (Beaudoin-Ollivier et al. 2003, Ranius 2006, Hedin et al. 2008, Svensson et al. 2011, Chiari et al. 2013, McCullough 2013, Le Gouar et al. 2015) families have been successfully studied through radio telemetry. Of particular interest is the study by Beaudoin-Ollivier et al. (2003) that used radio telemetry to successfully describe the flight behavior of *Scapanes australis* (Boisduval), a rhinoceros beetle in the same subfamily as coconut rhinoceros beetle (Dynastinae).

Given that adult coconut rhinoceros beetles utilize semiochemical communication (and potentially other cues) to find mates and aggregate at breeding sites, this species offers a good opportunity to test the Judas technique with an insect. Coconut rhinoceros beetles equipped with radio transmitters, released into the wild, and then tracked have the potential to enable the location of cryptic breeding sites that could then be treated, removed, or destroyed. Herein, we report on a field trial to determine the feasibility of radio-tracking coconut rhinoceros beetles to find cryptic breeding site aggregations at two locations on the island of Guam.

Materials and Methods

Release Sites and Experimental Conditions

Tagged coconut rhinoceros beetles were radio-tracked after release at two locations on Guam: the War in the Pacific National Historical Park in Asan (13.465972° N, 144.710944° E; Fig. 1A) and the University of Guam Agricultural Research Station in Yigo (13.532444° N, 144.873333° E; Fig. 1B). Asan Beach National Park is roughly triangular with the ocean bordering one side, coastal wetlands on another, and forested hillside on the third. The park itself is a large, open, grassy field, and includes coconut palms on the edges, many of which displayed coconut rhinoceros beetle damage at the time of the study. The release site (144.708537° E, 13.473904° N) was at the middle of a large, grassy field. Barrel traps ($n=16$) made from 45-gallon drums containing the coconut rhinoceros beetle pheromone oryctalure (Iriarte et al. 2015) were distributed throughout the park. The Yigo site is an inland agricultural experiment station farm bordered by residential areas and uncultivated forest areas that include coconut palms along with many other trees. At the time of the field trial, most coconut palms on the station were showing signs of coconut rhinoceros beetle damage. The release site (144.872750° E, 13.531333° N) was in the middle of an uncultivated field. Beetles were released in the vicinity of three types of



Fig. 1. The two release sites on Guam: (A) the War in the Pacific National Historical Park in Asan and (B) the University of Guam Agricultural Research Station in Yigo. Yellow dots mark the release sites (*), red triangles mark landing sites of radio-tagged coconut rhinoceros beetles (\diamond), and green arrows mark the last locations and headings lost coconut rhinoceros beetles (\rightarrow). Initial tracking was conducted at night with landing locations confirmed the following day.

pheromone traps baited with oryzalure at this location: standard vaned bucket traps ($n = 3$ traps; Hallett et al. 1995), barrel traps made from 45-gallon drums ($n = 31$), and DeFence traps made from plastic netting that entangles the coconut rhinoceros beetles ($n = 4$; Iriarte et al. 2015). Thus, both sites feature relatively accessible terrain that provides a variety of potential breeding sites as well as adult food sources.

Weather conditions during the experiment were mainly clear with occasional periods of rain and overcast skies. Over the release period, August 8 to August 14, average temperature ranged from 27 to 29°C, whereas relative humidity was 80–88%. Beetles were generally tracked under clear skies with the exception of August 9 during which light showers occurred.

Collection, Selection, and Preparation of Test Insects

Coconut rhinoceros beetles used for radio-tracking were wild caught in barrel traps containing oryzalure and collected within 1 wk of capture. These beetles were placed in tubs containing moist peat moss, fed fresh banana slices, and allowed to rest for at least 3 d.

After the rest period, captured beetles were flight tested at least 1 d prior to experimentation. The flight test chamber consisted of a 121-liter lidded garbage container. Within the chamber, about 30 beetles were placed in a smaller open metal bowl half filled with moist peat moss atop an upside down 19-liter bucket. Beetles could only exit the bowl by flying out of it; therefore, any beetle found on the bottom of the flight chamber container the next morning was considered flight capable. Only coconut rhinoceros beetles capable of flight were selected for radio tagging and release. Flight-capable coconut rhinoceros beetles were transported and stored until release in lidded plastic bins ~45 by 30 by 18 cm containing 10–15 cm of damp peat moss. Some beetles remained in storage for up to 6 d prior to radio-tracking.

Flight-capable beetles were marked with a unique four-digit identification number engraved on one elytrum using a laser engraver (Fenix Flyer, Synrad Inc., Mukilteo, WA). The ID number, sex, mass, and elytral dimensions of each beetle were recorded.

With a hot-melt glue gun, a transmitter was glued to the pronotum of each beetle using ~250 mg of glue per beetle (Supp. Fig. 1 [online only]). Prior to transmitter attachment, the beetle pronotum was abraded with sandpaper to improve adhesion.

Tracking Equipment

Beetles were tracked using a radio receiver (model R410) equipped with a three-element folding Yagi antenna (model 13863). Two receivers operated in the 148.641–148.992 MHz frequency band and two operated in the 164.032–164.409 MHz band. The approximate detection radius for the telemetry system was 500 m.

Transmitters (A2414) had a maximum battery life of 45 d with a warranty guarantee of 22 d. Each transmitter had a mass of ~300 mg. Transmitter frequencies for individual coconut rhinoceros beetles were recorded in conjunction with beetle identification numbers. All radio-tracking equipment was purchased from Advanced Telemetry Systems, Isanti, Minnesota. Handheld GPS units (Garmin Oregon 650, Salem, OR) were used to record locations where beetles were found or point of signal loss for each beetle.

Beetle Release and Tracking Procedure

Beetles were transported to release sites in plastic storage bins. The lid of the bin was removed at dusk (roughly 19:30), and the container was closed at roughly 21:30. Once the containers were opened, coconut rhinoceros beetle activity was carefully monitored using an infrared camera (model E4, FLIR Systems Inc., Wilsonville, OR). Observation with the infrared camera revealed that the thermal profile of the beetles would change just prior to flight. Thermally active beetles observed emerging from the peat moss were briefly viewed under red light to record the identification number and determine the frequency of the radio transmitter. Though nearly all beetles flew independently, several beetles that had not yet flown by the end of experimentation were encouraged to fly by removing them from the peat moss and throwing them into the air to facilitate takeoff.

Coconut rhinoceros beetles were pursued on foot following release and were tracked until a landing site was determined or until the transmitter signal was lost. In either case, a waypoint was recorded at the landing site or the last point of signal reception using a GPS unit.

Landing sites were visited on the following morning, and attempts were made to more precisely determine the location of each beetle. Beetle locations were monitored over several days, and beetles and transmitters were recovered when possible at the end of the experiment. Coconut rhinoceros beetles and transmitters were successfully recovered by digging up beetles that buried into soil or compost; however, the locations of coconut rhinoceros beetles tracked to coconut crowns could not be as exactly determined due to the density of the frond foliage.

Analysis

In assessing the flight patterns of beetles for trends with respect to sex and size, percent emergence weight (%EW) was calculated. The %EW describes coconut rhinoceros beetle mass at the time of measurement relative to its estimated mass upon emergence as an adult. This value can be estimated based upon a linear equation relating elytral measurements and emergence weight (EW; Vander Meer and McLean 1975). This value is significant in data analysis because %EW reflects the present life stage of a beetle and how much stored energy it has available; coconut rhinoceros beetles emerge at their heaviest weight and gradually lose weight over their life span.

GPS coordinates of the release sites at both experimental locations as well as each coconut rhinoceros beetle landing site were displayed as X, Y data in ArcMap Version 10.0 (ESRI 2011). The distance between the release site and the location where each beetle

was found was calculated by using the Point Distance tool in ArcMap.

Data were analyzed using SPSS Statistics software (IBM Corp. 2013) except where noted otherwise. All analyses of significance were made at the $P < 0.05$ level. All analyzed data were shown to be normally distributed by the Shapiro-Wilk test. Differences in weight, EW, and %EW means for coconut rhinoceros beetles that were successfully located or that were lost were compared with *t*-tests. The numbers of male and female coconut rhinoceros beetles that were successfully located or lost, as well as those tracked to above or below 1 m, were compared using Chi-square contingency tests with Yates correction. Linear regressions were used to analyze relationships between the distance beetles moved from the release point and beetle, weight, EW, %EW, and percent weight of the transmitter. Differences in the mean distance beetles moved at the two experimental sites and the mean distance male and female beetles moved were compared by *t*-tests.

Results

Tagging and radio-tracking of coconut rhinoceros beetles in this study led to the successful location of multiple cryptic breeding sites at both experiment locations. A total of 33 out of 34 beetles tagged for release flew during the course of this study. Of the 33 beetles that flew, 19 were successfully tracked to landing sites (Figs. 1 and 2). Most of coconut rhinoceros beetles tracked to landing sites were in tree tops (arboreal, $n = 11$), and the rest were on or below the ground (soil associated, $n = 8$).

Arboreal destinations were most commonly the crowns of coconut palms with coconut rhinoceros beetle bore holes; however, beetles also landed in the branches of other species of trees (Fig. 2). For

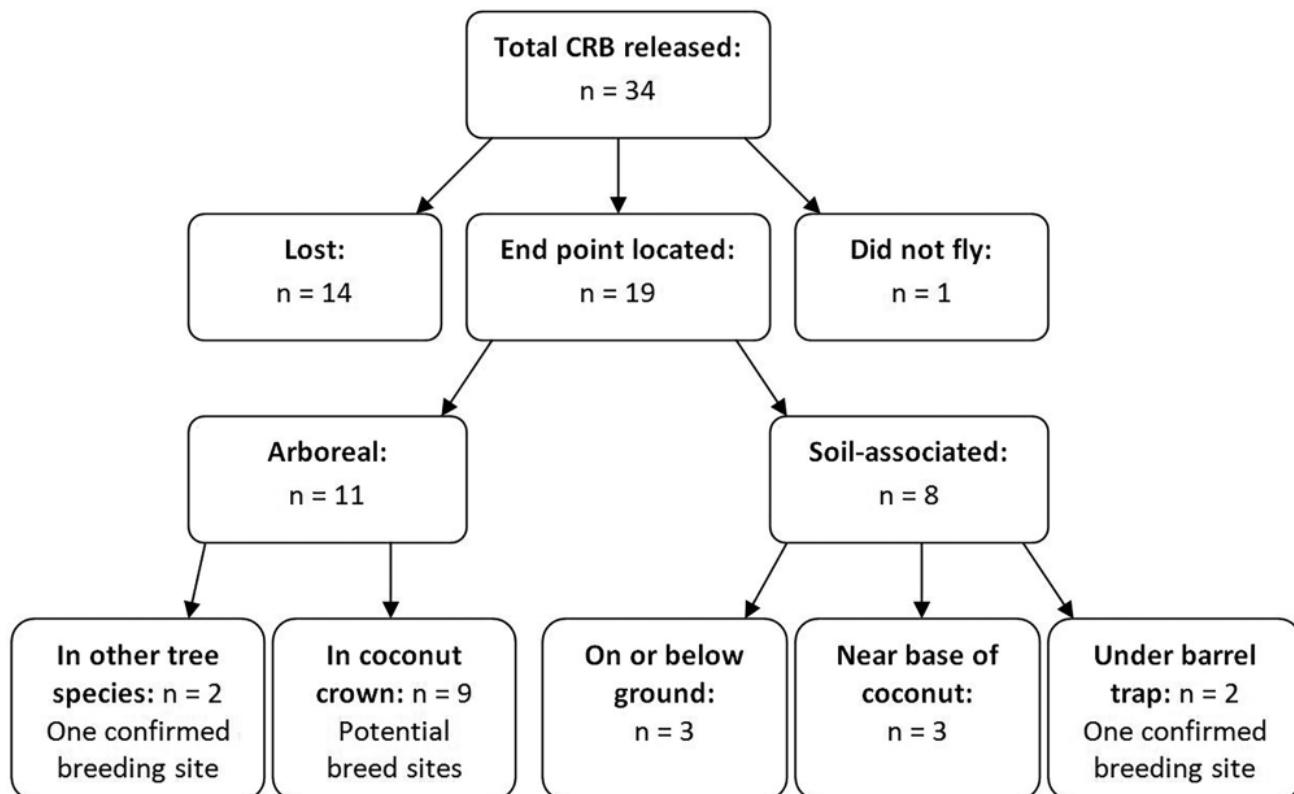


Fig. 2. Flowchart showing the fate of released radio-tagged coconut rhinoceros beetles (Judas beetles).

example, the first breeding site discovered by radio-tracking was in an extremely cryptic and unsuspected microhabitat: in a hole in a large rotting branch of a breadfruit tree (*Artocarpus altilis*) about 6 m above the ground. In this case, the transmitter became detached and the marked beetle was not recovered. However, three other coconut rhinoceros beetle adults and several larvae were found in the hole. It is highly likely that this breeding site was in a branch broken by high winds from Typhoon Dolphin, which passed over Guam in May 2015. In another instance, two beetles were tracked to the crown of the same highly damaged coconut tree independently of one another.

In soil-associated landing sites, coconut rhinoceros beetles tended to bury into the soil at depths up to ~15 cm upon landing. Typically, these sites were at the bases of trees. Four out of five of these landing sites were at the bases of coconut palms, though coconut rhinoceros beetles also landed in less predictable locations. For example, one beetle landed beneath a trailer parked on a grassy lawn in a residential area adjacent to the Yigo site. In two other examples of particular interest, single beetles were found beneath coconut rhinoceros beetle barrel traps baited with oryzcalure at each experimental site. At the Yigo site, only the tracked beetle was observed underneath the barrel trap. At the Asan site, other beetles and larvae were also found beneath the barrel trap.

Of the 33 released beetles that flew, only one was captured in a pheromone trap. This beetle was released at the Yigo site on August 11 and was radio-located the following day in the crown of a coconut palm 336 m from the release site. During the trapping period running from September 4 through September 11, this same beetle was eventually caught in a barrel trap.

The mean trap catch rate of pheromone traps at the Yigo experiment station during August 2015 was 0.03 beetles per trap-day for standard bucket traps ($n=3$ traps), 0.13 for barrel traps ($n=31$), and 0.15 for DeFence traps ($n=4$). In addition, no marked beetles were trapped in fish gill netting draped over a green waste pile at the Yigo site. This pile trapped 0.50 beetles per trap-day during August 2015.

Coconut rhinoceros beetles were most active from ~19:30 to 21:00, and flight activity did not appear to be heavily influenced by the prevailing weather conditions. Transmitters did not inhibit the flight mechanics of coconut rhinoceros beetles to an observable degree. Radio transmitters and adhesive accounted for between 8.5 and 17.8% of the coconut rhinoceros beetle weights at the time of release. There was no statistically significant correlation between coconut rhinoceros beetle flight distance and increasing percent transmitter/adhesive weight (as a ratio of total beetle weight; $R^2=0.048$;

$y=0.0004(4)x+12(1)$; $F(1,17)=0.864$; $P=0.366$). Over the course of experimentation, it was observed that beetles warmed flight muscles to ~37°C directly prior to flight. This observation allowed a reliable prediction of when beetles were about to fly by detecting thermal radiation with an infrared camera.

Distance between release sites and landing sites ranged from 52.8 m to 564.6 m for the 19 beetles tracked to landing sites. The remaining 14 beetles, termed lost, were not specifically located either due to inaccessible terrain (coastal wetlands or private property, Figs. 1 and 2) or due to loss of radio signal. Mean displacement for both successfully located and lost coconut rhinoceros beetles could not be estimated, but median displacement for all released beetles was 333 m.

The %EW, $78 \pm 2\%$, for coconut rhinoceros beetles that were successfully located differed significantly ($t(32)=2.418$; $P=0.021$) from coconut rhinoceros beetles that were lost, $72 \pm 2\%$. However, EW ($t(32)=0.226$; $P=0.822$) and weight ($t(32)=0.666$; $P=0.510$) did not differ between coconut rhinoceros beetles that were successfully tracked or lost after release. Additionally, there were no differences in the numbers of male and female coconut rhinoceros beetles that were successfully located or lost ($\chi^2(1, n=33)=0.041$; $P=0.839$).

No relationship was found between the distance beetles moved from the release point and beetle EW ($R^2=0.069$; $y=-0.003(2)x+6.4(6)$; $F(1,17)=1.252$; $P=0.279$), %EW ($R^2=0.046$; $y=0.01(1)x+75(3)$; $F(1,17)=0.824$; $P=0.376$), or weight ($R^2=0.047$; $y=-0.001(2)x+4.8(5)$; $F(1,17)=0.829$; $P=0.375$). There was no difference in the mean distance beetles moved at the two experimental sites, Yigo, 276 ± 42 m, and Asan, 215 ± 57 m ($t(17)=0.848$; $P=0.408$). Additionally, no differences were found between the mean distances male (246 ± 61 m) and female (258 ± 58 m) beetles moved ($t(17)=0.370$; $P=0.716$).

Landing locations of coconut rhinoceros beetles were categorized by microhabitats described as other trees, coconut crowns, traps, bases of trees, or soil unassociated with trees or traps (Fig. 3A). Microhabitats of coconut rhinoceros beetles were further clustered as arboreal (>1 m above ground) or soil-associated destinations (<1 m above ground; Fig. 3B). When microhabitats were grouped as either arboreal or soil-associated, the difference in mean %EW between the groups, arboreal, $74 \pm 2\%$, soil-associated, $82 \pm 3\%$, was found to be highly significant ($t(17)=4.175$; $P<0.001$). In addition, while EW was significantly different between arboreal, 6.5 ± 0.4 g, and soil-associated, 4.9 ± 0.5 g, microhabitats ($t(17)=2.566$; $P=0.020$), there were no differences in weight ($t(17)=1.468$; $P=0.160$) or distance traveled ($t(17)=0.118$; $P=0.908$) between these microhabitat groupings.

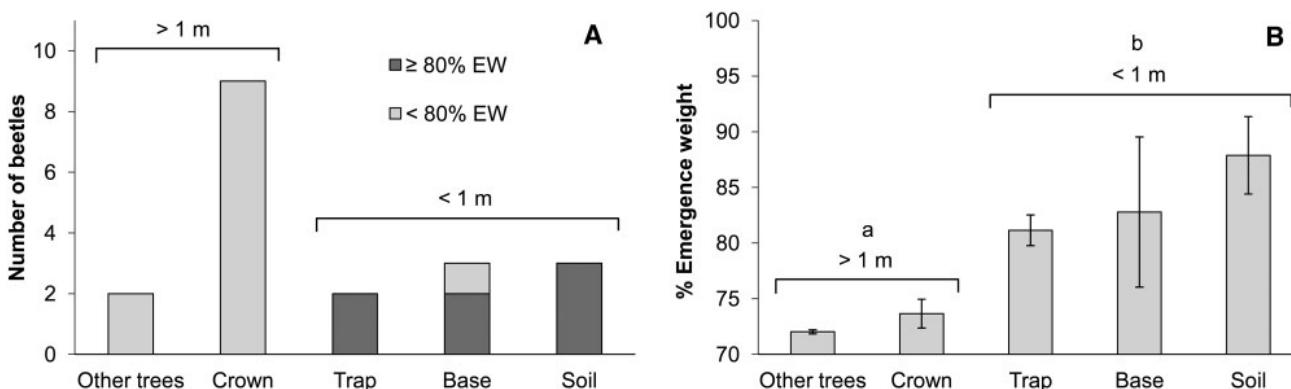


Fig. 3. Radio-tracked coconut rhinoceros beetles grouped by microhabitat of discovery location. (A) Numbers of beetles tracked to different microhabitats including an indication of percent emergence weight (%EW). (B) The %EW of beetles (mean \pm SE) grouped by microhabitat. Lower case letters represent significant differences between beetles tracked to arboreal microhabitats (>1 m above ground) or soil-associated microhabitats (<1 m above ground; t-test, $P<0.001$).

The numbers of male and female beetles did not vary between arboreal and soil-associated microhabitats ($\chi^2(1, n=19)=0.038; P=0.845$).

Discussion

Radio-tagged coconut rhinoceros beetles (Judas beetles) were successfully tracked to cryptic breeding sites at two locations on the island of Guam. The two areas where coconut rhinoceros beetles were tracked differed both in topography and vegetation, and the effective location of tagged beetles in these different environments shows promise for the applicability of this technique in the varied habitats where coconut rhinoceros beetle infestations may occur. Out of 33 released coconut rhinoceros beetles, a total of 19 were followed to final landing sites while 14 beetles were lost.

The use of radio telemetry to monitor flying species has generally been constrained by the weight of radio transmitters. This limitation is especially true when monitoring flying insects because a small increase in weight may severely hinder flight behavior. In recent years, the gradual miniaturization of transmitters has circumvented this obstacle, allowing for more precise monitoring of flying insects (Kissling et al. 2014). One of the factors determining the feasibility of this study was whether adult coconut rhinoceros beetles could fly undisturbed with the attached radio transmitters. Adult coconut rhinoceros beetles are excellent fliers and can exert force much larger than their body weight when boring, so it was reasonable to expect that the miniature radio transmitters would have little to no effect on coconut rhinoceros beetles flight capability. Indeed, observed flight capability of coconut rhinoceros beetles was seemingly unaffected by the extra weight of radio transmitters. Radio transmitters plus adhesive amounted to no more than 18% of the coconut rhinoceros beetle weight at the time of release, and there was no correlation between the increasing percent transmitter/adhesive weight and the single flight distance of coconut rhinoceros beetles, indicating that coconut rhinoceros beetles could fly carrying the extra burden of the radio transmitters. It is important to note that the addition of the radio transmitters and adhesive resulted in only one coconut rhinoceros beetle experiencing a %EW of over 100%. This coconut rhinoceros beetle had a 110.64%EW once the transmitter and adhesive were included. These observations are consistent with other studies monitoring members of the Scarabaeidae family that found that radio transmitters did not noticeably affect beetle flight capabilities (Beaudoin-Ollivier et al. 2003, McCullough 2013).

There is little information available on the natural flight range of coconut rhinoceros beetles. In a laboratory experiment, Hinckley (1973) observed that tethered beetles attached to a flight mill flew between 2 and 4 km with flight duration of 2–3 h. However, field observations indicate that natural flight is limited to a few hundred meters, and this distance is influenced by the availability of feeding or breeding sites. Kamarudin et al. (2007) performed a mark-release-recapture study in a small (4.5 ha) oil palm replanting area containing a grid of 49 pheromone traps. Displacement averaged 118 m with a range of 51–186 m. The authors acknowledge that these values may be below the actual flight potential.

Another important factor to consider is the distance over which coconut rhinoceros beetles can be monitored. Radio telemetry monitoring typically covers only short to medium displacement distances, usually limiting the applications of the technology (Kissling et al. 2014). The radio devices employed in this study had an effective range of localization that varied with topological conditions. In this study, the range of detection was appropriate for monitoring, since the overall

coconut rhinoceros beetle flight distance from release sites to landing sites ranged from 52.8 m to 564.6 m. This range also roughly delineates a radius for breeding site discovery from released coconut rhinoceros beetles; the Judas beetle must be released no further than ~500 m from breeding sites. This might present difficulties for eradication teams since the breeding sites in question occur in cryptic locations presumably unknown to those searching for them. The relatively short detection radius of the radio devices obligates teams to close in on the cryptic sites through other investigative means.

Radio telemetry to monitor flying insects was first employed by Hedin and Ranius (2002) to study the dispersal range of *O. eremita*, and since then several studies have adopted this technique to study the behavior of beetles (Beaudoin-Ollivier et al. 2003, Ranius 2006, Rink and Sinsch 2007, Hedin et al. 2008, Negro et al. 2008, Svensson et al. 2011, Chiari et al. 2013, McCullough 2013, Le Gouar et al. 2015). Flight distance is the main metric that elucidates components of the behavior of interest. In this regard, coconut rhinoceros beetles fell within comparable mean distances but lagged in maximum flight distances compared with Rink and Sinsch (2007) reporting a maximum flight of 1,700 m and Beaudoin-Ollivier et al. (2003) reporting a maximum distance of 3,000 m. McCullough (2013) reported a maximum male flight of 402 m and a maximum female flight of 99 m but noted that most females flew out of range reaching up to 3,000 m of flight distance. This observation contrasts with coconut rhinoceros beetle flight patterns because the proportion of lost coconut rhinoceros beetles was not statistically significantly different between male and female coconut rhinoceros beetles.

Beetle microhabitat analysis was an integral component of these studies as well. McCullough (2013) was able to observe mating and feeding patterns, while Rink and Sinsch (2007) pinpointed final beetle locations to underground, ground level, and above ground, each representing different beetle behaviors. Beaudoin-Ollivier et al. (2003) utilized a combination of radio transmitters and enzymatic light tags to obtain even more detailed microhabitat observations. Radio telemetry also distinguished coconut rhinoceros beetle microhabitat selection patterns effectively, and the utilization of visual cues could increase the efficacy of this method in discovering cryptic breeding sites.

Radio transmitters with masses between 200 and 400 mg and detection ranges between 300 and 800 m have proved to be the most useful method to monitor large beetles. Such transmitters are light enough for undisturbed flight and provide sufficient coverage for monitoring beetles. Only one of these studies (McCullough 2013) expressed a substantial need for improvement in radio transmitter technology for the feasibility of beetle monitoring, while the remaining studies expressed the efficacy of the technique even over varied topographical conditions.

Despite the fact that all radio-tagged beetles were released within proximity of several different kinds of pheromone traps, including standard bucket traps, barrel traps, and DeFence traps, only one of these released beetles was trapped. Currently, a grid of pheromone traps largely covers the island of Guam, making it difficult to release beetles into areas where they are unlikely to encounter synthetic aggregation pheromone. Initial expectations were that pheromone traps might affect coconut rhinoceros beetle flight behavior and that many radio-tagged beetles might be trapped. However, somewhat surprisingly, large numbers of released coconut rhinoceros beetles were not caught the pheromone traps, showing that this technique may be compatible with monitoring and mass trapping efforts. If it is assumed that wild beetles respond to traps in the same way as radio-tagged beetles, it can be estimated that the suite of traps in the vicinity of the release points catches about one in 33 (~3%) of wild beetles. This low trap performance is consistent with results from previous

mark-release-recapture data from Guam (A. Moore, unpublished data). It is possible that oryctalure is less attractive to individuals of the coconut rhinoceros beetle-Guam biotype than it is to individuals of other biotypes. Results indicate that none of the currently available coconut rhinoceros beetle trapping methods are useful for population suppression of the coconut rhinoceros beetle-Guam biotype.

Although the majority of released coconut rhinoceros beetles were successfully tracked to discrete locations, 14 coconut rhinoceros beetles were lost presumably due to out-of-range flights. Interestingly, those coconut rhinoceros beetles that flew out of range had statistically significantly lower %EW than those that stayed within the detection range of the radio devices: $72 \pm 2\%$ compared to $78 \pm 2\%$, respectively, suggesting that lighter coconut rhinoceros beetles fly further from initial release site presumably in search of food sources. This observation raises the ability to minimize the loss of coconut rhinoceros beetles while radio-tracking. Prior to release, the %EW of coconut rhinoceros beetles must be monitored to ensure that the selected individuals will remain within the detection radius. The distance that found coconut rhinoceros beetles flew from the release site had no statistically significant correlations with %EW. However, the distance flown by all 33 coconut rhinoceros beetles that were released, both lost and found, could correlate with %EW if the distance of the lost coconut rhinoceros beetles were determined.

Moreover, %EW of released coconut rhinoceros beetles had a strong association with the microhabitats in which tagged coconut rhinoceros beetles were found. Of the 19 retrieved coconut rhinoceros beetles, 11 landed in arboreal microhabitats, whereas 8 landed in soil-associated microhabitats. Coconut rhinoceros beetles that landed in arboreal microhabitats had a statistically significant lower %EW than those coconut rhinoceros beetles that landed in soil-associated microhabitats, $74 \pm 2\%$ compared to $82 \pm 3\%$, respectively. In many locations studied, adult coconut rhinoceros beetles tend to split their time between feeding on the crowns of palms or breeding in either soil or compost (Zelazny 1975, Bedford 1980). As coconut rhinoceros beetles alternate between these microhabitats, individuals fluctuate in their %EW, making it possible to determine the behavioral pattern in which coconut rhinoceros beetles will engage by noting their %EW (Vander Meer 1987). Coconut rhinoceros beetles at a higher %EW will very likely refrain from further feeding and will instead fly in search of breeding sites, whereas coconut rhinoceros beetles at a lower percentage of their EW will likely forage in search for food. This corresponds with the observation that coconut rhinoceros beetles that landed in terrestrial microhabitats, commonly associated with breeding, had statistically significantly higher average %EW than those that landed in palm crowns, generally associated with feeding sites. This characteristic of the coconut rhinoceros beetle life cycle offers the potential that this tracking method be highly specific and controllable. In order to increase the probability that coconut rhinoceros beetles fly predominantly to soil-associated breeding sites, only individuals with a high %EW might be selected for tracking. In doing so, monitoring and eradication teams might improve the likelihood that the released coconut rhinoceros beetles will lead them to breeding sites rather than feeding sites, which will increase the effectiveness of this method.

It is important to note, however, that in Guam, coconut rhinoceros beetles are known to breed in both compost and coconut crowns (Moore et al. 2015), making it difficult to assess whether beetles are going to trees to feed or mate and making the distinction between arboreal and soil-associated microhabitats less useful in this specific location. At least one of the arboreal locations to which coconut rhinoceros beetles were tracked in this study, a damaged breadfruit tree, appeared to be a potential breeding site. Previous

reports of coconut rhinoceros beetle breeding in coconut crowns on Guam were not from a random sampling but instead trees that showed coconut rhinoceros beetle damage and an accumulation of crown debris (Moore et al. 2015). However, in this study, all the coconut trees at the War in the Pacific National Historical Park in Asan were well maintained with minimal decaying organic matter in the crowns, suggesting that these beetles were more likely to be visiting coconuts to feed and not to breed. In contrast, several of the coconut trees to which coconut rhinoceros beetles were tracked at Yigo were not only heavily damaged by other coconut rhinoceros beetles but also had large amounts of decaying organic matter in the crowns. Therefore, these trees could be either breeding or feeding sites. In total, there were 10 landing sites in coconut palm crowns that might be potential breeding sites at the two locations. Due to limitations of time and equipment, the nature of beetle activity in coconut crowns was not further investigated, but this is an issue that could be taken up in future studies.

In terms of overall efficiency in time and materials, this Judas beetle method presents an effective technique that would improve existing control options for coconut rhinoceros beetles. Coconut rhinoceros beetle itself has several characteristics that lend themselves to the application of radio-tracking techniques with this species. First, it is a large, powerful beetle that can fly with the additional mass of a transmitter. Second, coconut rhinoceros beetle aggregates at breeding sites. And third, coconut rhinoceros beetles do not fly during the day, providing time to precisely locate landing points. Limitations of this technique encountered during this study include the cost of transmitters ($\sim \$180$ each) and the higher than anticipated number of lost beetles. Further refinement in tracking protocols and more experience for those tracking the beetles will likely lead to a lower percentage of lost beetles. Recapturing beetles in the field allows the reuse of transmitters and so a lower percentage of lost beetles also has the potential to reduce materials costs for a tracking program.

Beetle tracking to breeding sites required an input of ~ 30 min per coconut rhinoceros beetles immediately after release and about the same amount of time on the following day. The tactic of tracking coconut rhinoceros beetles to an approximate location during the night followed by more precise pinpointing during the daytime proved to greatly facilitate the retrieval of released coconut rhinoceros beetles, as it was much easier to visually track beetles and maneuver the terrain in the natural light of day. To further facilitate visual tracking, future experiments might include the use of small, lightweight LED lights in addition to radio transmitters.

Additionally, future experiments should consider the limitations of radio transmitter technology as they develop protocols for beetle tracking. In this field trial, the duration of commercially available radio transmitters (10–14 d) was appropriate for coconut rhinoceros beetle monitoring. Battery life of the transmitters is a major factor in determining monitoring protocol timelines. Therefore, in more extensive trials of this technique, experimenters should be aware of the time frame in which release and tracking should occur and must plan accordingly in order to achieve optimal coconut rhinoceros beetle tracking.

Future trials should also include visual monitoring of damage and trapping assessment of coconut rhinoceros beetle populations to effectively estimate possible locations of coconut rhinoceros beetle breeding sites. Based on such information for this trial on Guam, coconut rhinoceros beetle breeding sites clearly existed in the vicinity of chosen release sites. This protocol is particularly important in regions where coconut rhinoceros beetle is not yet widespread to ensure that tracking coconut rhinoceros beetles provides useful information. Once visual monitoring and trapping have indicated the existence of coconut rhinoceros beetles in a particular location, the Judas beetles would be

released in the vicinity to pinpoint the exact location of the breeding sites. This combination of monitoring methods would ease the control and eradication of coconut rhinoceros beetles, and since traps and visual monitoring are already widespread, it would not be complicated to craft an integrated strategic plan. Regions in which coconut rhinoceros beetle is not yet widespread may also present concerns related to the introduction of additional coconut rhinoceros beetle adults. In this case, sterilized males could be used in tracking to prevent any potential impact upon existing coconut rhinoceros beetle populations.

This study was a success in that all but one of the 34 radio-tagged beetles flew, and the majority of these beetles were tracked to final landing destinations including definite breeding sites. In further assessing the final landing locations of beetles that were tracked successfully, it is important to note the varied and cryptic nature of these sites. For example, the breeding site found in the top of a breadfruit tree was well hidden within the leaves and branches and was ~6 m above the forest floor. This example demonstrates an unexpected site that would be nearly impossible to locate with current tracking methods such as trained dogs. Breeding sites discovered in other unusual locations such as beneath barrel traps or buried in the soil also demonstrate how tracking with conspecifics provides an advantage that cannot be rivaled by present tracking techniques.

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Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

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5.4.3 A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros nudivirus* and is invading Pacific Islands

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A new haplotype of the coconut rhinoceros beetle, *Oryctes rhinoceros*, has escaped biological control by *Oryctes rhinoceros* nudivirus and is invading Pacific Islands

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ABSTRACT

The coconut rhinoceros beetle (CRB; *Oryctes rhinoceros*) is a major pest of coconut and oil palm, but the discovery and release of *Oryctes rhinoceros* nudivirus (OrNV) in the 1960s and 70s suppressed the pest such that no new invasions of uninfested islands by CRB were reported for over 30 years after implementation of the biocontrol programme. Surprisingly, a highly damaging outbreak was reported from Guam (2007), which could not be controlled by OrNV. Subsequently, new invasions have been reported from Port Moresby, Papua New Guinea (2009); O'ahu, Hawai'i (2013); and Honiara, Solomon Islands (2015). We have found that all of these outbreaks have been caused by a previously unrecognized haplotype, CRB-G, which appears to be tolerant to OrNV. PCR analysis shows that OrNV is generally present at high incidence in established populations of CRB, but is generally absent from the invasive CRB-G populations. CRB-G from Guam was not susceptible to OrNV infection by oral delivery, but injection of the virus did cause mortality. Further genetic analysis shows that CRB populations can be divided into a number of clades that coincide with the endemic and invasive history of the beetle. Analysis suggests that CRB-G originated in Asia, though the precise location remains to be discovered.

1. Introduction

Oryctes rhinoceros (Linnaeus 1758) (Coleoptera: Scarabaeidae: Dynastinae), commonly known as the coconut rhinoceros beetle (CRB), is endemic to the tropical Asia region (including South East Asia). CRB damages both coconut and oil palm, and can sometimes kill palms when adults bore into crowns to feed on sap (Bedford, 2013a, 2013b). The beetle was inadvertently introduced into the Pacific in 1909 when infested rubber tree plants were transported to Samoa from Sri Lanka (previously known as Ceylon) (Catley, 1969). The pest rapidly multiplied in Samoa and subsequently spread to several nearby Polynesian islands. Separate invasions further distributed CRB through Palau, parts of Papua New Guinea, and other Pacific nations through disruptions and uncontrolled movements during World War II (Catley, 1969; Gressitt, 1953). The invasive phase of the beetle was brought under control by the discovery and distribution of a viral biocontrol agent, *Oryctes rhinoceros* nudivirus (OrNV; previously known as *Rhabdiovirus oryctes* and *Baculovirus oryctes*). OrNV is currently present and causes

persistent population suppression on many of the CRB infested Pacific Islands (Bedford, 2013b; Huger, 2005).

Virus introduction into affected Pacific Island countries and territories suppressed and weakened the CRB populations such that its spread into the Pacific islands ceased and for 30 years there was no further expansion of the range of CRB (Secretariat of the Pacific Community, 2015). Outbreaks of the beetle can still occur in conditions that provide an abundance of breeding sites, such as after cyclones or felling of mature palms for plantation replanting. The strategy for CRB management has been sanitation, coupled with population suppression using OrNV as a biocontrol (Jackson, 2009). The use of PCR in monitoring has shown that virus is regularly found in adult beetle populations, where the incidence can be over 70% (Ramble et al., 2005).

After the success of the OrNV biocontrol programme (Huger, 2005) it was surprising to see a new CRB invasion on Guam in 2007. Following a failed eradication attempt, the beetle has since spread across the whole island (Moore, personal communication). The Guam CRB population has proven to be recalcitrant to infection using commonly

Abbreviations: CRB, coconut rhinoceros beetle; OrNV, *Oryctes* nudivirus; PNG, Papua New Guinea; PoM, Port Moresby; NI, New Ireland; NB, New Britain; USDA, United States Department of Agriculture; APHIS, Animal/Plant Health Inspection Service

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applied OrNV isolates that cause disease in other CRB populations (Moore and Jackson, unpublished). Additionally, new CRB invasions have also since been reported in Port Moresby (Papua New Guinea; 2009), O'ahu (Hawai'i, USA; 2013), and Honiara (Solomon Islands; 2015).

In this paper we report efforts to control the invasive population in Guam with biocontrol and characterization of the population. We report on the identification of a new, invasive haplotype of CRB and its distribution as well as attempts to control with OrNV. The implications of a new, invasive, form of *O. rhinoceros* in the Pacific that cannot be controlled by known isolates of OrNV are discussed.

2. Materials and methods

2.1. Molecular characterization of *O. rhinoceros* populations

2.1.1. Collection of *O. rhinoceros* tissue for DNA extraction

CRB tissue samples were obtained from live CRB adults collected from Guam and several other geographic locations across the tropical Asia-Pacific region (American Samoa, Diego Garcia, Fiji, Hawai'i, India, Indonesia, Malaysia, Palau, Papua New Guinea, Philippines, Samoa, and Solomon Islands). Specimens were collected using pheromone traps baited with oryctalure (ethyl 4-methyloctanoate; ChemTica Internacional, Costa Rica). Oryctalure is an aggregation pheromone that attracts both sexes of CRB. To ensure DNA quality was maintained, a 0.5–1 cm piece of the midgut tissue from each live CRB specimen was dissected (when gut tissue dissection was not possible 2–4 legs were removed) and immediately submerged in monopropylene glycol (PPG), and stored at –20 °C until required. DNA was extracted from CRB tissue using Isolate Genomic DNA Mini (Bioline) or ZR Genomic DNA Tissue MiniPrep (Zymo Research) kits. DNA elution was carried out using 100 µl of elution buffer from the appropriate kit, and aliquots of eluted DNA samples were subsequently used for further analyses.

2.1.2. DNA sequencing of the mitochondrial COI barcode region

The ‘universal barcode’ primers were used to amplify a region of the cytochrome C oxidase I (COI) gene: LCO1490 (5'-GGTCAACAAATCATAAAGATATTG-3') and HCO2198 (5'-TAAACTTCAGGGTACCAAAAAATCA-3') (Folmer et al., 1994; Simon et al., 2006). Each 50 µl PCR reaction contained 0.3 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10 × PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl LCO1490 (10 µM), 0.5 µl HCO2198 (10 µM), 2 µl undiluted DNA template, and 43.7 µl water. PCR amplifications were performed in a C2100 (BioRad) thermocycler with a cycling profile of 35 cycles of 94 °C denaturation (30 s), 52 °C annealing (45 s), 72 °C extension (1 min) with an initial denaturation of 3 min at 94 °C and a final extension of 5 min at 72 °C. A 5 µl aliquot of each PCR reaction was separated by agarose gel electrophoresis (1%, 0.5 × TBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIDOC HD2 gel doc (UVITECH). Successfully amplified PCR products were sent to Macrogen (www.macrogen.com/eng/) for purification and DNA sequencing. PCR amplicons were sequenced in both directions using the COI barcoding primers LCO1490 and HCO2198 (Folmer et al., 1994; Simon et al., 2006). Returned DNA sequences were imported into the Geneious version R8.0 software package (Kearse et al., 2012) for further sequence manipulation and analyses. Partial COI sequences from individual specimens were trimmed, edited, and assembled into unique contiguous sequences. The individual representative DNA sequences used have been deposited into GenBank as the following accessions: KY313828 (Malaysia-M1-1), KY313829 (PNG-NI216-1), KY313830 (PNG-ENB16-1), KY313831 (PNG-WNB16-1), KY313832 (PNG-PoM15-1), KY313833 (Malaysia-M3-2), KY313834 (India-A2), KY313835 (Malaysia-M1-10), KY313836 (Samoa-A35), KY313837 (India-A1), KY313838 (Indonesia-3), KY313839 (Palau-2), KY313840 (Palau-10), KY313841 (Indonesia-1),

KY313842 (Palau-6), KY313843 (PNG-PoM15-9), KY313844 (Solomon_Islands-3), KY313845 (Philippines-Da1), KY313846 (Guam-1), KY313847 (Hawaii-1), KY313848 (PNG-PoM16-1), KY313849 (Diego_Garcia-2), KY313850 (Fiji-ViL-N1), KY313851 (Fiji-Tav-TRDC4), KY313852 (AmSamoa-15), KY313853 (Fiji-Yas-Y1), KY313854 (Samoa-A15), KY313855 (India-G2), KY313856 (Malaysia-M2-4), KY313857 (Malaysia-M3-1), KY313858 (Palau-4).

Molecular species identification used BLAST analysis (Altschul et al., 1997) of CRB COI sequences against the NCBI Reference Sequence Database (RefSeq Release 26) databases (OLeary et al., 2016). Morphological species determination of Guam specimens collected during September 2007 was performed by Natalia J. Vandenburg of the USDA-ARS Systematic Entomology Laboratory. Specimens were compared with material in the US National Museum, male genitalia were dissected, and the key in Endrödi (1985) was used to determine species identity.

2.1.3. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for detecting the *O. rhinoceros* CRB-G haplotype

The following primer pair was designed and used to amplify a 523 bp fragment of the CRB COI gene: C1-J-1718Oryctes (5'-GGAGGTTTCGGAAATTGACTTGTCC-3') and C1-N-2191Oryctes (5'-CCAGGTAGAATTAAAATRTATACCTC-3'). A unique *Mse*I restriction site polymorphism within this amplified region allows the CRB-G haplotype to be identified. Each 25 µl PCR reaction contained: 0.2 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10 × PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (2.5 mM each), 0.5 µl C1-J-1718Oryctes (10 µM), 0.5 µl C1-N-2191Oryctes (10 µM), 1 µl undiluted CRB DNA template, and 19.8 µl water. PCR amplifications were performed in a C2100 thermocycler (BioRad) with a cycling profile of 35 cycles of 94 °C denaturation (30 s), 50 °C annealing (45 s), 72 °C extension (1 min) with an initial denaturation of 3 min at 94 °C and a final extension of 5 min at 72 °C. A 5 µl aliquot of each PCR reaction was checked by agarose gel electrophoresis (1%, 0.5 × TBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVIDOC HD2 gel doc (UVITECH). For RFLP analysis, successfully amplified COI PCR products (8 µl) were each combined with 0.2 µl *Mse*I (10U/µl; New England BioLabs, NEB), 1 µl 10 × NEB Buffer#4, 0.1 µl 100 × NEB BSA and 5.7 µl water, and incubated at 37 °C for 3 h. Digested samples (15 µl) were mixed with DNA loading dye and loaded onto a 2% agarose gel in 0.5 × TBE buffer. The gel was electrophoresed using 60 V for 1.5 h, stained with RedSafe dye, and DNA fluorescence detected over UV light. Photographs were taken using an UVIDOC HD2 gel doc. The DNA fragment sizes obtained following the *Mse*I digest are shown in Fig. 1.

2.1.4. Phylogenetic analysis of the *O. rhinoceros* COI barcode region

Assembled CRB COI barcode sequences were aligned using the MUSCLE algorithm (default parameters) as implemented within Geneious R8.0. After removal of redundant sequences from the alignment, a dataset of 31 geographically representative sequences remained. Further trimming of the alignment was done to minimize end gaps, which yielded a 676 bp sequence fragment from the COI gene. Tree construction was inferred from Bayesian phylogenetic analysis using an HKY85 model with a Gamma rate variation setting carried out in Geneious R8.0. Posterior probabilities were calculated over 2.0×10^6 generations.

2.2. Pathogen challenge bioassay

2.2.1. Collection, rearing, and maintenance of adult *O. rhinoceros*

Live adult *O. rhinoceros* were field collected from Guam using pheromone traps baited with oryctalure (ethyl 4-methyloctanoate; ChemTica Internacional, Costa Rica). In the lab individual beetles were

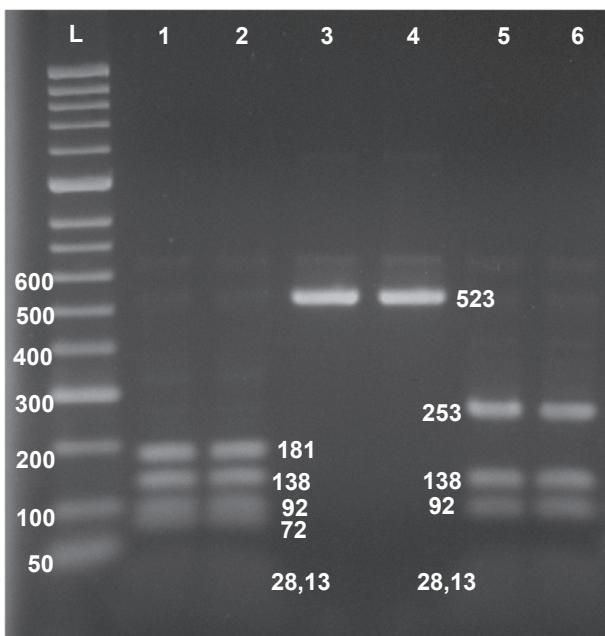


Fig. 1. Exemplar PCR-RFLP analysis results that distinguish the CRB-G haplotype from other CRB populations. Results shown are examples of CRB-S (lanes 1–3) and CRB-G (lanes 4–6) haplotype specimens. Following PCR amplification of a 523 bp fragment of the *COI* gene from *O. rhinoceros* DNA, the amplicons were digested with the restriction enzyme *Mse*I and the generated fragments subsequently separated on a 2% agarose gel. The ladder lane corresponds to a 100-bp DNA size ladder, lanes 1, 2, 5, and 6 are *Mse*I-digested CRB PCR amplicons from independent CRB specimens, and lanes 3 and 4 exemplify undigested PCR amplicons. Numbers on the figure next to the DNA bands indicate fragment size in base pairs.

incubated at 25 °C in individually labelled 500 ml glass Mason jars containing moist peat moss. The top of each jar was enclosed with a metal disk that had a single hole punched in it. A piece of cloth or paper towel was trapped between the top of the disk and the screw ring to prevent small insects from entering or leaving the jars. Slices of banana were provided as food and replenished as required.

2.2.2. Preparation of *O. rhinoceros* nudivirus (OrNV) isolates for inoculation of *O. rhinoceros*

The following OrNV isolates were used in the pathogen challenge assays: OrNV-X2B (commonly used within the Pacific region for augmentative release; isolated from Bugsuk Island, Philippines); OrNV-I (isolated from Kerala, India), OrNV-TAS (isolated from Upolo, Samoa), OrNV-TAP (isolated from Savaii, Samoa), and OrNV-MalB (isolated from Perak, Malaysia). The OrNV-MalB isolate was extracted from fresh gut tissue (supernatant from macerate passed through a 0.22 µm filter to sterilize) that had been confirmed to be the correct isolate (Crawford et al., 1986; Ramle et al., 2005). The OrNV-X2B, -I, -TAS, and -TAP were previously isolated and propagated using cell culture methods as described by Crawford and Sheehan (1984). Briefly, cells of the *Heteronychus arator* DSIR-Ha-1179 cell line (BB) (Crawford, 1982) were seeded into culture flasks and grown in PS100 medium (Grace's insect cell medium (Sigma), 2.95 g/l tryptose phosphate broth (Sigma), 1 ml/l TC-100 vitamins with the pH adjusted to 6.2 (using potassium hydroxide) and further supplemented with fetal bovine serum to 10% (Life Technologies) and gentamicin (25 µg/ml) (Sigma)). Culture flasks were incubated in air at 27°C. When the cell culture reached 25% confluence, OrNV isolates from sterile stocks were inoculated into appropriate flasks that were incubated for a further 10–14 days to allow virus multiplication. Virus was harvested by centrifugation of resuspended flask contents to obtain an OrNV containing cell-free supernatant. OrNV titer was quantified as infectious units per milliliter (IU/ml) by endpoint dilution analysis as previously described (Pushparajan et al.,

2013).

2.2.3. Bioassay treatments

Oral treatments of adult *O. rhinoceros* were carried out using a modification of standard methods previously described (Lacey, 2012; Zelazny, 1978). Oral treatments of OrNV isolates for dosing field collected *O. rhinoceros* adults were administered as two sequential 30 µl doses of virus (5×10^4 IU of virus per dose prepared in a sterile 10% (w/v) sucrose solution). A control treatment (sterile 10% (w/v) sucrose solution) was also included. The first dose involved immobilizing adults on their backs, applying a droplet of solution directly onto their mouth parts, and allowing the full dose to be consumed before placing each beetle individually into an empty container. The following day a second dose was absorbed into a slice of banana and placed into the container for consumption over 3–4 more days. Following this, moist peat moss was placed into the container, and fresh untreated banana slices were provided as food and replenished as required. CRB were inspected for symptoms of OrNV infection (reduced feeding, lethargy, mortality) at regular intervals over eight weeks, and observations recorded. On completion of the experiment, or after death, beetles were dissected for visual evidence of disease and gut tissue removed for PCR and histological examination.

Direct hemocoel treatments of adult *O. rhinoceros* were carried out using a modification of methods previously described (Lacey, 2012; Zelazny, 1978). To prevent accidental infection arising from the injection process, beetles were surface sterilized with 70% ethanol prior to inserting needle through the cuticle (junction of hind leg and body) parallel to the gut to avoid puncture. A single 30 µl treatment dose of sterile virus (5×10^4 IU in sterile PS100 medium) or control (sterile PS100 medium containing no virus) was provided using sterile 1 ml syringes fitted with 30-gauge needles. Treated insects were placed in individual containers and monitored over two days for signs of mortality caused by the injection process. Following this period, moist peat moss was added, and fresh untreated banana slices were provided as food and replenished as required. Assessments were made as above.

Bioassays were carried out as maximum challenge tests, with both oral, hemocoel and control treatments set up over two separate days on two different occasions. Maximum dose pathogen challenge bioassays were carried out using a range of isolates; X2B, TAS and TAP (isolated from Samoa), I (isolated from Kerala, India), and MalB (isolated from Perak, Malaysia). Observations were recorded at regular intervals over eight weeks to detect symptoms of OrNV infection (reduced feeding, lethargy, mortality). The data were analyzed with a generalized linear model for the proportion dead and the treatments as a factor using a binomial distribution as implemented by R software (R Core Team, 2017). Separate models were fitted to the data for the oral and hemocoel routes.

2.3. Diagnosis of *O. rhinoceros* nudivirus infection

2.3.1. PCR detection of *O. rhinoceros* nudivirus infected *O. rhinoceros* beetles

CRB gut tissue dissected from moribund or dead bioassay specimens had DNA extracted as described in Section 2.1.1. The PCR protocol for detection of OrNV was based on that described in Richards et al. (1999), and has been subsequently modified by using diluted DNA template (down to 1 in 5000) to better distinguish infection from mere presence (e.g. incidental contact contamination). The primer pairs used to amplify a 945 base pair (bp) fragment of the OrNV genome were OrNV15a (5'-ATTACGTCGTAGAGGCAATC-3') and OrNV15 b (5'-ATGATCGATTCTGTCTATGG-3') (Richards et al., 1999). Each 25 µl PCR reaction contained 0.2 µl i-StarTaq DNA Polymerase (iNtRON Biotechnology), 2.5 µl 10× PCR buffer (iNtRON Biotechnology), 0.5 µl dNTP mixture (10 mM), 0.5 µl OrNV15a (10 µM), 0.5 µl OrNV15 b (10 µM), 1 µl diluted DNA (paired reactions of 1 in 100 and 1 in 5000), and 19.8 µl water. PCR amplifications were performed in a C2100

(BioRad) thermocycler with a cycling profile of 35 cycles of 94 °C denaturation (30 s), 50 °C annealing (45 s), 72 °C extension (1 min) with an initial denaturation of 3 min at 94 °C and a final extension of 5 min at 72 °C. An 8 µl aliquot of each PCR reaction was separated by agarose gel electrophoresis (1%, 0.5× TBE), stained with RedSafe (iNtRON Biotechnology) and fluorescence visualized over UV light. Photographs were recorded using an UVidoc HD2 gel doc (UVItech). Detection of OrNV PCR product from the 1 in 5000 dilution was considered indicative of OrNV infection and has been validated (unpublished) by comparison with pathological effects such as gross visual inspection and histological analysis based on diagnostics described by Huger (2005).

2.3.2. Visual and histological observations of field collected and *O. rhinoceros nudiviruses* challenged *O. rhinoceros* beetles

When taking gut samples (Section 2.1.1) from field collected or bioassay challenged beetles, a visual diagnosis was carried out of gut condition to look for evidence of gut swelling and whitening typical of OrNV infection (Huger, 2005). In addition to gut samples for PCR, a subset of samples from field collected and moribund bioassay specimens was taken for histology. Samples were immersed for 48 h in FAA fixative (5% formaldehyde, 2.5% acetic acid, 50% ethanol as an aqueous solution) before paraffin embedding, serial sectioning, and hematoxylin and eosin (H & E) staining (Kiernan, 1990). Slides of gut tissue were examined under bright-field and differential interference contrast (DIC) optics with observations of OrNV infection status recorded based on pathology described by Huger (2005).

3. Results and discussion

3.1. Characterisation of the CRB Guam population and attempts to introduce OrNV

In 2007, beetles collected from Guam were recognized as *Oryctes rhinoceros*, based on morphological characteristics and damage to coconut palms (Berringer, 2007); there is no record of CRB from Guam prior to 2007 (Moore, personal communication). To confirm the invasive beetle present in Guam was in fact *O. rhinoceros* (as found in other Pacific regions) the universal barcoding region of the *COI* gene (bases 1490–2198) was PCR amplified and DNA sequenced. Sequences from all ten of the initial Guam specimens analyzed were identical across this gene region. When compared against DNA sequences obtained from other CRB specimens collected within Fiji, PNG, and Samoa, a level of less than 2% DNA sequence variation was observed within this region. As this fell within the accepted < 2 to 3% level of difference for a single species (Hebert et al., 2003a, 2003b; Meyer and Paulay, 2005), it validated the original supposition that the invasive Guam beetle population was species *O. rhinoceros*. Subsequent comparative morphological analysis by the USDA-ARS Systematic Entomology Laboratory (unpublished) further confirmed the insect identification as *O. rhinoceros*.

Following the successful use of using OrNV as a biocontrol agent to suppress CRB populations within the Pacific region (Huger, 2005), establishment of virus disease was attempted in Guam. However, attempts to infect CRB from Guam with the OrNV X2B isolate (commonly used in the Pacific region in augmentative release biocontrol programmes; isolated from Bugsuk Island, Philippines) were unexpectedly unsuccessful (Moore and Jackson, unpublished). Bioassays were repeated as maximum challenge tests using a range of isolates, with treatment doses delivered through either oral or hemocoel routes. Lack of impact of the virus on the Guam beetles was confirmed with no observed differential effect from the control on feeding or morbidity. Mortality results from the bioassay are presented in Table 1 (corrected mortality values are presented in Supplementary Table S1). Mortality following oral dosing with OrNV isolates ranged from 28.6% to 62.5%, with none of the isolates tested producing mortality statistically significantly different to the control mortality (28.1%). After hemocoel

Table 1
O. rhinoceros mortality data from OrNV pathogen challenge assays.

Route	Treatment	n =	# Dead	Mortality (%) ^a	SE (%) ^b	p-value ^c
Oral	Control	32	9	28.1	7.9	
	OrNV-I	14	4	28.6	12.1	0.975
	OrNV-X2B	22	11	45.7	7.3	0.106
	OrNV-TAS	10	4	40.0	15.5	0.081
	OrNV-TAP	8	5	62.5	17.1	0.481
	OrNV-MalB	46	21	50.0	10.7	0.121
Hemocoel	Control	23	7	30.4	9.6	
	OrNV-I	23	15	65.2	9.9	0.021*
	OrNV-X2B	24	15	70.0	14.5	0.031*
	OrNV-TAS	15	14	62.5	9.9	0.043*
	OrNV-TAP	10	7	93.3	6.4	0.002*
	OrNV-MalB	NA				

^a Proportion of mortality from observations.

^b SE, standard error of the proportional mortality.

^c p-values based on comparison of the control treatment to each of the OrNV treatments. p-values were calculated using a generalized linear model for the proportion dead and the treatments as a factor using a binomial distribution.

* Indicates significance at a > 95% confidence level.

injection, mortality ranged 62.5–93.3%, with all four isolates tested producing statistically significant mortality ($p < 0.05$) compared with the control (30.4%). On beetle death or at completion of the experiments beetles were dissected for visual diagnosis of the white and swollen midgut symptomatic of OrNV infection (Huger, 2005). There was no substantive visual evidence that the virus treated beetles were infected as most (> 98%) had normal coloured unswollen midguts. From a subset of 17 specimens analyzed by histopathology and PCR, signs of OrNV were detected in a total of nine specimens, with two out of nine found from orally dosed beetles (none with white swollen midguts), and seven out of eight identified dosed via the hemocoel route (three with white swollen midguts). These results are consistent with field observations as white and swollen midgut symptoms typical of OrNV infection have never been observed in wild-caught CRB from Guam despite virus treated beetle release, while they are routinely observed in susceptible populations of CRB from locations where OrNV is present (e.g. Fiji, Malaysia, Samoa).

Failure to produce significant levels of mortality from oral inoculation in the Guam CRB was surprising. OrNV has been widely used as a biocontrol agent (Huger, 2005). This is based on well-established evidence of its infection and pathogenicity, observations of reduced beetle populations and palm damage, and ease of establishment (Bedford, 2013a; Jacob, 1996; Jayawardena, 2013; Zelazny, 1973, 1979). Additionally, in recent concurrent tests using OrNV produced by cell culture, the virus was proven to be pathogenic (> 90% mortality by eight weeks after treatment) to a Malaysian population of CRB (Khudri et al., 2016). As the natural route of OrNV infection for CRB is oral ingestion (Huger, 1966; Zelazny, 1976), these observations strongly suggested that the Guam population is highly tolerant (if not completely resistant) to oral infection by the OrNV isolates tested.

3.2. CRB-G haplotype identification and distribution

Due to the unexpected difficulties in establishing *per os* OrNV infection within the Guam CRB population, the partial *COI* sequences were inspected for variable sites that could possibly distinguish it from the archetypal OrNV-susceptible CRB populations found elsewhere. A fixed base change was found to exclusively correlate with the Guam CRB population. This was located at nucleotide position 288 within the 676 bp sequence fragment examined. An A > G transition (nucleotide position 288) was centered on a *Mse*I restriction site. From this observation, a PCR-RFLP assay was developed and validated, which enabled populations related to the Guam CRB invasion (referred to as the CRB-G haplotype) to be distinguished from other CRB populations.

Table 2
Summary of *O. rhinoceros* haplotype and OrNV presence by location.

Location ^a	Haplotype ^e	OrNV present ^f	n = ^g	% CRB-G	% OrNV +
American Samoa	CRB-S	No	2	0	0
Tutuila	CRB-S	No	2	0	0
<i>Diego Garcia</i>	<i>CRB-S</i>	<i>ND</i>	2	0	ND
<i>Fiji</i>	<i>CRB-S</i>	<i>Yes</i>	34	0	47.1
Viti Levu	CRB-S	Yes	21	0	61.9
Vanua Levu	CRB-S	Yes	10	0	10
Yasawa	CRB-S	Yes	3	0	33.3
<i>Guam</i>	<i>CRB-G</i>	<i>No</i>	17	100	0
<i>Hawai'i</i>	<i>CRB-G</i>	<i>No</i>	14	100	0
O'ahu	CRB-G	No	14	100	0
<i>India</i>	<i>CRB-S</i>	<i>Yes</i>	4	0	50
Kerala	CRB-S	Yes	4	0	50
<i>Indonesia</i>	<i>CRB-G, CRB-S</i>	<i>No</i>	7	57.1	0
Sumatra	CRB-G, CRB-S	No	7	57.1	0
<i>Malaysia^b</i>	<i>CRB-S</i>	<i>Yes</i>	31	0	45.2
Type A OrNV ^b	CRB-S	Yes	24	0	45.8
Type B OrNV ^b	CRB-S	Yes	7	0	42.9
<i>Palau^c</i>	<i>CRB-S, CRB-G</i>	<i>Yes (CRB-S, -G)</i>	11	72.7	72.7
Aimeliik	CRB-S, CRB-G	Yes (CRB-S, -G)	8	62.5	75
Ngarraard	CRB-G	No	1	100	0
Airai	CRB-G	Yes (CRB-G)	2	100	100
<i>Papua New Guinea^d</i>	<i>CRB-S, CRB-G</i>	<i>Yes (only CRB-S)</i>	143	6.3	49
New Ireland	CRB-S	Yes	86	0	62.8
West New Britain	CRB-S	Yes	31	0	22.6
East New Britain	CRB-S	Yes	13	0	46.2
Port Moresby	CRB-S, CRB-G	Yes (only CRB-S)	13	69.2	23.1
<i>Philippines</i>	<i>CRB-G</i>	<i>No</i>	12	100	0
Negros	CRB-G	No	12	100	0
<i>Samoa</i>	<i>CRB-S</i>	<i>Yes</i>	31	0	64.5
Upolo	CRB-S	Yes	25	0	72
Savai'i	CRB-S	Yes	6	0	33.3
<i>Solomon Islands</i>	<i>CRB-G</i>	<i>No</i>	10	100	0
Honiara	CRB-G	No	10	100	0

^a Countries locations are indicated using in bold and italics. Where appropriate, specimen collection points within particular regions of a country are listed underneath.

^b CRB collected from Johor and Terengganu (OrNV Type A), and from Perak (OrNV Type B), as defined in Ramle et al. (2005).

^c *O. rhinoceros* first entered Palau in 1942 (Gressitt, 1953), with CRB-G likely to be a second invasion (ca 2000s) due to the recent reports of increased levels of severe damage. OrNV was detected in both CRB-S (3 of 3 from Aimeliik) and CRB-G (3 of 5 from Aimeliik, and 2 of 2 from Airai) in Palau.

^d *O. rhinoceros* invaded the outer islands of PNG from 1942 to 1960 (Catley, 1969). CRB was first detected near Port Moresby (ca 2009), with both OrNV susceptible (CRB-S) and OrNV tolerant (CRB-G) haplotypes detected. OrNV was not detected in any CRB-G specimens from Port Moresby.

^e The CRB-G haplotype designation was based on DNA analysis showing similarity to specimens identified from Guam, with CRB-S represent CRB specimens associated with known susceptibility to OrNV infection.

^f Based on dilution PCR assays optimized to distinguish OrNV infection from gut tissue versus simple presence. ND, not determined as detection of OrNV infection based on tissue from legs has an unreliable association of OrNV presence.

^g Individual specimens included were analyzed for both haplotype and virus detection.

Fig. 1 provides a representative example of the PCR-RFLP results observed. This PCR-RFLP technique offers a relatively quick, simple, and cheap molecular technique to distinguish the CRB-G populations from other CRB populations found in the Pacific.

To begin demarcating the range for CRB-G and identify potential

source populations, the PCR-RFLP technique was used to survey several hundred CRB specimens obtained from areas harboring both established CRB populations and newly invaded sites. A library of 367 CRB population profiles was assembled using DNA extracted from CRB tissue specimens collected from 13 diverse geographic locations within the Asia-Pacific region (American Samoa, Diego Garcia, Fiji, Guam, Hawai'i, India, Indonesia, Malaysia, Palau, Papua New Guinea, Philippines, Samoa, and Solomon Islands). Table 2 summarizes these results and revealed that the CRB-G haplotype was present in Guam, Hawai'i, Indonesia, Palau, Papua New Guinea (Port Moresby region), Philippines, and Solomon Islands. All specimens obtained from Guam, Hawai'i, Philippines, and Solomon Islands shared the CRB-G PCR-RFLP pattern. Four of 7 specimens from Indonesia, 8 of 11 from Palau and 14 of 17 from Port Moresby were also found to be of the CRB-G haplotype. The remaining specimens from Indonesia, Palau, Port Moresby, and all other locations sampled displayed the more commonly observed CRB-OrNV susceptible PCR-RFLP haplotype pattern.

To gain preliminary insight into the relationships between the various CRB populations that were sampled, 152 CRB specimens were sequenced across the COI ‘universal barcode’ region, which encompasses the small region used in the PCR-RFLP haplotype assay. A subset of 31 sequences representative of each location was used to create a multiple alignment across a 676 bp region of the COI gene, which was followed by analyses of the variation observed at this locus among the CRB populations. From the unrooted tree construction shown in Fig. 2, four main groupings can be distinguished (clades I to IV). The CRB-G haplotype formed a distinct grouping separate from the other CRB haplotypes (clades II-IV). Within the CRB-G haplotype grouping (clade I) evidence for two further divisions were observed. Members of CRB-G^A subtype appeared to originate from a single original source population (100% sequence identity observed; seen as a group of eight sequences in Fig. 2) and are represented by populations identified from Guam, Hawai'i, Indonesia, Philippines, Port Moresby, Solomon Islands, plus a subset of specimens from Palau. The second cluster (CRB-G^B) was represented solely by a second subset of Palau specimens (seen as a group of two sequences in Fig. 2). This observation potentially represents a recombination event (Tsaousis et al., 2005) between the CRB-G^A group and a third subset population in Palau; putatively the original invasive CRB population into Palau that may be represented within clade IV. Clade IV also includes specimens obtained from Malaysia, India, and Indonesia. Alternatively, Palau may have been invaded by a second CRB-G-like population from a different source to the CRB-G^A subtype.

Of the two other main groupings, clades II and III correlated well with the reported historical pattern of CRB invasion into the Pacific (Catley, 1969). Clade II is composed of specimens collected from PNG and Malaysia. When the CRB-G specimens identified from Port Moresby were excluded, all remaining PNG specimens shared 100% sequence identity. Clade III contained members from Samoa, Fiji, Diego Garcia, India, and Malaysia. Specimens within Clade III displayed a 100% sequence conservation for all specimens except for one from Samoa (A35) and one from Malaysia (M1-10). The minor division within clade III involving the individual Malaysia and Samoa specimens (seen as two sequences in Fig. 2) may be due to a recent secondary introduction of a Malay CRB into Samoa. However, it is of more interest to point out that historically Sri Lanka was acknowledged as the original source of CRB for the 1909 Samoan introduction. From Samoa, CRB then spread into American Samoa, Fiji, and other surrounding Polynesian Islands (Catley, 1969). Here we provide genetic evidence supporting this claim, since it is widely held that CRB entered Sri Lanka from India. Records also showed that CRB invaded Diego Garcia from India during World War I via troop movements (Bedford, 1980; Orian, 1958). Clade IV nominally encompassed CRB specimens from India, Indonesia, Malaysia, and Palau. As opposed to the case in Polynesia, the origin of the CRB introductions into Palau and the outer islands of PNG (New Britain, New Ireland and Manus) were less clear, likely due to the

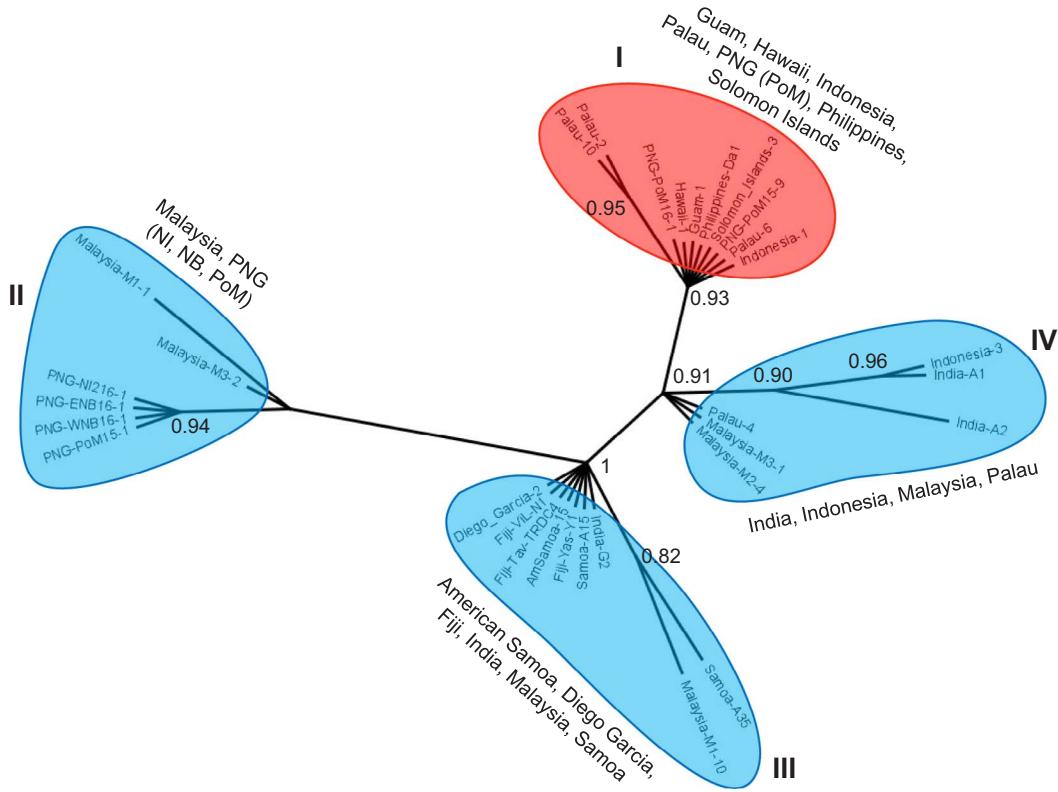


Fig. 2. Phylogeny of *O. rhinoceros* based on partial *COI* gene sequence. The unrooted majority rule consensus tree includes 31 representative CRB partial *COI* sequences (676 bp fragment between positions 1490–2198 (Folmer et al., 1994; Simon et al., 2006) from specimens obtained at various geographic locations (see Section 2.1.2 for associated Genbank accession numbers). The constructed tree was inferred from Bayesian phylogenetic analysis as implemented in Geneious R8.0. Posterior probability values are shown at branch nodes. Individual clade groupings are labelled with roman numerals. Red shading (clade I) highlights the OrNV-tolerant CRB-G haplotype (based on DNA sequencing) with A and B subtypes also indicated, while the blue shading (clades II–IV) emphasizes CRB populations associated with susceptibility to OrNV infection. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

chaotic nature of the wartime activities. The data summarized in Fig. 2 and Table 2 has suggested the possibility that accidental introduction of CRB into Micronesia and Melanesia arose from a location either within Malaysia or another nearby region.

With the center of origin for CRB believed to be located somewhere near the area bordering Continental and South East Asia (Bedford, 1976), an important component of this research was to begin identifying possible native sources for the invading OrNV-tolerant CRB-G haplotype. In addition to improving management of biosecurity risks, knowing the native range for CRB-G would provide an opportunity to search for candidate biocontrol agents able to deliver effective management of the invasive CRB-G biotype by reducing population number, which would reduce palm damage in infested areas, and prevent further spread into new regions. The data summarized in Fig. 2 suggests that the locations sampled from within Malaysia and India are unlikely to be candidate sources for CRB-G, which has assisted in excluding some regions from the search.

3.3. *O. rhinoceros* population-virus interactions

Concurrent with the CRB-G investigation, dilution PCR analysis of CRB tissue extracted DNA was used to detect the incidence of OrNV infection from 318 CRB specimens of confirmed haplotype. Results (see Table 2) revealed that 100% of the CRB-G specimens from the new outbreak areas of Guam, Hawai'i, Indonesia, Philippines, Port Moresby, and Solomon Islands, were negative for OrNV, indicating no detectable OrNV infection was present in these locations. Within the Palau specimens analyzed, five of the eight CRB-G specimens were positive for OrNV, while all three of the non-CRB-G haplotype specimens (i.e. non-CRB-G haplotype) were positive for OrNV infection. Interestingly, in

Port Moresby both CRB-G and CRB-S were also found together; however, no OrNV was detected in CRB-G (nine specimens), while three of the four CRB-S collected were positive for OrNV. Moreover, no OrNV was detected in specimens from Indonesia or Philippines, which are both known to have widespread OrNV presence. From all of the other non-CRB-G regions sampled, where greater than five individuals were collected, OrNV infection was detected in a proportion of the CRB individuals ranging from 45.2% to 64.5% (Table 2).

Fig. 3 presents a map summarizing the current known distribution for CRB-G populations in the Asia-Pacific region and reveals that most locations with confirmed CRB-G populations are generally not infected by OrNV, i.e. Guam, Hawai'i, Port Moresby, Solomon Islands, Indonesia, and Philippines. Furthermore, this absence of OrNV infection in CRB-G was correlated with severe to lethal levels of palm damage being reported in these areas (see Supplemental Fig. S1 for exemplar photos). OrNV infection is known to be associated with all other CRB populations (Table 2, Fig. 3), and with palm damage being reported as light to moderate in these areas (data not shown). Palau appears to be the exception to the observation that CRB-G beetles are uninjected by OrNV, although the PCR positive results could be due to cross contamination. Further validation will be required to confirm these observations, but it is notable that Palau has also reported increased palm damage since 2010, and the severity of palm damage has significantly increased over time. In other areas where CRB-G was found to cohabit with other (OrNV susceptible) CRB populations (e.g. Port Moresby), or where OrNV presence has been historically widespread (e.g. Indonesia, Philippines) (Hajek et al., 2007; Jackson, 2009), OrNV was not detected from the CRB-G specimens.

The results defining the pattern of CRB-G distribution together with observations of scarce OrNV infection in CRB-G (even when OrNV is

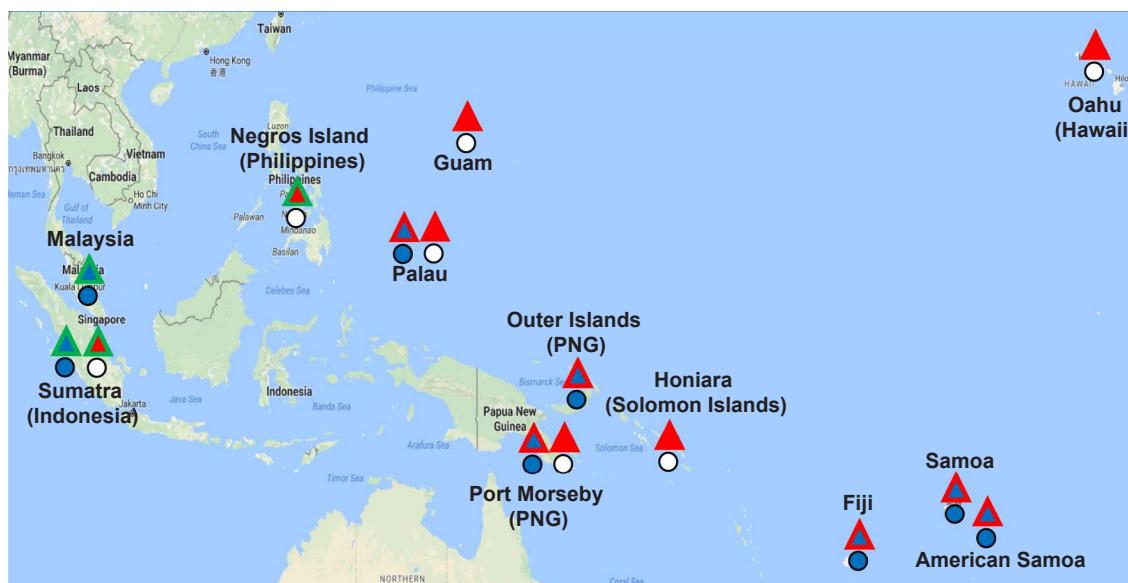


Fig. 3. Distribution of *O. rhinoceros* and *Oryctes rhinoceros nudivirus* from specimen collections in the South East Asia-Pacific region. Triangles indicate reported presence of *O. rhinoceros* in a location whereby a green outline indicates native CRB range, a red outline signifies CRB invaded area, red shading indicates CRB-G present, and blue shading indicates non-CRB-G populations. Blue filled circles represent OrNV infection detected in CRB specimens, while white filled circles indicate no OrNV infection was detected. Note that, except for Palau, OrNV was not detected in any CRB-G specimens, even when virus was known to be present within region. The background map is a screen shot from Google Maps (MapData 2016, www.google.co.nz/maps). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

present in the area) and correlated high levels of palm damage in the outbreak areas, are suggestive of OrNV tolerance in CRB-G populations as they are not being incapacitated by infection from virus currently used to manage CRB in the Pacific. The widespread heavy damage to palm trees caused by CRB-G (see Fig. S1) is reminiscent of observations of CRB impact in the Pacific prior to the release of OrNV in the biological control campaigns of the 1960s and 70s (Huger, 2005). The re-emergence of the coconut rhinoceros beetle problem with severe damage associated with a virus-free beetle also provides validation of the long-term impact of the original OrNV releases where virus has been maintained in the treated populations and damage has remained low (Bedford, 2013b; Huger, 2005). Interestingly in relation to resistance, Zelazny et al. (1989) reported lower than expected mortality when CRB collected from the Philippines were challenged with OrNV isolates known to infect CRB collected from Samoa. From these observations they postulated that an OrNV resistant population of CRB may be present within the Philippines. The current data presented here appears to corroborate their hypothesis as the CRB-G haplotype was orally tolerant to infection by several OrNV isolates, and potentially the dominant (if not sole) population within a localized area of the Philippines. Observations within this area did not show signs of severe CRB palm damage as is seen in other CRB-G infested regions (unpublished data).

Irrespective of the specific mechanism limiting infection of CRB-G by the OrNV isolates tested so far, future research is essential to identify effective biocontrol agents to assist with management of CRB-G. Of relevance with respect to improving CRB-G population control within invaded Pacific regions was the identification of CRB-G specimens from Indonesia and the Philippines, which are both considered native locations for CRB. Aside from CRB palm damage resulting from felled vegetation due to recent cyclones (Philippines) or development of new oil palm plantations (Indonesia), severe CRB damage has not been recently reported. Native habitat provides a good opportunity to identify candidate pathogens or other organisms for use as effective CRB-G biocontrol agents; this approach was previously successful in achieving CRB population control from the original series of CRB invasions (Huger, 2005). Although the OrNV isolates tested here did not orally infect CRB-G, considerable genetic variation has been documented among OrNV isolates, and research within new island release areas

have shown rapid evolution of the virus (Crawford et al., 1985, 1986; Crawford and Zelazny, 1990). This suggests there is a good chance to identify an effective OrNV isolate or other control option. Recognition of the CRB-G haplotype having escaped control from the commonly used OrNV isolates has highlighted the importance of actively overseeing and maintaining management programmes for important established insect pests, even when it appears a robust solution has been found.

4. Conclusion

The CRB-G haplotype identified here is genetically distinct from other CRB populations already established in the Pacific region and is highly damaging to palms. The evidence provided demonstrates CRB-G is not appreciably affected by the OrNV isolates commonly used for biocontrol management of other CRB populations. Conversely, identification of the CRB-G haplotype has highlighted how effective and important the use of the OrNV biocontrol agent was for effective management of the other CRB populations that invaded the Pacific region. Further invasion and spread of CRB-G poses a serious threat to the Pacific islands and states, particularly through its potential to damage and kill the culturally iconic and economically vital palm trees. Over the past decade, CRB-G has spread to uninfested islands at a rate of about one new island every two years. This is especially worrying for atolls and small islands, where coconut is an essential source of food and building material. However, the current findings have identified the cause of the problem and indicate that new biocontrol agents/strains will be needed. Furthermore, we have identified candidate locations to begin the search for potential biocontrol agents to assist with establishing effective CRB-G population control. This information also provides an important base for developing future biosecurity policies and improving CRB management efforts for the Pacific region and beyond.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jip.2017.07.006>.

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5.4.4 Movement of Packaged Soil Products as a Dispersal Pathway for Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) and Other Invasive Species

Please see next page.

Movement of Packaged Soil Products as a Dispersal Pathway for Coconut Rhinoceros Beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae) and Other Invasive Species

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The coconut rhinoceros beetle (CRB), a major pest of coconut and other palms, was first detected on Guam in 2007. Adults damage and may kill palms when they bore into crowns to feed on sap. Grubs do no damage, but feed on decaying plant material which includes dead trees and piles of compost, sawdust, or manure. Here we report that CRB grubs can feed and develop in a variety of packaged commercial soil products and movement of these products is a high-risk pathway for dispersal of CRB.

On April 19 2016, a retired officer of the Guam Customs and Quarantine Agency reported finding CRB-like grubs in a bag of soil (Miracle-Gro Garden Soil for Flowers and Vegetables; 2 cu. ft. bag; UPC 032247345248) purchased from a local garden center. Upon inspection, 57 third instar grubs were found in this bag. Mass of the grubs was 11.74 ± 0.75 g (mean \pm SD). DNA analysis confirmed the grubs were CRB.

This incident and multiple previous reports of Guam residents finding large scarab grubs in packages of soil products purchased from local garden centers prompted us to visit one on April 20, 2016. This garden center, part of a large hardware store franchise, imports many types of soil products from the United States mainland, typically packaged in plastic bags. Upon arrival, plastic-wrapped

pallets of these products are stored on industrial shelving in a fenced, outdoor garden shop.

We noticed several unopened bags of potting mix (Miracle-Gro Nature's Care Organic Potting Mix; 32 qt. bag; UPC 071645168318) with multiple holes (about 2 cm dia.) in the outer packaging. Contents of these bags were inspected on the spot. One bag harbored 5 CRB-like grubs (2 second instar and 3 third instar). DNA analysis confirmed the grubs were CRB. The remaining two bags did not contain CRB grubs.

Discussion

We know that packaged soil products may be a good food source for CRB grubs because we use one of these products (Earthgro Steer Manure Blend; 1 cu. ft.; UPC 036865175119) to rear them from egg to adult in the laboratory. We have had many complaints from clients who claimed that they had purchased soil products prepopulated with CRB grubs but we were not able to discount the possibility of infestation after purchase. We now have proof that unopened bags are infested before sale.

In addition to being a pathway for intra-island movement of pests, importation of soil is clearly a pathway for accidental introduction of invasive species. Live insects, mites, nematodes, and weed seeds

have been found in unopened bags of soil imported to Guam (AM, personal observation). This pathway could be closed by enforcing existing Guam law which prohibits importation of soil. According to the Guam Administrative Rules and Regulations (Government of Guam 1998), Title 9 – Animal Regulations; Division 1 – Care and Conservation of Animals; Chapter 3 – Animal Control:

§ 3304. Soils, Snakes, Injurious Insects, Etc.

Importation Prohibited. All persons are prohibited from receiving for transportation, bringing, or causing to be brought to the Territory, for the purpose of debarkation or entry there into, any of the following named articles:

(1) Soil, provided that limited quantities of soil may be imported into the Territory for experimental or other scientific purposes, under permit with conditions prescribed by the department.

“Soil” and “import” are previously defined in the document as follows:

§ 3301. Definition.

(17) Soil means that part of the upper layer of earth in which plants can grow; this material may or may not contain organic matter and includes such planting media as deteriorated peat, except clean coral, sand, pottery and industrial clay, volcanic cinders and other similar soil-free material.

(18) Import means shipment into the Territory from any point outside of the Territory.

Enforcement of the existing Guam law with respect to importation of soil is a priority action item in the Regional Biosecurity Plan for Micronesia and Hawaii

(United States Department of the Navy 2015):

Enforce the Guam law prohibiting the importation of soils for all soil and similar materials, specifically the bags of soil and soil like products soil[sic] in hardware stores. Either these items should not be imported or should need to be sterilized prior to import.

It is probable that movement of commercial soil products contributed to the rapid spread of CRB throughout Guam. Unfortunately, closing this pathway will do nothing to mitigate damage from the current CRB outbreak on this island. However, we suggest that regulatory actions to reduce the risk of accidental transport of CRB in packaged soil products should be considered on islands recently invaded by this pest. For example, banning outdoor storage of packaged soil products in CRB infested areas would almost eliminate the risk of bags of soil products becoming miniature, mobile CRB breeding sites.

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5.4.5 Coconut rhinoceros beetles (Coleoptera: Scarabaeidae) develop in arboreal breeding sites in Guam

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Coconut Rhinoceros Beetles (Coleoptera: Scarabaeidae) Develop in Arboreal Breeding Sites in Guam

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Coconut rhinoceros beetles (Coleoptera: Scarabaeidae) develop in arboreal breeding sites in Guam

Aubrey Moore^{1,*}, Trevor Jackson², Roland Quitugua¹, Paul Bassler¹, and Russell Campbell³

The coconut rhinoceros beetle (CRB), *Oryctes rhinoceros* (L.) (Coleoptera Scarabaeidae), is a major pest of coconut palm, *Cocos nucifera* L. (Arecales: Arecaceae). Adult beetles defoliate and kill palms when they bore into crowns to feed on sap. When Pacific Islands are invaded by this pest, coconut palm mortality may reach greater than 50% within a few years (Gressitt 1953). In contrast to adults, CRB larvae feed on decaying vegetation and do no economic damage. They usually are found in dead standing coconut palms, fallen coconut logs, and rotting coconut stumps. They also are found commonly in piles of sawdust and manure where these materials are available.

CRB first was detected in Guam in the Tumon Bay tourist hotel area in Sep 2007, and an eradication project was launched. The project relied on pheromone trapping, using ethyl 4-methyloctanoate (Oryctalure, P046, ChemTica International, Heredia, Costa Rica), to capture adults, and sanitation to remove rotting vegetation used as breeding sites. Despite these efforts, CRB damage in central Tumon Bay remained high, with about 50% of coconut palms in this area showing signs of recent attack.

The major source of CRB adults in Tumon Bay was presumed to be breeding sites in several unmanaged, vacant lots interspersed among hotel properties. Dead, standing coconut palms and severely damaged palms were felled and removed along with many tons of rotting coconut debris from vacant lots. Even though thorough searches of the Tumon Bay area detected no new breeding sites after the sanitation campaign, there was not a significant reduction in trap catch, and the incidence of new damage to palms remained high.

CRB eggs and larvae had occasionally been found developing in detritus captured in the crowns of coconut palms in Guam, but we had considered this to be a rare occurrence. To investigate the potential of arboreal sites as a significant source of CRB adults, we felled mature coconut palms and dissected their crowns at 2 sites. The 1st site, the former Fujita Hotel site, is in the area where the CRB first was discovered in Guam. This lot had been sanitized thoroughly, and we had not found new breeding sites on the ground for several months. The 2nd site, Agana Springs, is a swampy area that had been infested by the CRB for about 2 yr. We had begun sanitation work in this area, but it was not complete, and we were still discovering new breeding sites on the ground. At both sites, palms were not selected at random. We chose plants that had CRB injury and an accumulation of debris in their crowns.

We found all life stages of the CRB in crowns of 26 palms that we felled (Table 1). Larvae were found feeding in accumulated detritus

held in the crowns. We saw no evidence of immatures feeding on live tissues. The proportion of trees harboring immature CRBs was 50% (6 out of 12) at the old Fujita Hotel site and 29% (4 out of 14) at the Agana Springs site. These proportions were not significantly different ($P = 0.42$; Fisher's exact test). However, the mean number of immatures per tree differed significantly between sites (8.5 immatures per tree for the 12 trees felled at the old Fujita Hotel site; 0.6 immatures per tree for the 14 trees felled at the Agana Springs site; $P = 0.015$; Wilcoxon rank sum test).

Most adults recovered from crowns appeared very healthy. They were very active and strong, and their exoskeletons showed no signs of wear. Due to their pristine appearance, we suspected that at least some of these beetles had recently developed in situ. To test this hypothesis, we applied a method developed by Vander Meer (1986). This method estimates physiological age of CRB adults using size and mass measurements. According to Vander Meer (1986), CRB adults are at a maximum mass at eclosion, and they pass through 3 behavioral phases, which are correlated with body mass. During the 1st phase, lasting about 30 d, the beetle does not feed and continually loses weight. When the adult's weight is down to about 65% of its emergent weight, the beetle flies up to a coconut palm crown, bores into the stem, feeds on sap, and increases its weight to about 80% emergence weight. The 2nd phase, which starts at first feeding, lasts about 120 d during which the beetle goes through several flight and feeding episodes. Body weight oscillates between about 60% and 80% of its emergent mass. In a final, senescent stage, the beetle stops feeding, and its weight continually declines until death occurs at about 40% of its emergent mass. Estimated emergent mass (EEM) was calculated by an equation from Vander Meer & McLean (1975), which expresses emergent weight as a linear function of elytral area. We classified beetles collected from palm crowns into the 3 behavioral phases based on percentage of EEM. Beetles weighing greater than 80% EEM were placed in the "Pre-flight" category, beetles between 60 and 80% EEM were placed in the "Active" category, and beetles less than 60% EEM were placed in the "Senescent" category. Our estimates indicated that 9 out of 16 adults from coconut palm crowns were in the "Pre-flight" phase of their adult life (Table 2). Thus, they probably had developed from egg to adult in situ. Whereas CRB adults typically fly up into palm crowns, it is hard to explain how larvae and pupae would arrive in this microhabitat other than having developed from eggs laid in the crown.

Development of CRB larvae in palm crowns has been reported previously but seems to be rather a rare behavior except in Guam. In the Palau

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Table 1. Coconut rhinoceros beetle life stages and other animals found during dissection of crowns of mature coconut palms in Guam.

Tree number ^a	Coconut rhinoceros beetle life stages ^b								Other inhabitants
	E	L1	L2	L3	PP	P	Am	Af	
1	0	0	0	0	0	0	0	0	brown treesnake (<i>Boiga irregularis</i>), crab, centipedes (<i>Scolopendra</i>), ants
2	12	7	0	1	4	0	2	0	
3	0	0	5	0	0	0	1	0	
4	2	15	6	1	0	0	4	2	ants, crab
5	0	0	0	3	0	0	1	1	
6	0	1	0	1	1	0	5	0	brown treesnake (<i>Boiga irregularis</i>), bird nest
7	1	1	0	0	0	0	1 ^c	0	ants
8	0	0	0	0	0	0	0	0	ants
9	0	0	0	0	0	0	0	0	
10	0	0	0	0	0	0	1 ^c	0	ants, flower beetle (<i>Protaetia pryeri</i>)
11	0	0	0	10	0	0	0	0	ants, click beetle (<i>Lanelater bimaculatus</i>)
12	6	17	3	5	0	0	1	0	
13	0	0	0	0	0	0	0	1 ^c	
14	0	1	0	0	0	0	4	0	
15	0	0	0	0	0	1	0	0	Brown treesnake (<i>Boiga irregularis</i>), ants
16	0	0	0	2	0	0	0	0	ants, roaches, praying mantis
17	0	0	0	5	0	0	0	0	
18	0	0	0	0	0	0	0	0	
19	0	0	0	0	0	0	0	0	
20	0	0	0	0	0	0	1	2	termites, ants, lizard
21	0	0	0	0	0	0	0	1	
22	0	0	0	0	0	0	0	0	roaches, ants, lizard
23	0	0	0	0	0	0	0	1	roaches, ants, lizard
24	0	0	0	0	0	0	0	0	centipedes (<i>Scolopendra</i>)
25	0	0	0	0	0	0	0	0	ants, roaches
26	0	0	0	0	0	1	1		ants, roaches, termites

^aTrees 1–12 were felled at the old Fujita Hotel site, Tumon Beach, on 2–4 Jun 2010. Trees 13–26 were felled at Agana Springs on 17–24 Jun 2010.^bCoconut rhinoceros beetle life stages: E = eggs, L1–3 = larval instars 1–3, PP = prepupae, P = pupae, Am = adult males, Af = adult females.^cFound dead.

Islands, Gressitt (1953) found larvae developing in live coconut palms, which had been injured seriously either by adult feeding, or by some injury to the palm that caused local rotting, or by the accumulation of de-

bris among petiole bases of fronds that were delayed in falling. In India, Nirula (1955) reported that CRB larvae occurred in rubbish in the axils of living palms when ground breeding sites were unavailable.

Table 2. Size, mass, and behavioral status of coconut rhinoceros beetle adults removed during dissection of crowns of felled coconut palms at the old Fujita Hotel site, Tumon Beach, Guam, on 2–4 Jun 2010.

Beetle number	Sex ^a	EL Elytra length (mm)	EW Elytra width (mm)	EEM ^b Estimated emergent mass (g)	Mass (g)	Estimated percentage emergent mass	Behavioral status
904	m	22.06	17.44	4.499	4.558	101%	pre-flight
900	m	19.98	15.79	3.045	2.953	97%	pre-flight
908	m	21.16	17.29	4.103	3.922	96%	pre-flight
889	m	22.29	16.44	4.115	3.869	94%	pre-flight
906	m	24.85	20.39	7.061	6.211	88%	pre-flight
888	m	25.44	20.14	7.180	6.166	86%	pre-flight
911	m	21.30	17.29	4.154	3.505	84%	pre-flight
896	f	23.79	18.93	5.877	4.828	82%	pre-flight
905	m	22.31	18.01	4.858	3.959	81%	pre-flight
903	m	23.99	19.10	6.042	4.651	77%	active
902	m	23.76	19.76	6.279	4.595	73%	active
898	m	24.69	21.08	7.350	5.339	73%	active
897	m	25.93	20.92	7.812	5.547	71%	active
901	f	23.98	20.29	6.638	4.216	64%	active
890	m	24.87	19.17	6.432	3.923	61%	active
899	m	23.58	18.57	5.615	3.412	61%	active

^aSex: f = female, m = male.^bEEM = 0.021 ' EL ' EW – 3.580.

Our observations indicated that arboreal development of CRB grubs in Guam is a common occurrence. This habitat extension may be due to almost total absence of predation by insectivorous birds and mammals as a result of heavy predation of the latter by the brown tree snake, *Boiga irregularis* (Bechsterin) (Squamata: Colubridae). Elsewhere, rats are commonly found in the crowns of coconut palms and are known to prey on CRB larvae (Gressitt 1953; Hinkley 1967). In the crowns we dissected, we found 3 tree snakes and no evidence of rats (Table 1). There are rats in Guam, but they are rare. Ten rat snap traps baited with peanut butter were nailed to trunks of coconut palms at the Fujita Hotel site. No rats were caught during a 2 wk trapping period. It should be noted that arboreal breeding sites were not found in palms that had inflorescences, nuts, and old fronds removed, which is a standard management practice in hotel and golf course landscapes.

Following the discovery that arboreal breeding sites were common in Guam, we included removal of unmanaged coconut palms in our sanitation program. Palms were felled, inspected for the CRB, chipped, and disposed of by deep burial or burning. Stumps also were removed. In 121 coconut palms felled in our sanitation program, we found 510 CRB (99 eggs, 40 first instars, 72 second instars, 201 third instars, 25 pupae, 34 adult males, and 30 adult females).

This study was conducted by the Guam Coconut Rhinoceros Beetle Eradication Project with funds from the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service, the USDA Forest Service, and the Government of Guam.

Summary

Coconut rhinoceros beetle (CRB; *Oryctes rhinoceros* [L.]; Coleoptera: Scarabaeidae) grubs feed only on decaying vegetation and usually are found in dead standing coconut trees and decaying coconut debris on the ground. However, in Guam, a significant proportion of the CRB population develops in detritus caught within the crowns of live coconut palms. This habitat extension may be due to almost total absence

of predation by insectivorous birds and mammals as a result of heavy predation by the brown tree snake, *Boiga irregularis* (Bechsterin) (Squamata: Colubridae).

Key Words: brown tree snake; *Cocos nucifera*; *Oryctes rhinoceros*; detritus; emergent weight; physiological age; rat

Sumario

Las larvas del escarabajo rinoceronte del coco (ERC; *Oryctes rhinoceros* [L.]; Coleoptera: Scarabaeidae) se alimentan sólo de la vegetación en descomposición y por lo general se encuentran en palos de coco muertos pero todavía parados y en los escombros de coco en descomposición en el suelo. Sin embargo, en Guam, una proporción significativa de la población de ERC se desarrolla en detritus atrapado dentro de las coronas de las palmas de coco vivas. Esta extensión de hábitat puede ser debido a la ausencia casi total de la depredación por aves y mamíferos insectívoros, como resultado de la depredación pesada por la culebra marrón de árbol, *Boiga irregularis* (Bechsterin) (Squamata: Colubridae).

Palabras Clave: culebra marrón de árbol; *Cocos nucifera*; *Oryctes rhinoceros*; detritus; peso emergente; edad fisiológica; ratas

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5.4.6 First record of eggplant mealybug, *Coccidohystrix insolita* (Hemiptera: Pseudococcidae), on Guam: Potentially a major pest

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First record of Eggplant Mealybug, *Coccidohystrix insolita* (Hemiptera: Pseudococcidae), on Guam: Potentially a major pest

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Abstract

The eggplant mealybug, *Coccidohystrix insolita* (Green) (Hemiptera: Pseudococcidae), is recorded from the island of Guam in the Mariana Islands for the first time. Factors indicating that this introduced mealybug has the potential to become a pest of economic importance for agriculture and horticulture on Guam are discussed.

Keywords

Coccidohystrix insolita, eggplant mealybug, invasive species

Introduction

The eggplant mealybug, *Coccidohystrix insolita* (Green) is broadly distributed in the tropics and subtropics and well known as a agricultural and horticultural pest (Lit et al. 1998,

Williams 2004, Williams and Watson 1988). This article documents the first detection of *C. insolita* on Guam.

Mealybugs producing long ovisacs were found infesting the lower leaf surfaces of eggplant, *Solanum melongena* L., in a farmer's field on Guam on December 4, 2013 (Figs 1, 2, 3). Samples of infested leaves were preserved in ethanol for subsequent identification at the California Department of Food and Agriculture Diagnostic Center in Sacramento, California. Diagnosis was based on morphology of adult females mounted on microscope slides.



Figure 1.
Adult *Coccidohystrix insolita* females with ovisacs.



Figure 2.
Coccidohystrix insolita nymphs.



Figure 3.

Eggplant (*Solanum melongena* L.) leaf underside infested with *Coccidohystrix insolita*.

Materials and methods

Infested leaves were collected into bags and taken to the laboratory. Color photographs were taken to record the appearance of the insects in life using a Leica EZ4HD dissection microscope. Infested leaf fragments were preserved in 70% ethanol and sent to the Plant Pest Diagnostic laboratory of California Department of Food and Agriculture (CDFA-PPDC) for diagnosis. A total of seven adult female specimens were selected and prepared on three microscope slides using the method given by Sirisena et al. 2013. The specimens were studied using a Nikon Eclipse 80i compound microscope with phase contrast illumination and $\times 30$ – $\times 600$ magnification, and were identified using the keys in Williams and Watson 1988 and Williams 2004. Slide-mounted voucher specimens will be deposited in the California State Arthropod Collection at CDFA-PPDC in Sacramento, California.

Taxon treatment

Coccidohystrix insolita (Green, 1908)

Material

- a. islandGroup: Mariana Islands; island: Guam; municipality: Dededo; locality: near Swamp Road; decimalLatitude: 13.539981; decimalLongitude: 144.83435; samplingProtocol: eggplant leaf samples; eventDate: 2013-12-04; sex: 7 slide-mounted adult females were examined; catalogNumber: AM20131204.002; occurrenceRemarks: on eggplant leaves; recordedBy: Jesse Bamba; identifiedBy: Gillian W. Watson; dateIdentified: 2013-12-13; collectionID: ESUG; institutionCode: UGUAM; basisOfRecord: LivingSpecimen; source: <http://guaminsects.myspecies.info/node/2623>

Diagnosis

9-segmented; posterior ostioles present, anterior ostioles absent; cerarii on margins numbering 17 pairs, numerous dorsal cerarii present also, each cerarius consisting of 1–15 large conical setae situated on a sclerotized prominence, without any associated trilocular pores; legs well developed, each claw with a denticle present on plantar surface; circulus absent; anal lobes well developed, each with a sclerotized ventral bar; quinquelocular pores numerous on venter; multilocular disc pores numerous on venter of abdominal segments III-IX, a few also present on the venter of segments I and II and on the dorsum of segment VII; ventral oral collar ducts present on submargins of abdominal segments V-VIII; oral rim ducts absent entirely.

Diagnosis was based Williams 2004 which includes a good taxonomic illustration of *C. insolita*.

Distribution

C. insolita has been recorded in the literature from the following regions and countries:

Afrotropical: Kenya, Madagascar, Rodrigues Island (Mauritius), South Africa, Tanzania, Zanzibar; **Australasian:** Western Samoa; **Oriental:** Bangladesh, Burma (=Myanmar), India, Laos, Pakistan, Philippines, Sri Lanka, Thailand, Vietnam; **Palaeartic:** China, Saudi Arabia (Ben-Dov 2013).

In addition, a Japanese quarantine inspector found *C. insolita* on *Alternanthera* (Amaranthaceae) imported from Singapore (Tokihiro 2006).

Prior to our discovery on Guam, *C. insolita* was known only from two Pacific island nations: the Philippines and Western Samoa. *C. insolita* was first detected in the Philippines during 1994 (Lit et al. 1998) and in Western Samoa in 1966 (Williams and Watson 1988).

Biology

Coccidohythrix insolita lives on the leaves (Fig. 3).

Notes

The appearance of *C. insolita* in life is unusual for a mealybug because the adult female has very little dorsal wax and secretes a white, waxy ovisac up to 6 times as long as the body of the female (Fig. 1), which is more typical of some Coccoidea. The immature stages do not secrete a thick layer of mealy wax, the body being shiny yellow-green with submedian grey spots on 2 abdominal and 1 thoracic segments (Fig. 2). This contrasts with the in life appearance of the solenopsis mealybug, *Phenacoccus solenopsis* Tinsley, in which all developmental stages develop a thick layer of white

mealy wax except for two longitudinal lines of bare cuticle that expose dark submedian spots on 3 or 4 segments on the abdomen and 1 or 2 on the thorax.

Host Plants

C. insolita is polyphagous and is recorded from the following families of host plants (Ben-Dov 2013): Acanthaceae, Amaranthaceae, Apocynaceae, Araceae, Arecaceae, Aristolochiaceae, Asteraceae, Chenopodiaceae, Cucurbitaceae, Euphorbiaceae, Fabaceae, Malvaceae, Menispermaceae, Moraceae, Poaceae, Rhamnaceae, Rubiaceae, Solanaceae, Sterculiaceae, Tiliaceae, Zygophyllaceae.

Many plants belonging to these families are important to agriculture and forestry on Guam.

Parasitoids

Twenty-three species of hymenopterous parasitoids are associated with *C. insolita* (Noyes 2013):

Aphelinidae: *Coccophagus pseudococci*; **Encyrtidae:** *Adektitopus longipennis*, *Anagyrus gracilis*, *Apolectomastix bicoloricornis*, *Blepyrus insularis*, *Gyranusoidea signata*, *Homalotylus albiclavatus*, *Homalotylus hemipterus*, *Homalotylus indicus*, *Homalotylus turkmenicus*, *Leptomastix nigrocincta*, *Leptomastix nigrocoxalis*, *Neocharitopus orientalis*, *Paranathrix tachikawai*, *Prochiloneurus albifuniculus*, *Prochiloneurus pulchellus*; **Eulophidae:** *Aprostocetus ajimerensis*, *Aprostocetus annulicornis*, *Aprostocetus jaipurensis*; **Pteromalidae:** *Catolaccus crassiceps*; **Signiphoridae:** *Chartocerus hyalipennis*, *Chartocerus kerrichi*, *Chartocerus kurdjumovi*.

None of these species are known to exist on Guam and there were no signs of parasitism in the specimens examined.

Other Natural Enemies

The following natural enemies have been recorded attacking *C. insolita*: **Fungi:** *Metarhizium anisopliae*; **Insecta:** **Coleoptera:** **Coccinellidae:** *Anegleis cardoni* (Weise); *Hyperaspis maindronia*; *Nephus regularis*; **Lepidoptera:** **Lycaenidae:** *Spalgis epeus* (Ben-Dov 2013). None of the insect predators are known to exist on Guam.

Attendant Ants

Three species of attendant ants are associated with *C. insolita*: *Dolichoderus bituberculatus*, *Solenopsis geminata*, *Anoplolepis gracilipes* (Ben-Dov 2013). The latter two species are abundant on Guam but so far, we have not yet seen any ant associations with *C. insolita*.

Discussion

Guam, like all small tropical islands, is susceptible to damage from invasive species because of a warm climate with no winter, coupled with a lack of natural enemies for many new arrivals. It is difficult to predict the eventual pest status of any newly detected invasive insect species, but *C. insolita* has the hallmarks of being a major pest on Guam for several reasons:

1. **Plant hosts.** Many of the known host plants of *C. insolita* are commonly grown as crops on Guam. In addition several others, such as coconut palm, *Cocos nucifera*, and *Hibiscus* spp. are major components of natural and ornamental vegetation.
2. **Escape from natural enemies.** None of the parasitoids or predators known to attack *C. insolita* are known to exist on Guam. Despite the fact that several parasitoids and predators pre-existed in the Philippines prior to arrival of *C. insolita*, this species became a major agricultural pest (Lit et al. 1998). It is likely that implementation of biological control will be required to prevent major economic and environmental damage by this pest on Guam.
3. **Attendant ants.** Two of the three ants known to form a commensal relationship with *C. insolita*, namely *Anoplolepis gracilipes* and *Solenopsis geminata*, are common on Guam. In addition, several other ant species which readily establish associations with mealybugs are present. Attendant ants protect mealybugs from parasitism and predation, making it difficult to establish biological control.
4. **Origin.** Guam is an unincorporated territory of the United States of America (U.S.). Experience has shown that invasive species which originate from outside of the U.S., such as this one, are harder to deal with than those accidentally imported from the U.S. mainland or Hawaii. For invasive insect species already present in the U.S., control resources are usually readily available. Often research has been done, control methods have been developed, biological control agents have been identified, an exploratory entomologist has been sent out to collect candidate species, and these have been imported, cultured and tested, and are available for use on Guam. However, resources are scant when it comes to responding to invasive species of non-U.S. origin.
5. **Rapid Response Capacity.** There is currently a critical lack of capacity to deal with entomological problems on Guam and in the rest of Micronesia. The number of Ph.D. level entomologists practicing on Guam and in the rest of Micronesia has decreased from nine during the mid-1990s to only three at present.
6. **Biological Control Agent Import Permits.** Guam is required to comply with U.S. Department of Agriculture regulations for importing biological control agents. These requirements are far more stringent for organisms originating outside of the U.S. than for those imported from within the U.S. Delays in the permitting process and a lack of capacity to comply with permit conditions sometimes impede rapid progress towards establishment of biological control in time to prevent major economic and environmental damage. Often, there is a pest population explosion prior to

implementation of biological control. During this initial outbreak, risk of accidental export to trading partners is high.

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5.4.7 Status and biological control of cycad aulacaspis scale

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General News

Status and Biological Control of Cycad Aulacaspis Scale

According to the IUCN/SSC* Cycad Specialist Group's action plan (<http://data.iucn.org/dbtw-wpd/edocs/2003-010.pdf>), cycads are the most ancient seed plants still living today and the extant c. 297 taxa form one of the world's most threatened groups. The invasive cycad aulacaspis scale, *Aulacaspis yasumatsui* (CAS), is a threat to native cycad populations worldwide as well as a costly pest for horticultural industries. The diaspidid or armoured scale, CAS, is already documented as threatening the extinction of the endemic cycads *Cycas microneesica* in Guam and *C. taitungensis* in Taiwan (www.cycadsg.org/pages/CAS.htm). A few species – notably *C. revoluta* – are important ornamentals globally and trade in whole plants has been the pathway for CAS spread between countries, although local spread can occur via wind dispersal of first-instars also. This article outlines the limitations of all but the most stringent import restrictions, and then looks at the current status of CAS and its control in some invaded countries, focusing on biological control and its prospects.

CAS attacks primarily *Cycas* species and is native to an area of South-east Asia between Thailand and peninsular Malaysia in the west and Vietnam in the east. It has spread within Asia, becoming a pest in southern China in the 1990s through the importation of infested *C. inermis* from Vietnam; later spreading to Hong Kong in 1992, where it caused significant damage to cycads; to Taiwan in the early 2000s; and to Singapore, Malaysia and Indonesia¹.

Outside Asia, CAS was first identified in south Florida in 1996 following accidental introduction on cycads legally imported for botanical gardens. By the time it was identified it had infested 22 cycad species. It has since spread north to South Carolina and west as far as Texas. Cycads of Florida origin have probably been responsible for the scale's spread to some Caribbean islands and Costa Rica, and to Hawaii (first reported in 1998), Guam (2003), Rota in the North Marianas (2007) and Palau (2008) in the Pacific. It has also been reported from a few European countries (France, Croatia and Bulgaria)¹. In its introduced range, CAS is highly destructive. Long-term infestations lead to sequential defoliations, with leaves of each successive flush being smaller and fewer, ultimately leading to plant death, even of mature cycads and sometimes within only a few months of becoming infested.

The IUCN/SSC Cycad Specialist Group proposes preventing introduction as the most effective way of combating this pest. Its three alternatives for preventing entry centre on quarantine measures: (i) prohibiting entry of host cycad plants from countries known or suspected of having infestations; (ii) mandatory insecticide treatment as a condition of entry for host plants coming from infected countries; or (iii) close inspection of host plants for scale infestations with subsequent insecticide treatment if infestations are found (www.cycadsg.org/publications/CAS/Cycad-Aulacaspis-Scale-Pest-Alert.pdf). However, the minute size of the first-instar crawlers allows them to reach plant tissues not accessible to inspection². The unique morphology of *C. revoluta* stems and cataphylls (thickened leaves that protect the apical meristem), together with hidden root surfaces, mean that even the most thorough quarantine inspection will not find all the scales. The efficacy of insecticide treatments cannot be guaranteed for the same reason. Thus the best hope for prevention is prohibition of entry from infested areas. However, even where this is implemented – as in Palau – there is no guarantee that CAS will be kept out indefinitely. Strict quarantine is also essential: CAS infestation progresses rapidly and scale covers are easily detected on leaves within a few weeks of crawlers settling. Imported cycads truly need to be quarantined, rather than just inspected and released. If CAS evades quarantine, by the time this becomes apparent in the field and its identity is confirmed the insect may have been established for a year or more, by which time all hope of eradication is long past.

Continental USA

In Florida, the thriving, multi-million-dollar cycad nursery industry combines mechanical, cultural, chemical and biological control methods to mitigate CAS damage. Natural enemies already present, including the predatory beetle *Cybocephalus nipponicus* and the coccinellid *Rhyzobius lophanthae* (introduced previously from Australia to control diaspidid pests) did not provide adequate control.

From surveys in Thailand, Richard Baranowski (University of Florida), with Banpot Napompeth (National Biological Control Research Center, Thailand), identified and released in Florida two natural enemies of CAS: *Cy. nipponicus* and an aphelinid parasitoid, *Coccobius fulvus*.

Ronald Cave (University of Florida) and Ru Nguyen (Florida Department of Agriculture) found *Co. fulvus* in northern Vietnam. This was released (after climate matching) in northern Florida in 2007, and established and spread. From 2003 to 2011, *Co. fulvus* was the only parasitoid observed in CAS in Florida; although high rates of parasitism by it have been seen recently in northern Florida, it seems unable to provide satisfactory control. The impact of both *Cy. nipponicus* and *Co. fulvus* may be affected by hyperparasitism although the extent is unknown: *Co. fulvus* is hyperparasitized by *Ablerus* sp. and *Cy. nipponicus* is attacked by the prepupal-pupal parasitoid *Aphanogmus albicoxalis*.



An encyrtid parasitoid commonly found by Cave and Nguyen in China, Vietnam and Thailand in 2006, 2007 and 2009 surveys was *Arrhenophagus chionaspidis*. This wasp had been reported attacking another diaspidid, white peach scale (*Pseudaulacaspis pentagona*), in Florida many years before but had not at that time been recorded from CAS there. It was found in CAS in Florida in 2012 but although rates of parasitism were in some cases very high, it has no apparent control impact on CAS populations.

During the survey in Thailand in 2007, an undescribed coccinellid (subsequently named *Phaenochilus kashaya*) was discovered at two localities where scale infestations were light. This species is under investigation in Florida. No-choice testing indicated it would feed on early instars of some other groups, including aphids and mealybugs, and small larvae of *Cy. nipponicus*, *R. lophantheae*, and CAS mummified by *Co. fulvus*, but choice tests suggest it is normally a specialist on armoured scales including CAS. Mixed colonies of *P. kashaya* and *Cy. nipponicus* perform well in the laboratory. It is notable that *Cy. nipponicus*, *Co. fulvus* and *P. kashaya* are all found in Thailand, but during exploration only *A. chionaspidis*, *Cy. nipponicus*, and *P. kashaya* were seen on plants at the same locality. Another factor makes *P. kashaya* an exciting discovery: preliminary results suggest that the reproductive rate of CAS outstrips the consumption rate of *Cy. nipponicus*, *Co. fulvus* and *R. lophantheae*; but *P. kashaya* consumes CAS faster than the scale reproduces (which does create problems for quarantine testing, as the predator wipes out the scale colony).

Among other natural enemies tested, the fungus *Isaria fumosorosea* performed well in the laboratory³, as did entomopathogenic nematodes, but similar results could not be obtained in the field and funding is not currently available to pursue this work.

CAS was detected in Texas in 2006, but is being controlled by fortuitous establishment of *R. lophantheae* and the aphelinid parasitoid *Aphytis lingnanensis*⁴. Nurseries in California very occasionally find CAS but it is always eradicated when found.

Hawaii

CAS was first detected in Hawaii in 1998 and found to be under biological control by a pre-existing population of *R. lophantheae*⁵. It was only a decade later, in 2008, that two parasitoids were first seen attacking CAS there. They were identified as the aphelinids *A. lingnanensis* and *Pteroptrix* n. sp. near *leptocera*, with *A. lingnanensis* being the most numerous⁶. *Aphytis lingnanensis* was purposefully introduced to Hawaii as a biocontrol agent in 1952. The parasitoid attacking CAS in Hawaii may be a recent, fortuitous introduction of a new biotype of *A. lingnanensis*⁶.

Guam

Since its discovery on ornamental cycads in Guam in 2003, CAS has decimated imported cycads there. However, of greater concern is its spread to the

native *Cycas micronesica*, a dominant component of Guam's limestone and ravine forests, which it has also devastated. Without treatment, the mortality rate for the native cycads is 100% within one year of infestation. After six years of CAS attack no recruitment is occurring among the survivors, even after the introduction of a biocontrol agent. *Rhizobius lophantheae* was introduced from Hawaii in 2005 and is protecting mature plants, but seedlings are still severely impacted. Research has uncovered a number of factors contributing to control breakdown: there is a disparity in size between pest and predator which means that CAS occupying tiny spaces escape predation². In addition, CAS feeding on *C. revoluta* are protected from predation by the plant's trichomes⁷. Possibly seedlings and young cycads are particularly susceptible to mortality from CAS infestation because *R. lophantheae* predation rate is reduced near the ground⁸. Although many mature trees died in the interval between arrival of the scale and establishment of the beetle, *R. lophantheae* has been responsible for the survival of mature trees; but no seedlings are surviving⁹.

Long-term prospects are not good. A study monitoring *C. micronesica*, which was initially a healthy, stable population, concluded that Guam has already lost over 90% of its *C. micronesica* to CAS, and the trend line predicts extinction in the wild by 2019¹⁰. This is a first-class ecological disaster that few people know about, and one that was predicted as far back as 2000¹¹ but unfortunately discounted at the time. In a 2002 forest survey of Guam, *C. micronesica* was listed as the most numerous plant with a stem diameter greater than five inches [12.5 cm]. By 2006, the plant was on the IUCN Red List.

Small numbers of a second biocontrol agent, *Coccobius fulvus*, were imported to Guam from Florida and released in 2005 but the parasitoid did not establish. Current efforts are focused on trying to import *A. lingnanensis* from Hawaii, selected because it coexists with *R. lophantheae* as a natural enemy of CAS in Texas and Hawaii. This parasitoid is much smaller than *R. lophantheae*; it is hoped that it will attack CAS in refuges too small for the coccinellids to access, and will do a better job at protecting cycad seedlings.

Palau

News from Palau is currently more promising, although there is concern about the future of native cycads there also. Following the arrival of CAS in Guam in 2003, Palau banned imports of cycads in an effort to prevent invasion, but the scale eventually evaded quarantine restrictions and was found in Palau in 2008. While introduced cycads on the main islands of Koror and Babedao were initially infested, the main concern was for the future of the abundant native cycads found on the isolated Rock Islands.

Rhizobius lophantheae was obtained from Guam and released in Palau in 2009 (but not in the Rock Islands, as erroneously reported in BNI 31(1), March 2010, pp. 2N-3N); it established and dispersed well. Once the biocontrol agent had begun to disperse, no

further releases were necessary and project activity ceased. The last project survey, conducted in October 2009, found no scales on the native cycads in the Rock Islands, so no releases were made there. Awareness activities also ceased in December 2009, and the biocontrol agent rearing programme was wound down.

As of December 2012, ornamental cycads (mainly *Cycas revoluta*) in Koror and elsewhere show no obvious signs of scale infestation or damage: plants which were severely infested in 2008–09 now look healthy. The native cycads in the Rock Islands remain at risk, but there is little that can be done to protect them except regular checks for CAS infestation.

Taiwan^{1,2}

First found in Taiwan in 2000, CAS has since spread throughout the island. An estimated 110,000 cycads (of various ages) in nurseries in Taoyuan County were killed by the pest during the first year. Of particular concern is the scale's impact in Taitung Cycad Nature Reserve (290 ha), where it was first reported in 2004. This area was designated in 1986 specifically to protect the endemic Taitung cycad, *C. taitungensis*, a species on the IUCN Red List.

A preliminary survey in 2005 showed that 90% of Taitung cycads in sampling plots were infested, and accumulated mortality in the plots reached 37% by May 2010. The huge number of first instars sampled indicated a continuously growing and dispersing population of CAS and an increasing potential for damage to Taitung cycad. However, a high proportion of CAS (37%) was found to be parasitized by the encyrtid parasitic wasp *Arrhenophagus chionaspidis*, although there was a disparity between the sexes with parasitization rates for female scales averaging only 7% (maximum 14%).

The predatory beetle *Cybocephalus nipponicus* was introduced from Thailand in 2005. However, it was not an effective agent against the scale and interest is now focused on *A. chionaspidis*.

Indonesia

CAS was recorded from Indonesia recently; cycads in Bogor Botanical Garden and Bogor City were found to be heavily infested in late 2011¹. The pest was recorded from various sites in Indonesia in the past, and herbarium specimens from the Bogor *Cycas* collection from over 100 years ago contain armoured scales that may be CAS; but there is no mention of infestations on cycads in the early Dutch literature. If the scale was in Bogor years ago, it disappeared for several decades, possibly due to a lack of host plants there, and has either re-emerged or been re-introduced recently. There are five endemic species of *Cycas* in Indonesia which could be threatened with extinction by CAS.

Existing natural enemies appear unlikely to contribute to control in Indonesia. *Arrhenophagus chionaspidis* mainly parasitizes male CAS and its impact is reduced by a hyperparasitoid, *Signiphora bifasciata*¹.

Since the scale is already established in West Java and has probably spread further afield, a survey of CAS throughout the country is warranted. It is critical for the government to be alerted to the seriousness of this pest, and for the importance of saving the endemic cycads to be publicized. Given the difficulty of implementing quarantine inspections in Indonesia's many islands, the most effective action would be to establish classical biological control to maintain CAS populations at low levels that do not endanger the native cycad species.

*IUCN/SSN: The World Conservation Union/Species Survival Commission.

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5.4.8 Vertical stratification in predation of armored scale on *Cycas micronesica* seedlings

Please see next page.

Vertical Stratification of Predation on *Aulacaspis yasumatsui* Infesting *Cycas micronesica* Seedlings

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Abstract. Container-grown *Cycas micronesica* seedlings were purposefully infested with *Aulacaspis yasumatsui* and then installed at 0, 75, or 150 cm above the ground to investigate effects of infestation height on predation by *Rhyzobius lophanthae*. Significantly more scales on elevated seedlings were attacked by the predator. Our results indicate that lower predation at ground level by *R. lophanthae* may partly explain why the predator is not effectively controlling this armored scale epidemic on Guam. Ephemeral outbreaks of *A. yasumatsui* documented in quarterly surveys from Sept. 2006 until Aug. 2012 confirm the inadequate biological control. Our results illuminate the importance of fully evaluating the reliance on an alien predator as a solo biological control agent for an alien pest.

Aulacaspis yasumatsui Takagi (Hemiptera: Sternorrhyncha: Diaspididae) is a cycad-specific armored scale that invaded Guam in 2003 (Marler and Muniappan, 2006). This pest has combined with several other invasive insects to threaten *Cycas micronesica* K.D. Hill and *Cycas revoluta* Thunberg trees. These two *Cycas* species were widely planted throughout the urban landscape before the pest invasions, and the regionally endemic *C. micronesica* was a dominant forest species. *Coccobius fulvus* Compere & Annecke (Hymenoptera: Aphelinidae), an endoparasitoid, and the beetle *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae), a predator, were introduced as efforts to establish a biological control program (Moore et al., 2005). The endoparasitoid failed to establish despite multiple introductions, but the predator proliferated.

Cycas micronesica plant mortality after the scale invasion was epidemic, but slowed down after the widespread establishment of *R. lophanthae* (Marler and Lawrence, 2012). After seven years, the biological control by this predator has not proven to be completely effective, although the beetle is widespread and both adults and larvae feed on *A. yasumatsui* (Fig. 1). The decline in population of the

endemic *C. micronesica* elicited an endangered status by the International Union for Conservation of Nature by 2006 (Marler et al., 2006), and continuing mortality predicts extirpation from local forests by 2019 (Marler and Lawrence, 2012). A few *C. micronesica* and *C. revoluta* trees linger in commercial and home landscapes, but they do not display their former aesthetic appeal and are thus less valuable in the horticulture trade.

In attempts to understand why *R. lophanthae* has not been completely effective in the urban and natural landscapes, we have noted that the beetle does not effectively protect *C. micronesica* seedlings from the armored scale (Marler and Terry, 2011). An understanding of the causal effects of the limited predation is required to inform ongoing

horticultural management and biological control strategies. Toward that goal, our objective was to determine scale predation on seedlings positioned at the natural ground level and artificially elevated to the height of mature tree leaves. To improve interpretation, we monitored pest and predator incidence two years before and four years after the elevation experiment at the study site.

Materials and Methods

We initiated quarterly surveys to determine the extent of *A. yasumatsui* infestation and presence of *R. lophanthae* within a *C. micronesica* population on the east coast of Guam in Sept. 2006. We selected a forest fragment that was positioned between a private golf course and a heavily trafficked forest trail maintained by the golf course. This ecotone served as a source of natural enemies and/or pests that influenced maintenance procedures of landscape managers for this golf course and many home landscapes adjacent to the fragment. During each survey, the cycad trees along a 115-m transect were observed. There were 173 mature trees initially within the transect, and 18 trees remained at the final observation period in Aug. 2012. First, a ranking of 0 to 5 was assigned with 0 indicating no observed *A. yasumatsui* and 5 indicating acute levels of infestation. This ranking was defined by integrating the number of leaves with scale infestation and the observed scale density within the stem and leaf infestations. Second, the number of adult *R. lophanthae* beetles observed on one leaf per tree was recorded.

Two-year-old *C. micronesica* seedlings growing in 2.6-L containers were moved from an open nursery to a Lucite screen cage in July 2008. Each seedling had two to three 30- to 45-cm long leaves. We placed *C. micronesica* leaves infested with *A. yasumatsui* on top of the seedlings for several hours to allow scale crawlers to infest the containerized plants. We repeated this procedure several times and

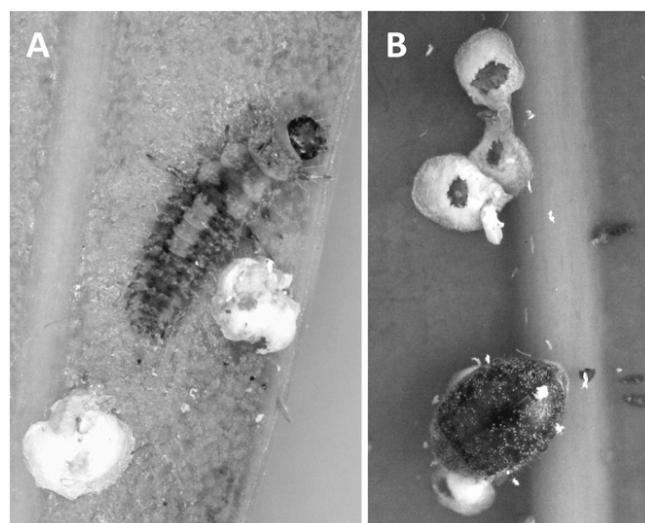


Fig. 1. *Rhyzobius lophanthae* larva (A) and adult (B) consuming *Aulacaspis yasumatsui* scales feeding on *Cycas micronesica* leaves.

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then kept the cage closed to exclude *R. lophantheae* predators. When the scale density reached ≈ 50 scales per cm^2 , we initiated the field test.

A total of 30 scale-infested container-grown seedlings were transported to the study site that was used for the quarterly surveys on 5 Sept. 2008. At each of 10 locations separated by a minimum of 20 m, we installed one containerized seedling at ground level, one containerized seedling at 75 cm height, and one containerized seedling at 150 cm height. The ground-level seedlings were inserted into a hole such that their position was natural, and none of the leaflets touched the ground or adjacent vegetation. Containers for the elevated seedlings were affixed to sapling trees by wire. Approximately once per week from 11 Sept. until 30 Oct., up to seven random leaflets per seedling were harvested, placed in plastic bags, and transported to the Univ. of Guam. Toward the end of the experiment, all leaflets on some of the seedlings had been harvested. Male and female scales and scales preyed on were counted under a stereomicroscope by inspecting 10 random 25-mm² squares per leaflet. Predation was ascertained if the covering of the male or female scale had been chewed open and the insect removed. There were no other scale predators in the system, so predation was restricted to *R. lophantheae*.

Statistical analysis was performed using R (R Development Core Team, 2011). We performed a linear regression of the ratio defined by total scales divided into predated scales as a function of height aboveground. We tested the zero slope null hypothesis using a permutation test using 10,000 Monte Carlo simulation trials. In each trial, values for the height variable were shuffled, ratio of predated scale covers at each height was recalculated, and a linear regression of the ratio as a function of height aboveground was performed. The number of trials yielding a slope exceeding the observed slope was used as an estimate of the *P* value associated with the null hypothesis.

Results and Discussion

Scale infestations of *C. micronesica* trees and observed presence of *R. lophantheae* adults exhibited classic predator-prey numerical response cycles from 2006 until 2012 (Fig. 2). Well after *R. lophantheae* was established in the habitat, outbreaks of the cycad pest occurred, as evidenced by scale outbreaks in mid-2009 and late 2011. These long-term trends indicate that biological control is successful on some levels but that outbreaks of the pest are still possible. The nature of these cycles with pest outbreaks and suppression and how they fit into a successful biological control program present difficult questions to answer.

We examined a total of 23,282 scales on leaflets sampled from cycad seedlings positioned at ground level, 75 cm, or 150 cm above the ground. Damage to *A. yasumatsui* scale covers by *R. lophantheae* increased with

height (Fig. 3). A linear regression of the ratio of damaged scale covers as a function of height in centimeters yielded a slope of 0.001278 (predation increased $\approx 13\%$ per meter). A permutation test rejected the zero slope null hypothesis (*P* = 0.0083). Predation was increased 29% at 75 cm in height and 42% at 150 cm in height above that at ground level.

Vertical stratification in tropical forests is known to influence general arthropod assemblages (Basset et al., 2001, 2003), including beetle assemblages (Stork and Grimbacher, 2006). Generalist predator arthropods are among the examples exhibiting disparity between ground and elevated strata (Beaulieu et al., 2010). Most of the published reports on coccinellid behavior in relation to spatial effects have focused on prey density effects (e.g., Schellhorn and Andow, 2005; Turchin and Kareiva, 1989). However, Ewert and Chiang (1966) and Ulyshen and Hanula (2007) trapped coccinellids during flight to reveal an influence of trap height on number and diversity of predators trapped. We have shown that seed set is not influenced by vertical stratification of *C. micronesica* female megastrobili, and used the results to infer that Guam's entomophilous pollinators do not exhibit a preferred vertical strata (Marler and

Niklas, 2011). Herein we show that a measurable dichotomy occurs between predation of *A. yasumatsui* feeding on *C. micronesica* seedling leaves positioned at ground level and those artificially positioned at 75 and 150 cm above the ground. Reduction of predation near the ground may partly explain why *R. lophantheae* has not protected small cycad plants from lethal infestations of the armored scale since the 2005 introduction of the predator.

Cycas revoluta and *C. micronesica* were prominent elements of the urban landscape before the invasion of this armored scale. Today, less than a decade later, they are rarely seen as a result of ongoing mortality. Furthermore, the unsightly appearance and poor health of the lingering specimens have removed the horticultural appeal of these trees. Guam experiences more tropical cyclones than any other region of the United States (Marler, 2001). The ability of these *Cycas* species to withstand tropical cyclones with little damage, combined with their resilience to recover quickly after damage by a tropical cyclone, continues to render them as ideal landscape candidates. The nursery and landscape industries will not return to promoting their use, however, until damage by

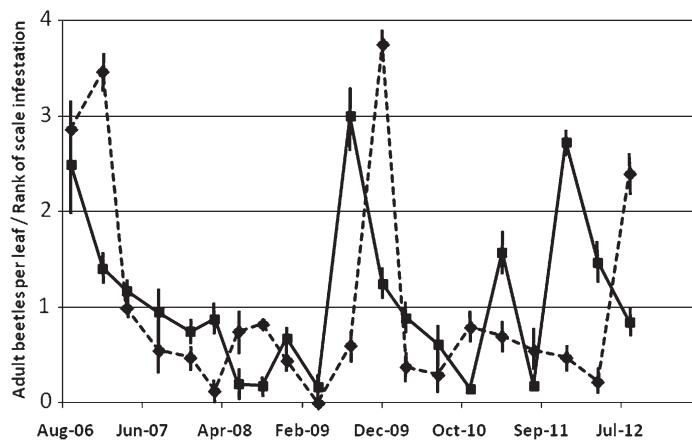


Fig. 2. The extent of *Aulacaspis yasumatsui* infestation on *Cycas micronesica* trees within an east coast habitat on Guam (square; 0 = no scales, 5 = heavy infestation) and the number of adult *Rhynchobius lophantheae* adults observed per leaf (diamond) on the same trees (from Sept. 2006 until Aug. 2012). Mean \pm SE.

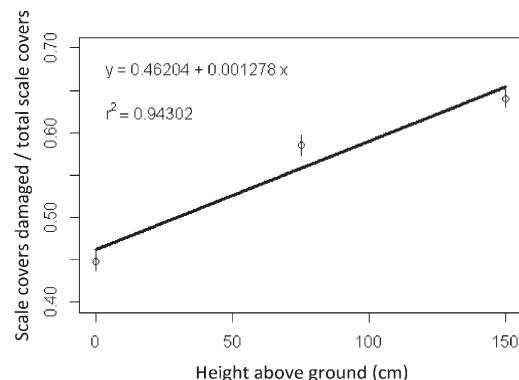


Fig. 3. The ratio of *Aulacaspis yasumatsui* scale covers damaged by *Rhynchobius lophantheae* in relation to total number of scales as a function of height aboveground. Vertical bars are 95% confidence limits for each estimated ratio.

the invasive pest is brought under sustainable biological control.

In the managed landscape and conservation plantings, the use of chemical protection likely will remain mandatory for several years after planting *C. micronesica* seedlings. However, after the seedlings have gained stature, the established *R. lophantheae* control may be sufficient to implement an integrated program that relies on the predator control as the foundation supplemented by frequent scouting to identify all nascent *A. yasumatsui* outbreaks. Expensive chemical protection may therefore be avoided until the surveys indicate the need. Our study is the first to our knowledge that validates a stratification of the interactions among insects and any cycad species.

We have shown that *C. revoluta* plants harbor *A. yasumatsui* infestations that are not visible during plant inspections (Marler and Moore, 2010). These cryptic infestations result from an inability of *R. lophantheae* to access scales hidden within crevices on the plant surfaces (Marler, 2012). Although biological control practitioners have long debated the merits of introducing single vs. multiple agents (see Ehler, 1990), the inability of the established predator to fully protect Guam's *Cycas* plants indicates additional biological control is urgently needed on Guam to augment that of the predator. Wiese et al. (2005) likewise reported that the parasitoid *Coccobius fulvus* did not sufficiently control *A. yasumatsui* in Miami, FL, as a sole biological control agent.

The causes of reduced scale predation by *R. lophantheae* near the ground are unknown, but a parasitoid biological control agent may not exhibit these same limitations. Furthermore, because a parasitoid would be much smaller than *R. lophantheae*, it would likely be better able to access scale infestations within

cracks and crevices on *C. micronesica* and *C. revoluta* trees.

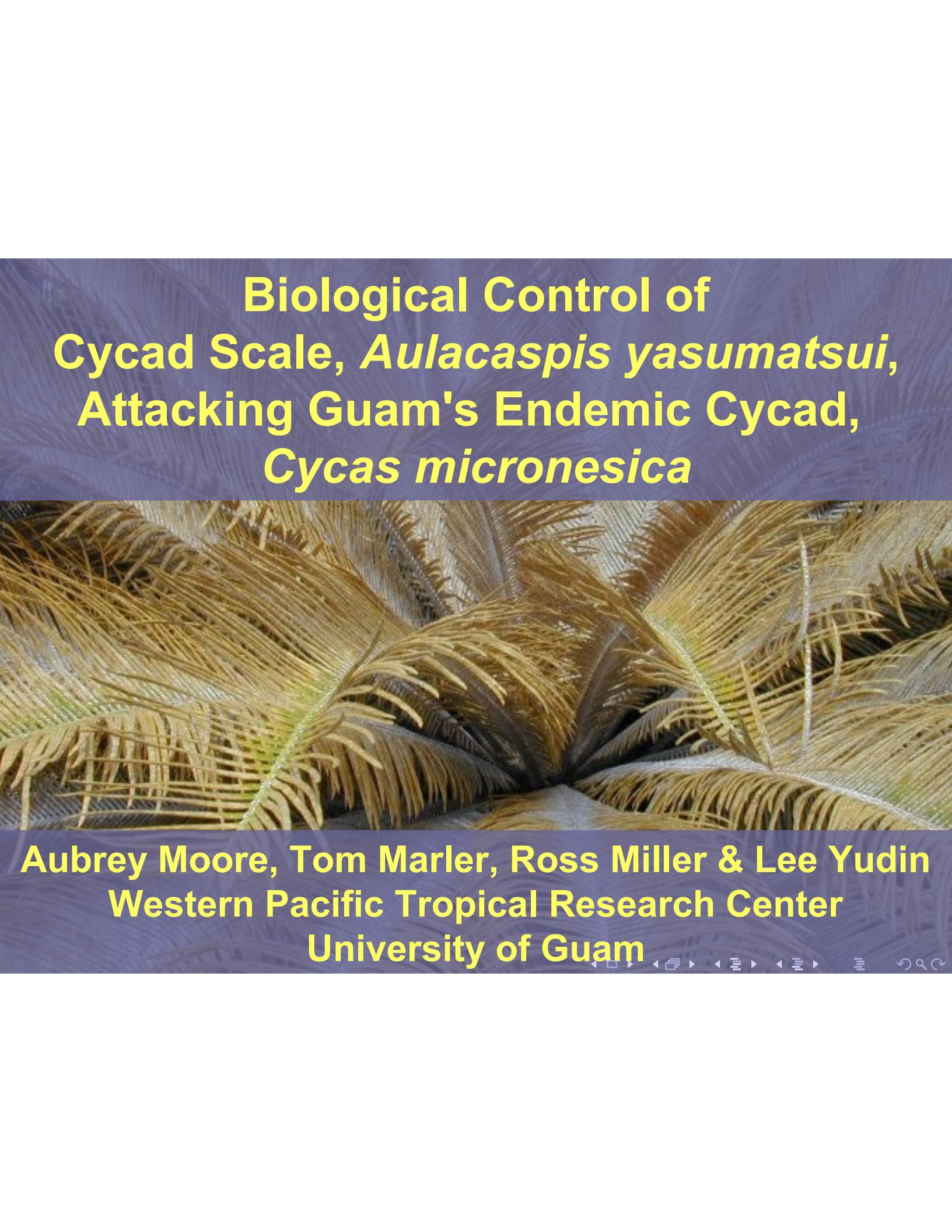
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5.5 Evidence: Presentations

5.5.1 Biological control of cycad scale, *Aulacaspis yasumatsui*, attacking Guams endemic cycad, *Cycas micronesica*

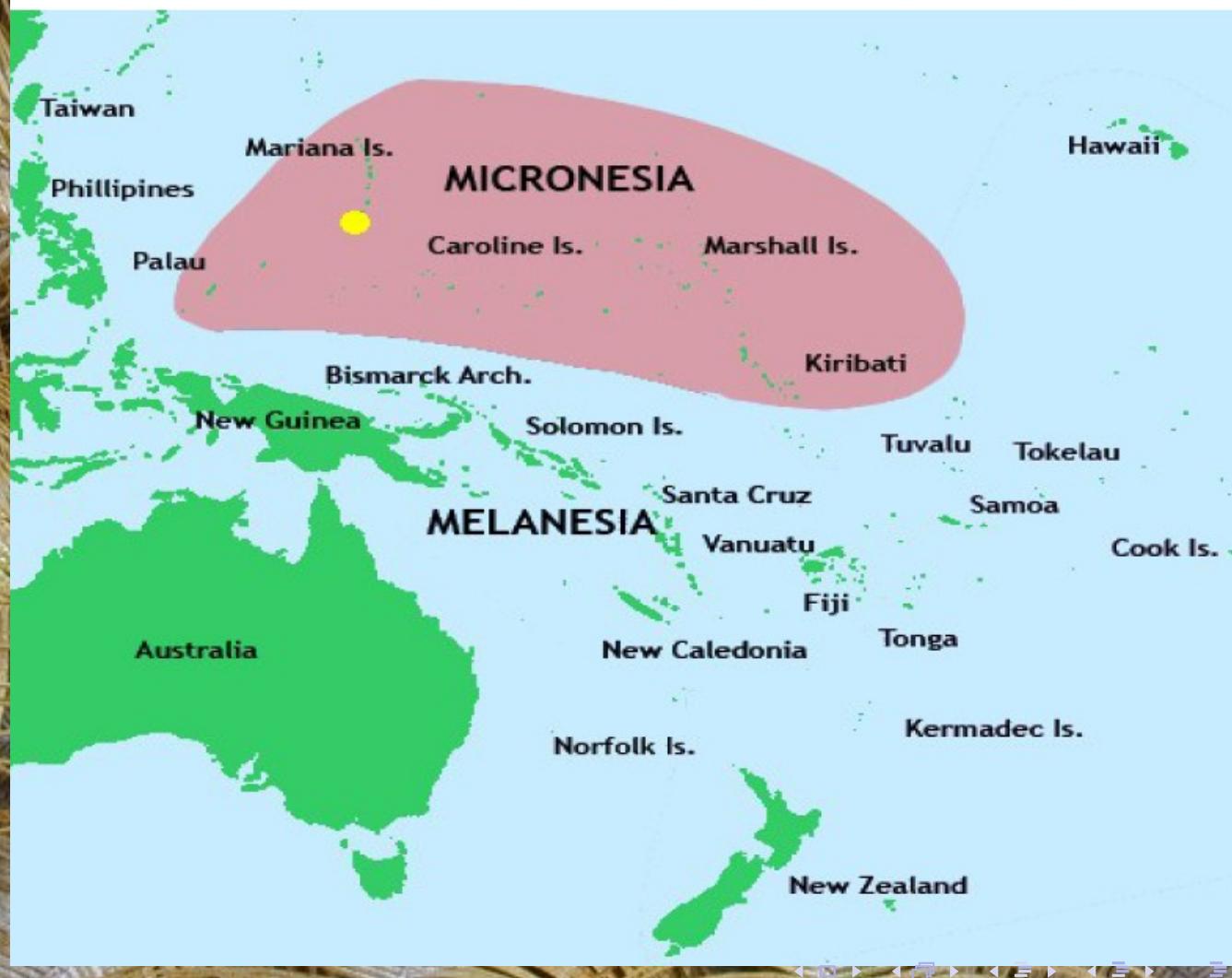
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Biological Control of Cycad Scale, *Aulacaspis yasumatsui*, Attacking Guam's Endemic Cycad, *Cycas micronesica*

**Aubrey Moore, Tom Marler, Ross Miller & Lee Yudin
Western Pacific Tropical Research Center
University of Guam**

Where is Guam?



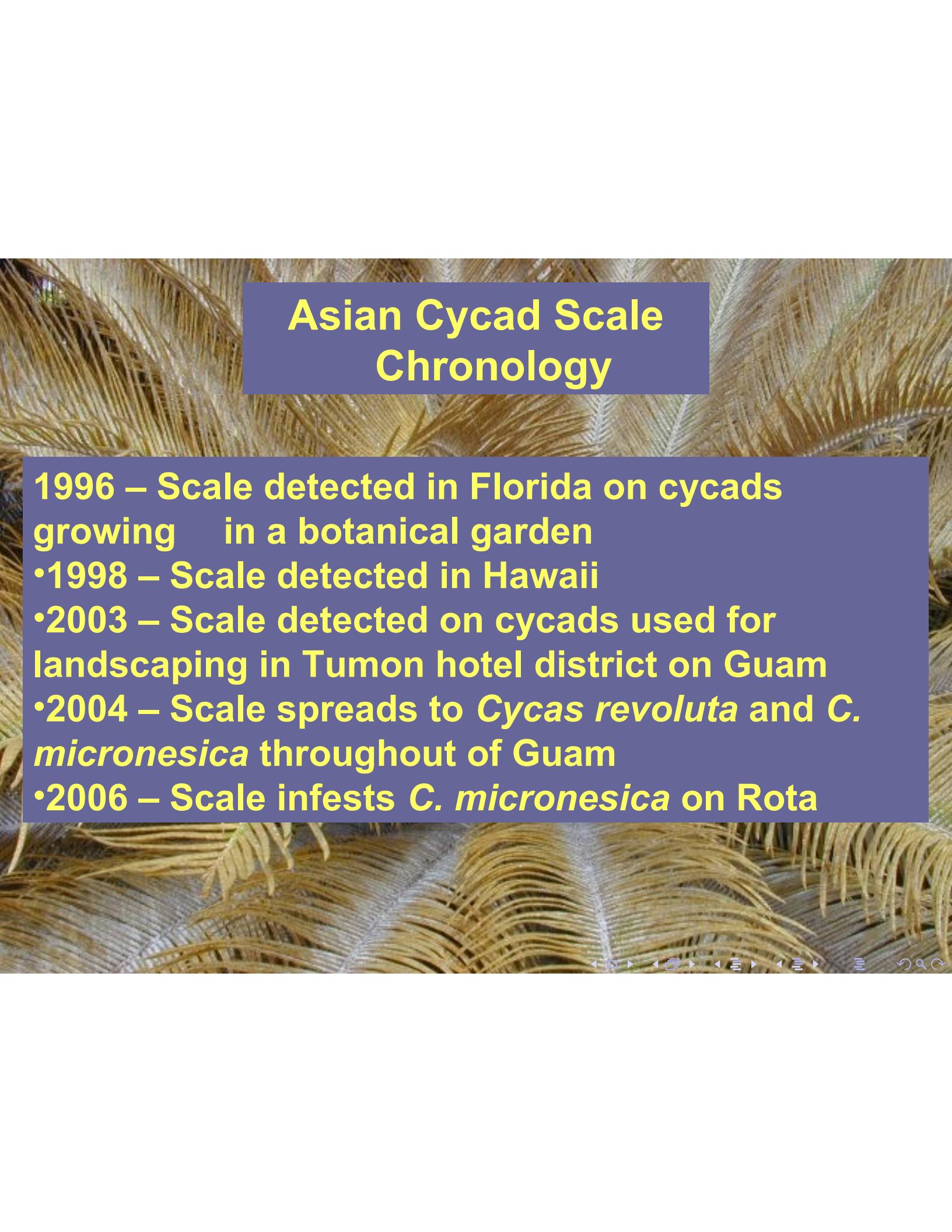
Major Biological Invasions on Guam

- ▶ Brown Treesnake (arrived around 1945)
 - ▶ Killed most of Guam's birds and small mammals. Caused 7 bird extinctions.
- ▶ Asian Cycad Scale (detected 2003)
 - ▶ Threatens survival of Guam's endemic *Cycas micronesica*, listed as most numerous tree in the 2002 Guam Forest Survey
- ▶ Coconut Rhinoceros Beetle (detected 2007)
 - ▶ Threatens Guam's coconut palms, listed as 2nd most numerous tree in 2002 Guam Forest Survey
- ▶ Little Fire Ant (detected 2011)
 - ▶ Threatens most animals remaining in Guam's forests



A close-up photograph of several cycad leaves, showing their characteristic pinnate venation and golden-brown color. The leaves are overlapping, creating a dense, textured background.

Asian Cycad Scale
***Aulacaspis yasumatsui* Tagaki 1972**
DIASPIDIDAE



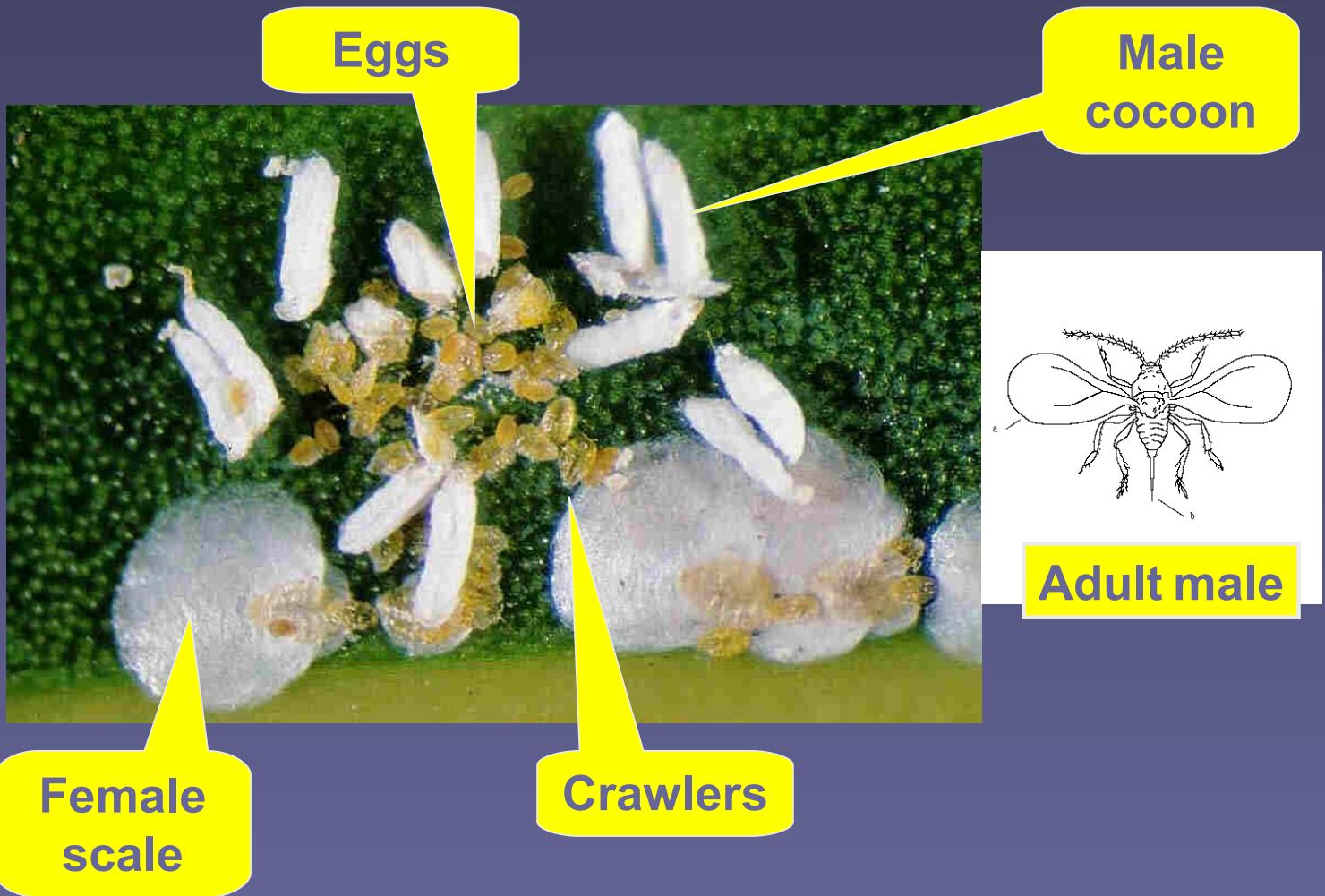
Asian Cycad Scale Chronology

- 1996 – Scale detected in Florida on cycads growing in a botanical garden
- 1998 – Scale detected in Hawaii
- 2003 – Scale detected on cycads used for landscaping in Tumon hotel district on Guam
- 2004 – Scale spreads to *Cycas revoluta* and *C. micronesica* throughout of Guam
- 2006 – Scale infests *C. micronesica* on Rota





Scale Morphology & Life History





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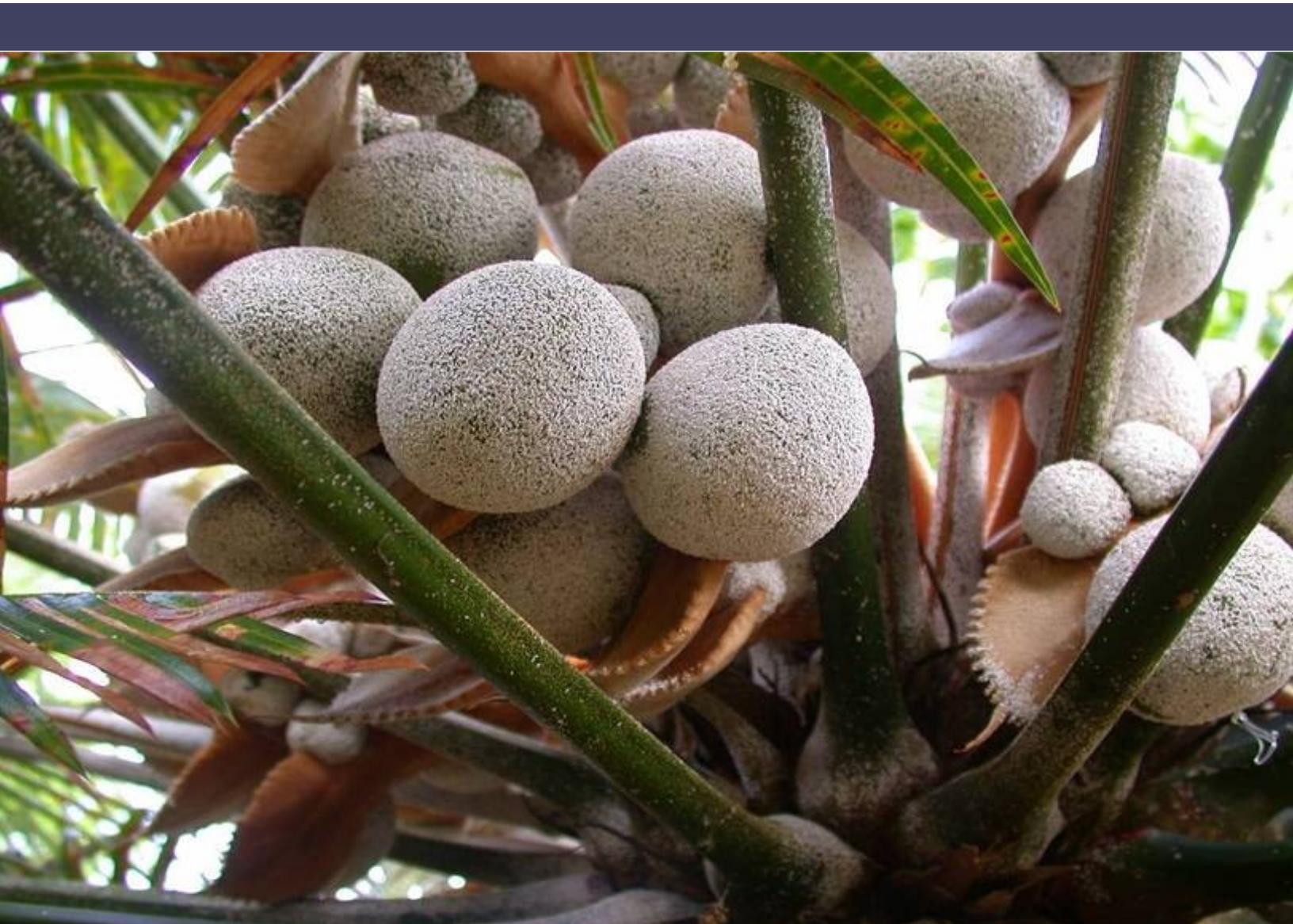














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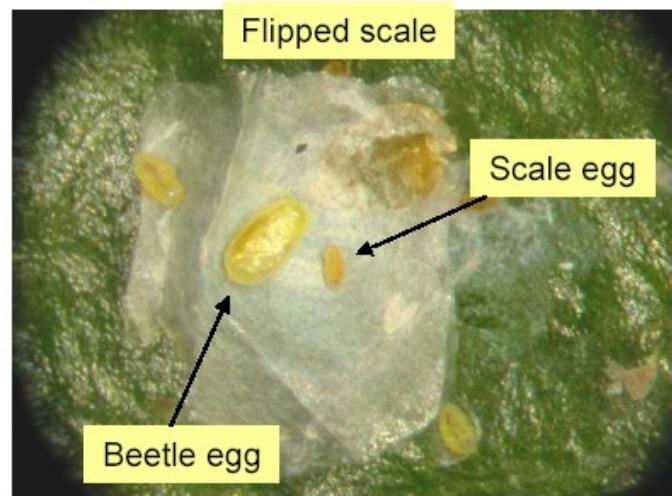
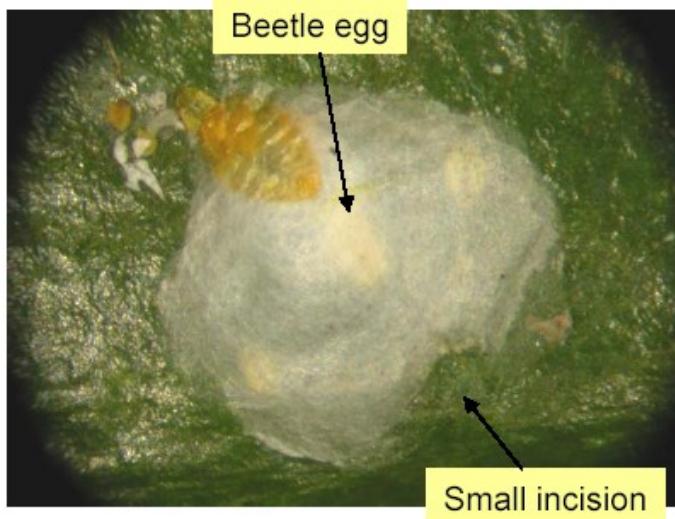
Biocontrol Attempts

- ▶ COCCINELLIDAE: *Rhyzobius lophantheae* imported from Hawaii 2004
 - ▶ Single attempt; Established Immediately
- ▶ APHELINIDAE: *Coccobius fulvus* from China via Florida starting in 2005
 - ▶ Several attempts; lab colony died; field releases did not establish
- ▶ APHELINIDAE: *Aphytis lignanensis* imported from Hawaii 2012
 - ▶ Single attempt; lab colony died prior to field release

Rhizobius lophanthae (COCCINELLIDAE), 'purple scale destroyer'

- ▶ both adults and larvae feed on Diaspidids (armored scales)
- ▶ introduced from Australia to California in 1892; from California to Hawaii in 1894
- ▶ Released on Guam in 1925 & 1926, but was never recovered

Top view of the Asian Cycad scales



Photos courtesy of Stacey Chun, University of Hawaii, Hilo









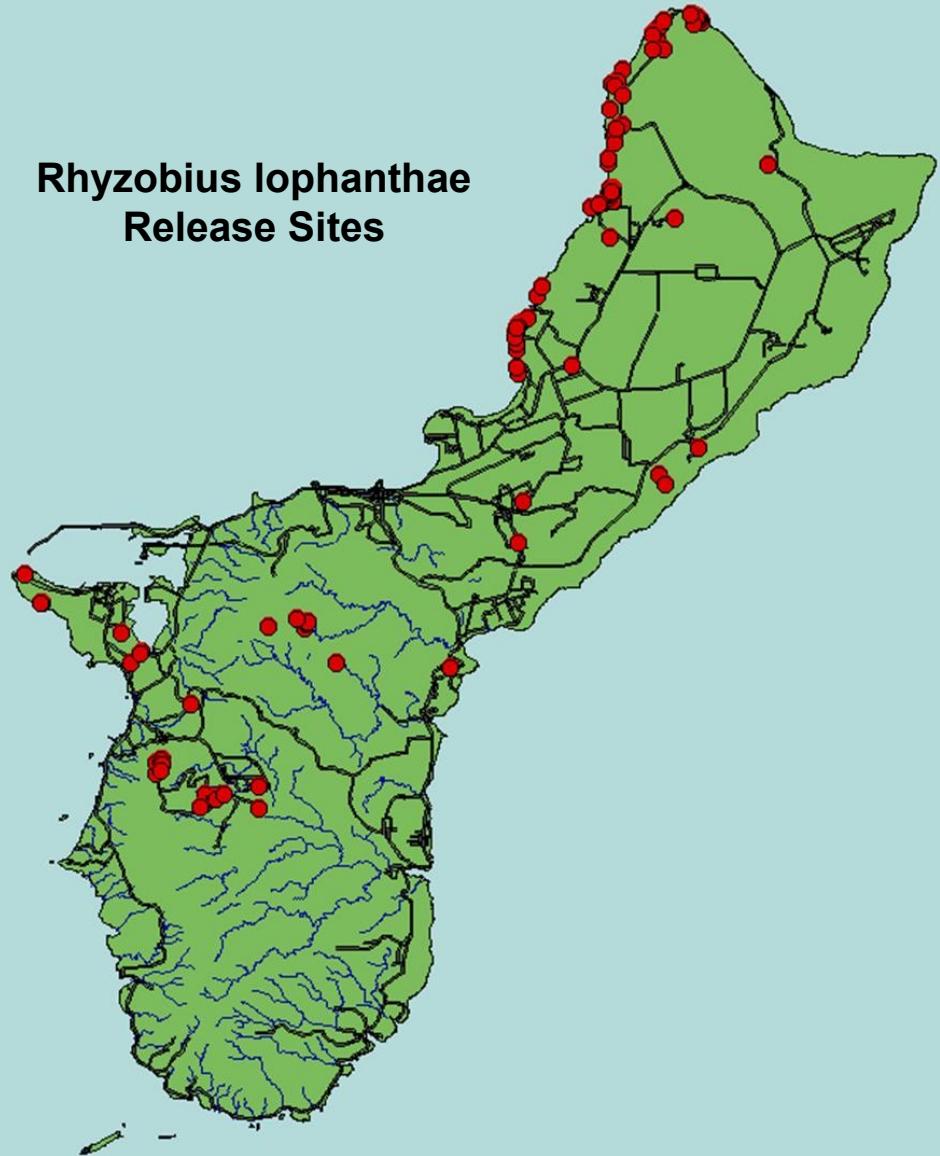








Rhyzobius lophanthae Release Sites



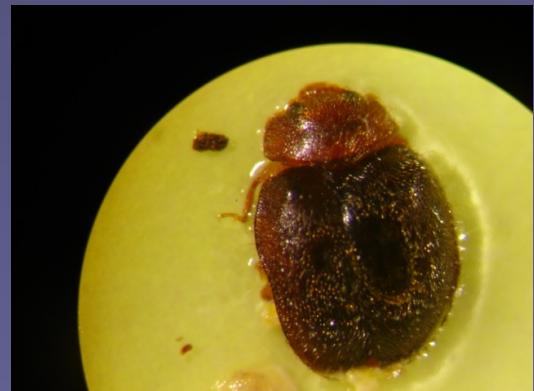
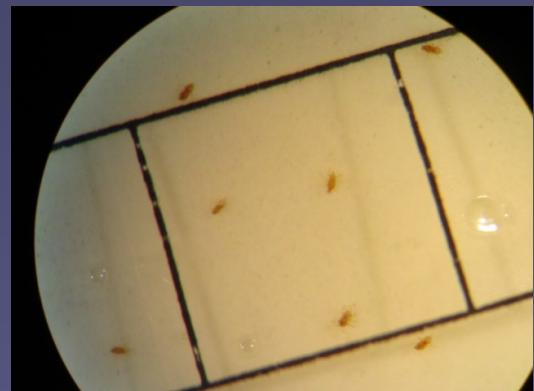
A photograph of a yellow sticky trap mounted on a grey pole, positioned in front of a tropical landscape. The background features dense green foliage, a sandy beach, and a vast blue ocean under a sky with scattered white clouds.

Sticky Traps

Stereoscope



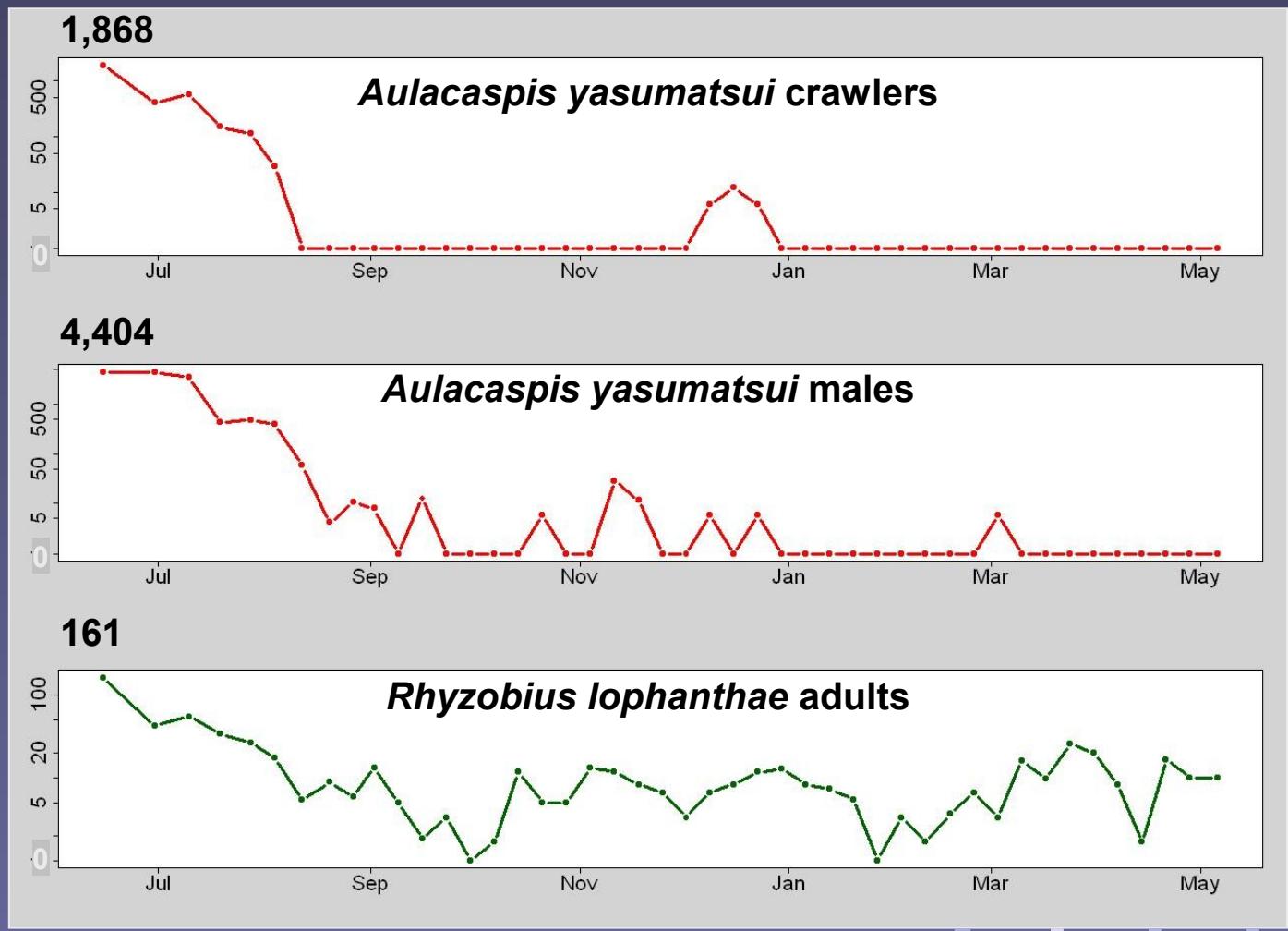
Aulacaspis yasumatsui
Adult males & Crawlers



Rhyzobius lopanthae
Adults

Insects per m² per day on Sticky Traps

Ritidian Pt.; June 2005 - May 2006



Current *Rhyzobius lophanthae* Status

- ▶ *R. lophanthae* is ubiquitous on Guam. It is almost impossible to find Asian cycad scale which is not being attacked by larvae and adults.

So the biocontrol program was a success and the cycads must be recovering by now, right? ...

Current *Cycas micronesica* Status

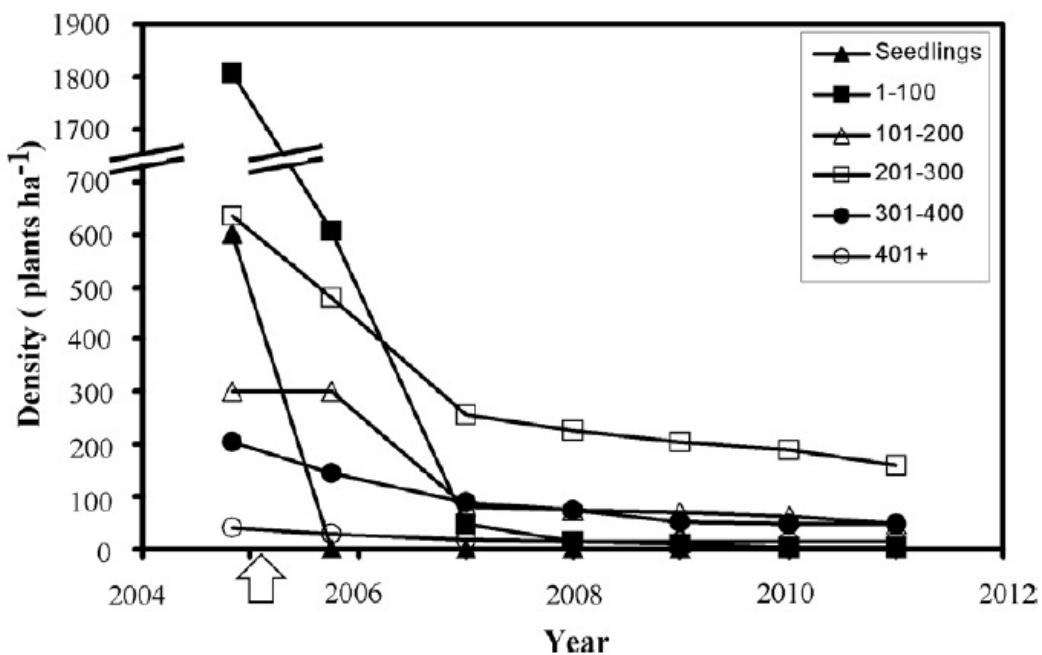


Figure 2. The influence of plant height size categories (cm) on survival of *Cycas micronesica* following the establishment of *Aulacaspis yasumatsui* in western Guam. The x-axis refers to January of each calendar year. Arrow on x-axis marks the initial infestation of *A. yasumatsui* in the study habitat.

Marler, T.E. and J.H. Lawrence. 2012. Demography of *Cycas micronesica* on Guam following introduction of the armoured scale



Current *Cycas micronesica* Status

The *C. micronesica* population is still in decline.

- ▶ In 2006, *C. micronesica* was placed on the IUCN Red List of Threatened Species and it remains on this list
- ▶ No reproduction on Guam has been observed since 2005.
- ▶ Only 7% of the original plants survive as of January 2013.
- ▶ Local extirpation predicted in 2019 if current trend persists.

Failure Analysis

R. lophantheae fails as a stand-alone biocontrol agent for Asian Cycad Scale because:

- ▶ *R. lophantheae* is too big to reach a significant proportion of the scale insects which live in small cracks and voids within plant structures
 - ▶ Marler, T.E. and A. Moore 2010. Cryptic scale infestations on *Cycas revoluta* facilitate scale invasions. Hort. Sci. 45: 837-839.
- ▶ *R. lophantheae* does not prey on scale insects living beneath trichomes on *C. revoluta*
 - ▶ Marler, T.E. 2012. Boomeranging in structural defense: Phytophagous insect uses cycad trichomes to defend against entomophagy. Plant Signaling & Behavior 7:1484 –1487.
- ▶ *R. lophantheae* predation decreases with proximity to the ground.
 - ▶ Marler, T.E., R. Miller, and A. Moore 2013. Vertical stratification of predation on *Aulacaspis yasumatsui* infesting *Cycas micronesica* seedlings. HortScience 48: 60–62.

Current Cycad Scale Biocontrol Objectives

- ▶ We are currently attempting to introduce a parasitoid in the hope that its smaller size will allow it to attack scale insects which escape beetle predation by hiding in small spaces within the plant structures.
- ▶ *Aphytis lignanensis* has been chosen as a candidate because it coexists with *R. lophnathae* in Hawaii and Texas.

Concluding Comments

- ▶ The predaceous lady beetle, *R. lophantheae* has failed as a stand-alone biocontrol agent for Asian cycad scale, even though it established readily and has become ubiquitous.
- ▶ Presence of *R. lophantheae* has thwarted our attempts to establish parasitoids as biocontrol agents for Asian cycad scale.
- ▶ If you wish to introduce predators and parasitoids, it may be easier to establish parasitoids first, then predators.

**Invasive species aren't all bad.
They provide job security for biologists.**



5.5.2 Biological Control of Cycad Scale, *Aulacaspis yasumatsui*, Attacking Guam's Endemic Cycad , *Cycas micronesica*

Please see next page.

5.5.3 A coalition of invasive species attacks Guams native cycads

Please see next page.



A Coalition of Invasive Species Attacks Guam's Endemic Cycad, *Cycas micronesica*

Aubrey Moore, Ross H. Miller, Thomas E. Marler and Lee S. Yudin

Western Pacific Tropical Research Center, University of Guam, Mangilao, Guam 96923, USA

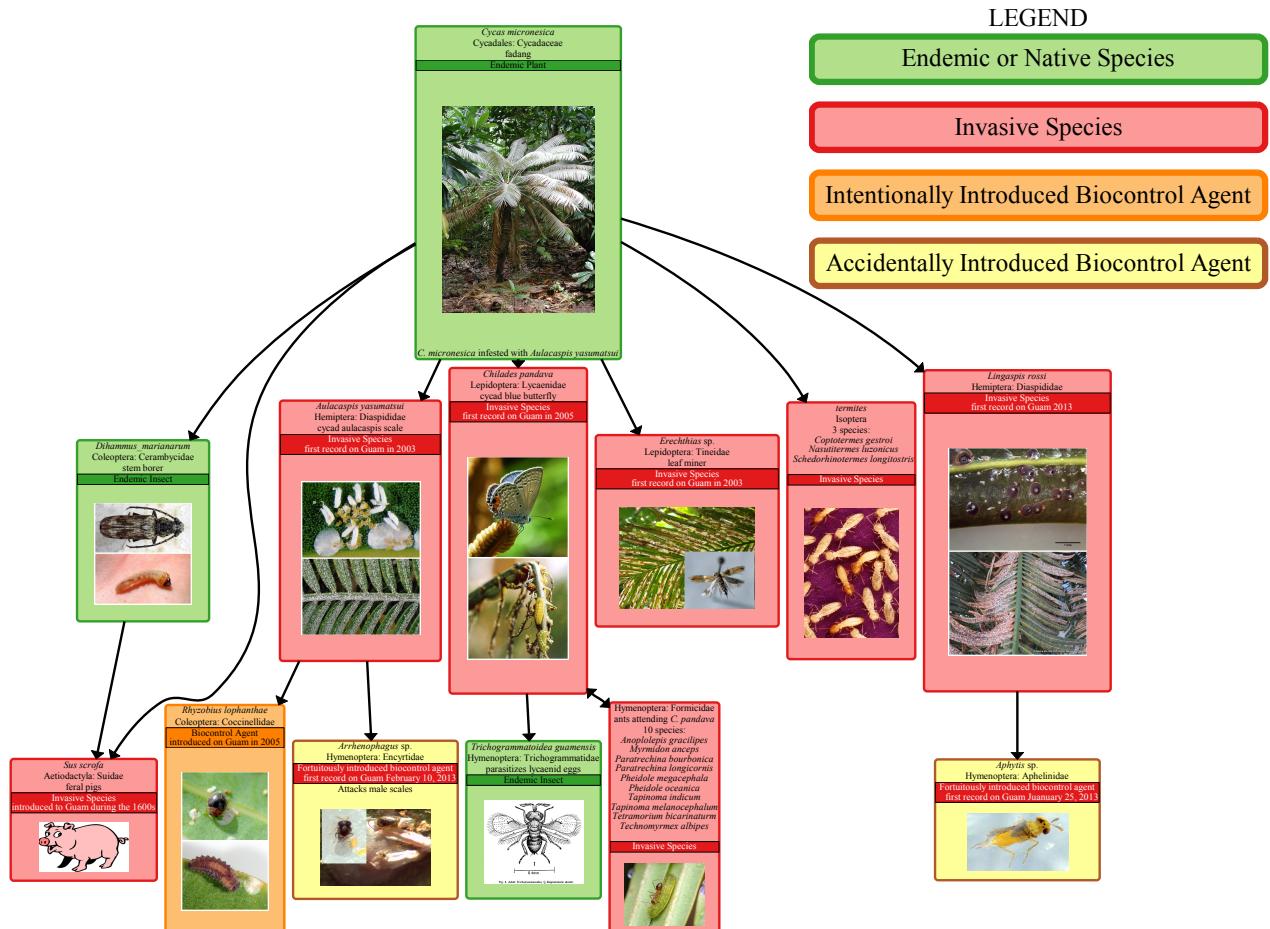


Figure 1: Summary of ecological relationships between *C. micronesica* and invasive species which threaten its existence. Arrows indicate which species benefit from relationships.

A 2002 forest survey listed *Cycas micronesica*, locally known as "fadang", as the most numerous tree-sized plant in Guam's forests. In 2006 *C. micronesica* was placed on the IUCN Red List of Threatened Species in response to high mortality from simultaneous attack by recently introduced invasive species including the cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui*, the cycad blue butterfly, *Chilades pandava*, and a lepidopteran leafminer, *Erechthias sp.* The coccinellid, *Rhyzobius lophanthae* was established as an effective biological control agent for CAS. However, the cycads continue to decline due to damage from CAS and other herbivores. In some areas of Guam, 90% of *C. micronesica* have been killed and the plant could be extirpated from the wild by 2019 if current trends persist.

References

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Marler, T.E. and R. Muniappan. 2006. Pests of *Cycas micronesica* leaf, stem, and male reproductive tissues with notes on current threat status. Micronesica 39: 1-9.
Marler, T. E. and A. Moore 2010. Cryptic scale infestations on *Cycas revoluta* facilitate scale invasions. HortScience 45(5): 837839.
Marler, T. E., L. S. Yudin and A. Moore 2011. *Schedorhinotermes longirostris* (Isoptera: Rhinotermitidae) on Guam adds to assault on the endemic *Cycas micronesica*. Florida Entomologist 94(3): 702-703.

Marler, T.E. and J.H. Lawrence 2012. Demography of *Cycas micronesica* on Guam following introduction of the armoured scale *Aulacaspis yasumatsui*. J. Trop. Ecol. 28:233242.

Marler, T. E. 2013. Temporal variations in leaf miner, butterfly, and stem borer infestations of *Cycas micronesica* in relation to *Aulacaspis yasumatsui* incidence. HortScience 48(10):13341338.

Marler, T. E. & J. H. Lawrence 2013. Phytophagous insects reduce cycad resistance to tropical cyclone winds and impair storm recovery. HortScience 48(10):12241226.

ACKNOWLEDGMENTS

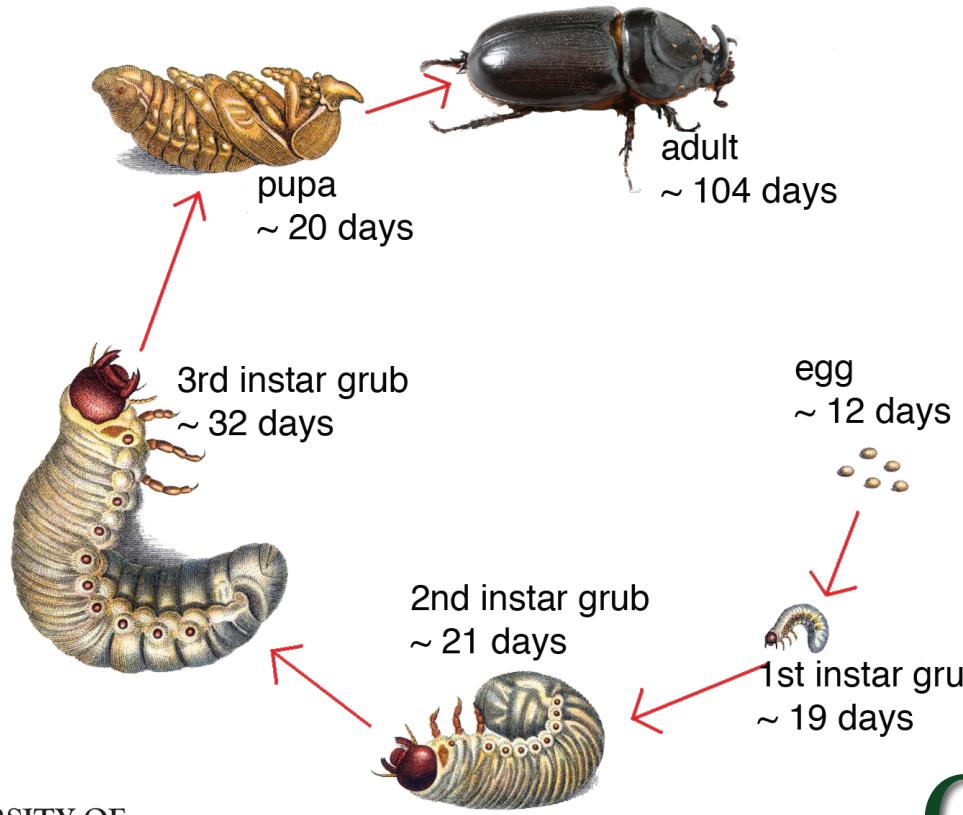
Data are from projects supported by grants from the US Forest Service, the US Fish and Wildlife Service, and USDA-APHIS.

5.5.4 Poster: Life Cycle of the Coconut Rhinoceros Beetle, *Oryctes rhinoceros*

Please see next page.

Life Cycle of the Coconut Rhinoceros Beetle

Oryctes rhinoceros



UNIVERSITY OF
GUAM
UNIBETSEDĀT GUAHAN

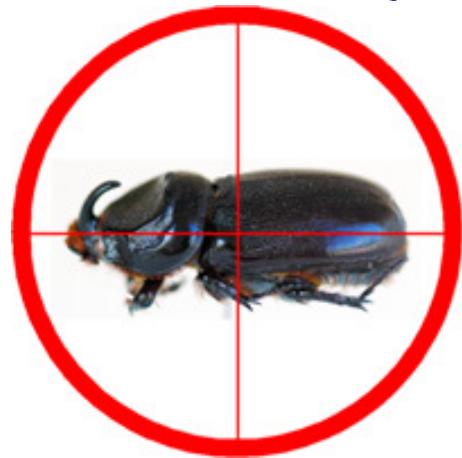
Olympia Terral, Roland Quitugua, Dr. Aubrey Moore
UOG Cooperative Extension Service
Agriculture & Natural Resources
Tel: 671-725-2080

CNAS
college of natural
& applied sciences

5.5.5 Overview of the Guam coconut rhinoceros beetle eradication project

Please see next page.

Overview of the Guam Coconut Rhinoceros Beetle Eradication Project



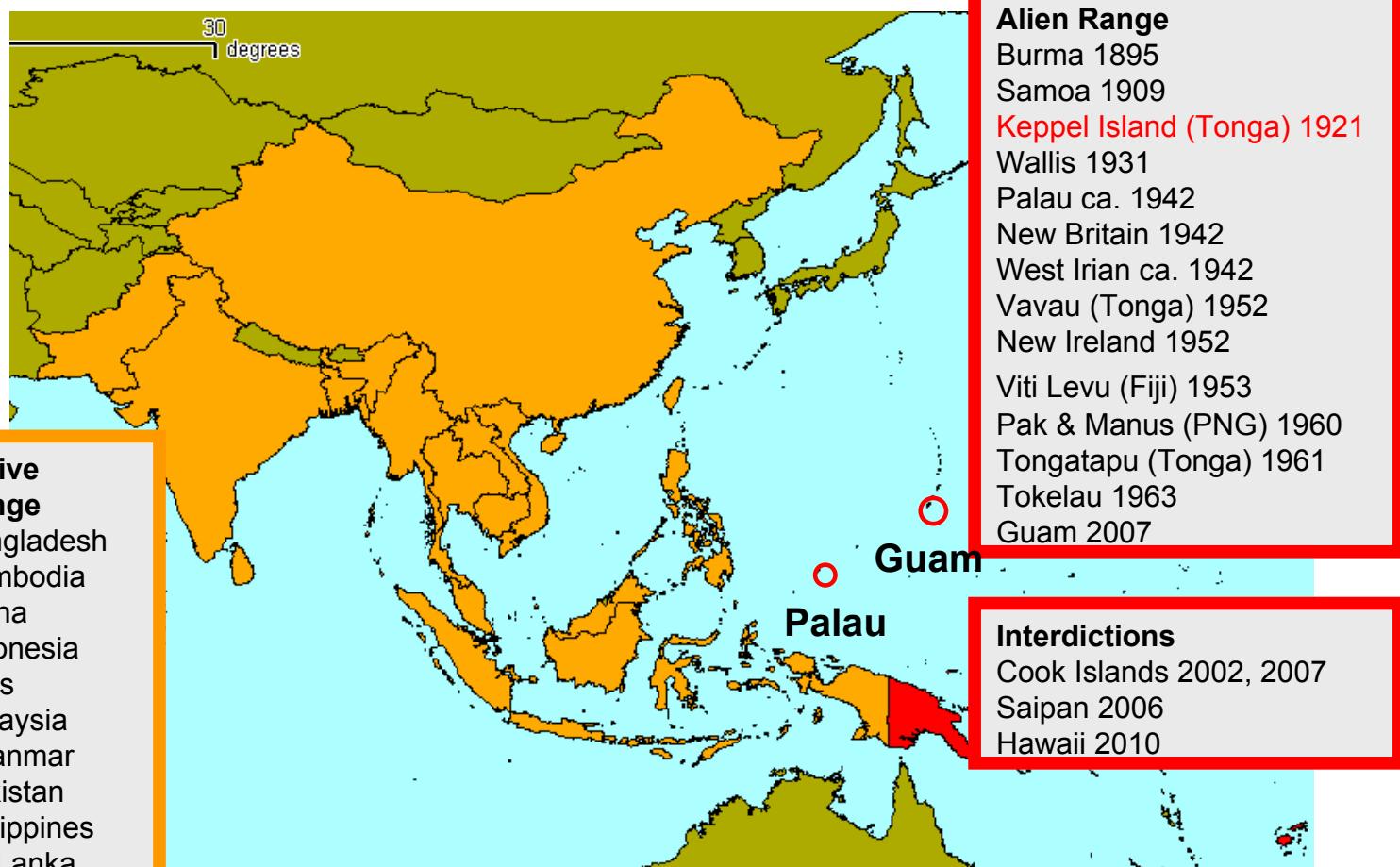
Hawaii CRB ICS, January 22, 2014

Aubrey Moore and Roland Quitugua
University of Guam Cooperative Extension Service

First Coconut Rhinoceros Beetle Collected on Guam 11-Sep-2007, Tumon Bay



Oryctes rhinoceros Distribution













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Please Don't Do This



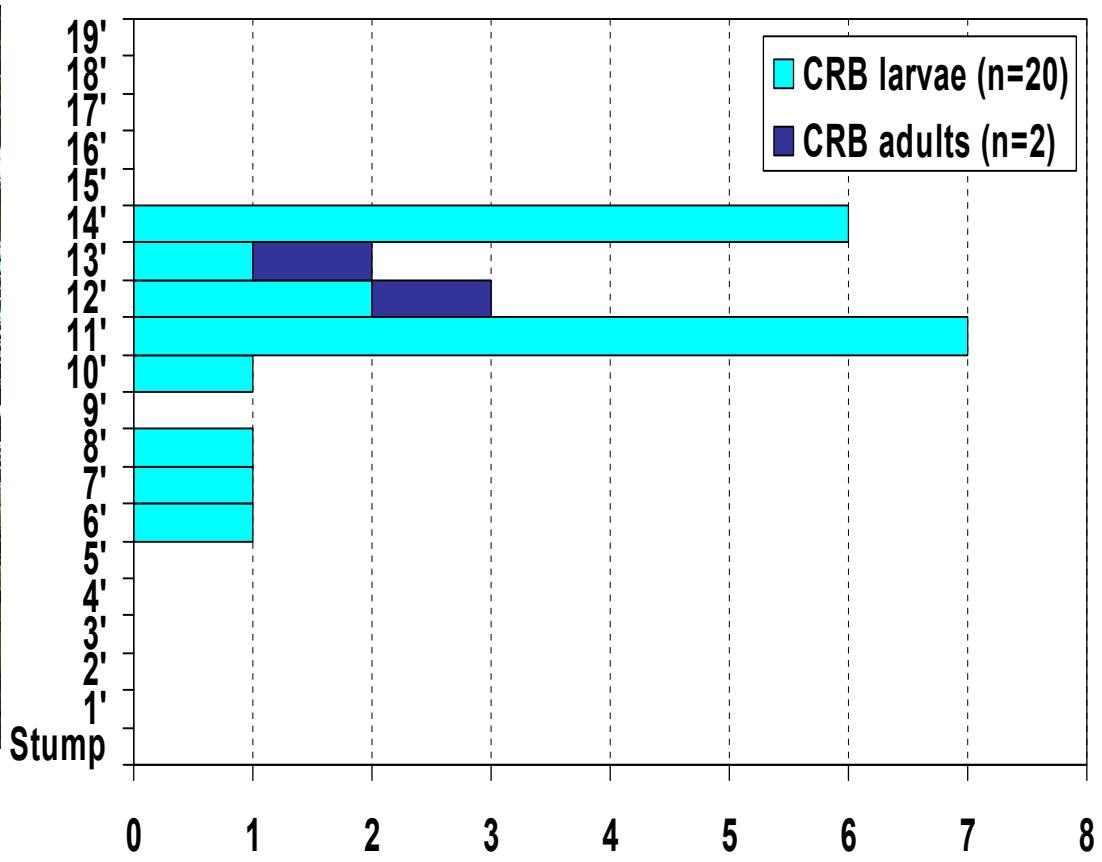
Or This







Vertical Distribution of CRB Larvae & Adults in Standing Dead Coconut Trankilidat, Guam; 25 Oct 2007



Novel CRB Behavior on Guam: Arboreal Development

CRB extracted from the crowns
of 121 felled coconut palms



Eggs	99
L1	40
L2	72
L3	210
Pupae	25
Adult males	34
Adult females	30
Total	510
Mean per tree	4.21

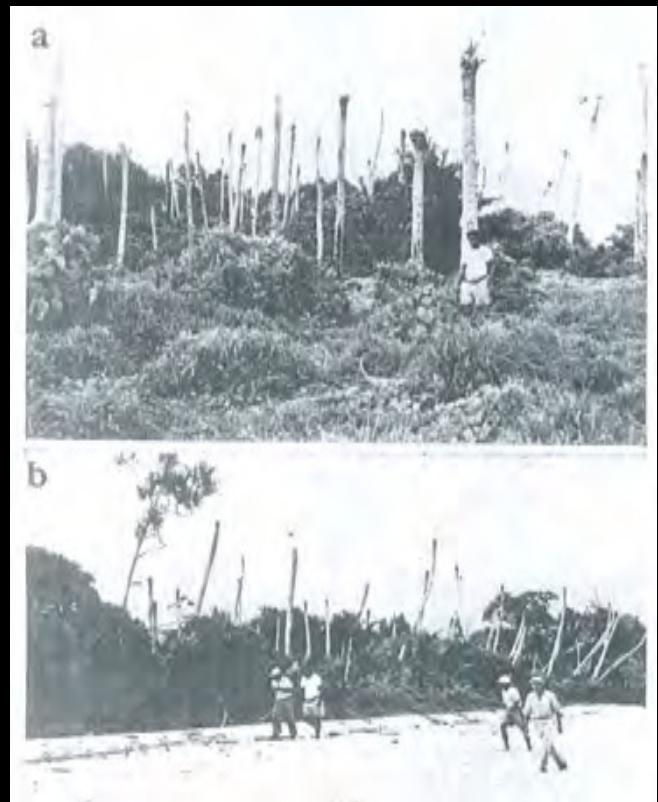


ADULTS KILL TREES

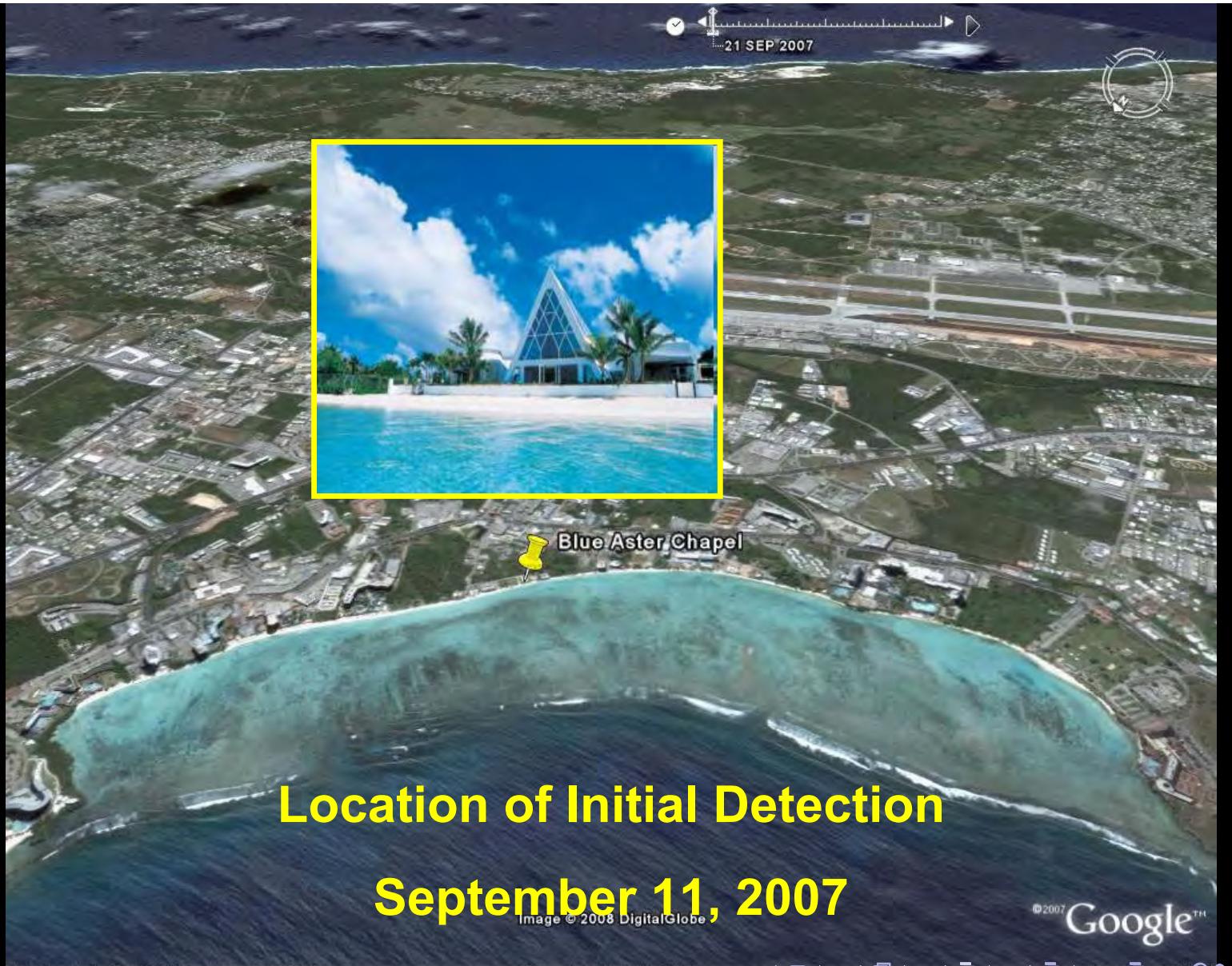
LARVAE FEED ON
DEAD TREES



Coconut palms killed by *Oryctes rhinoceros*; Viti Levu Island, Fiji; 1973
Source: ?



Coconut palms killed by *Oryctes rhinoceros*; Peleliu Island, Palau 1951
Source: Gressitt 1953



Location of Initial Detection

September 11, 2007

Image © 2008 DigitalGlobe

©2007 Google™

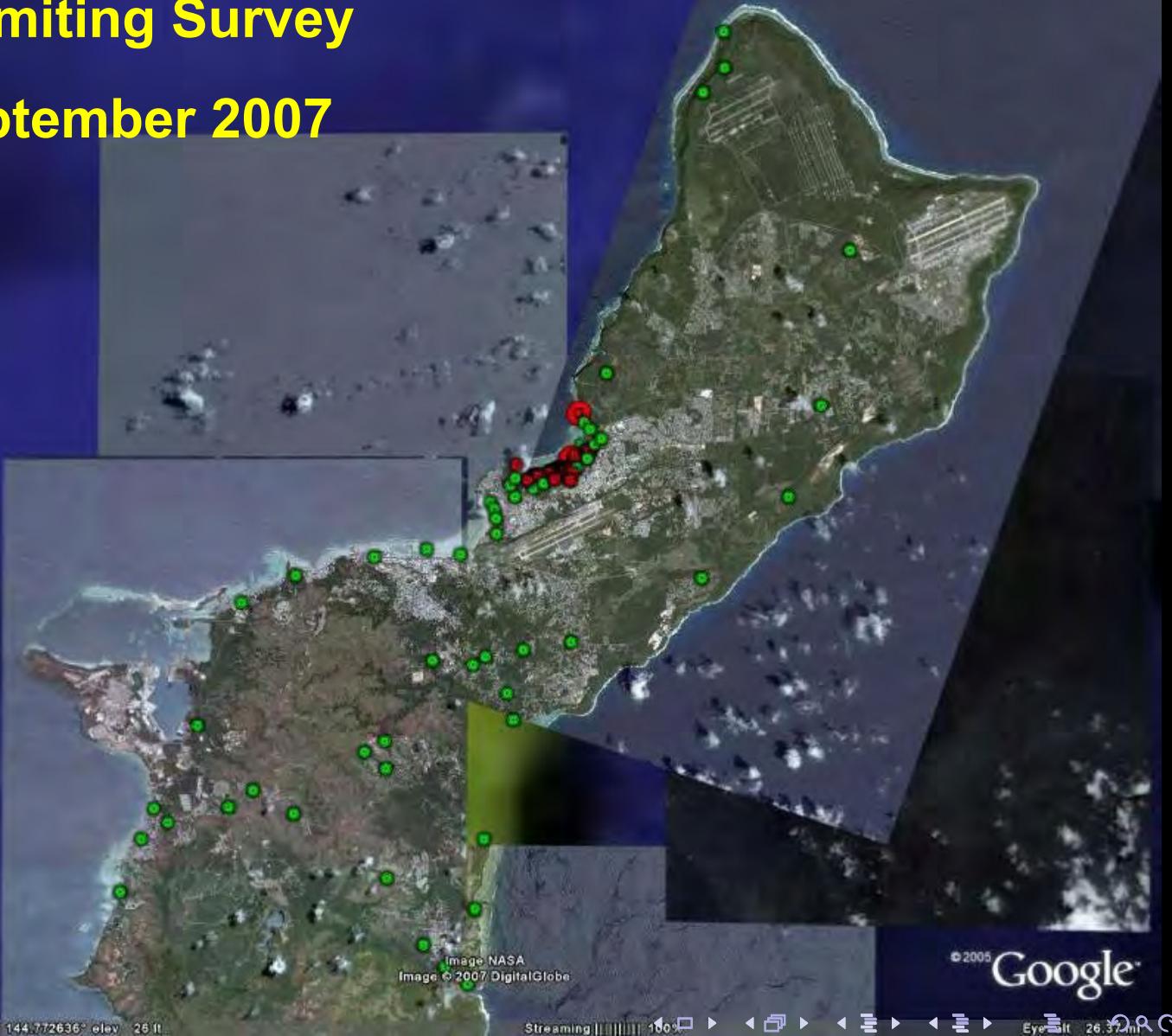
Pointer lat 13.505226° lon 144.802428°

Streaming ||||||| 100%

◀ □ ▶ ◀ □ ▶ ◀ □ ▶ ◀ □ ▶ Eye a 64 150

Delimiting Survey

September 2007



Guam Coconut Rhinoceros Eradication Project **ORGANIZATION**

Partners:

USDA-APHIS

Guam Dept. of Agriculture

University of Guam

Funding:

USDA-APHIS

US Forest Service

GovGuam

Guam Coconut Rhinoceros Eradication Project

TACTICS

Quarantine

Limit accidental transportation to uninfested parts of Guam.

Pheromone Traps

Capture adults and detect spread of the beetle population

Sanitation

Kill immatures and remove breeding sites

Detector Dogs

Efficient discovery of breeding sites.

Chemical Control

Injectable systemics for adults; spot treatments for breeding sites.

Biocontrol

Autodissemination of *Oryctes* virus



Initial Quarantine Area

September 2007





PHEROMONE TRAPS

- Mass trapping unsuccessful
- Traps useful for monitoring

Trap Data Entry Form

Mozilla Firefox

File Edit View History Delicious Bookmarks Tools Help

http://guaminsects.net/oryctes/upload_site_visit_gpx_3.php

New guinea_sugarcane Encyclopedia of Life F... webftp UOG mail Guam mail label printer weather Insect World Agriculture and Natural... We Are Guahan

http://guaminsects.e_visit_gpx_3.php

Upload Trap Visit GPX file to Database

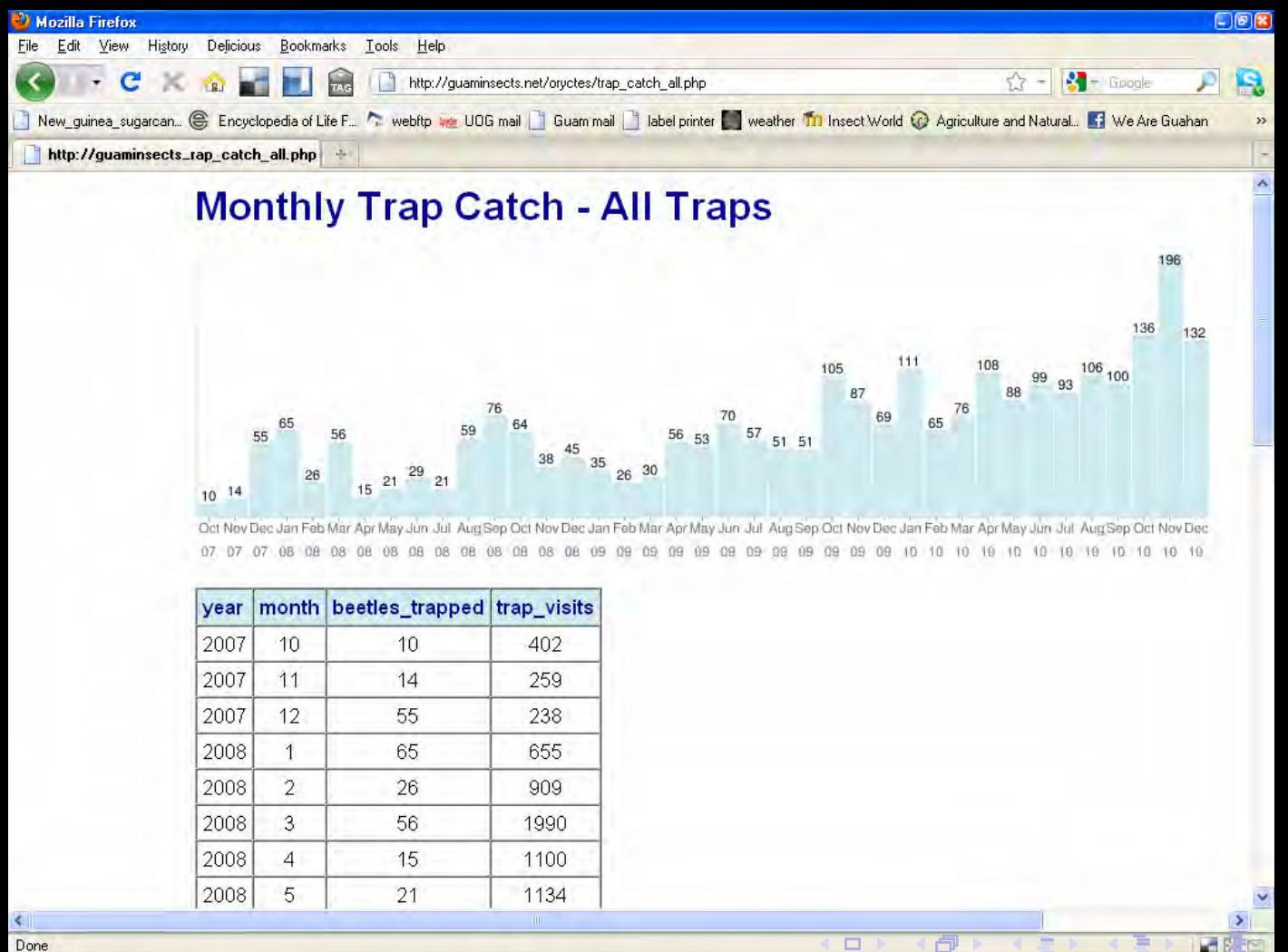
Trapper(s):

Trap Visit Date:

Choose a GPX file to upload:

Done

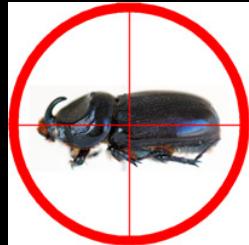
Online Trap Data Report



Visualization of Trap Catch Data

Aubrey Moore

Guam Coconut Rhinoceros Beetle Eradication Project



Generated 2014-01-08 20:23:57

Path: C:/Documents and Settings/Administrator/My Documents/CRB monthly surveillance reports/map dev

R script: makeMaps.R

Brew file: makeBeamer.txt

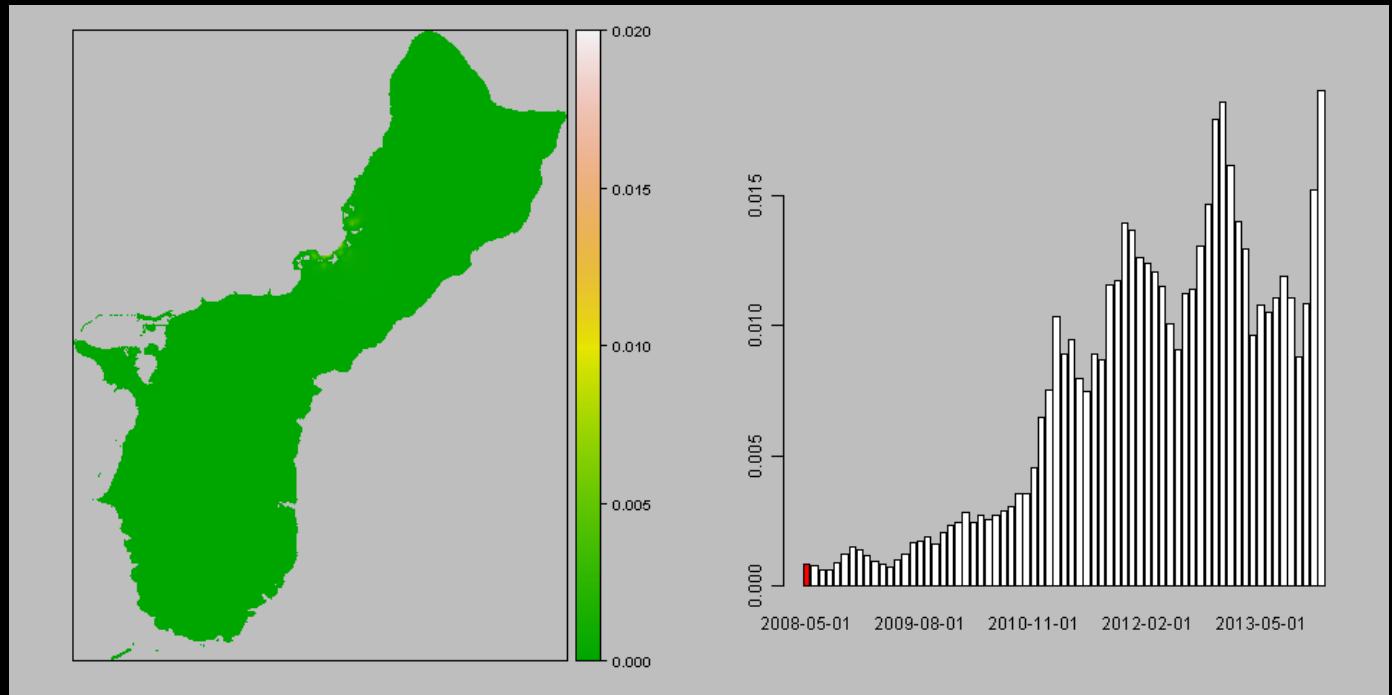
Introduction

- ▶ The following frames show spatial-temporal changes in numbers of CRB adults caught in pheromone traps.
- ▶ Note that trap catches on Guam are very low: the scale runs from 0 to only 0.02 beetles per trap day, a trap rate of only one beetle every 50 days.

Methods

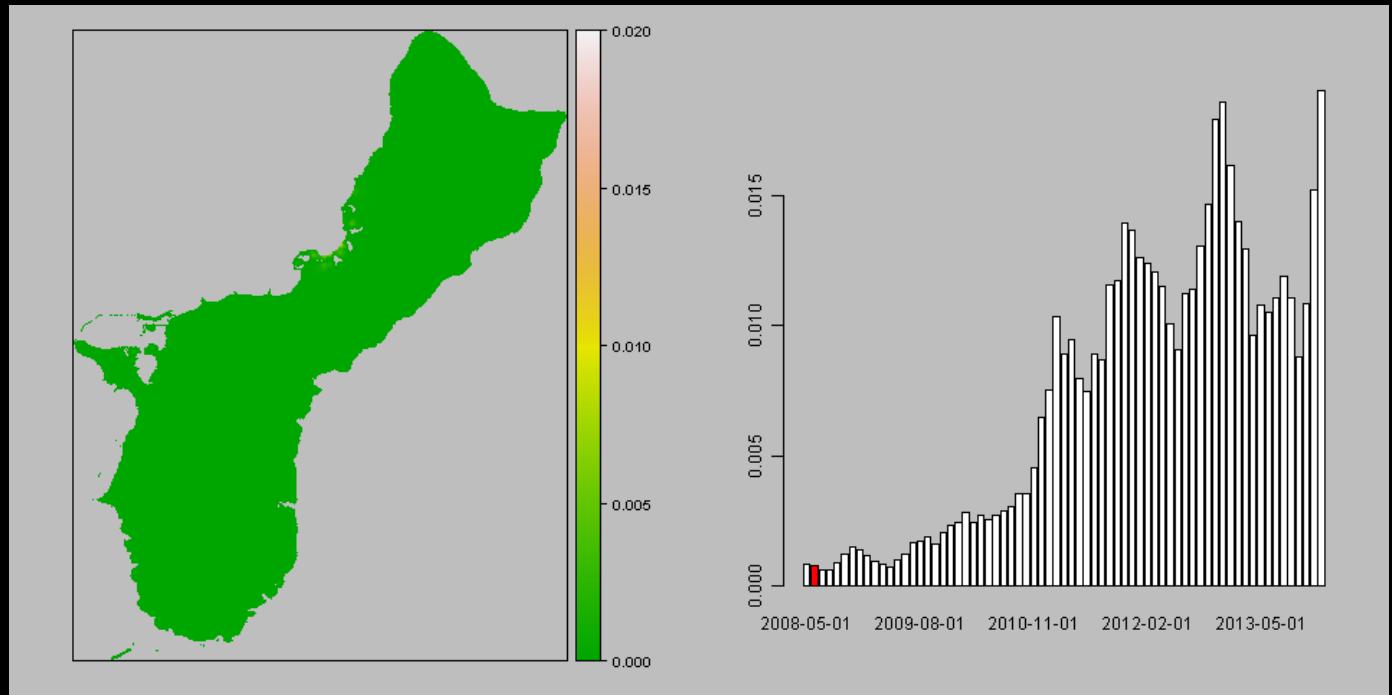
- ▶ Interpolated raster maps were made using an R script which:
 1. Accesses georeferenced data stored in the CRB project's online MySQL database.
 2. Processes the data using the GRASS6 GIS
 3. Writes the \LaTeX code which generated this PDF document.

90 day trapping period ending on 01 May 2008



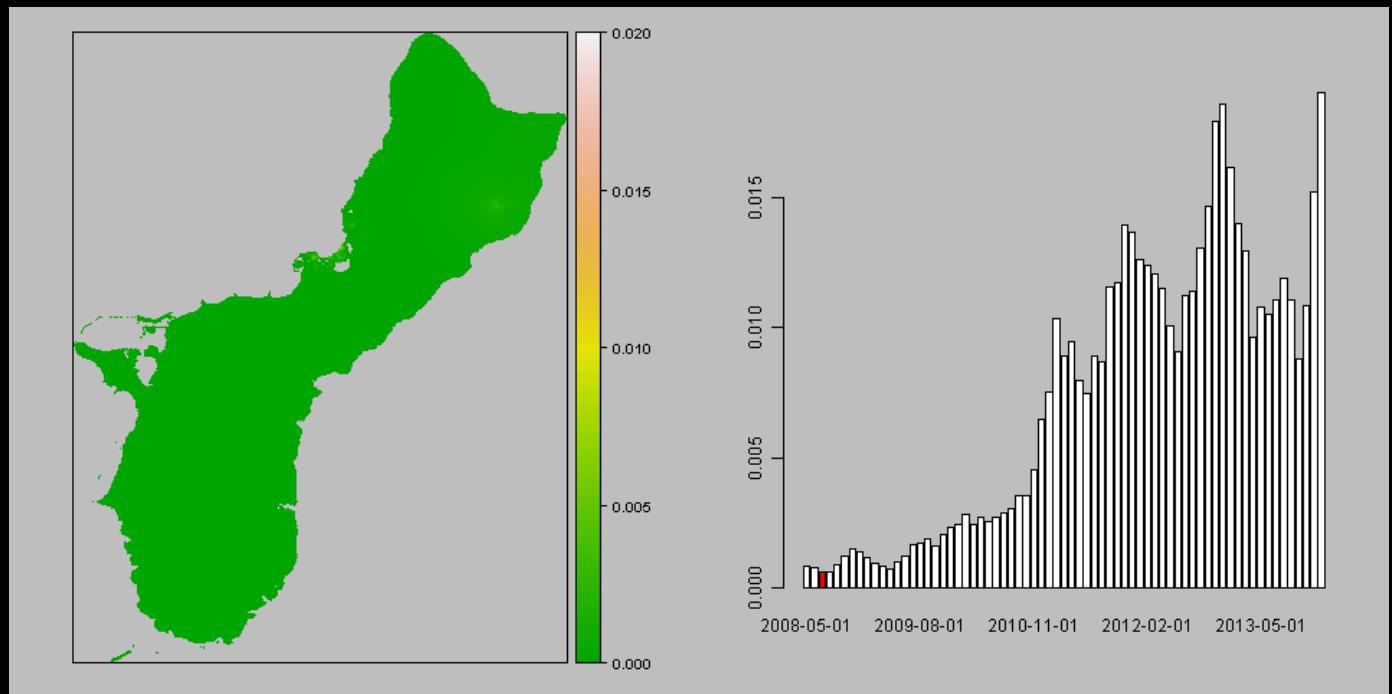
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2008



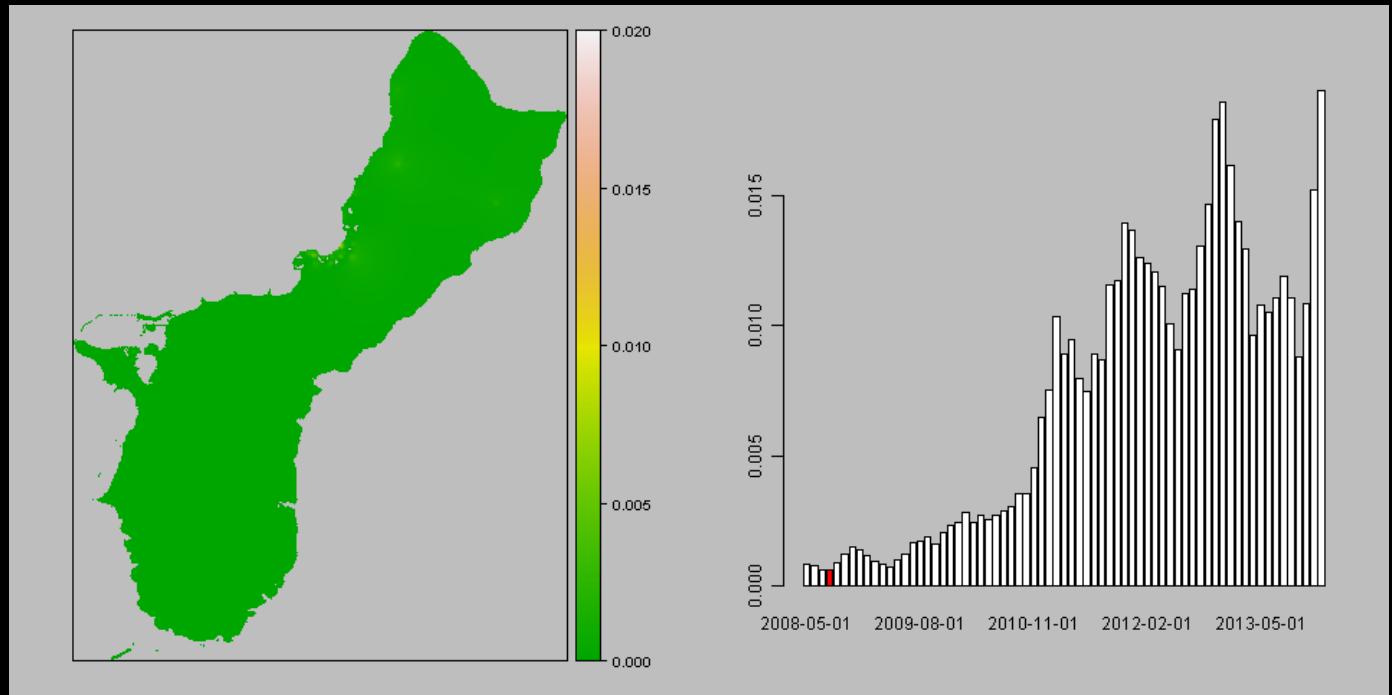
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jul 2008



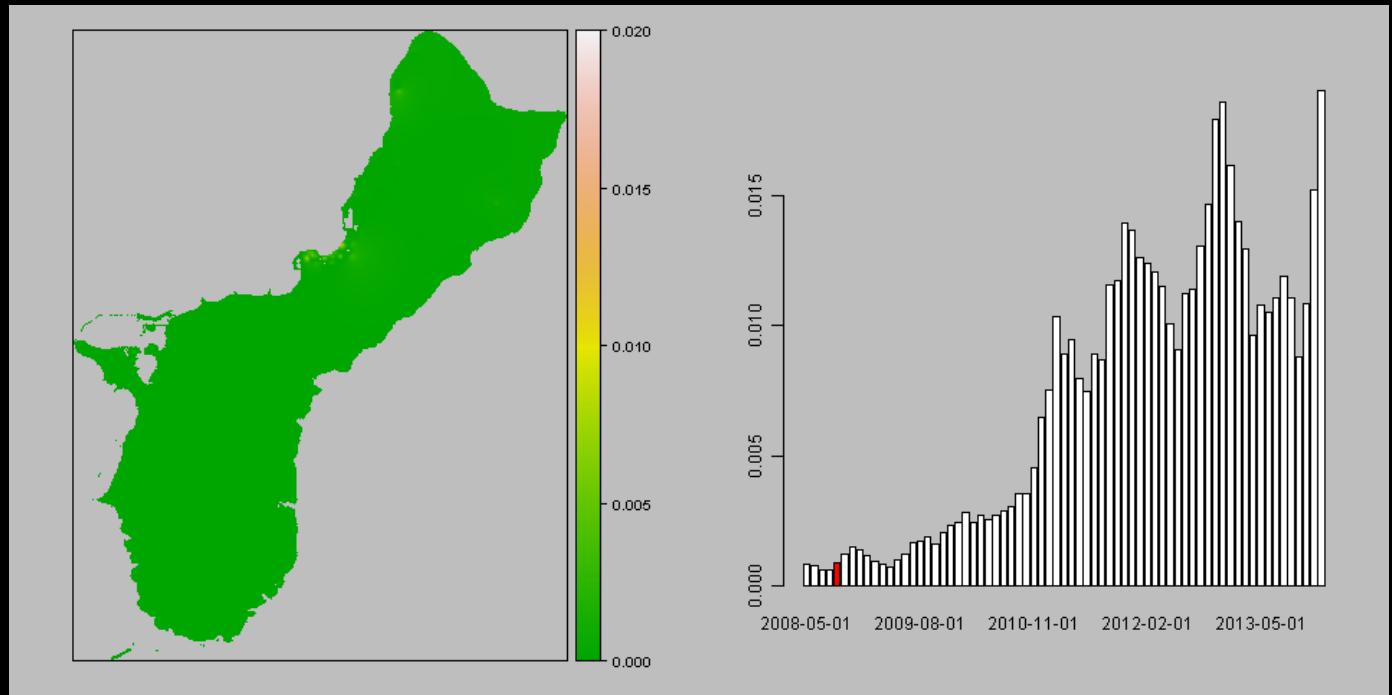
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2008



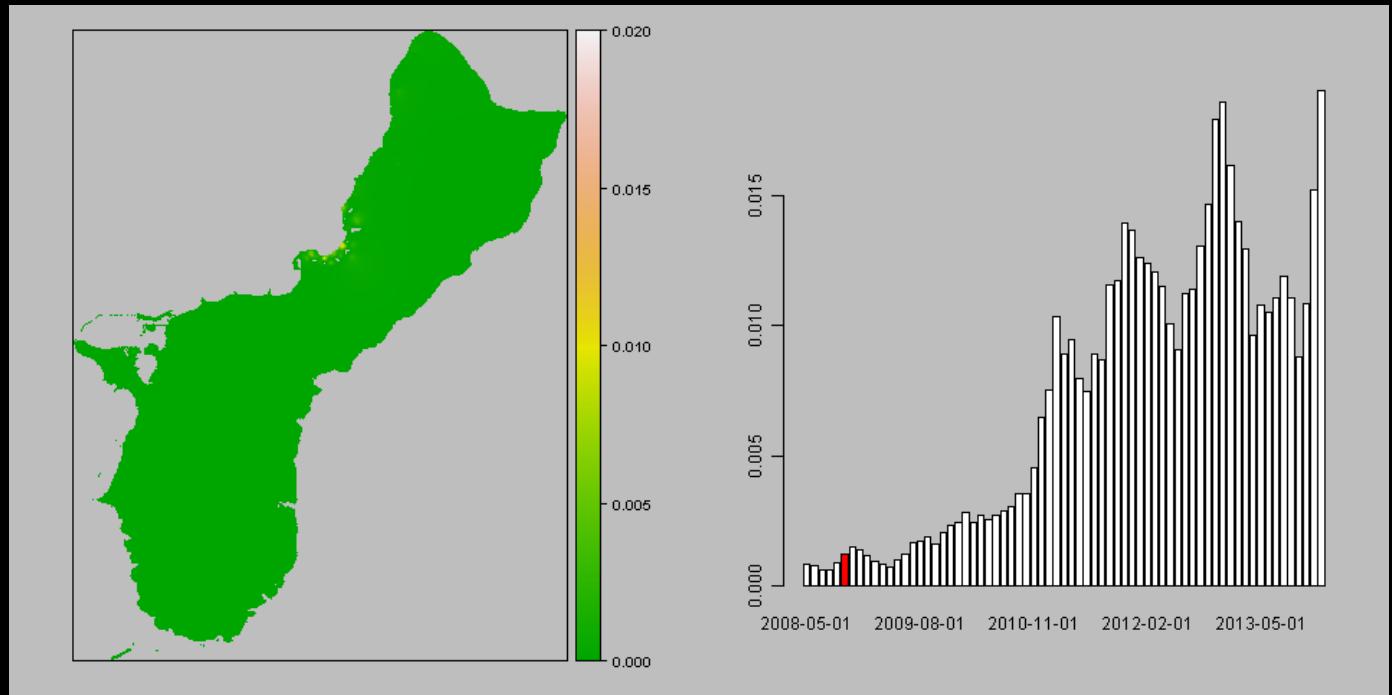
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2008



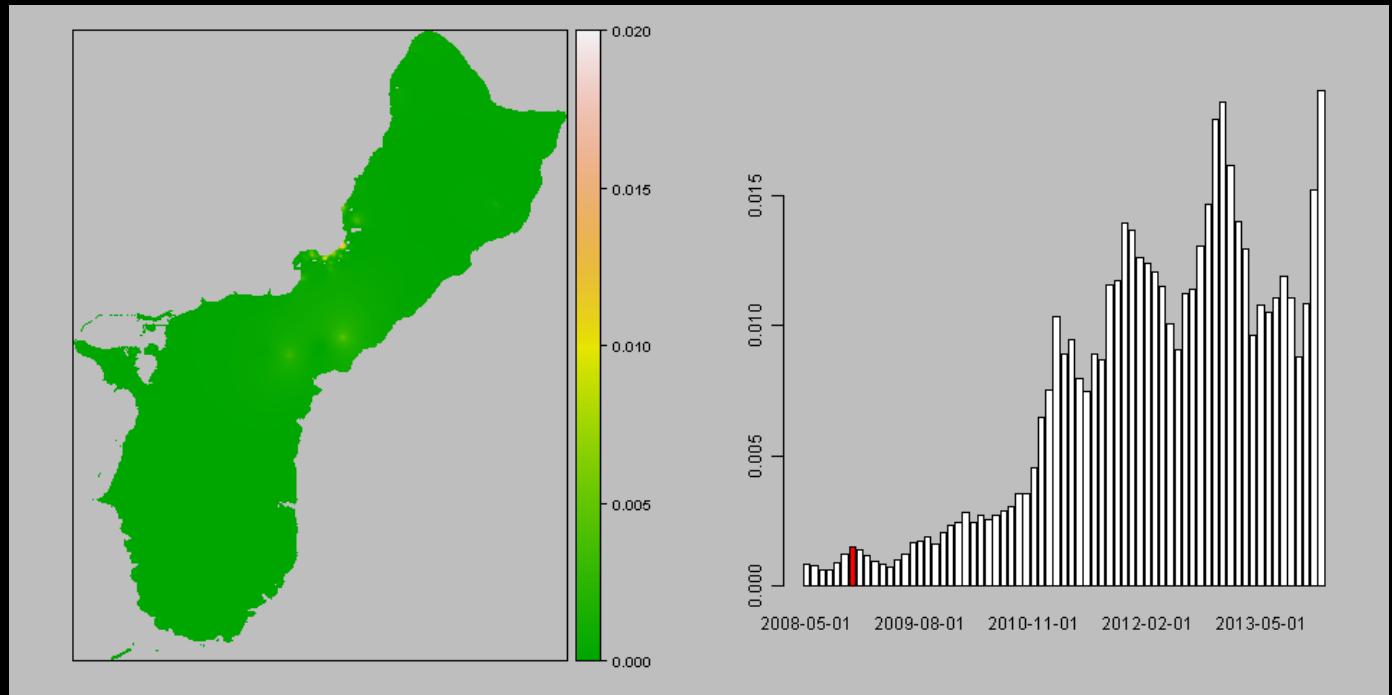
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2008



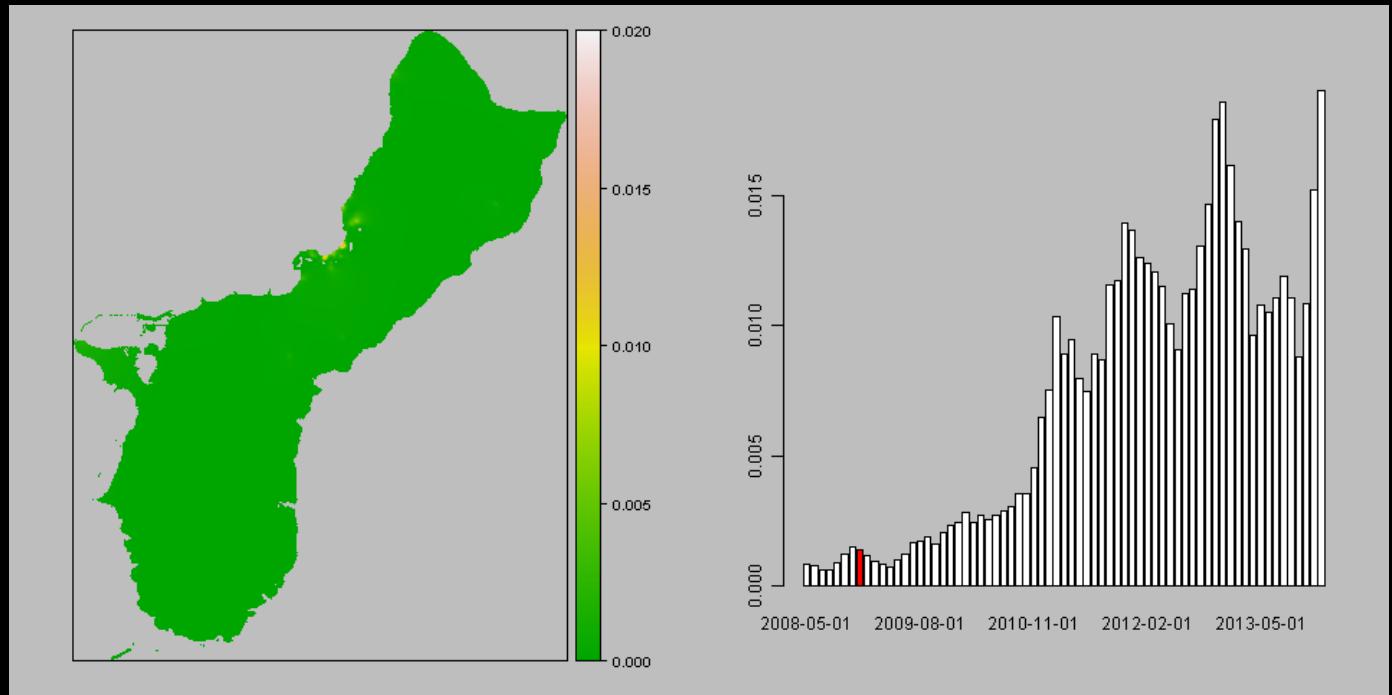
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2008



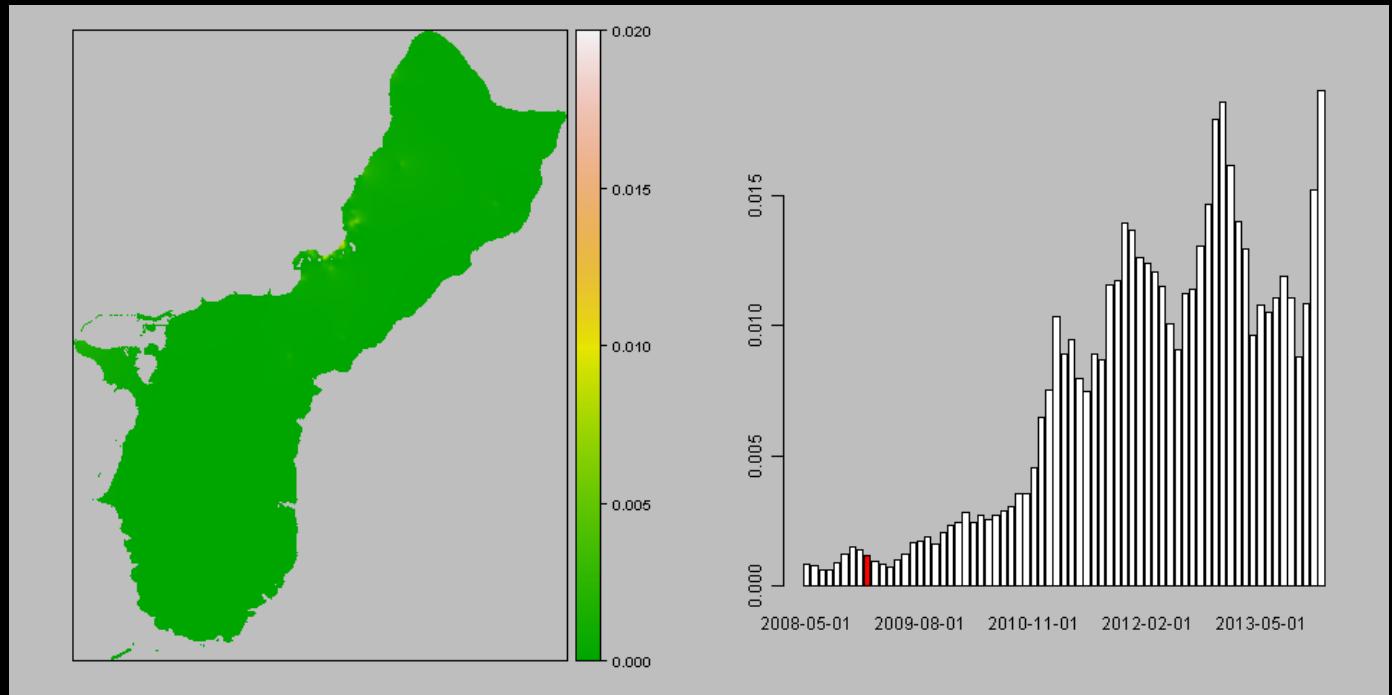
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2008



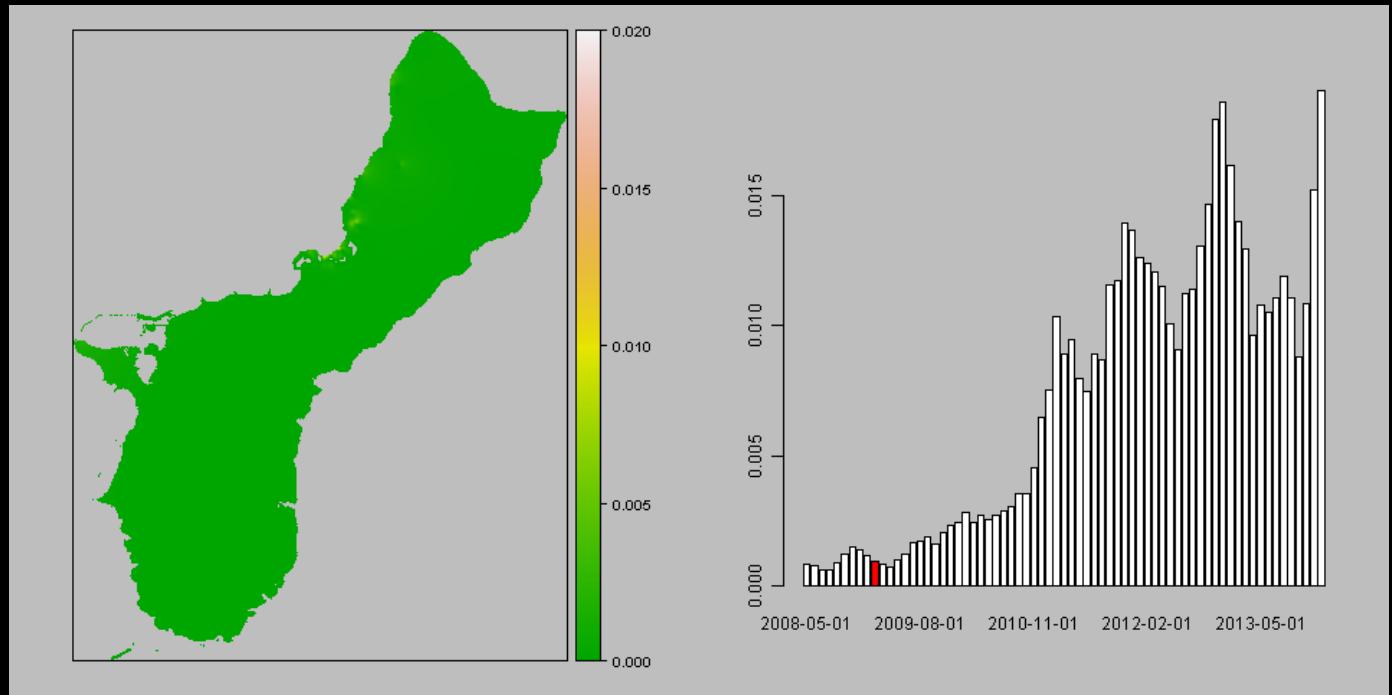
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2009



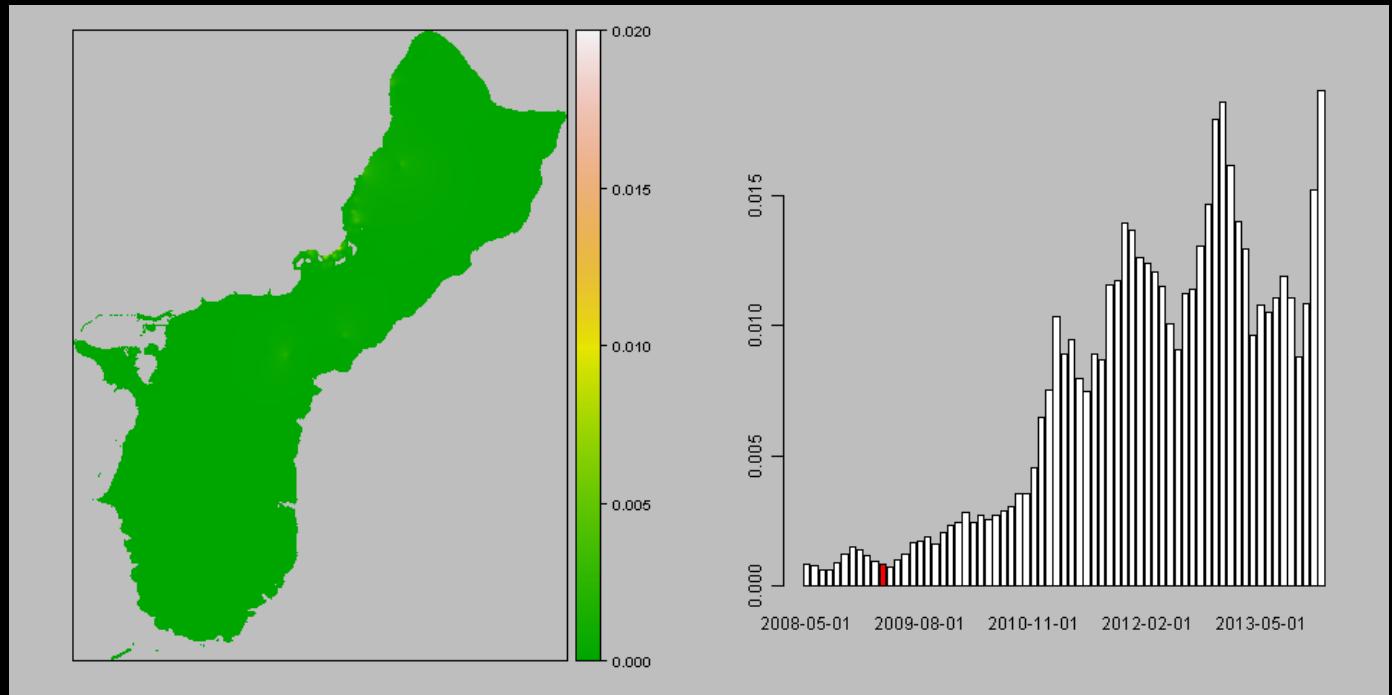
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Feb 2009



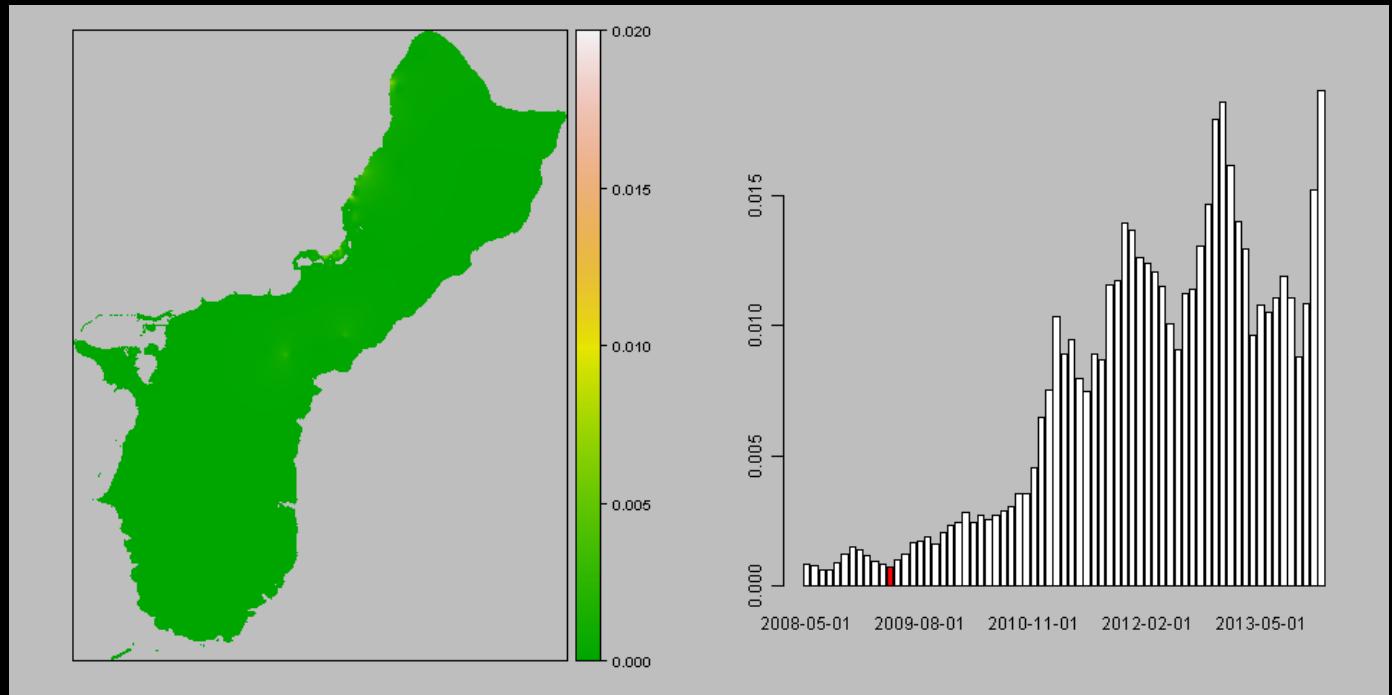
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Mar 2009



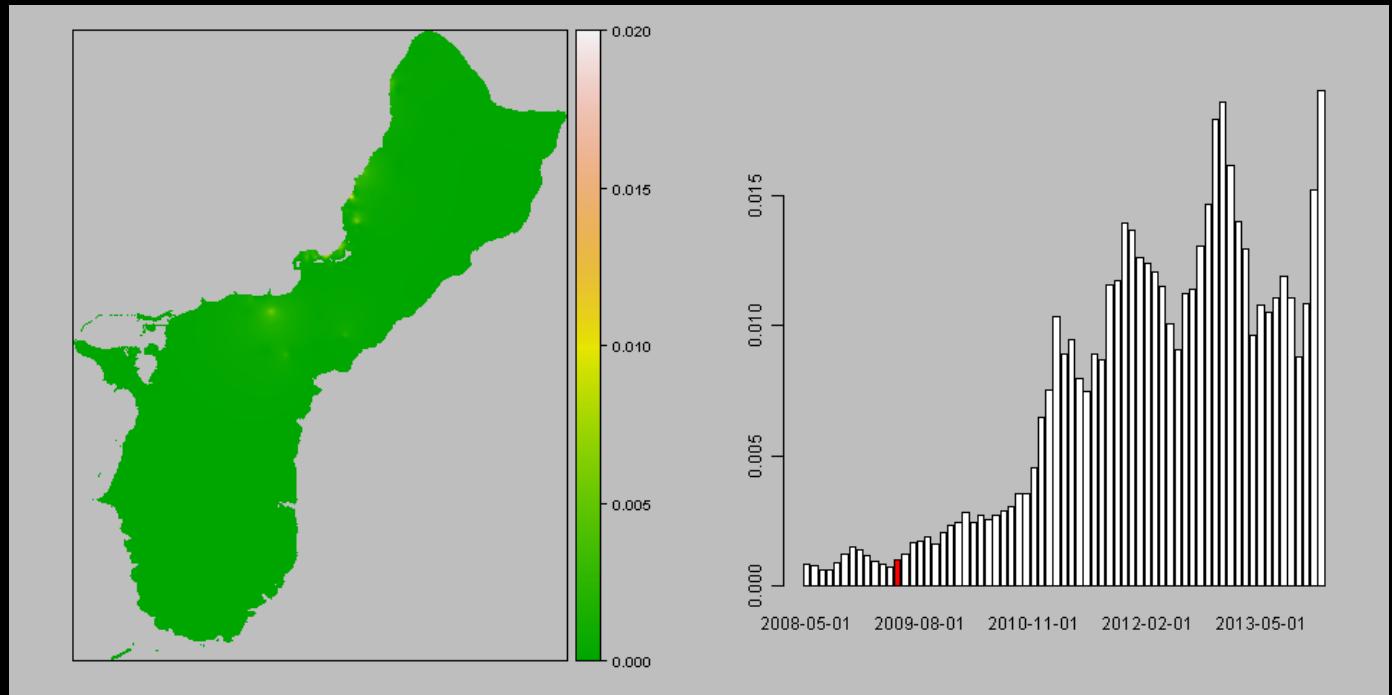
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Apr 2009



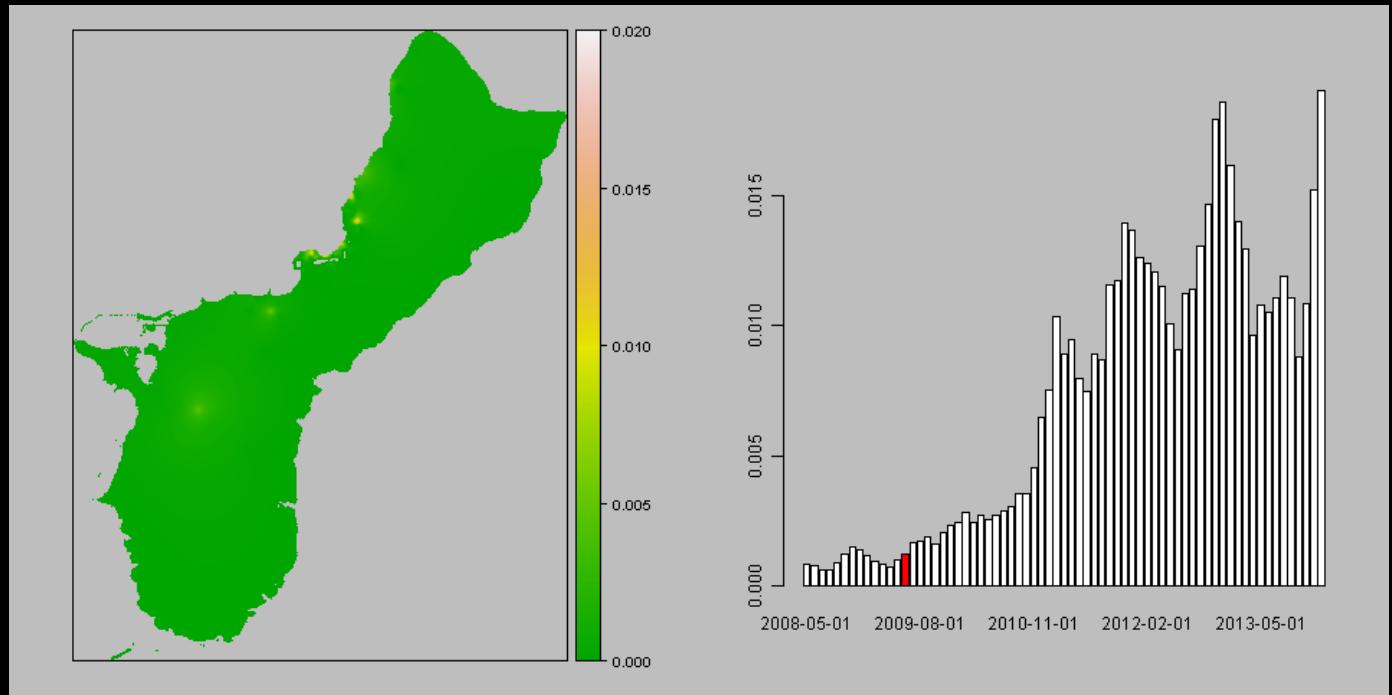
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 May 2009

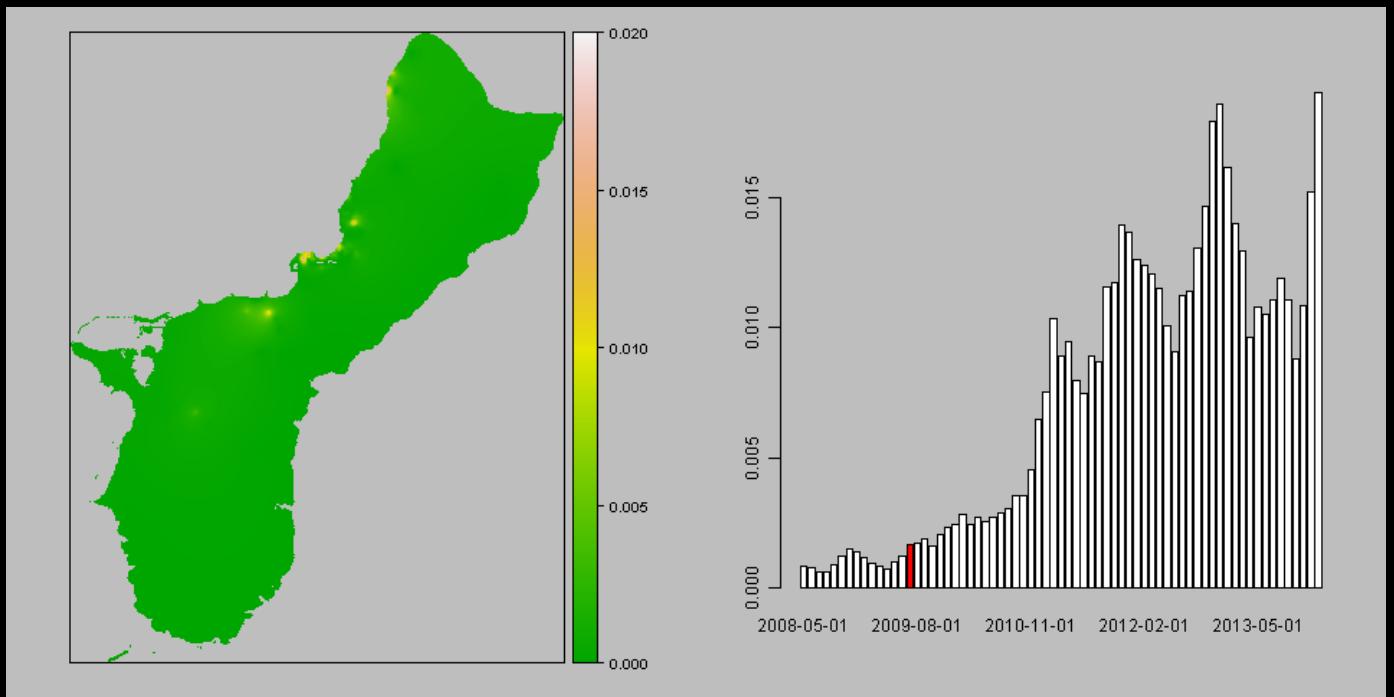


Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2009

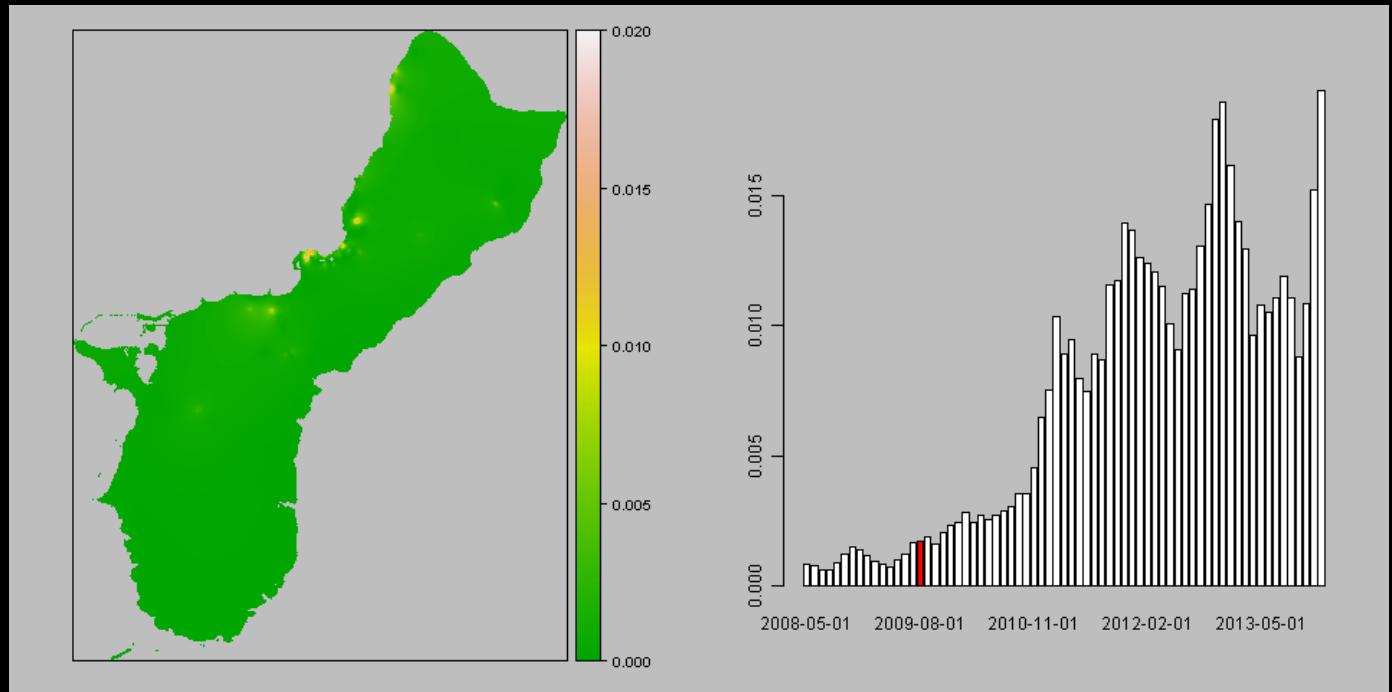


90 day trapping period ending on 01 Jul 2009



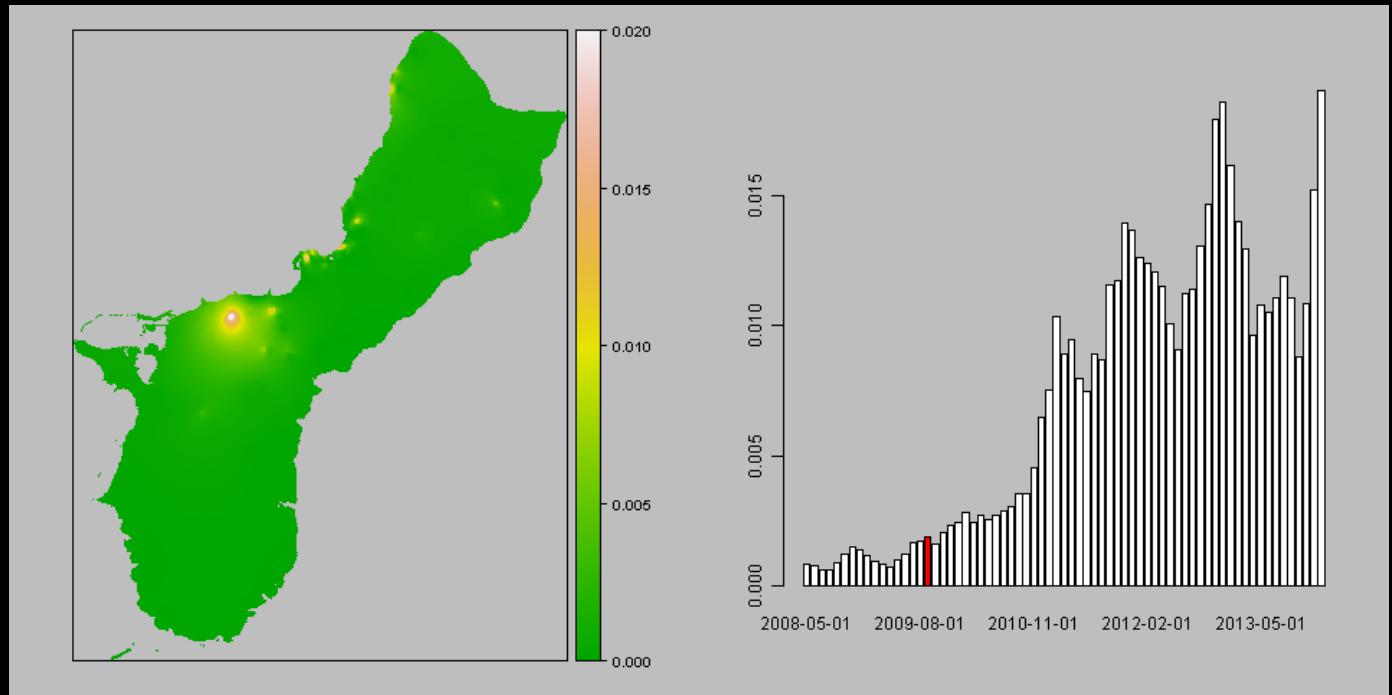
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2009



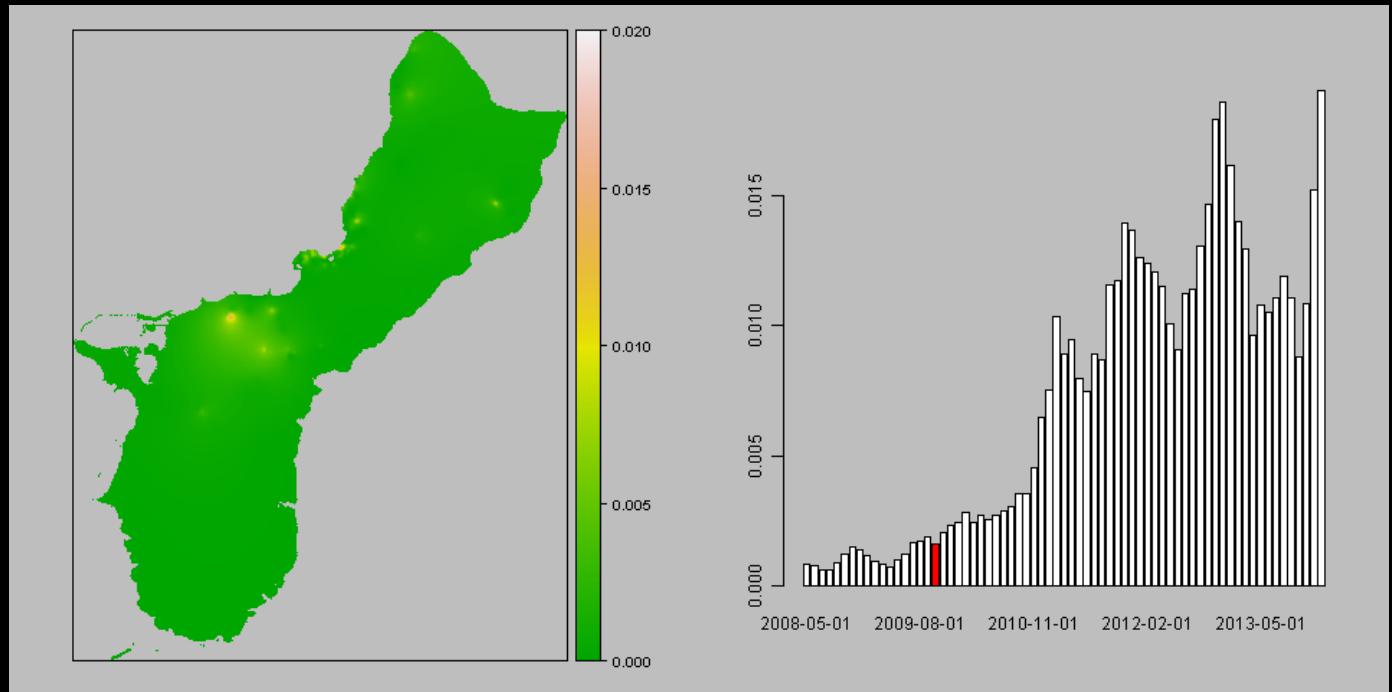
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2009



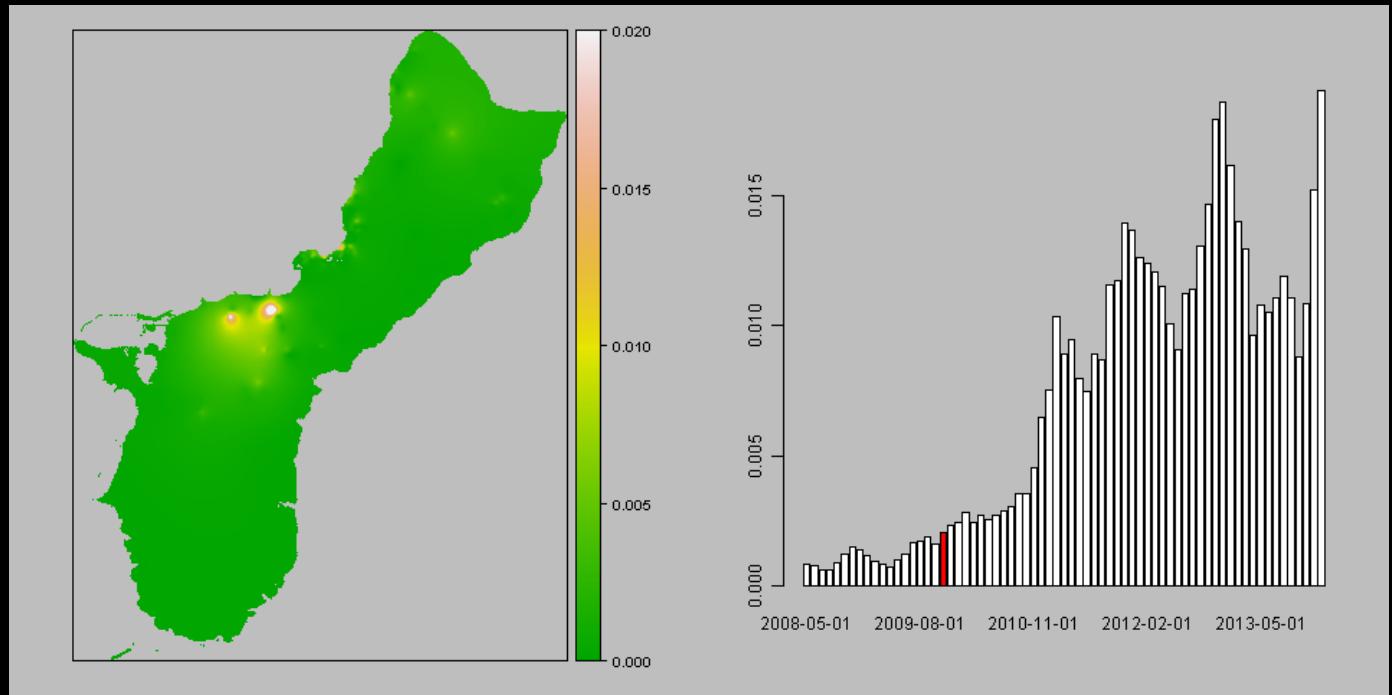
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2009



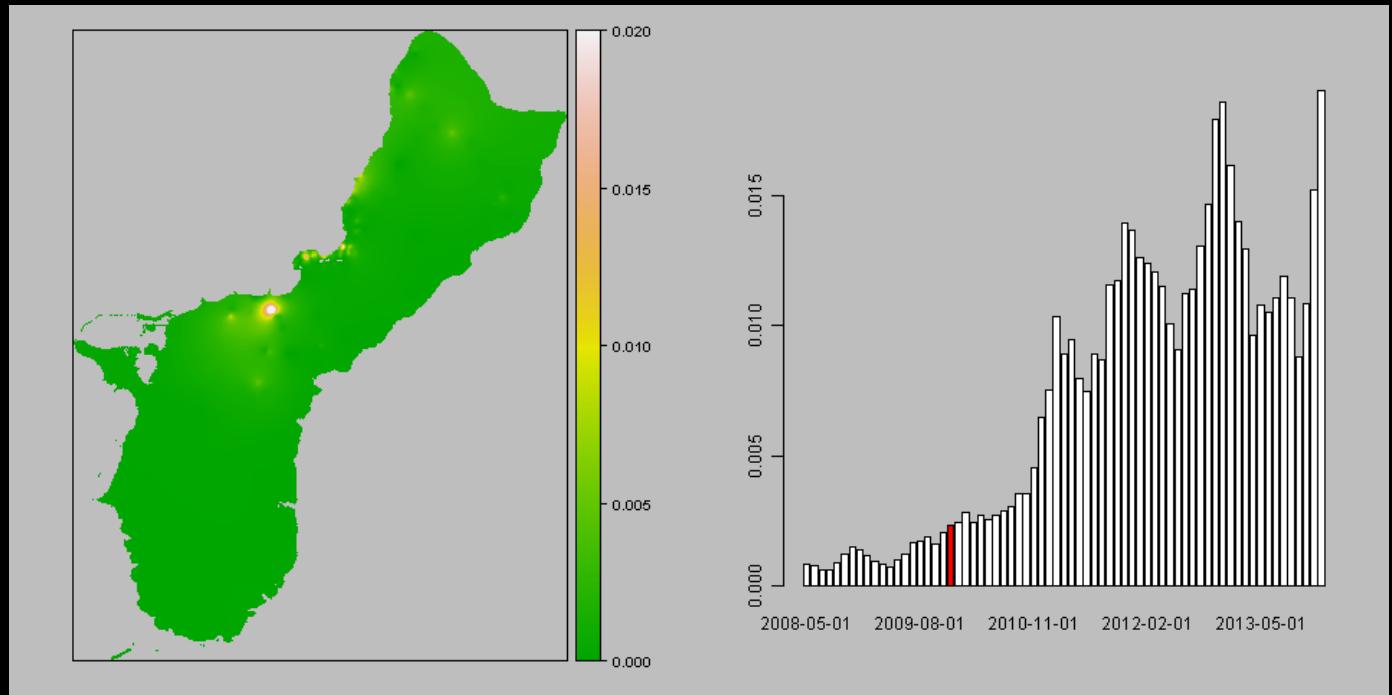
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2009



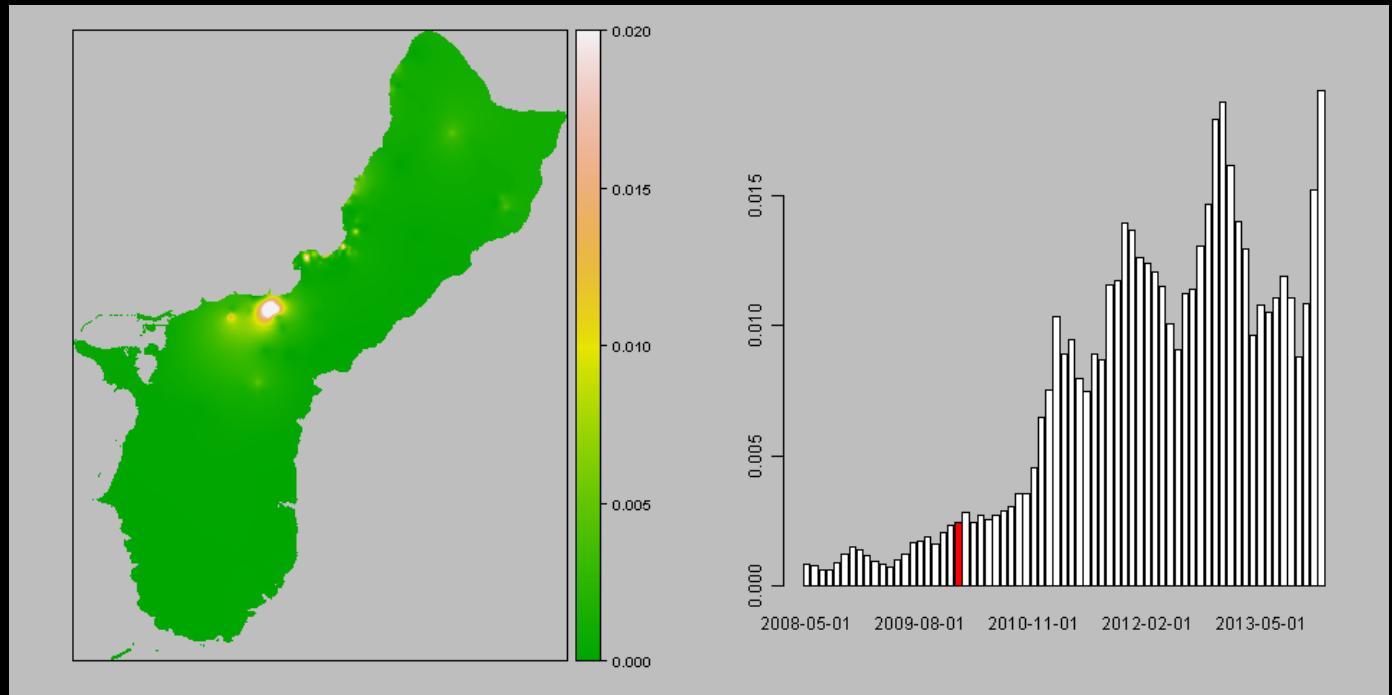
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2009



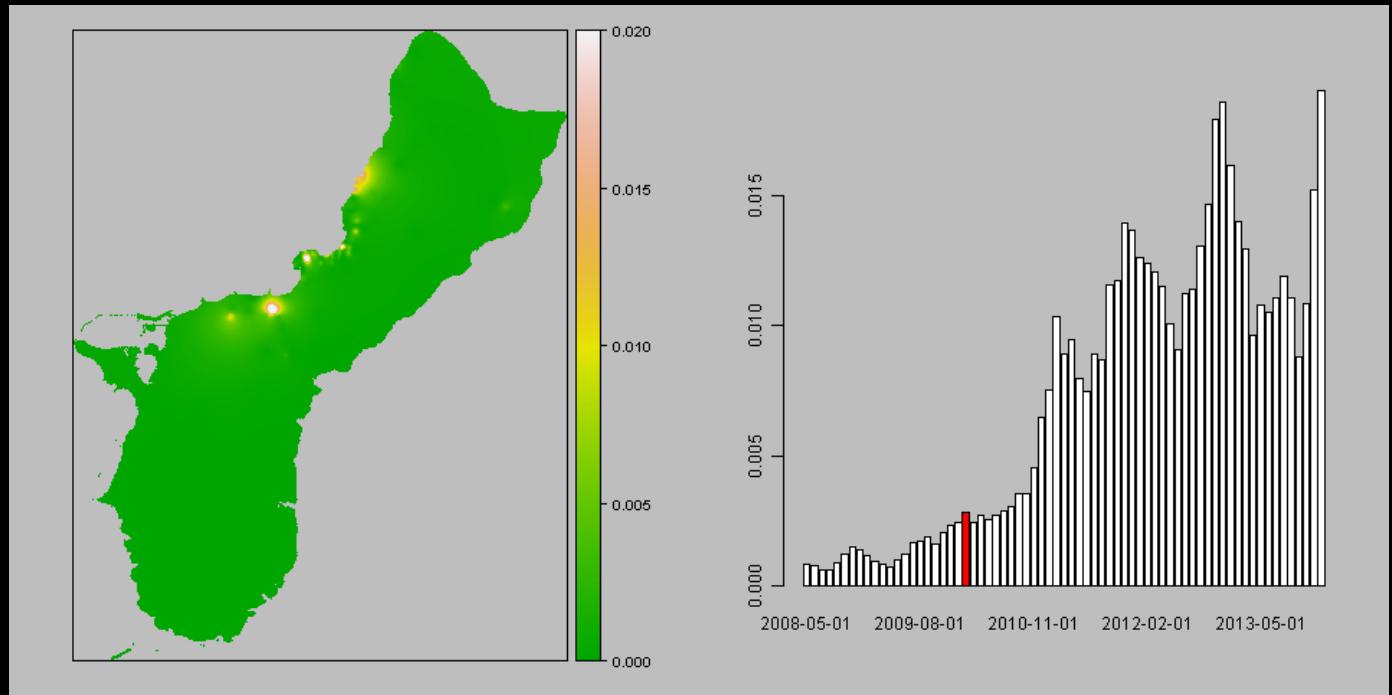
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2010



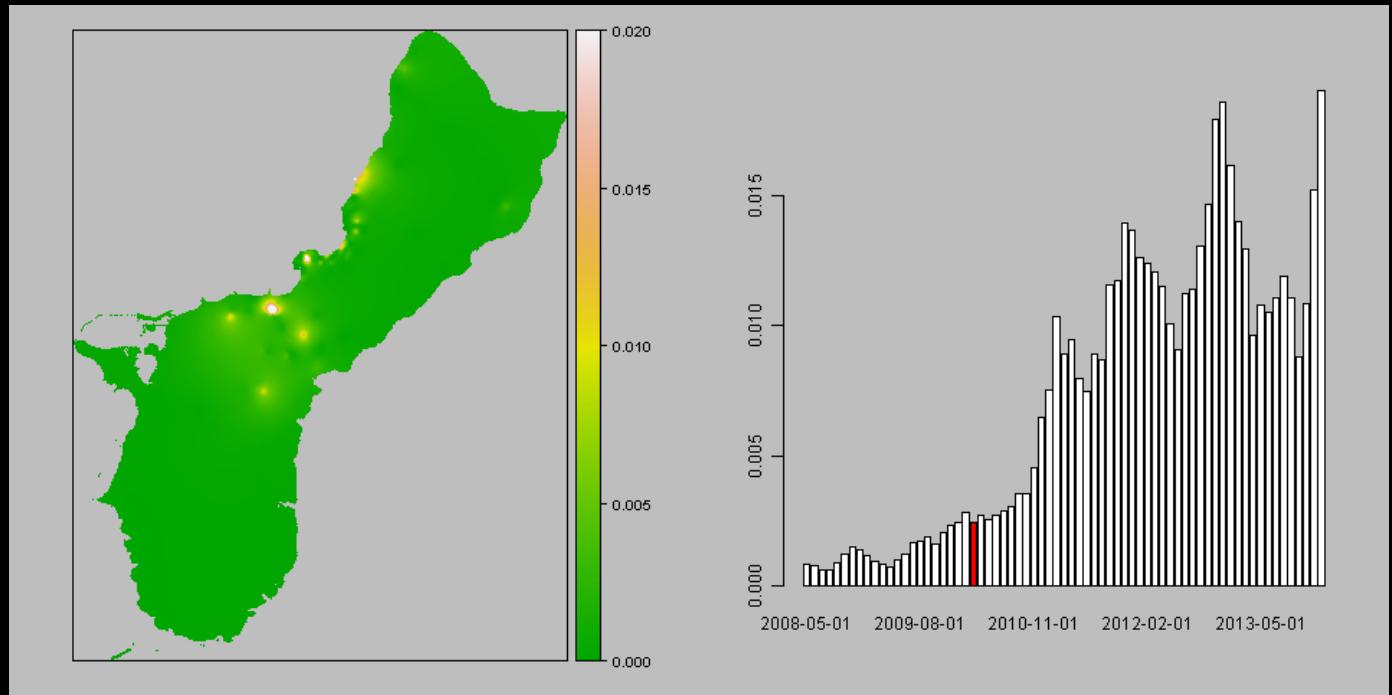
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Feb 2010



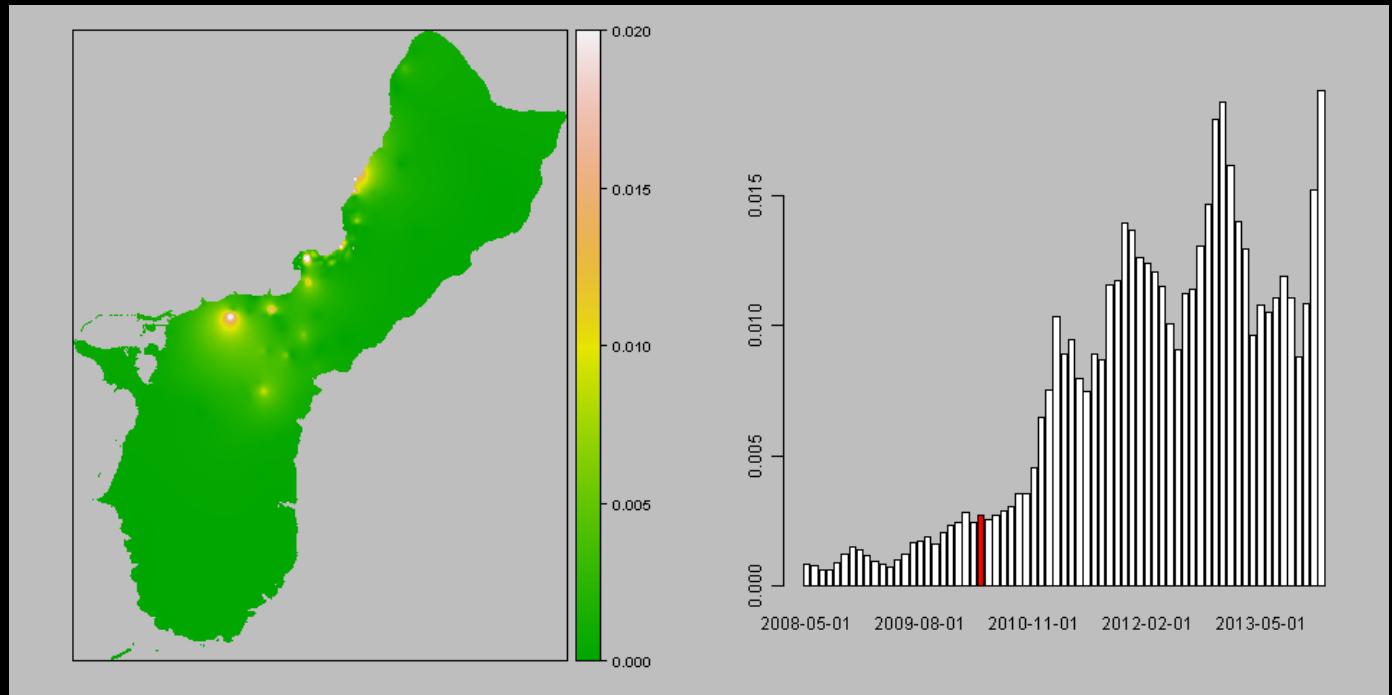
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Mar 2010



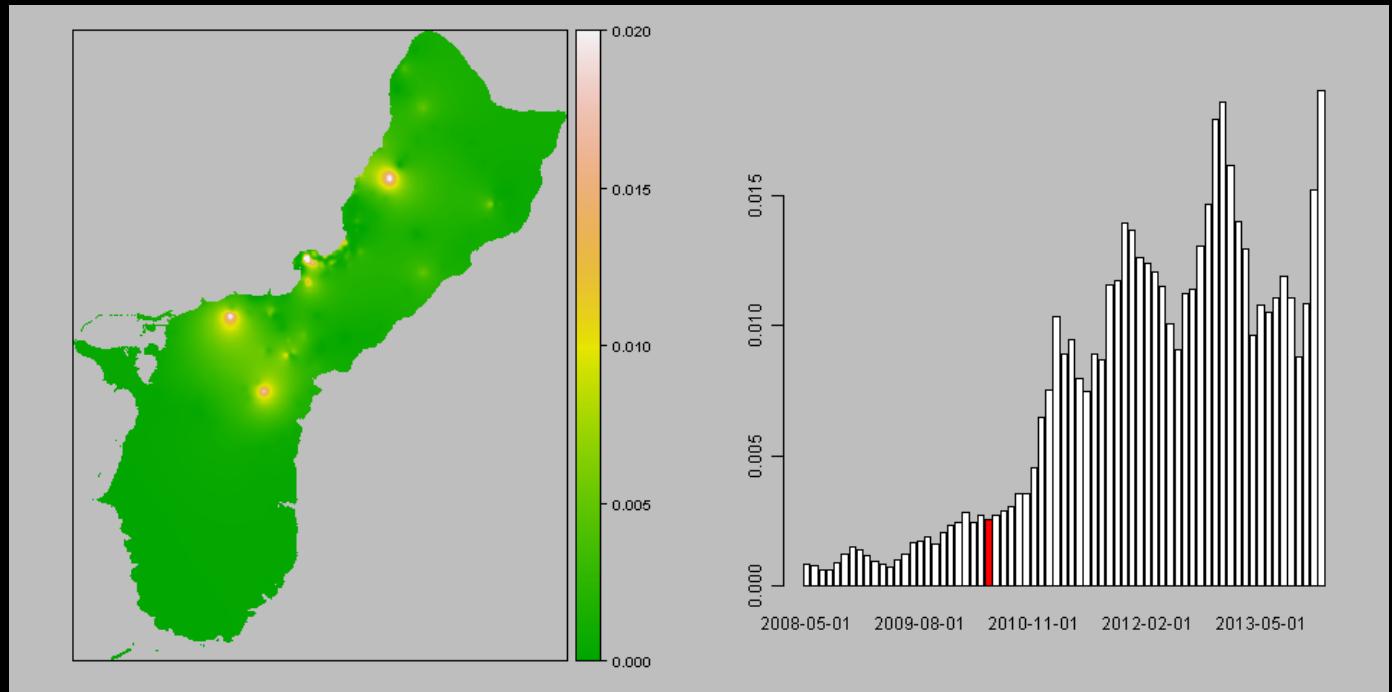
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Apr 2010



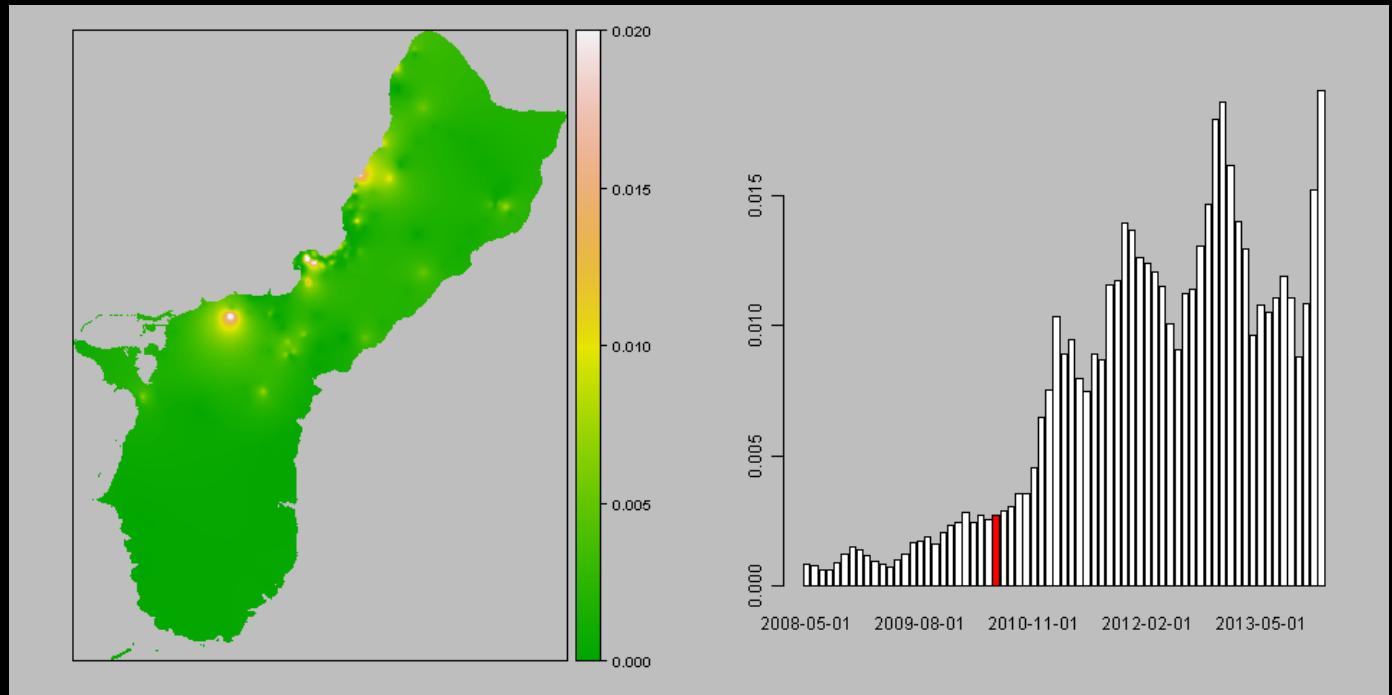
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 May 2010



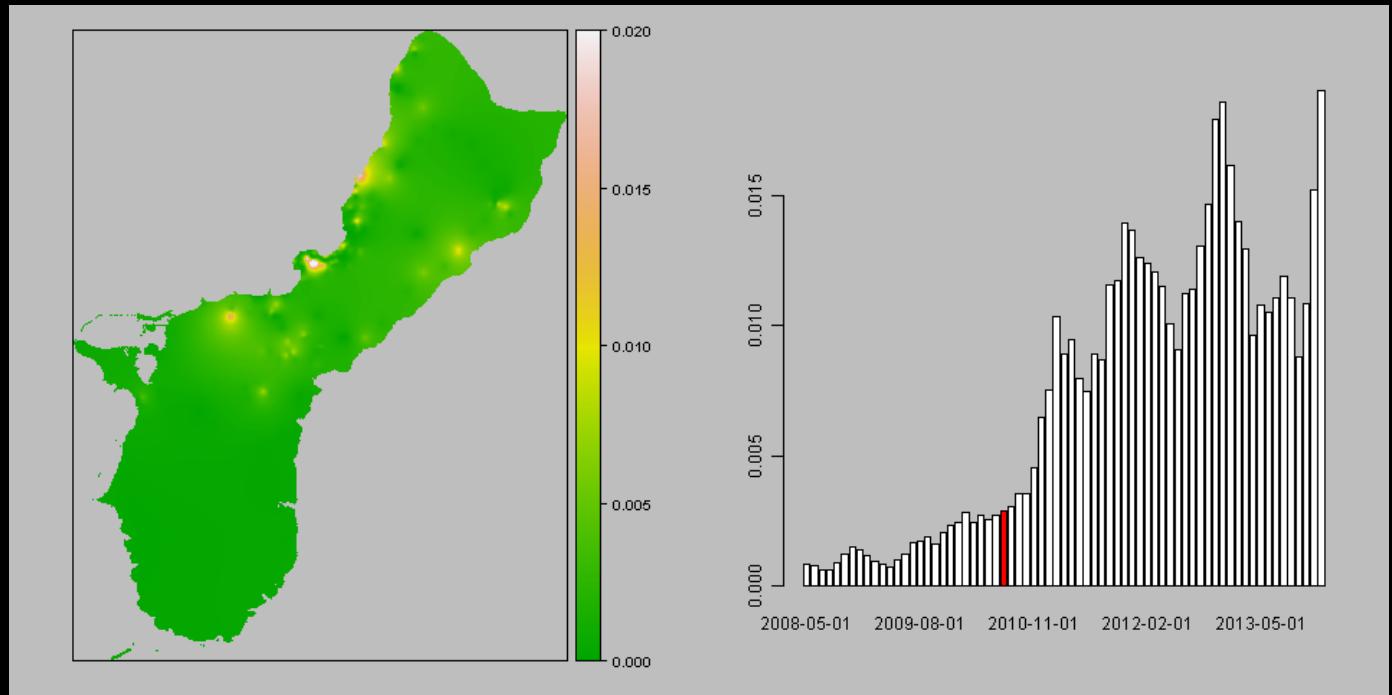
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2010



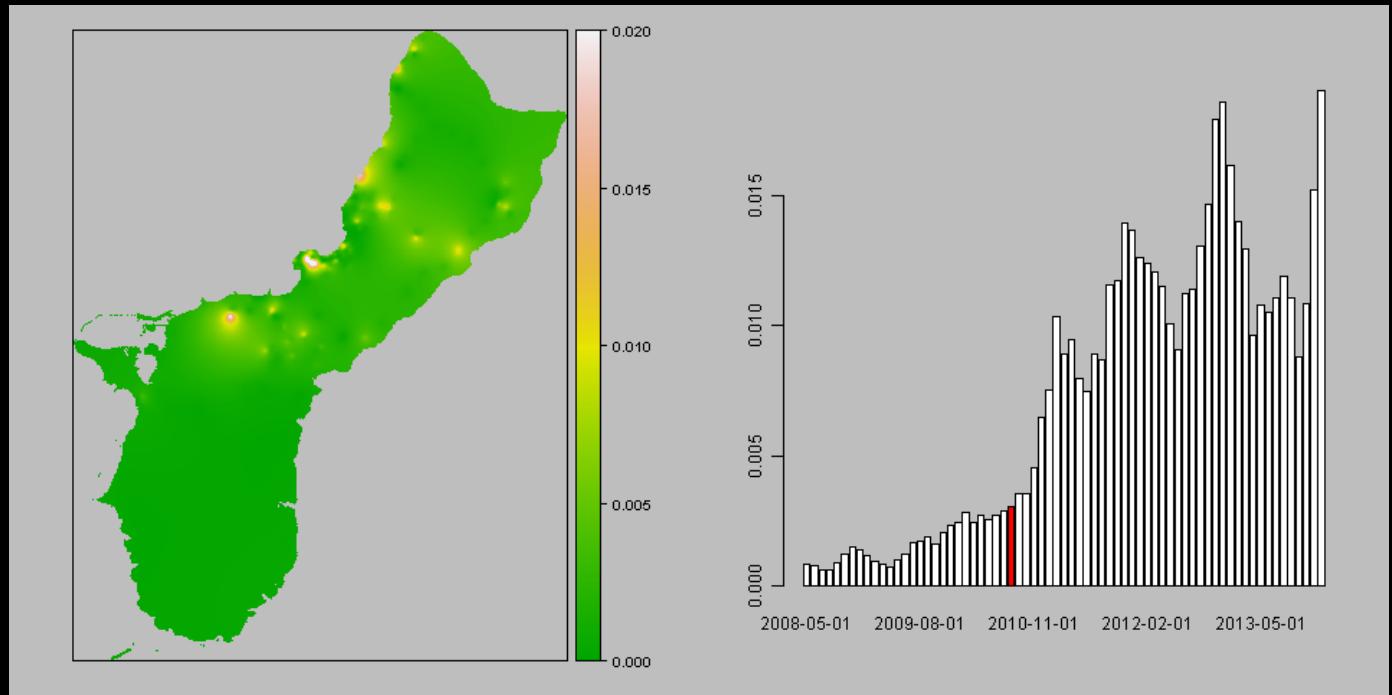
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jul 2010



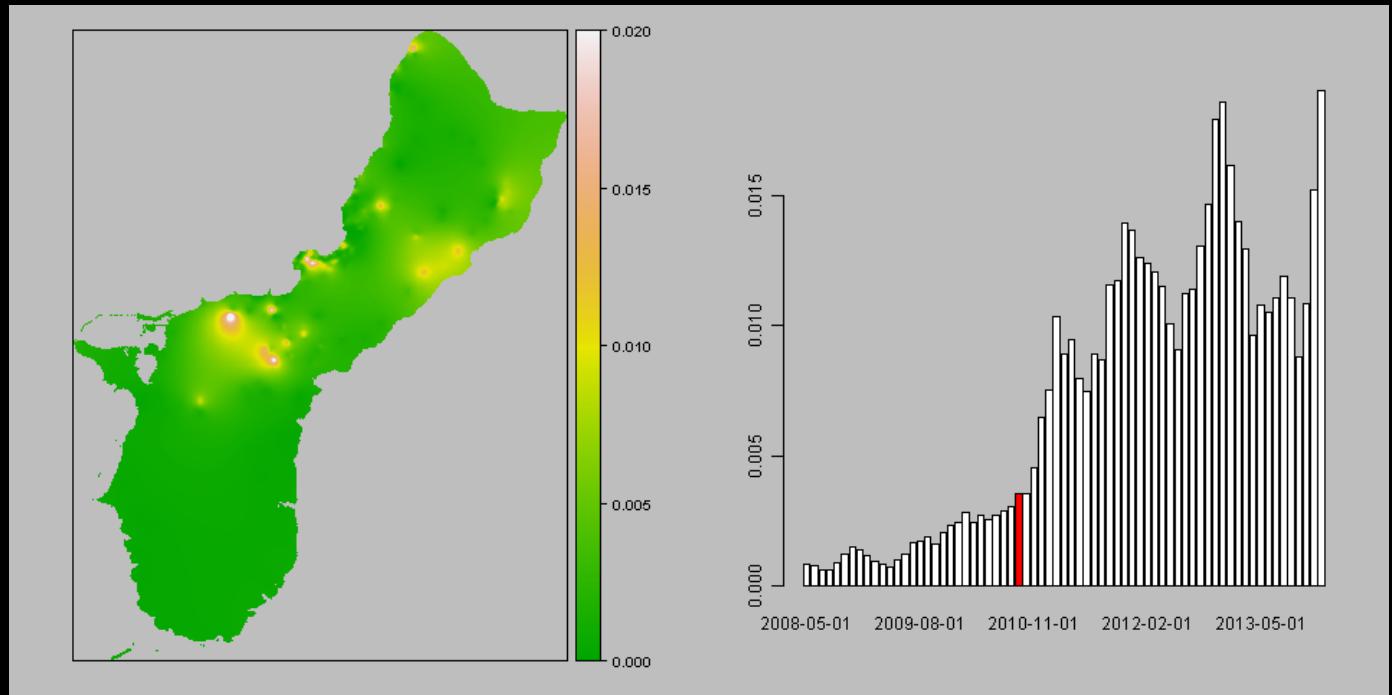
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2010



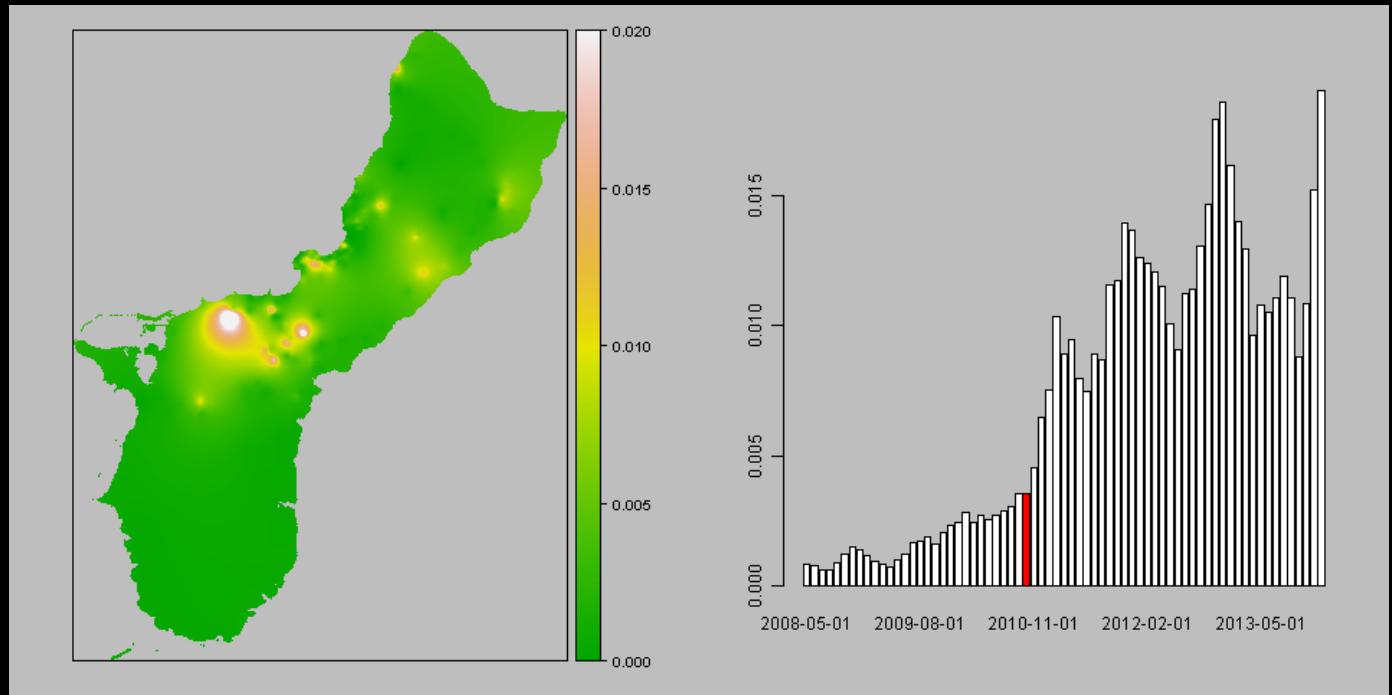
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2010



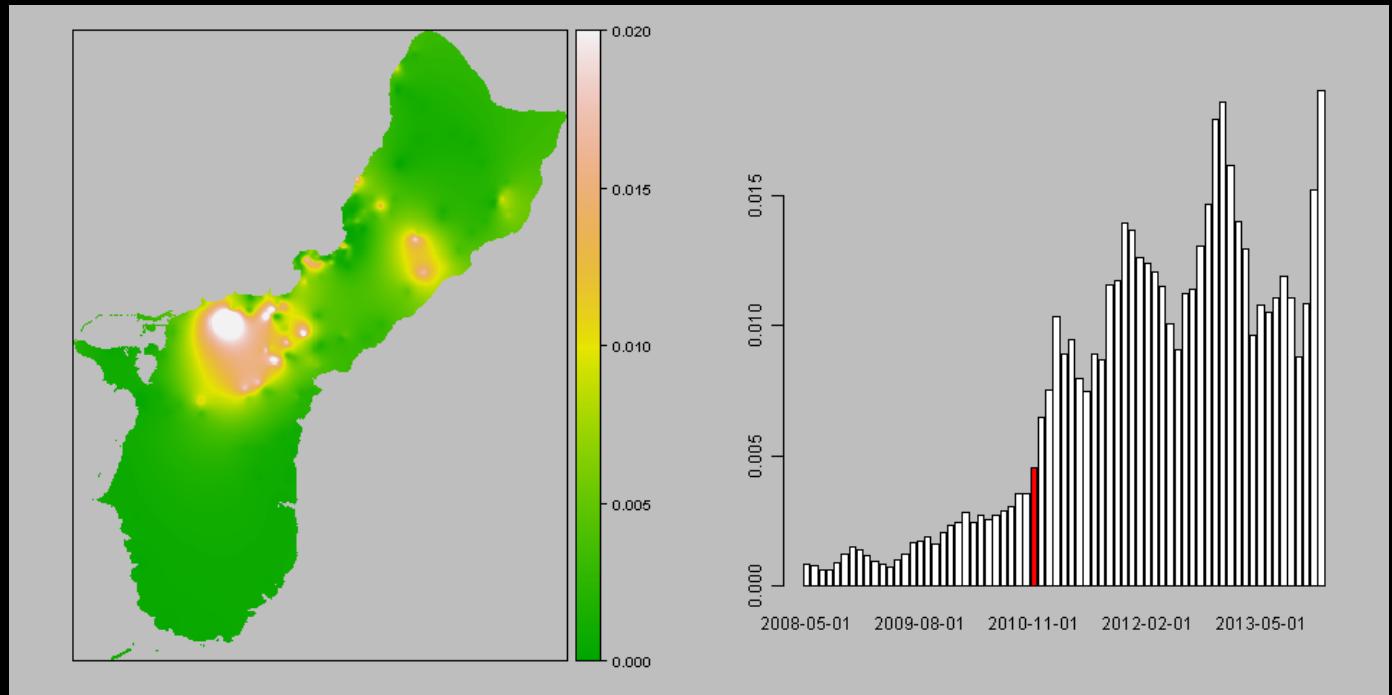
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2010



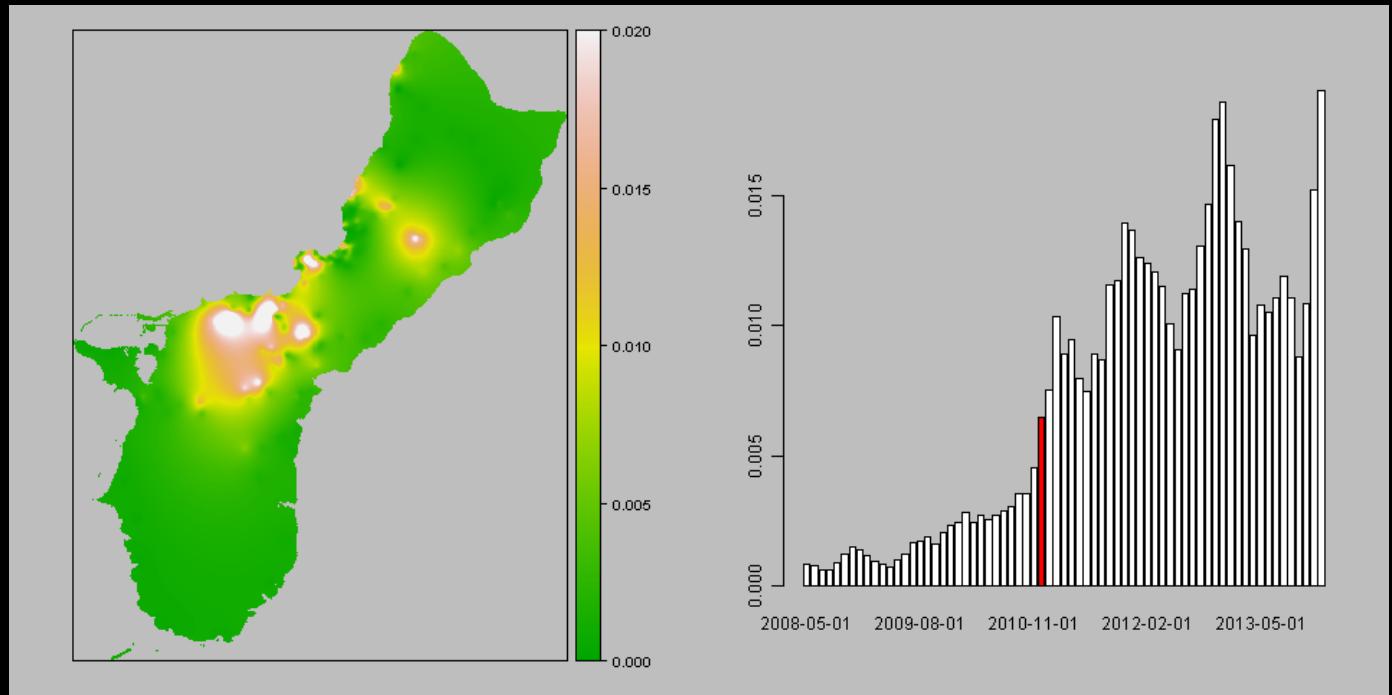
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2010



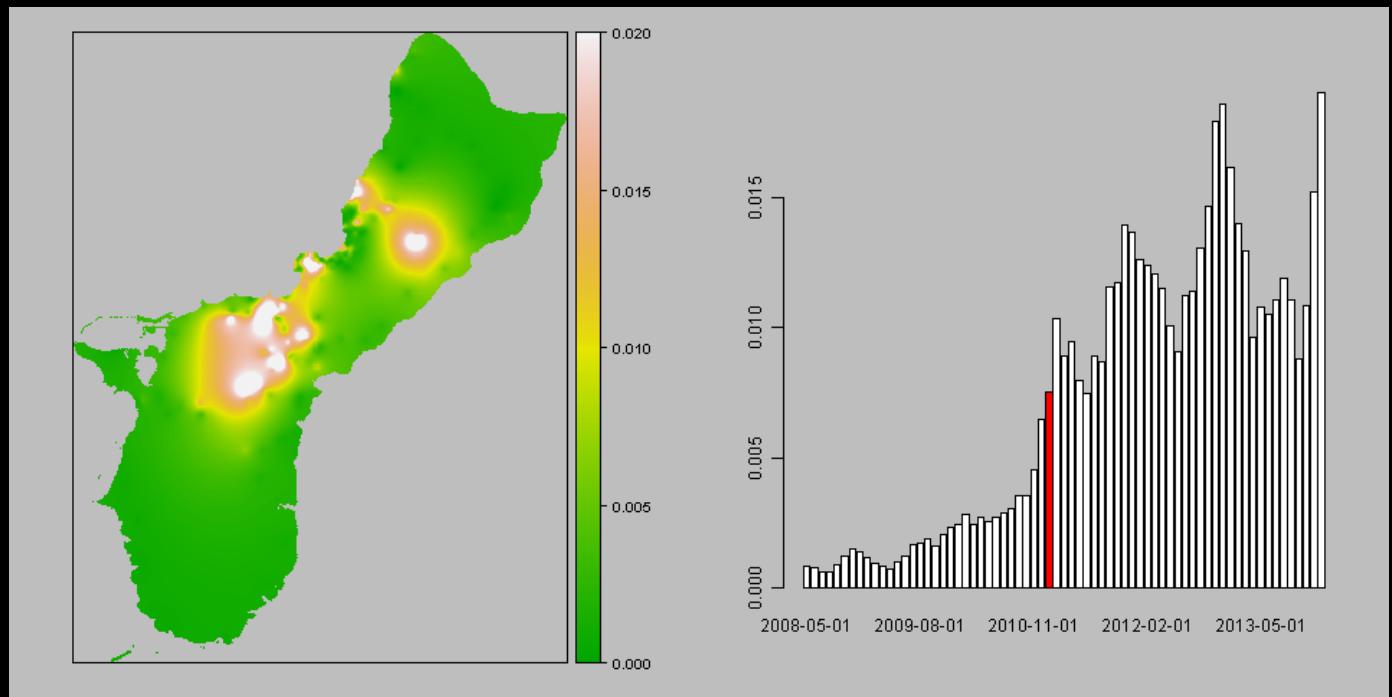
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2010



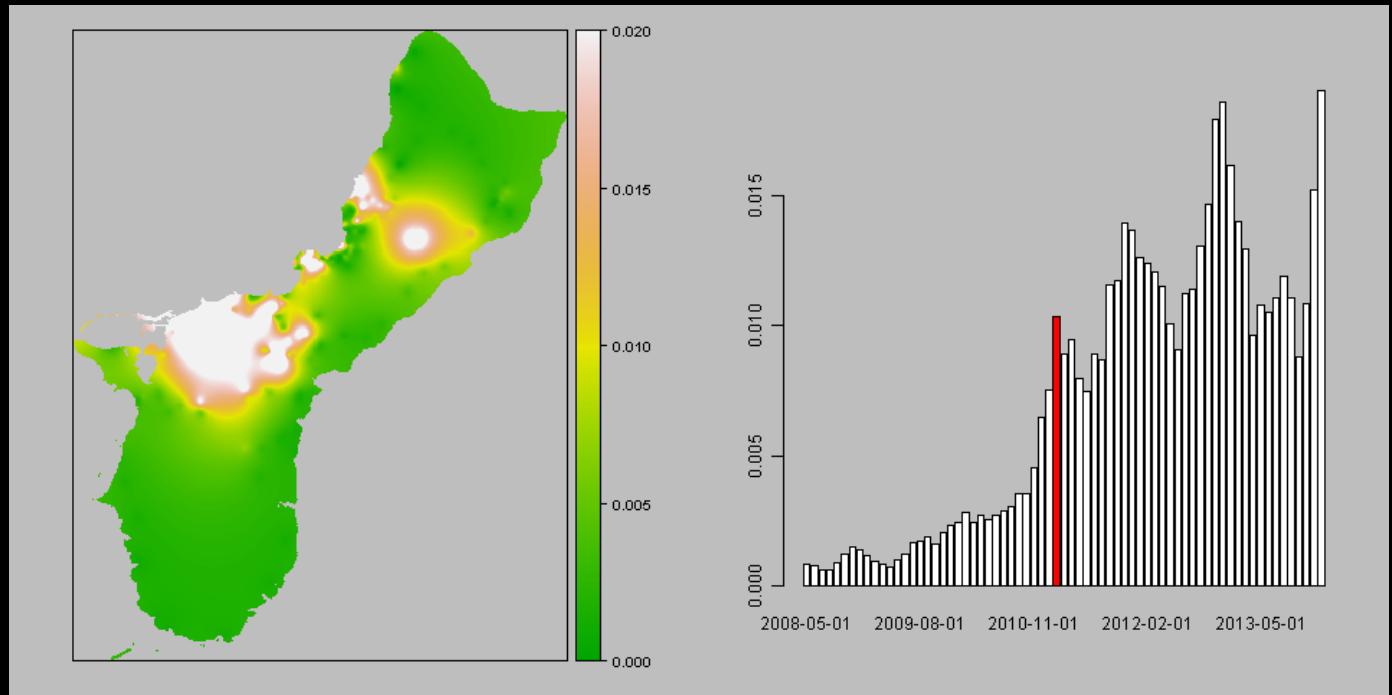
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2011



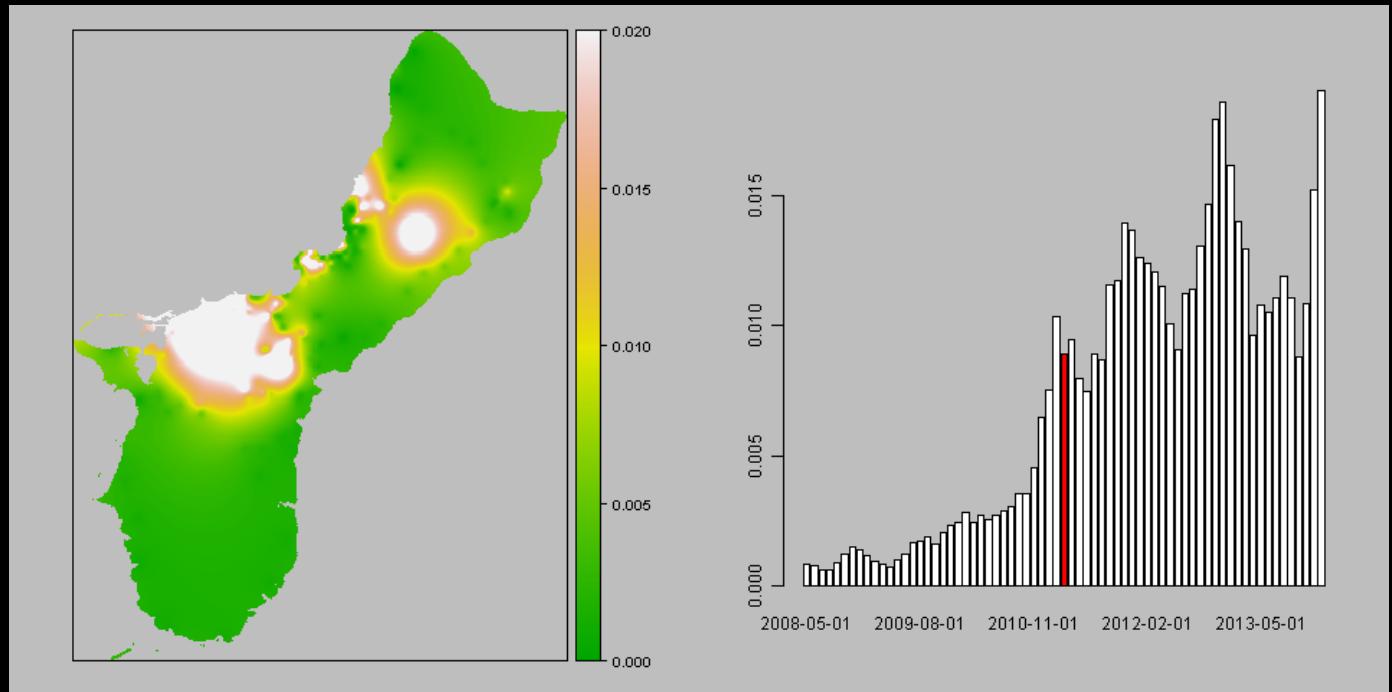
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Feb 2011



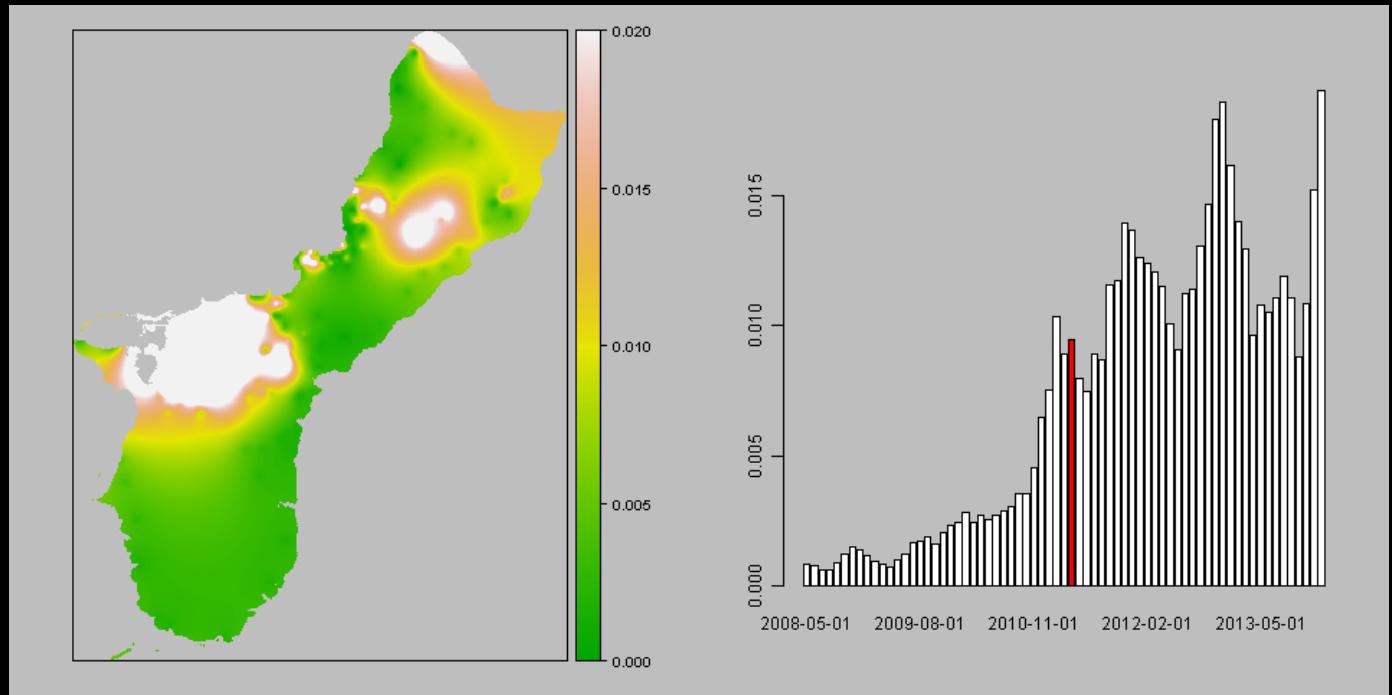
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Mar 2011



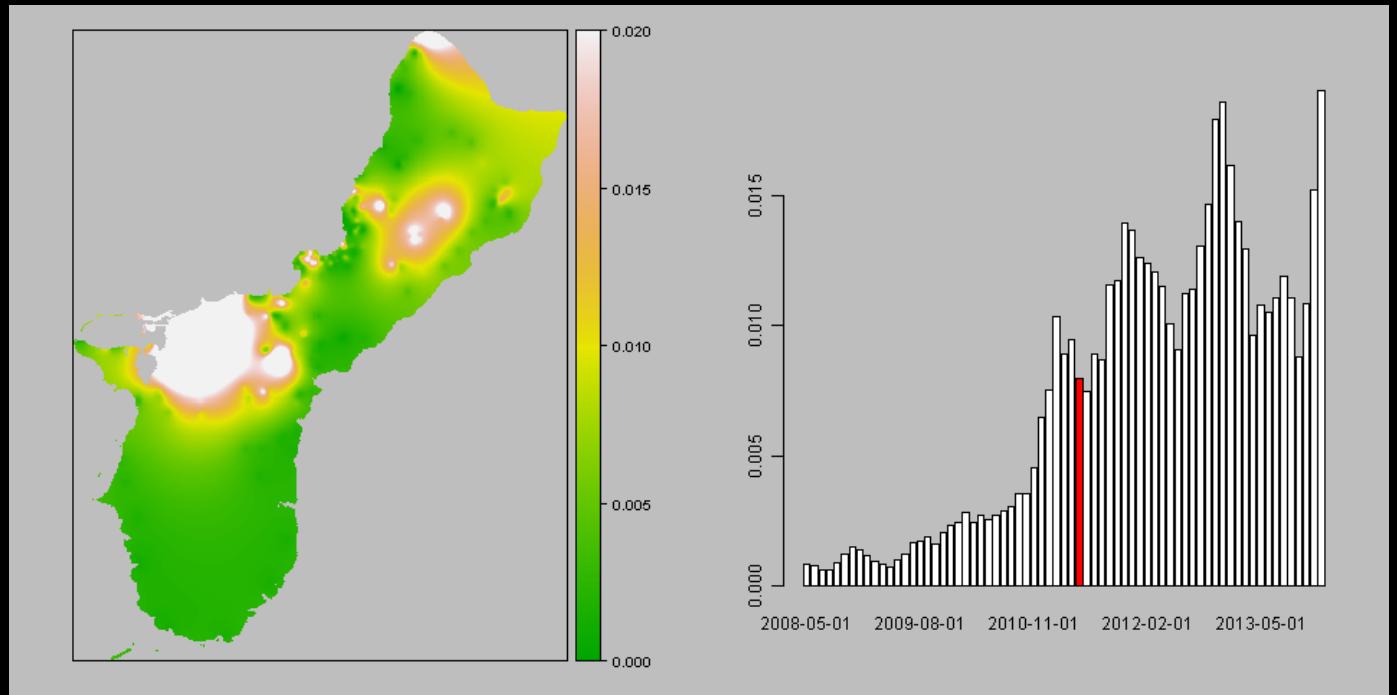
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Apr 2011



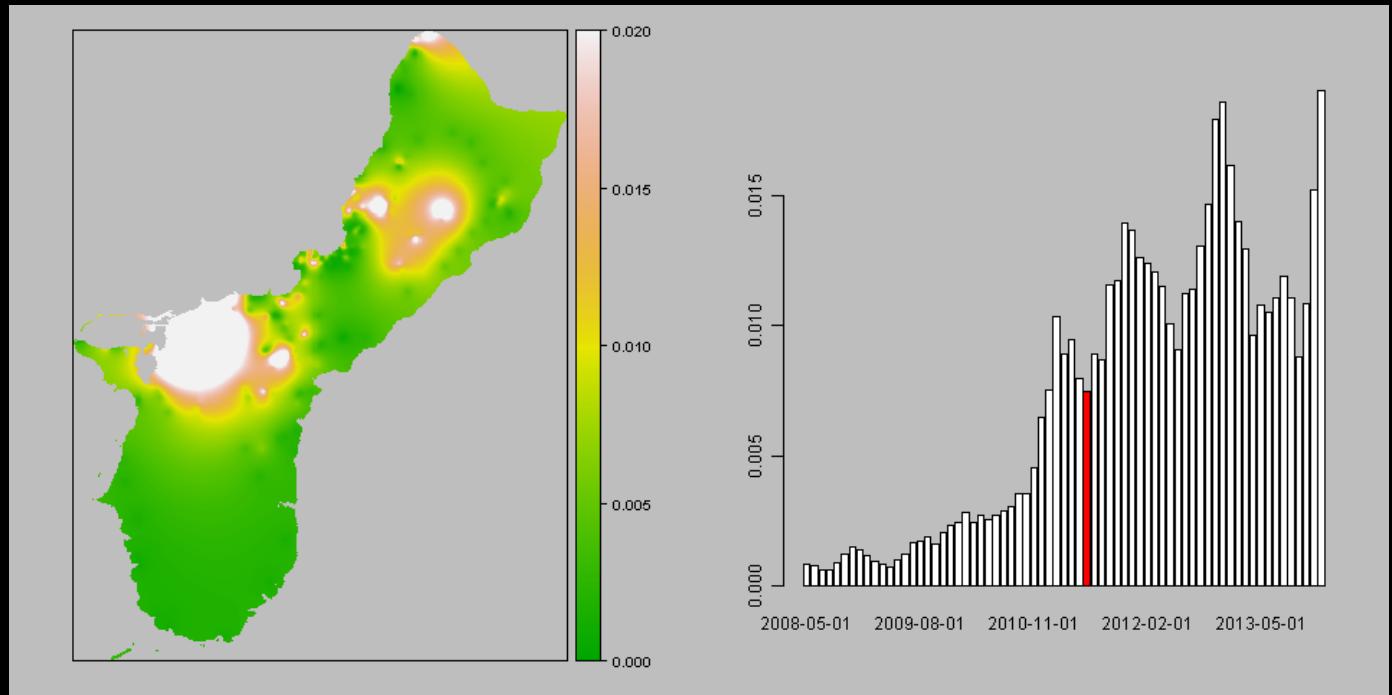
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 May 2011



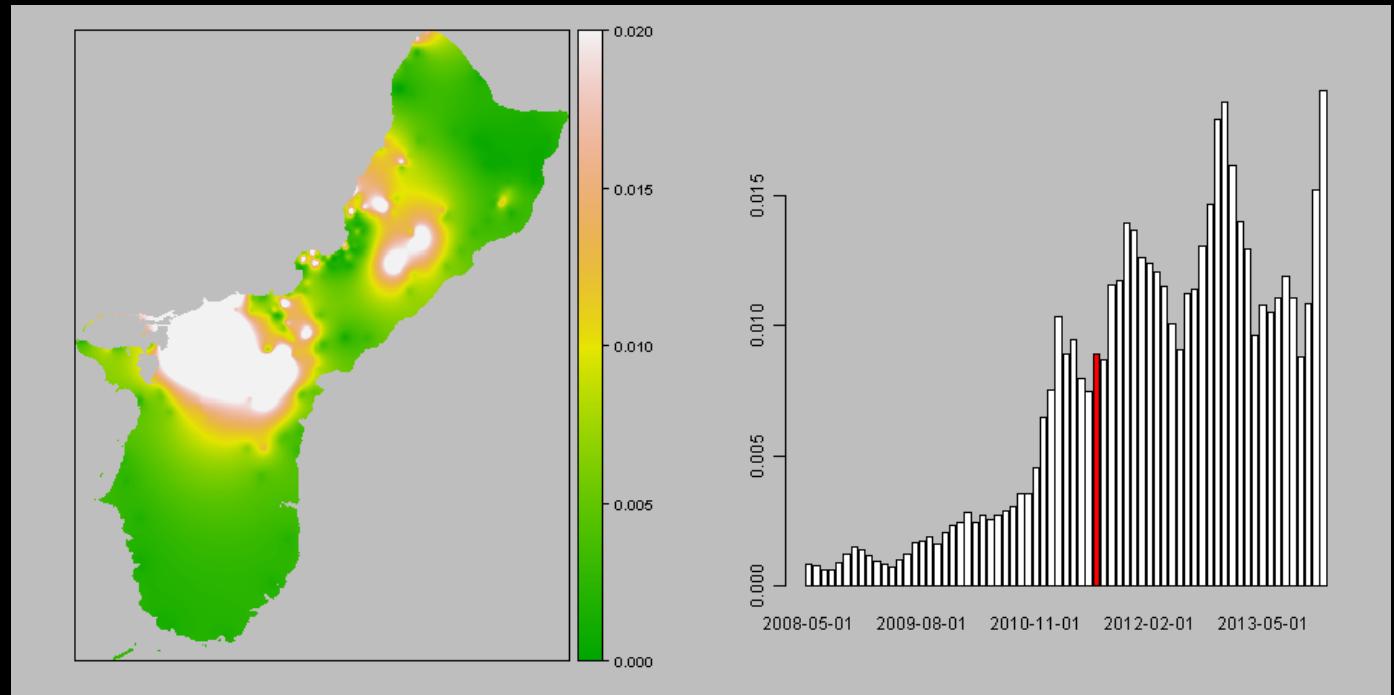
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2011



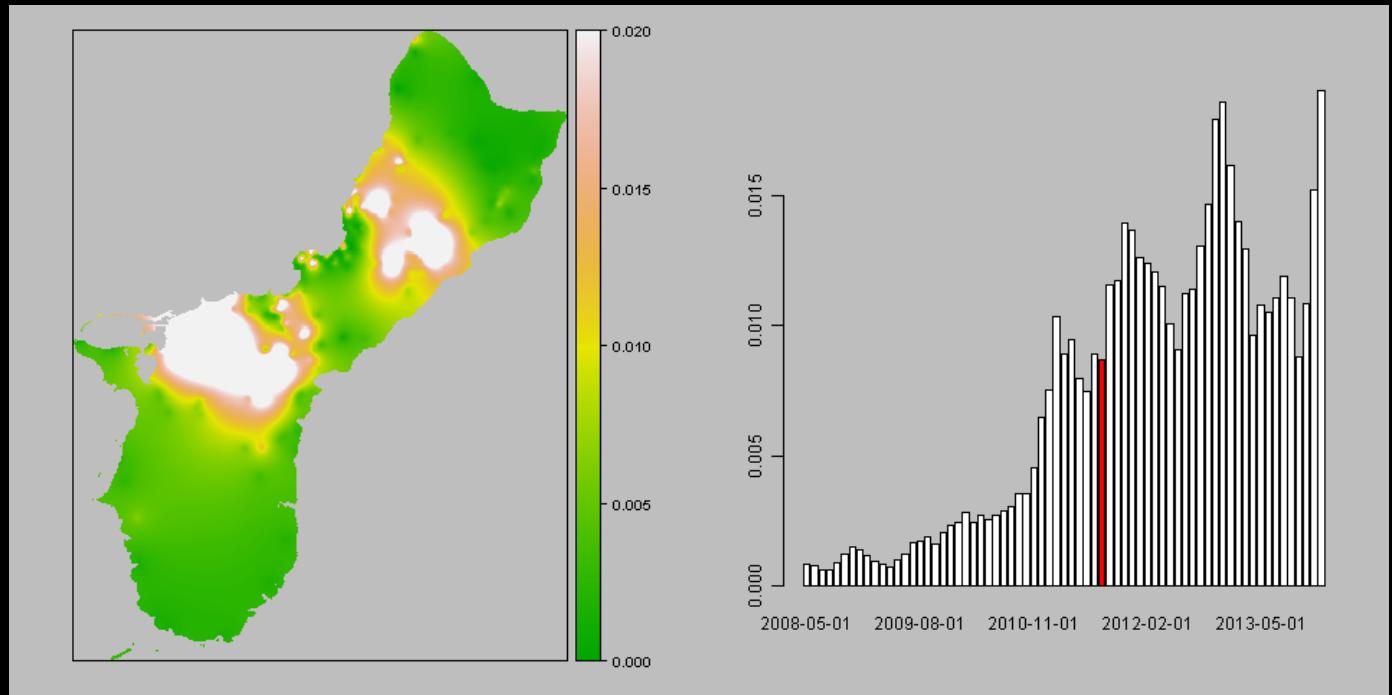
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jul 2011



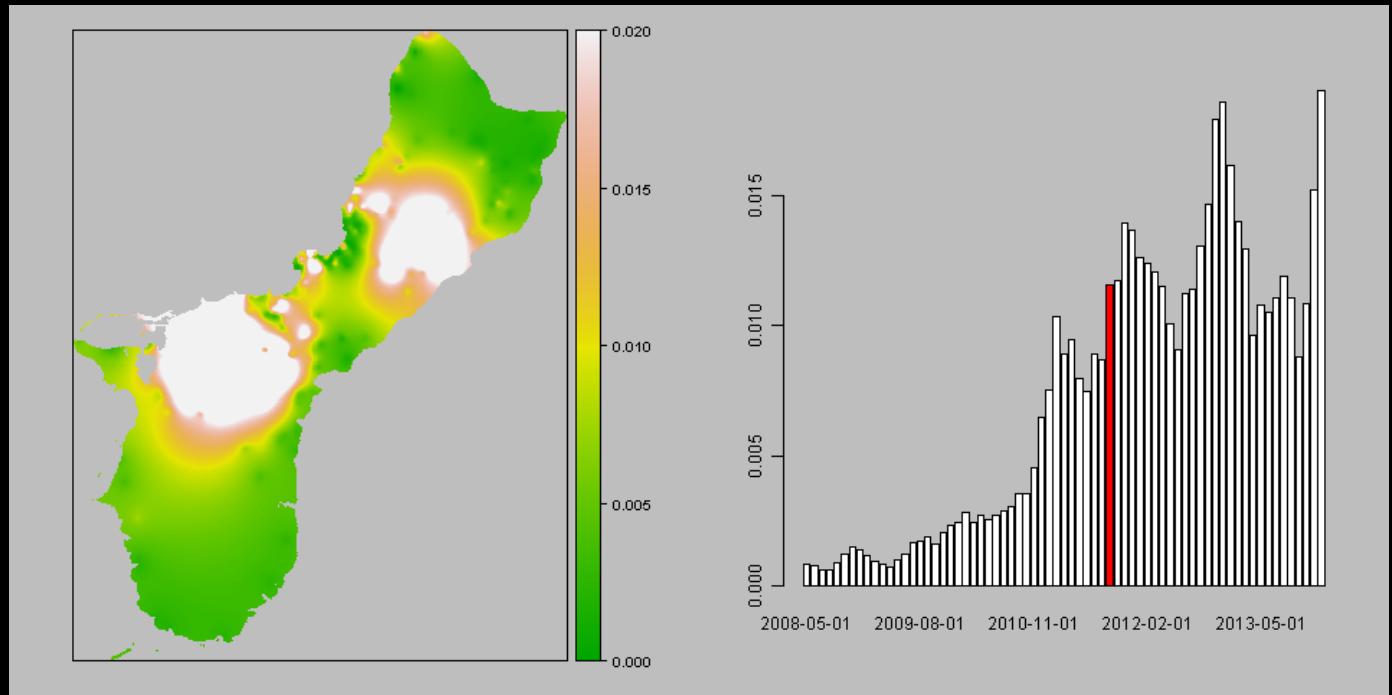
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2011



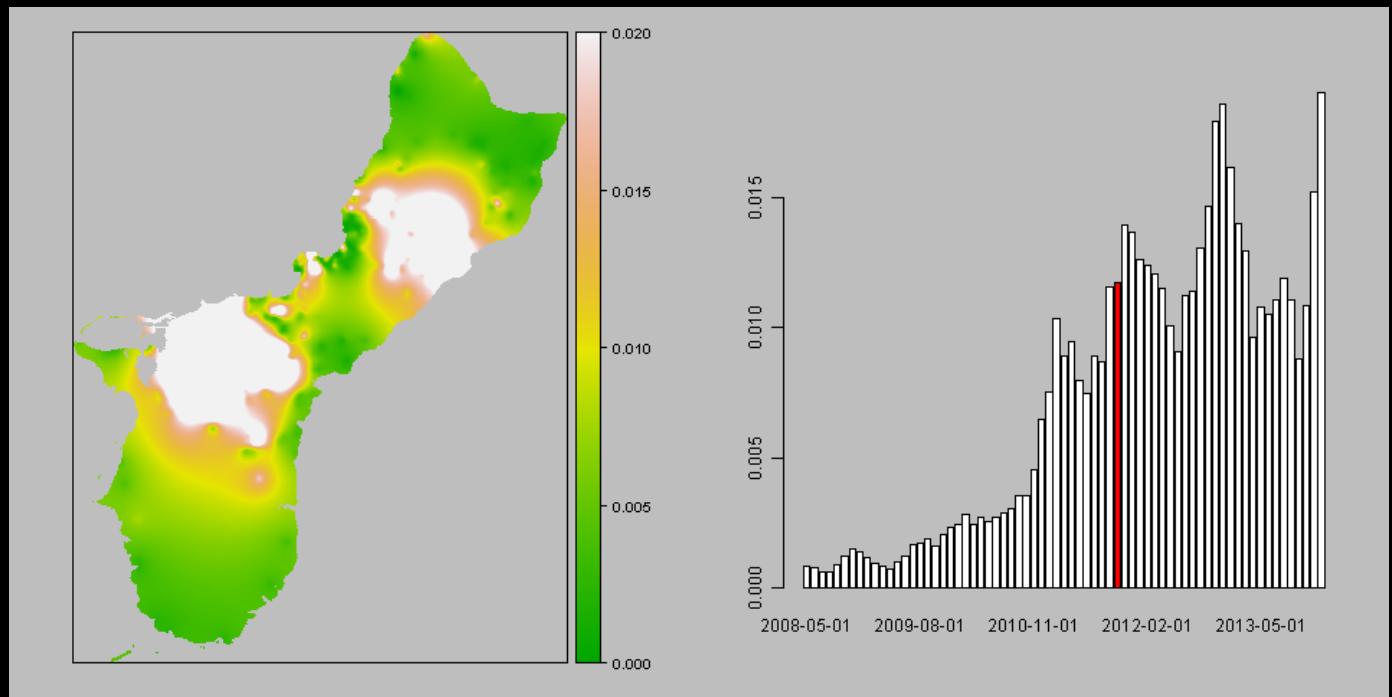
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2011



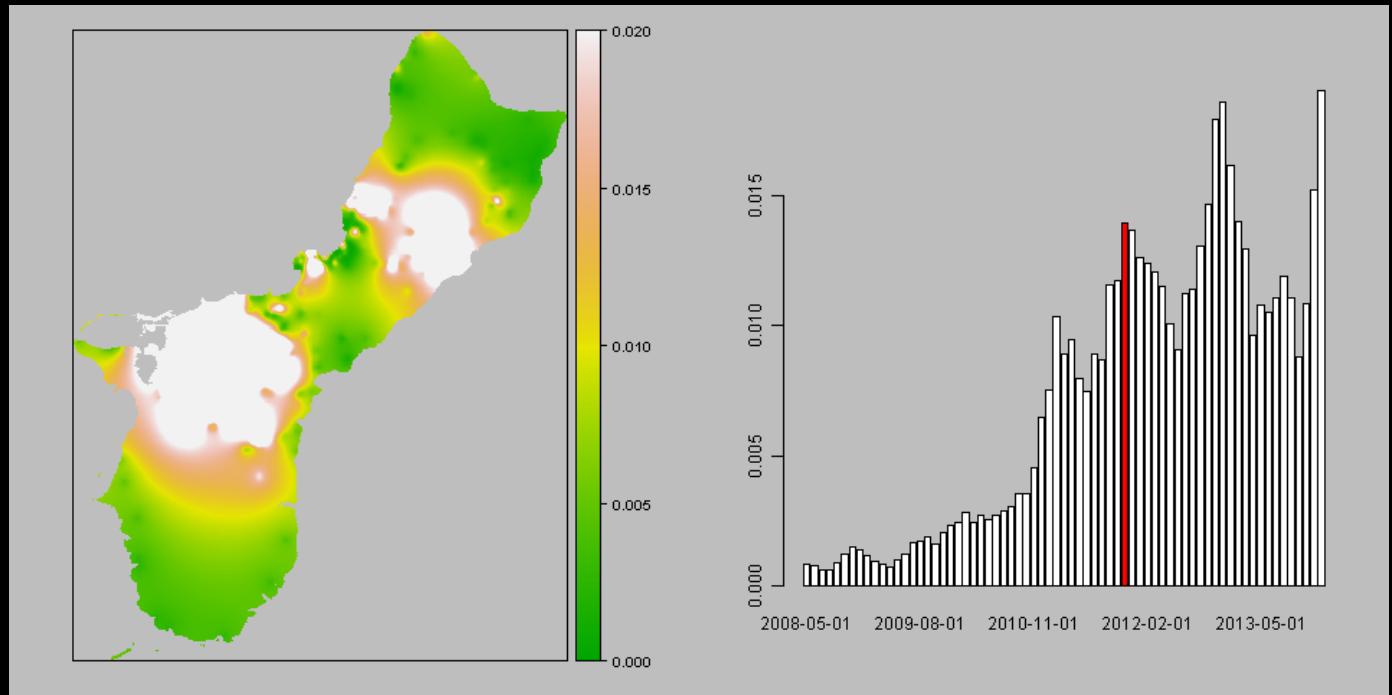
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2011



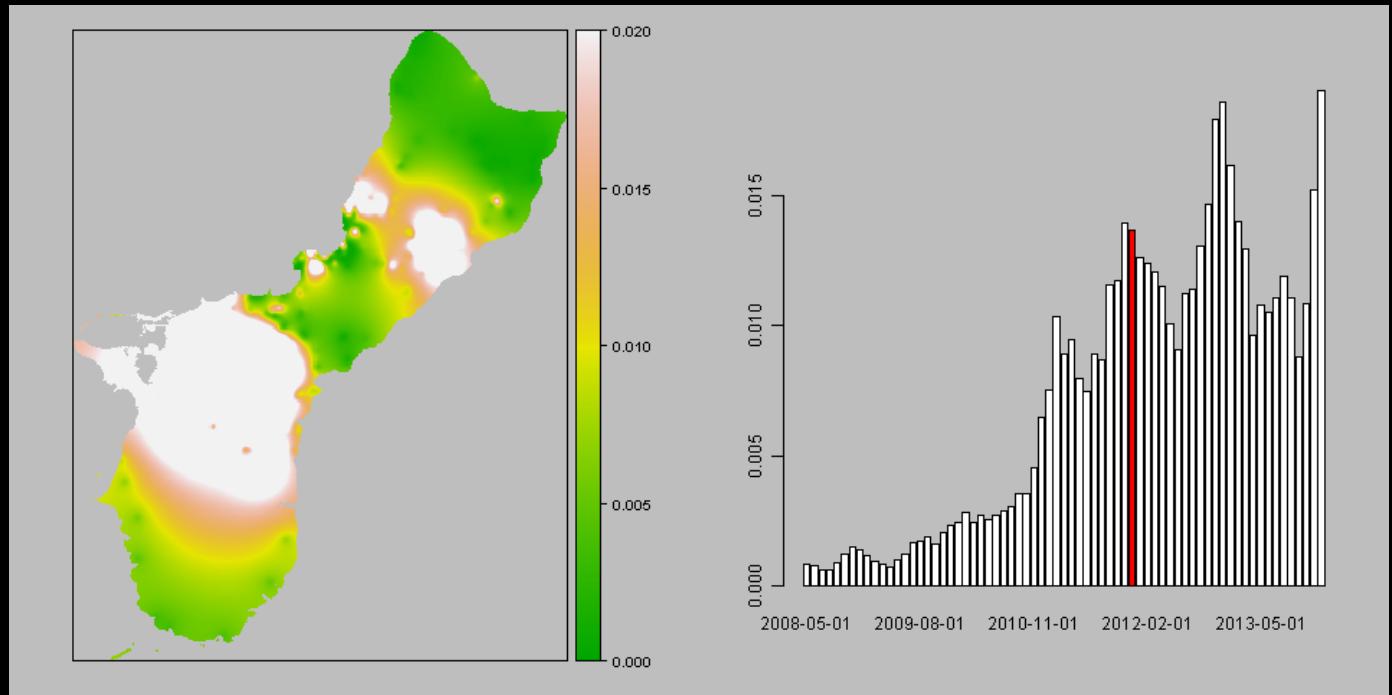
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2011



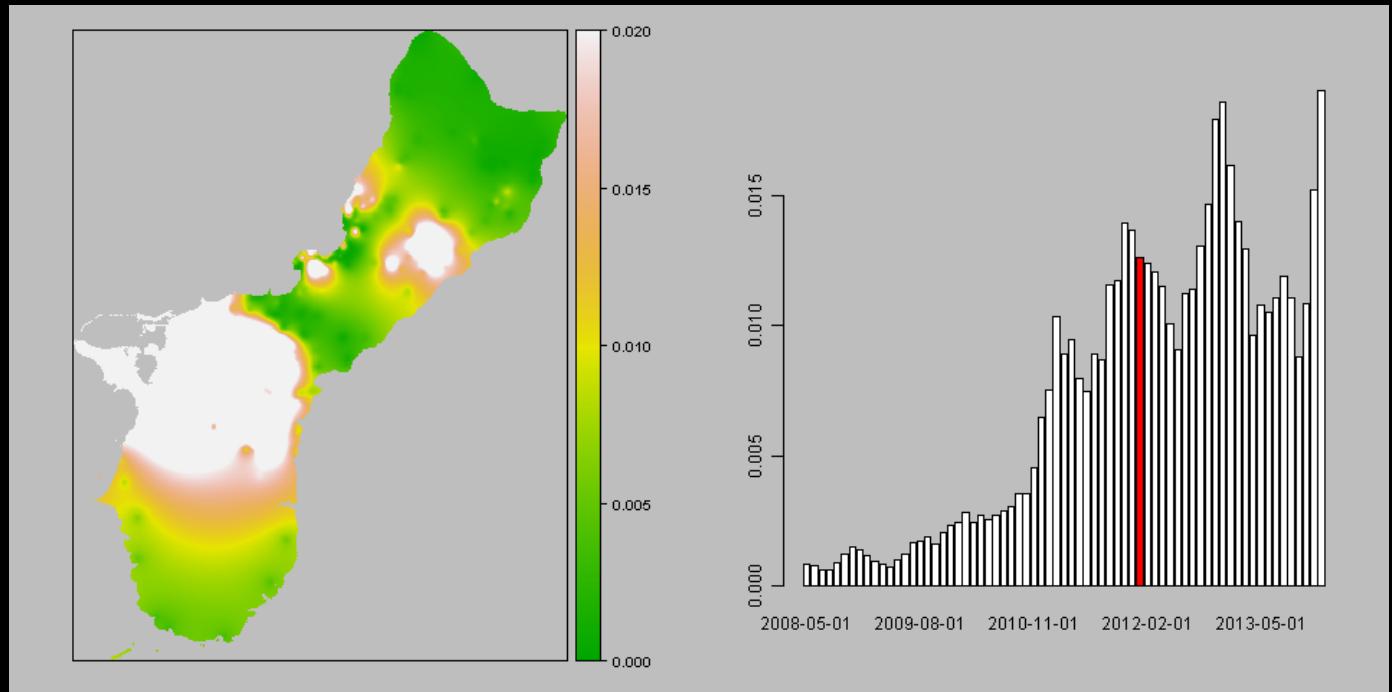
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2011



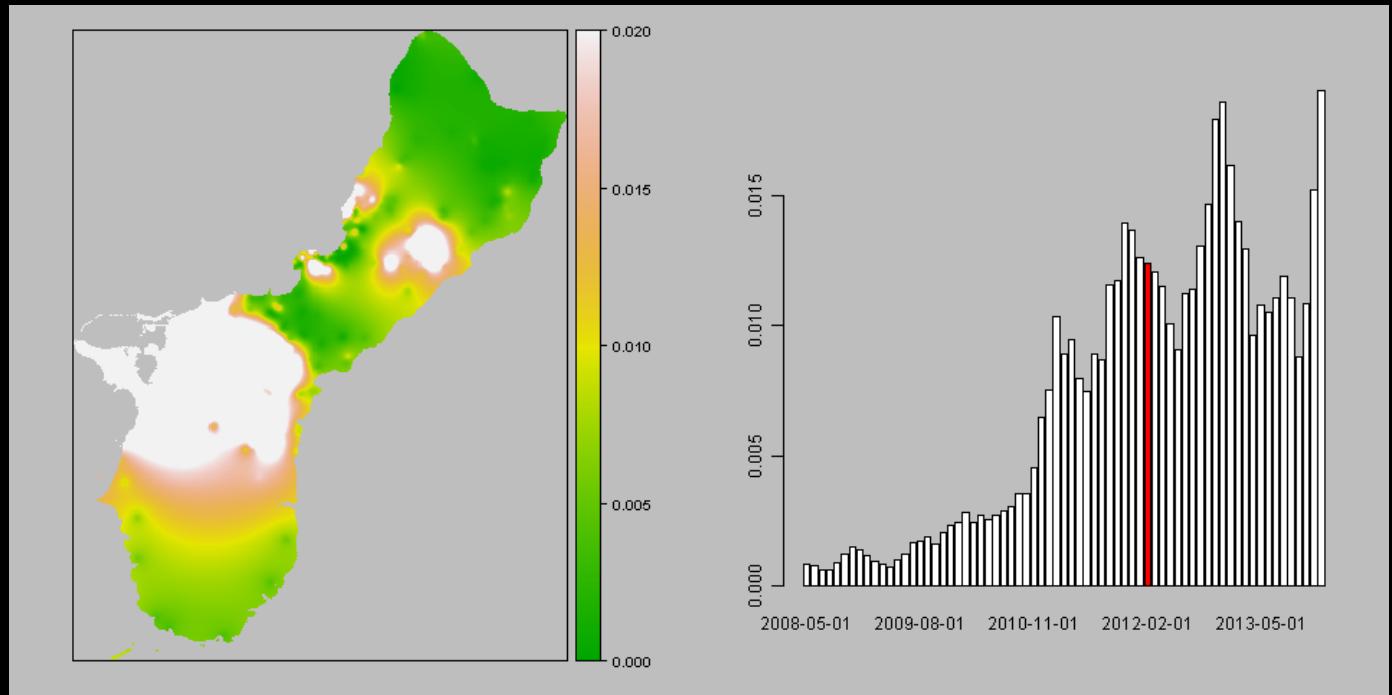
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2012



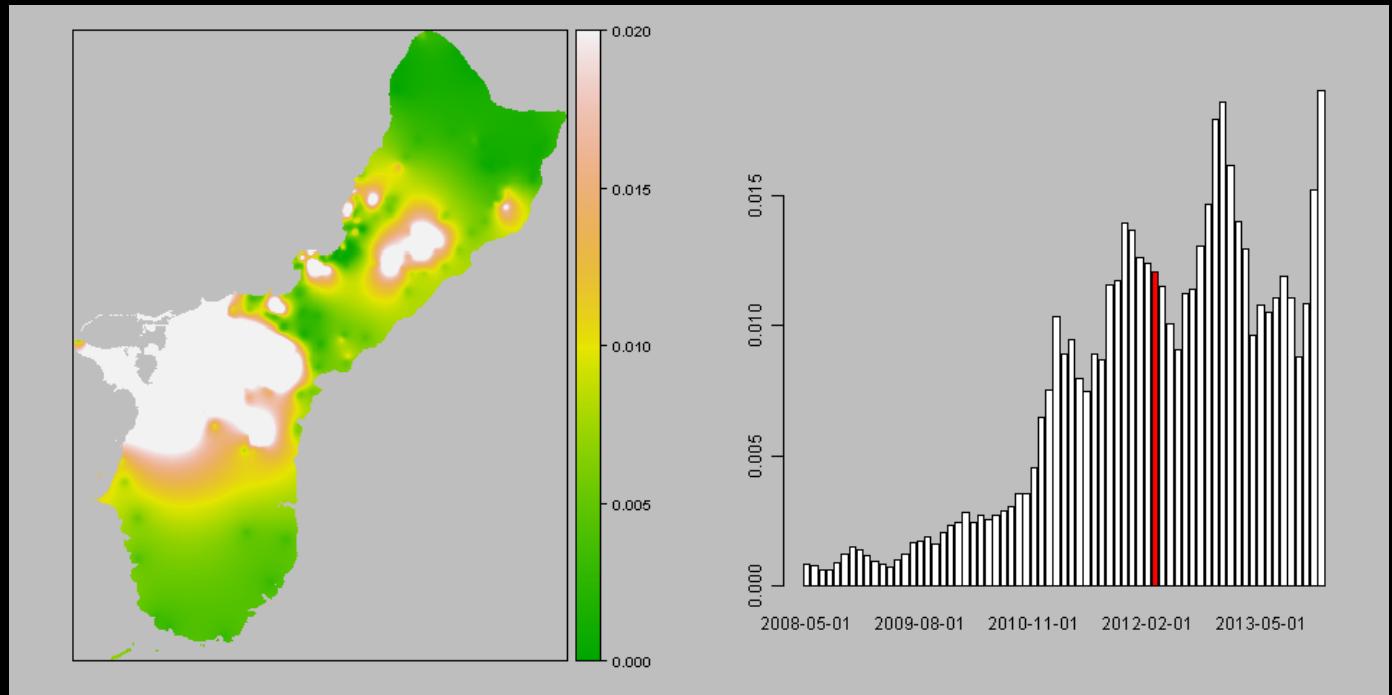
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Feb 2012



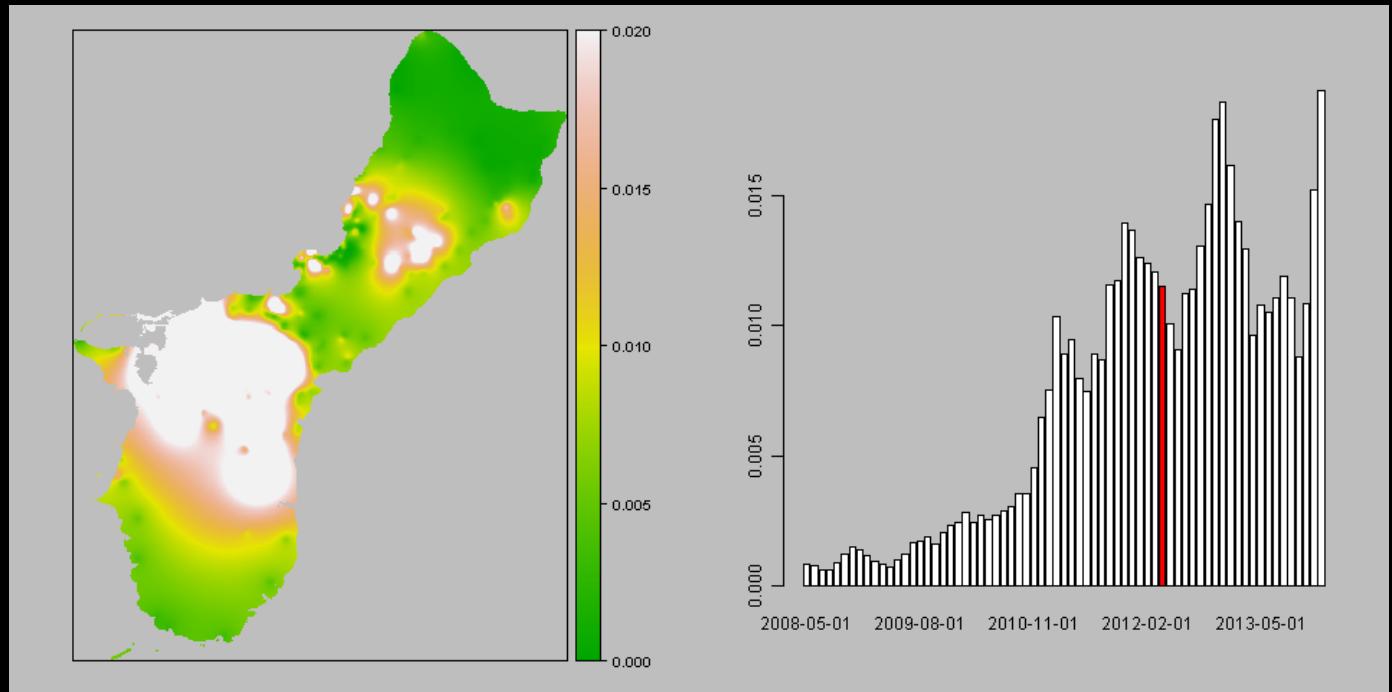
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Mar 2012



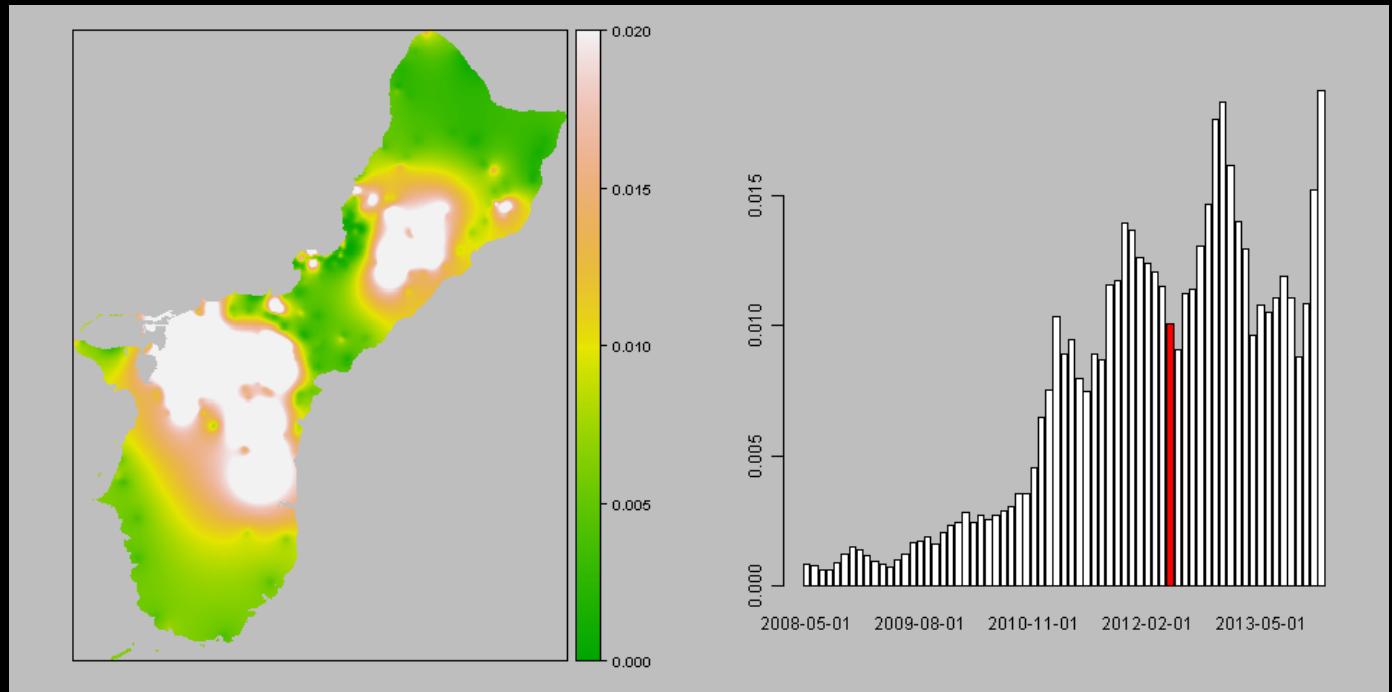
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Apr 2012



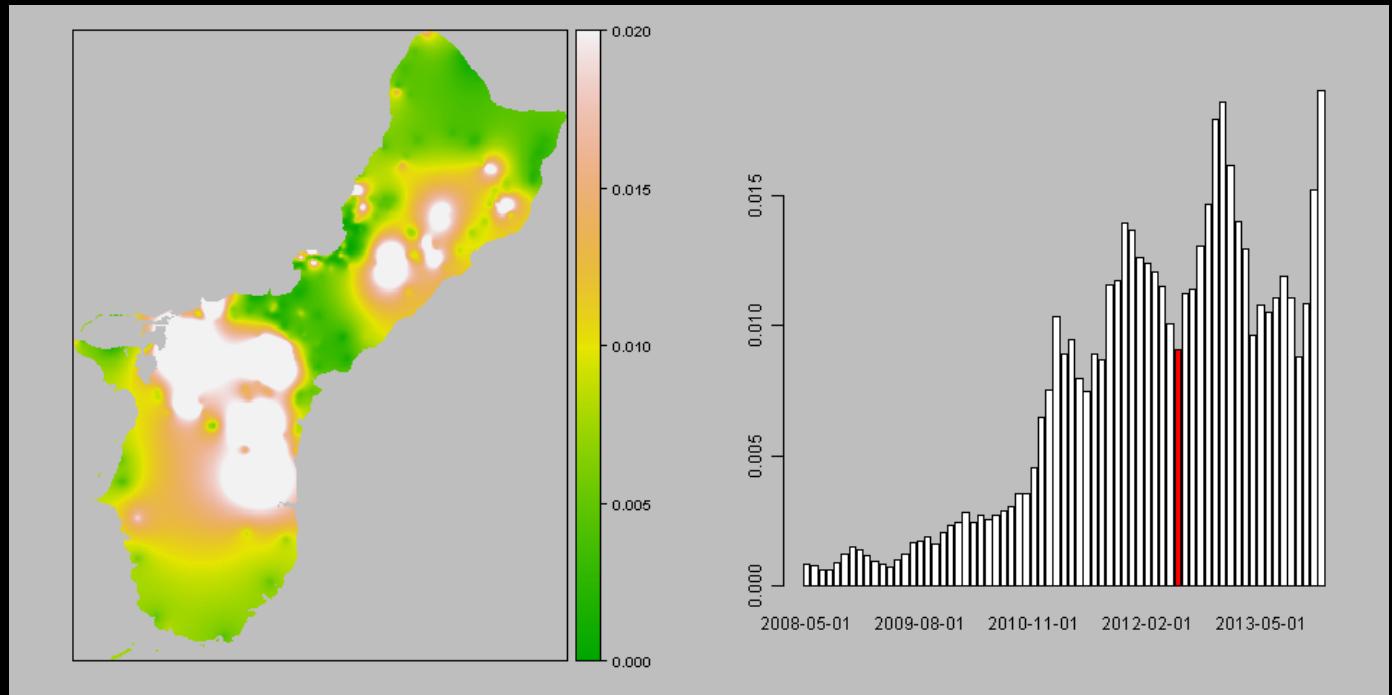
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 May 2012



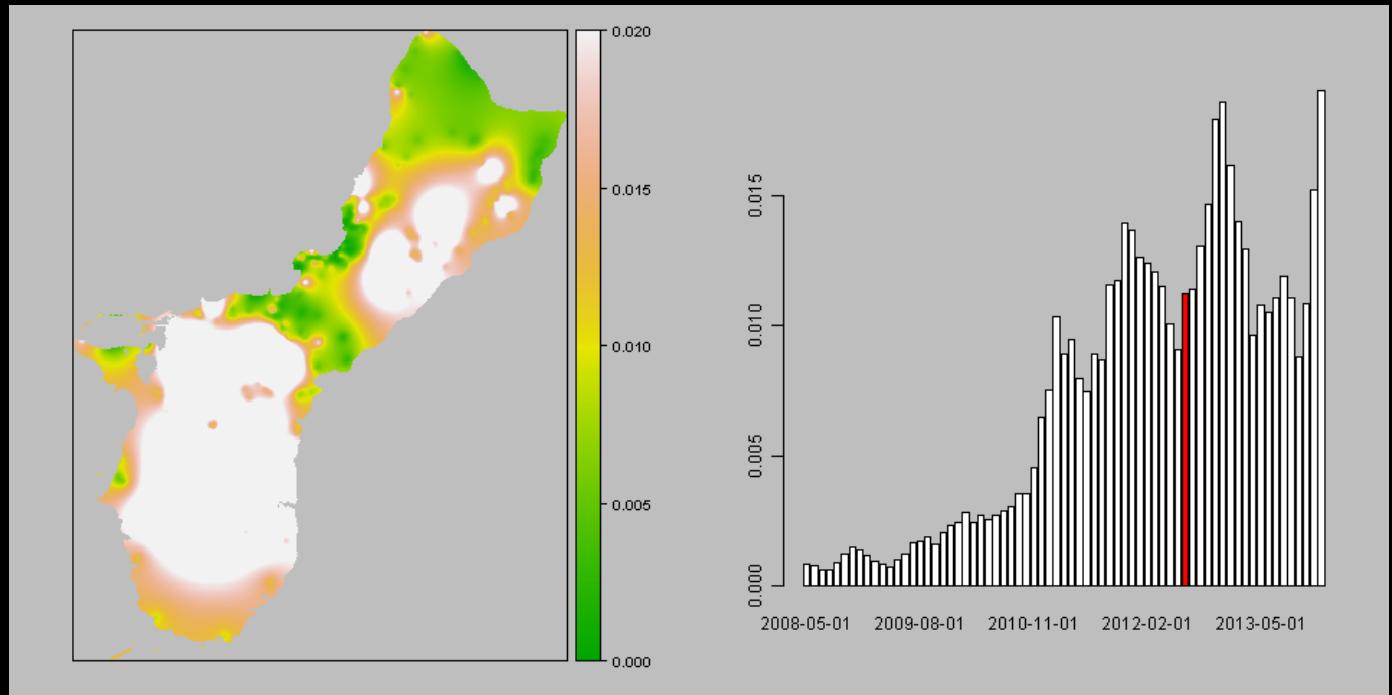
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2012



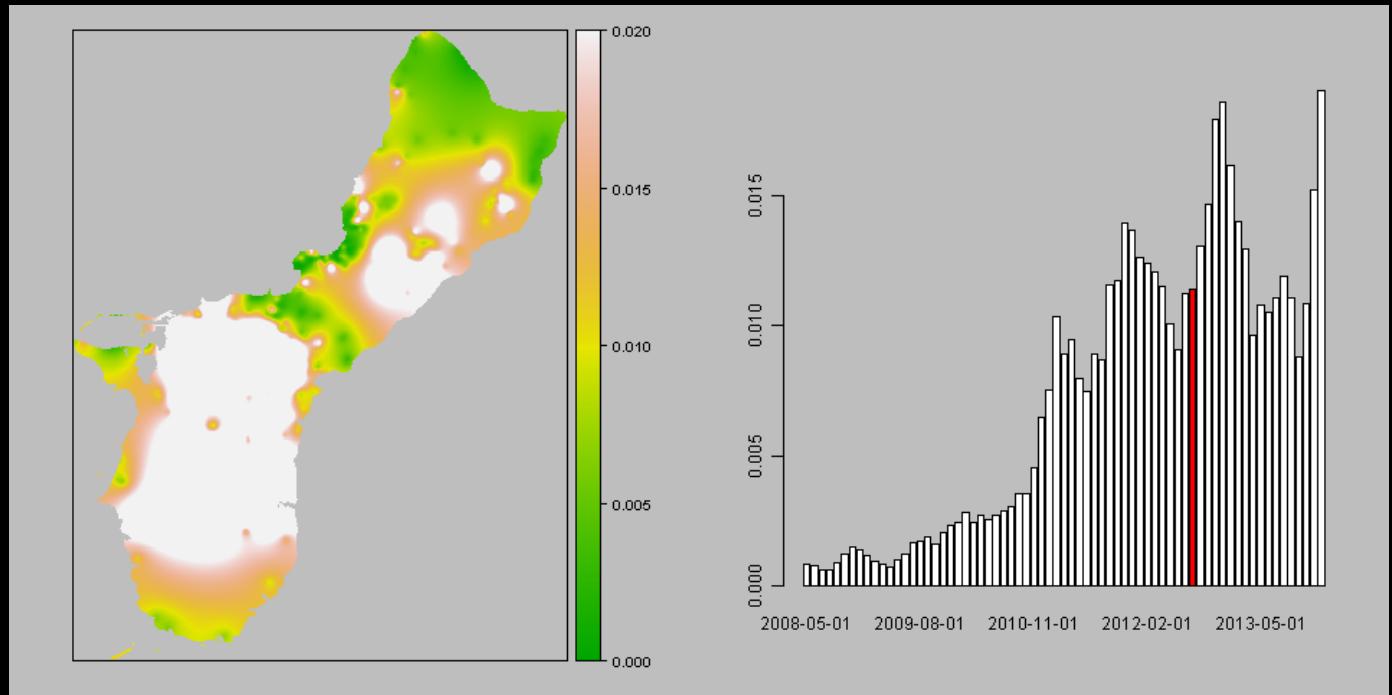
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jul 2012



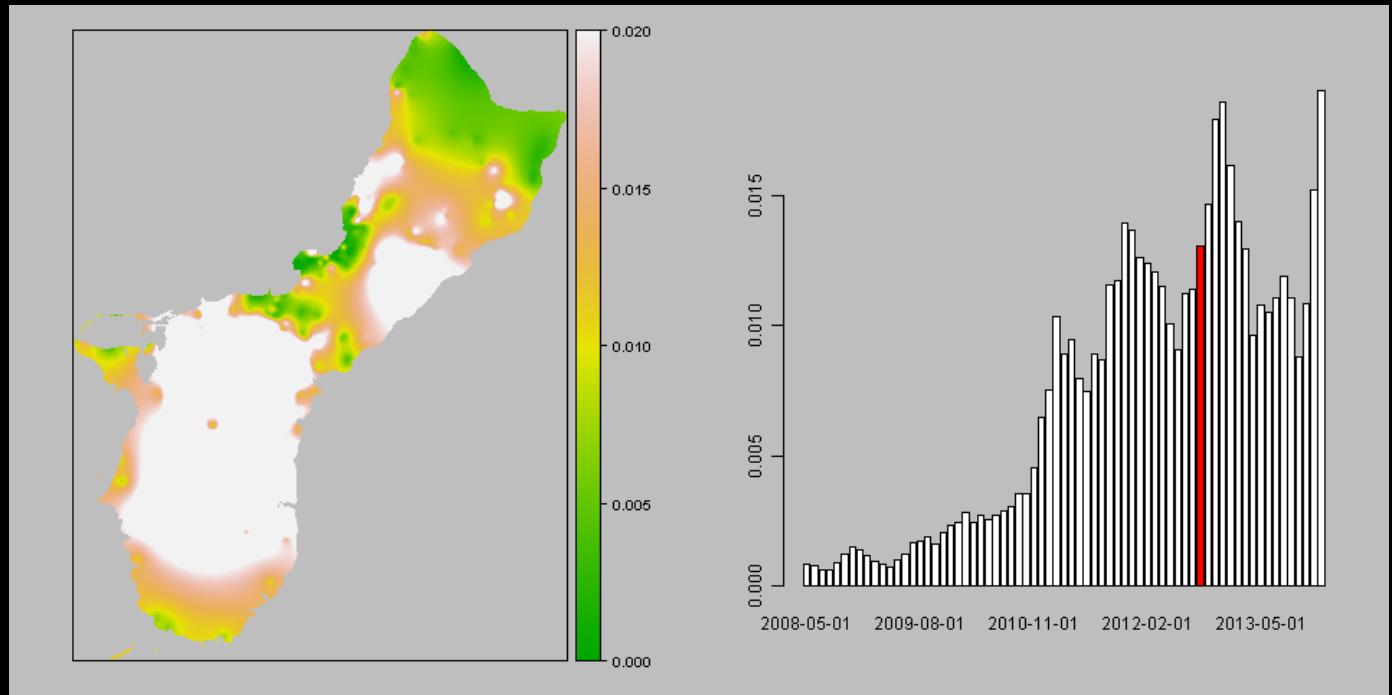
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2012



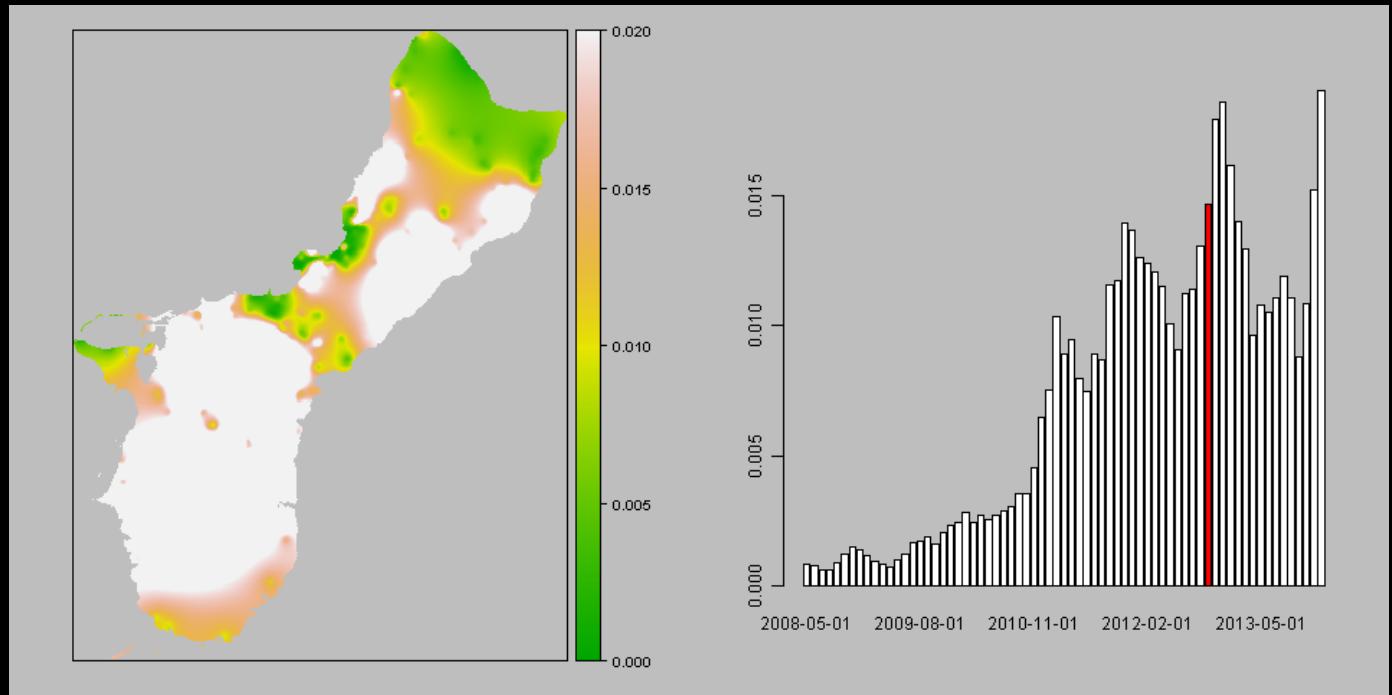
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2012



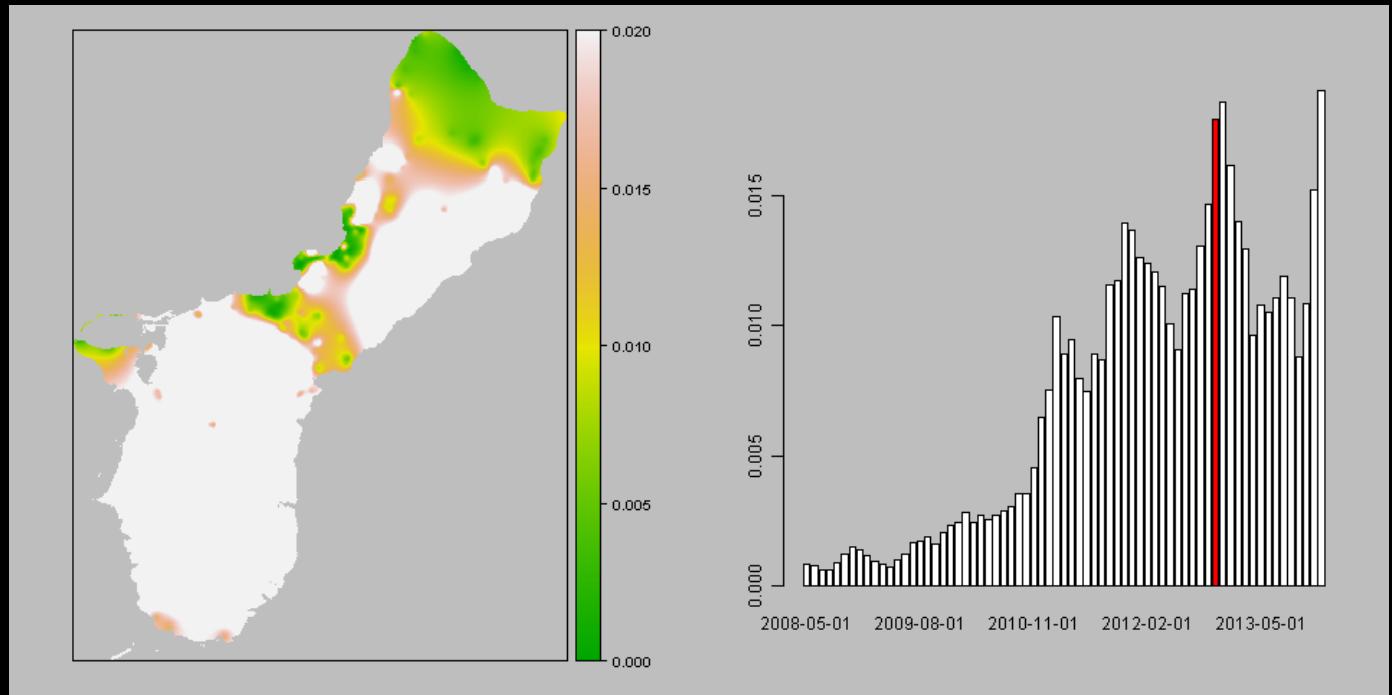
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2012



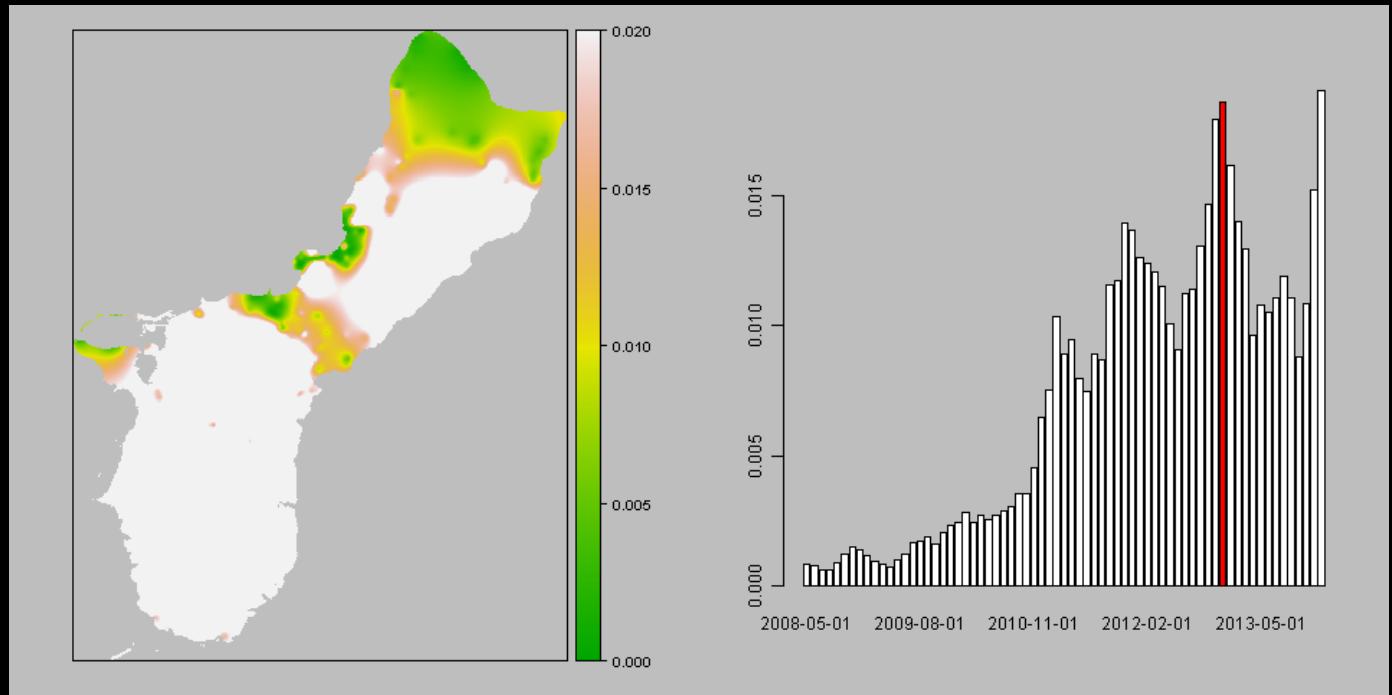
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2012



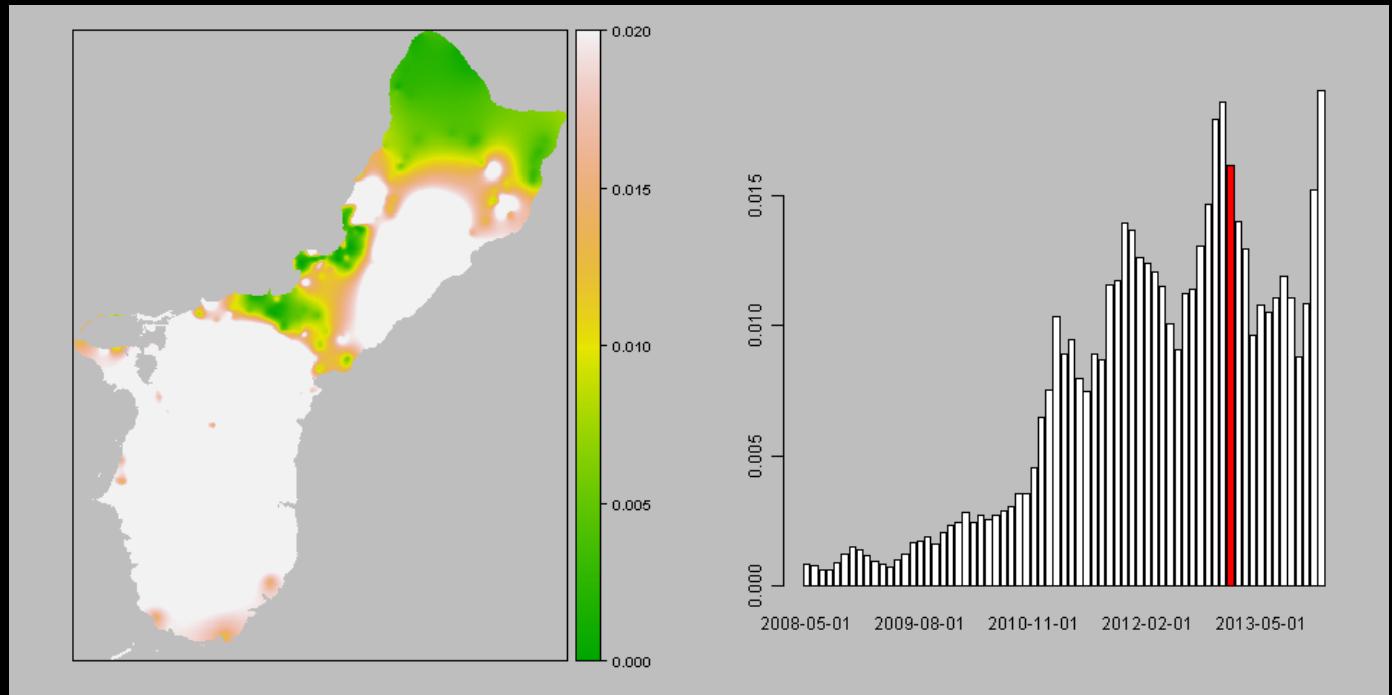
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2012



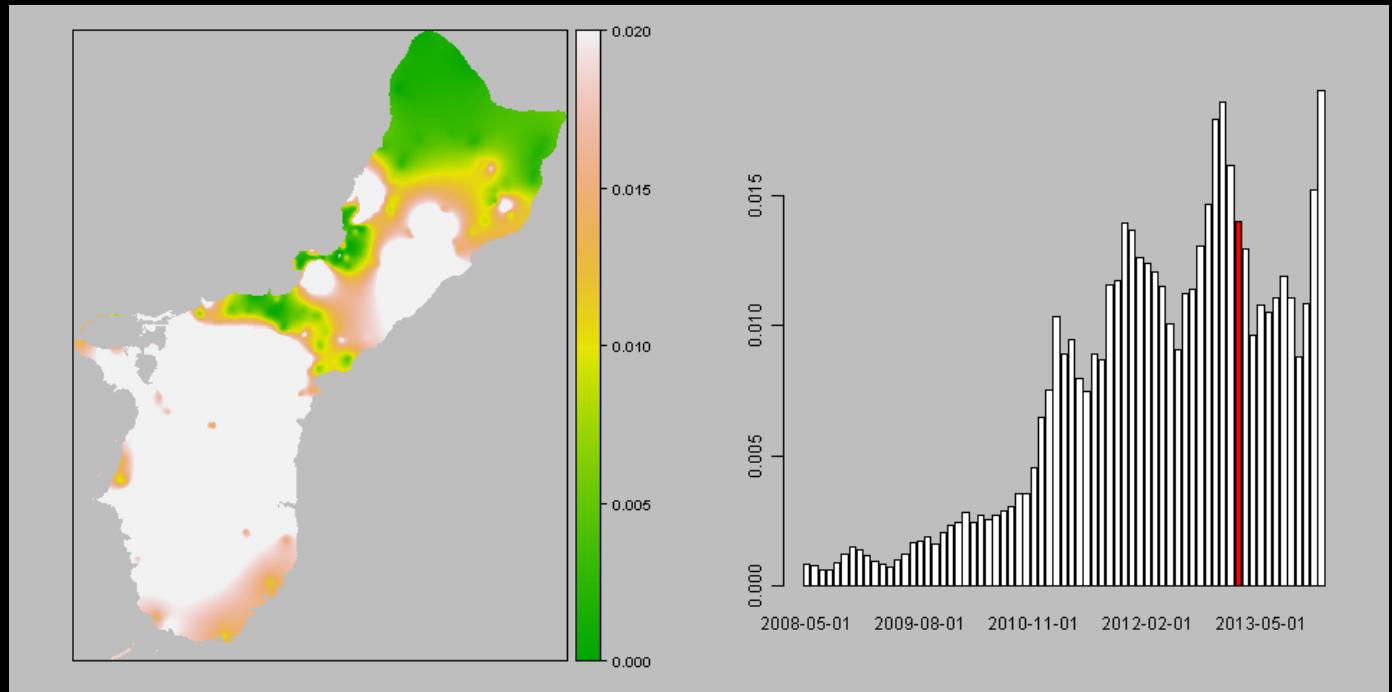
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2013



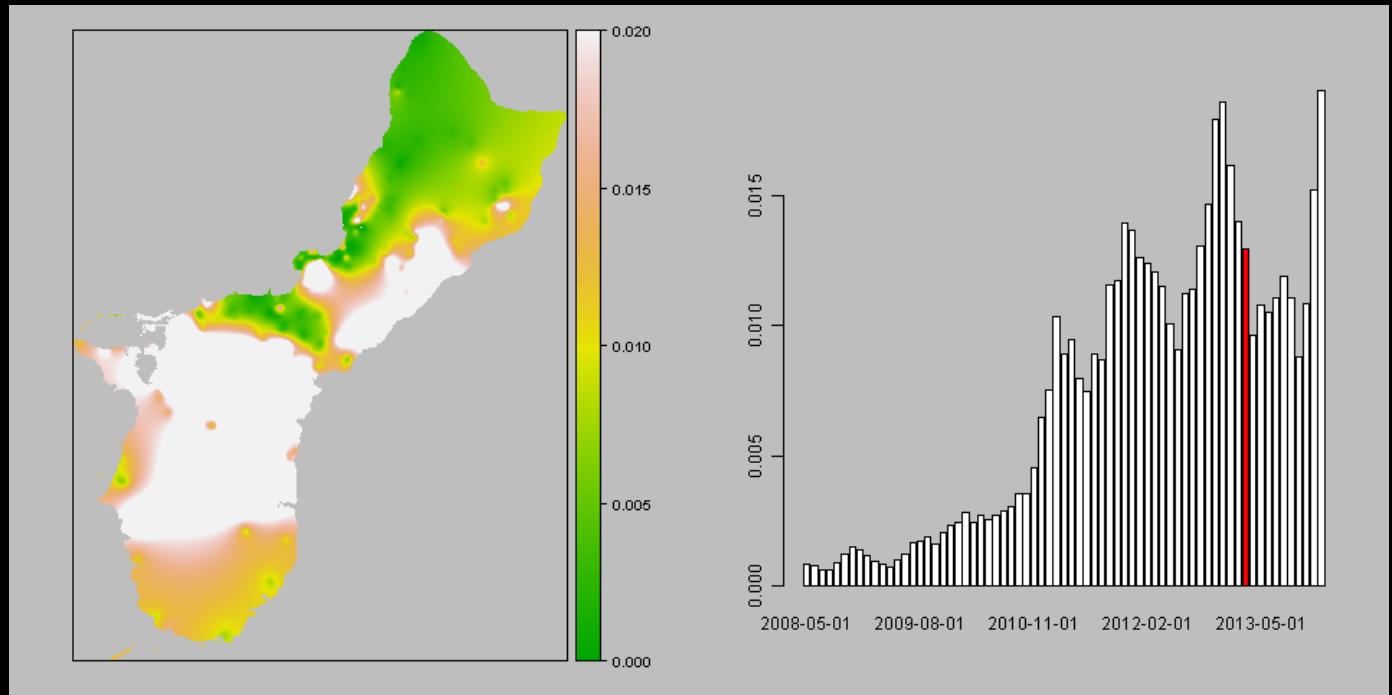
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Feb 2013

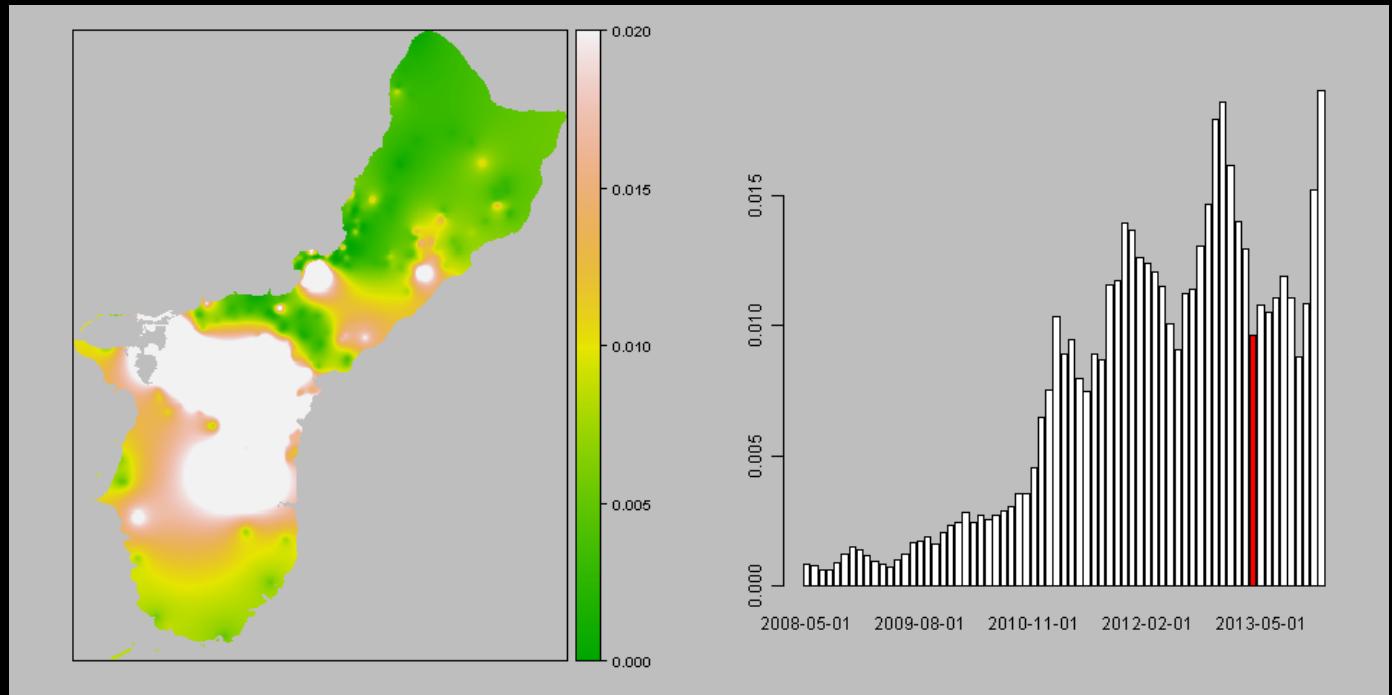


Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Mar 2013

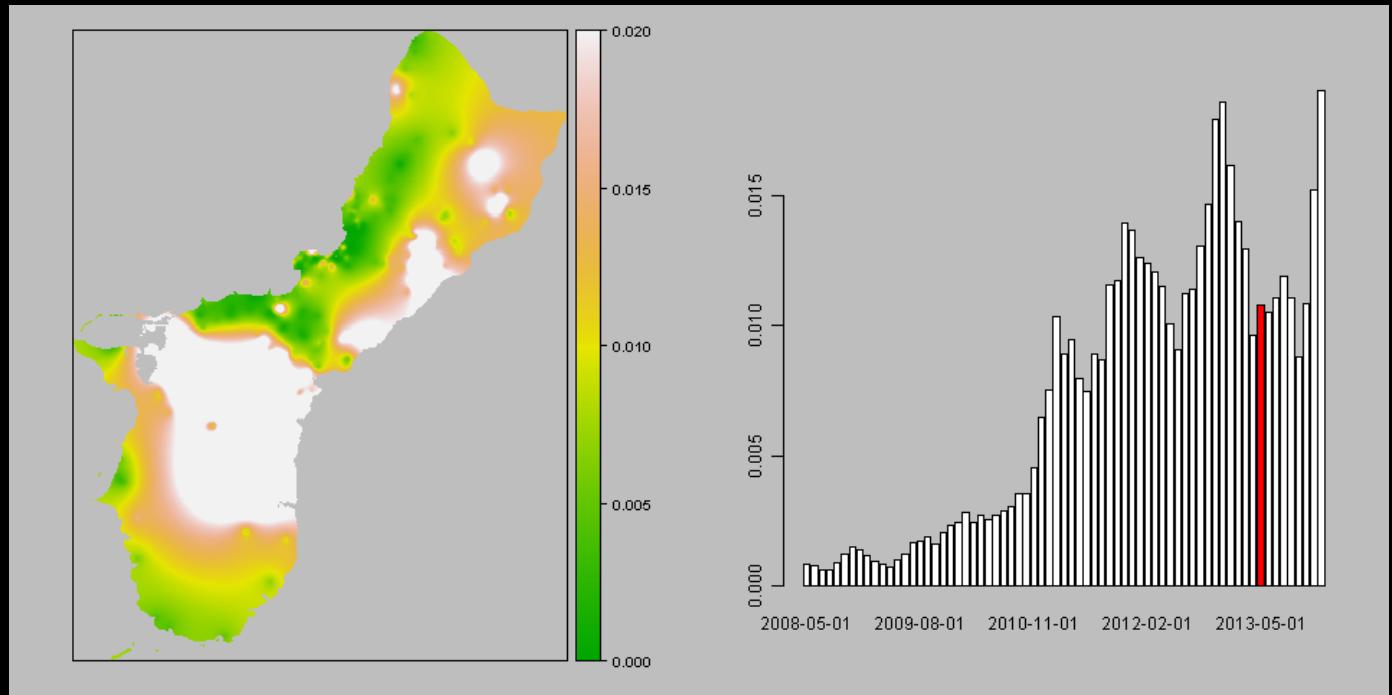


90 day trapping period ending on 01 Apr 2013



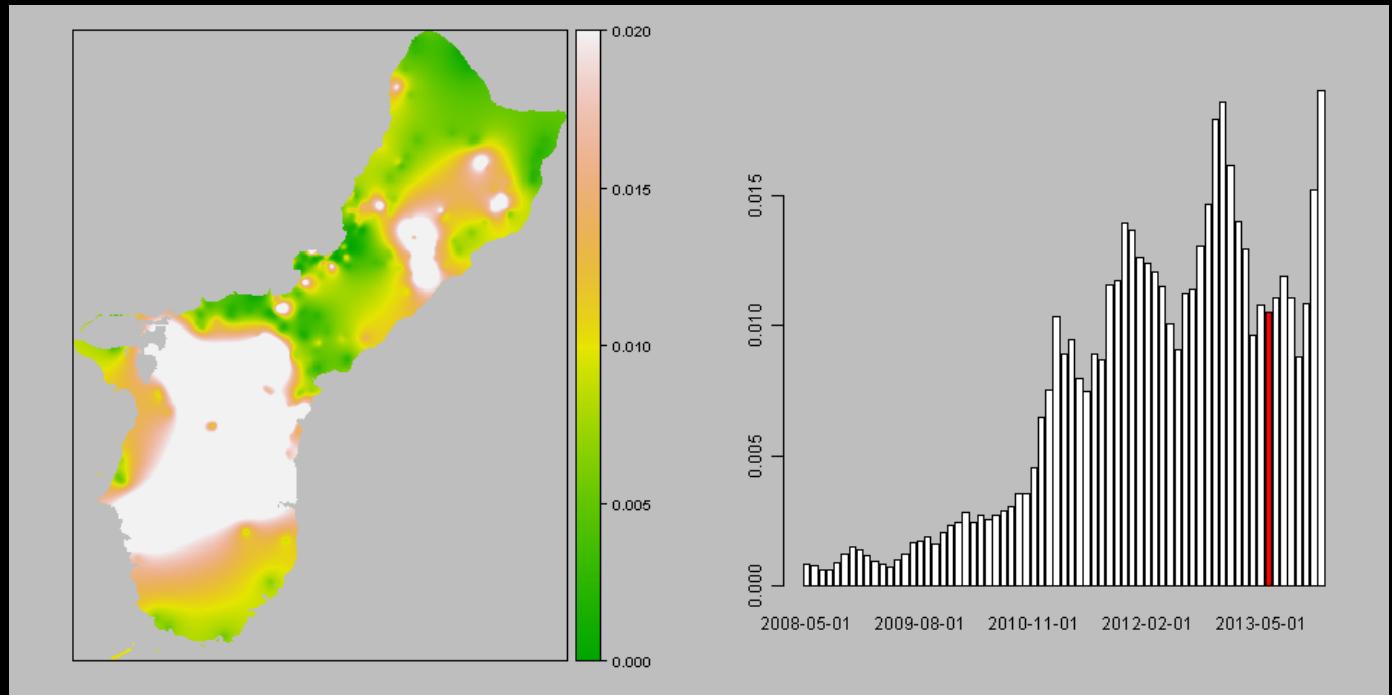
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 May 2013



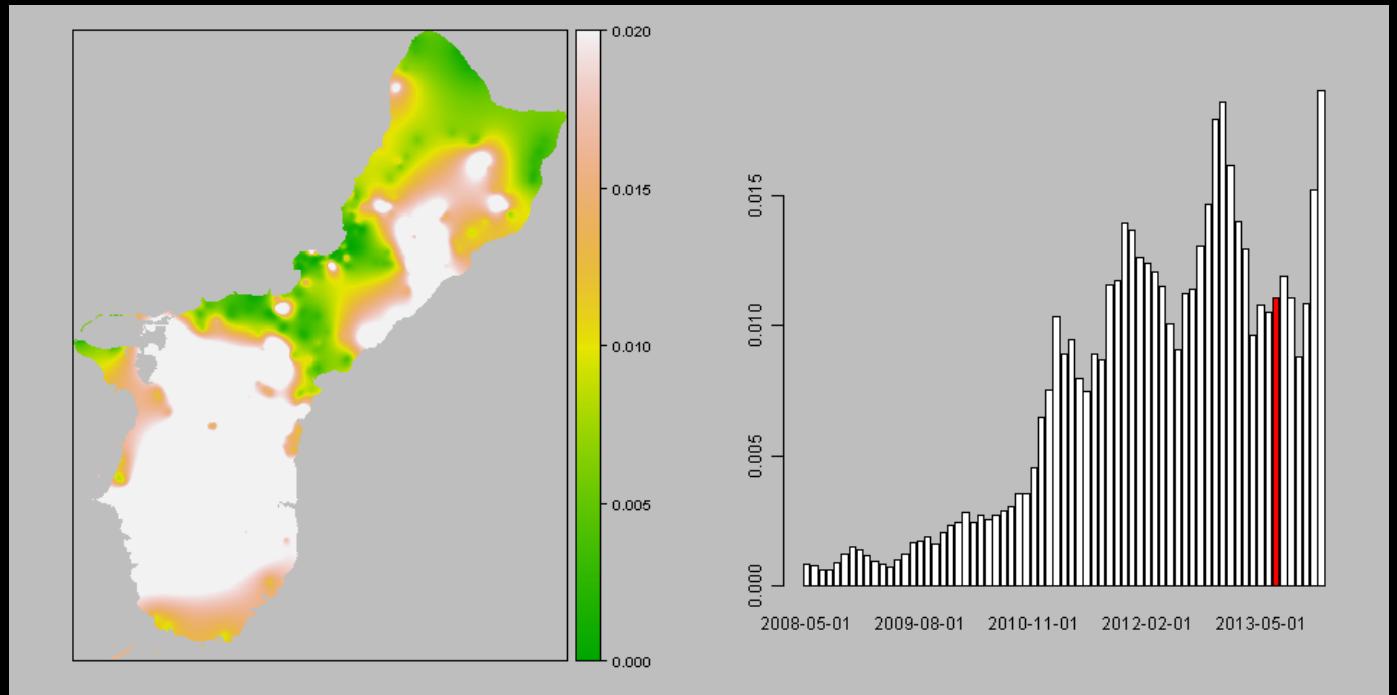
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jun 2013



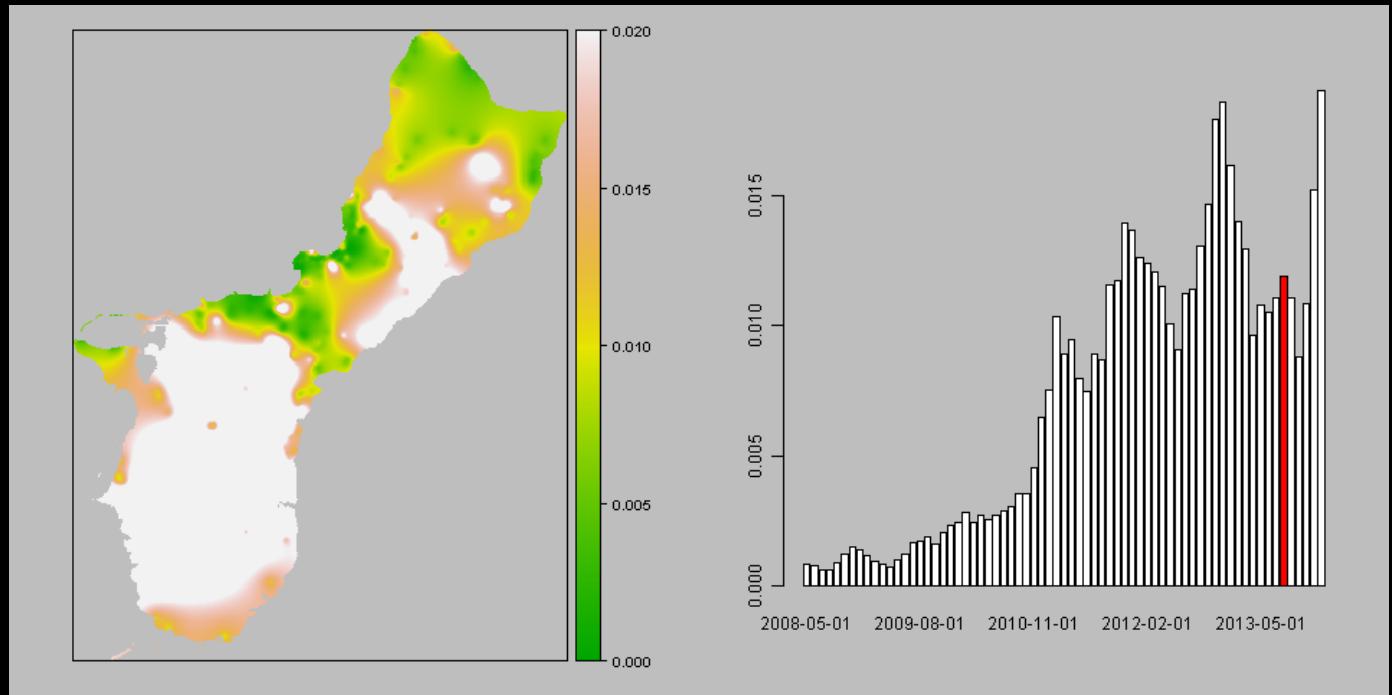
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jul 2013



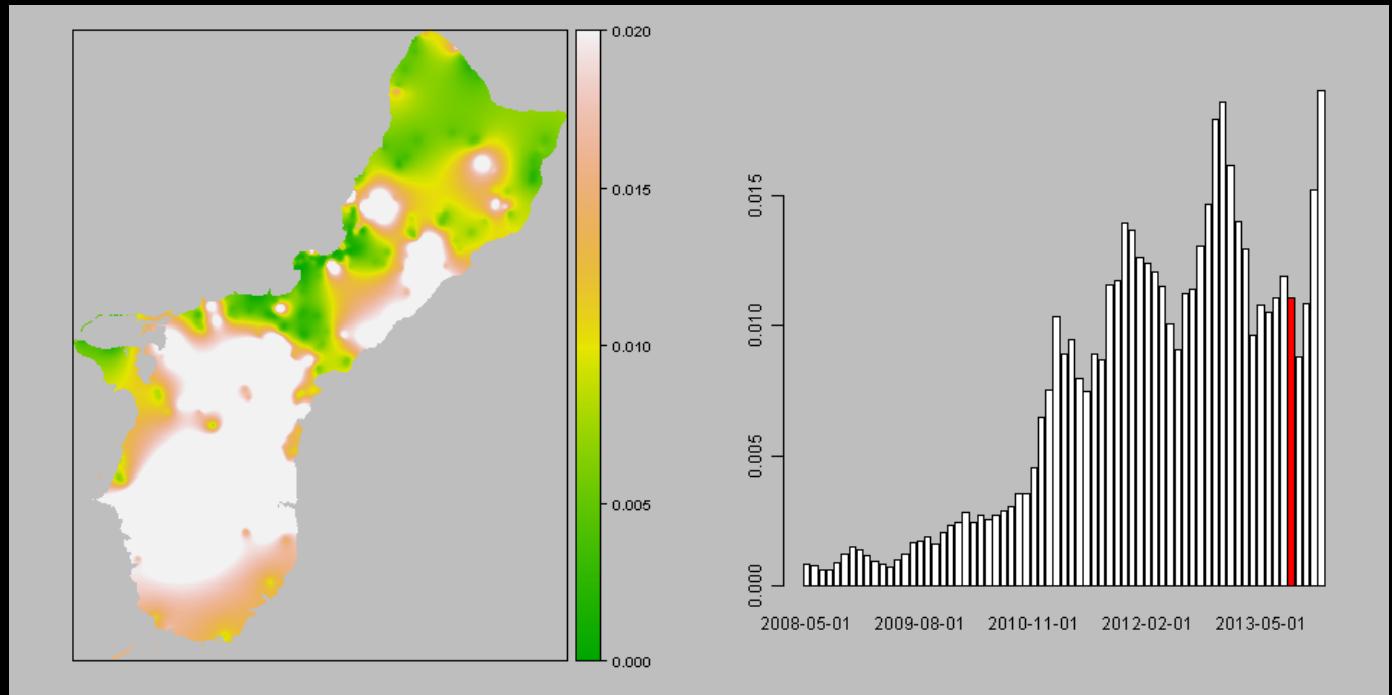
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Aug 2013



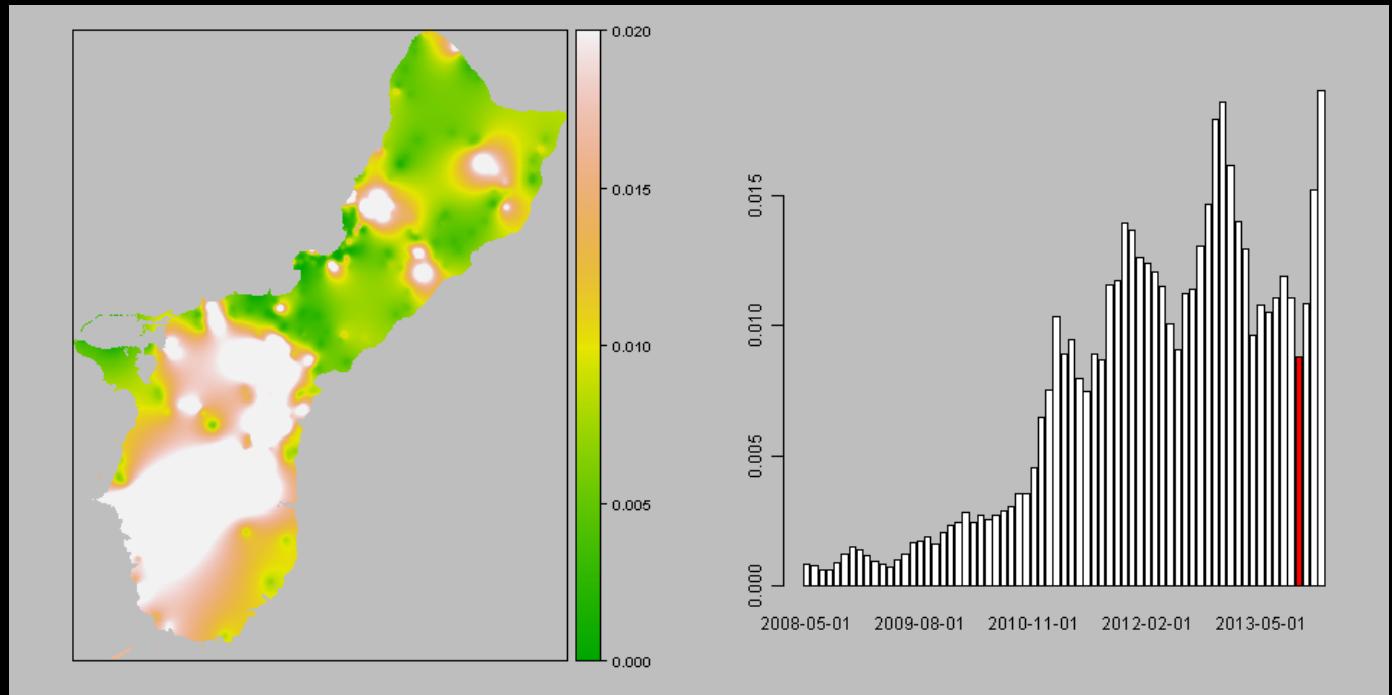
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Sep 2013



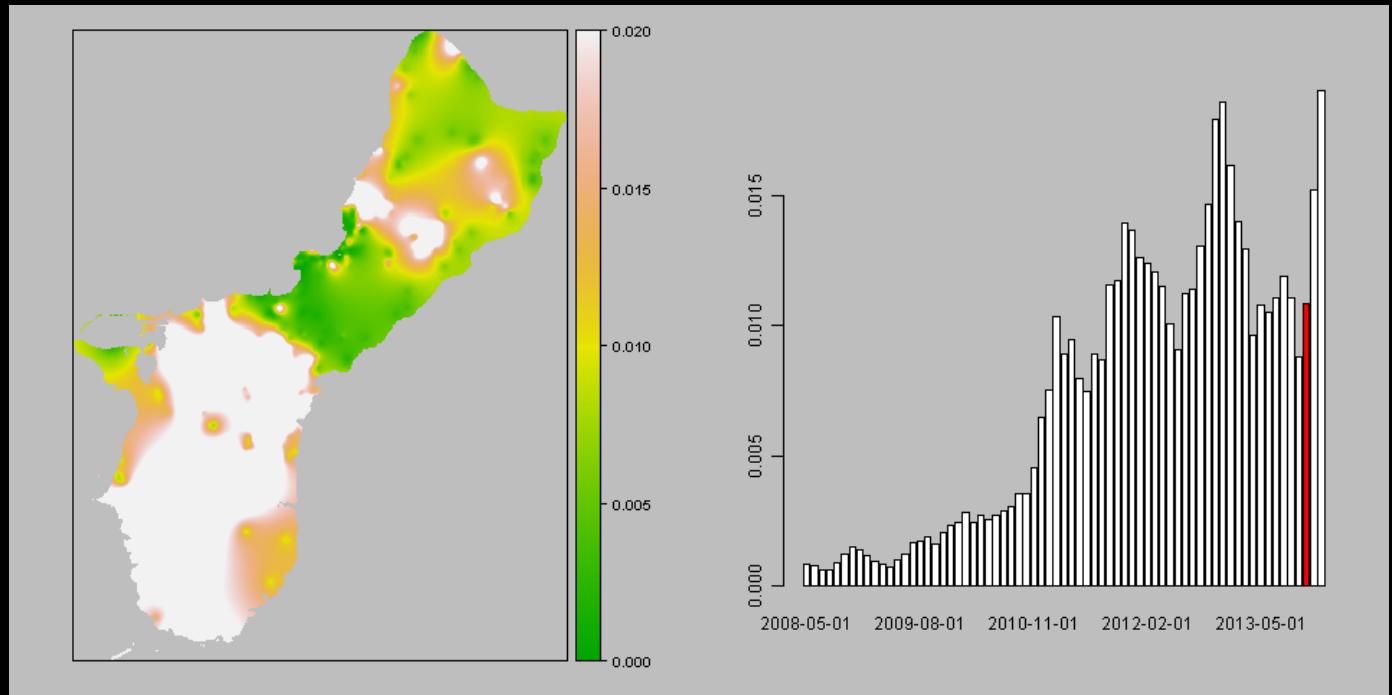
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Oct 2013



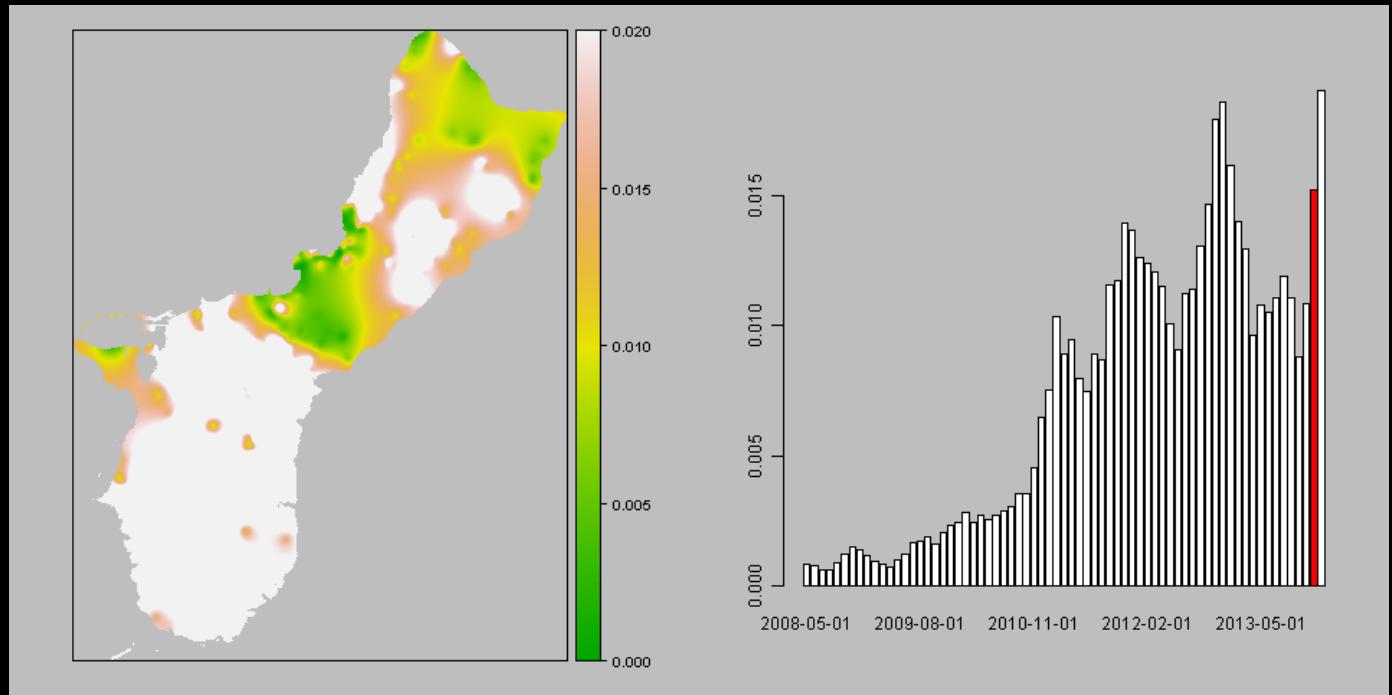
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Nov 2013



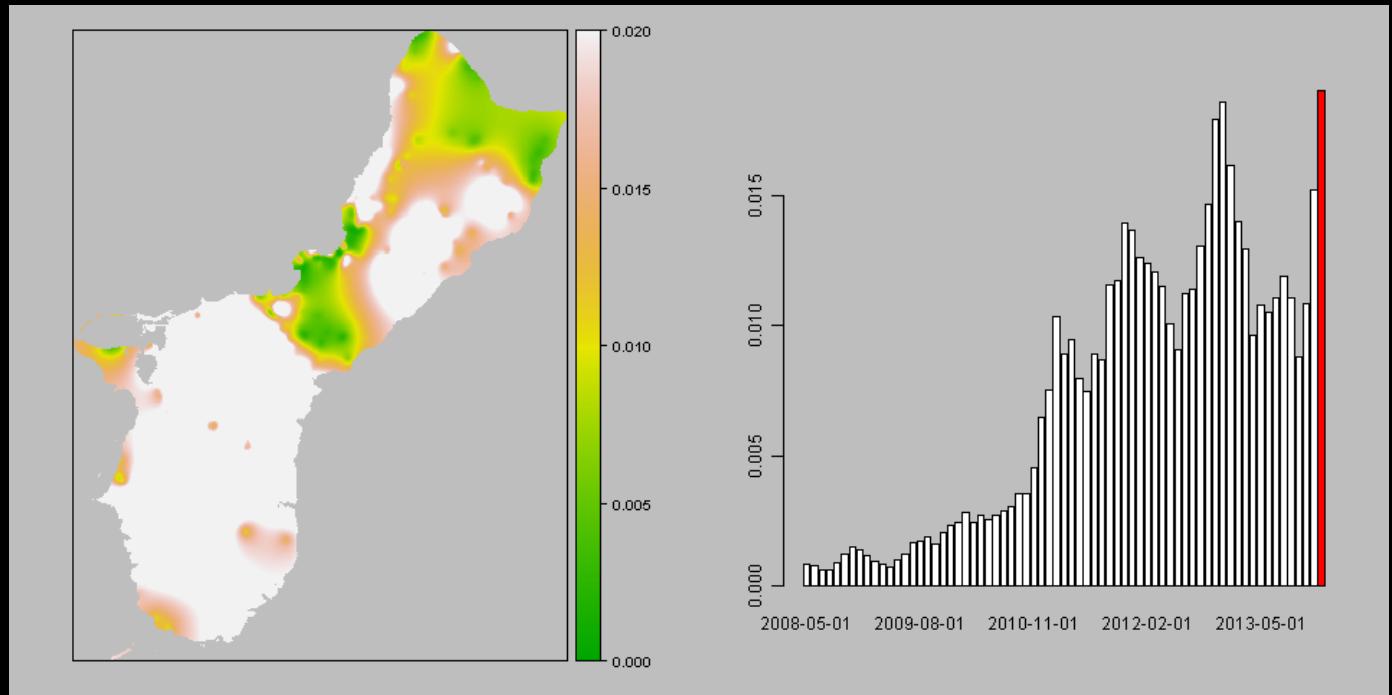
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Dec 2013



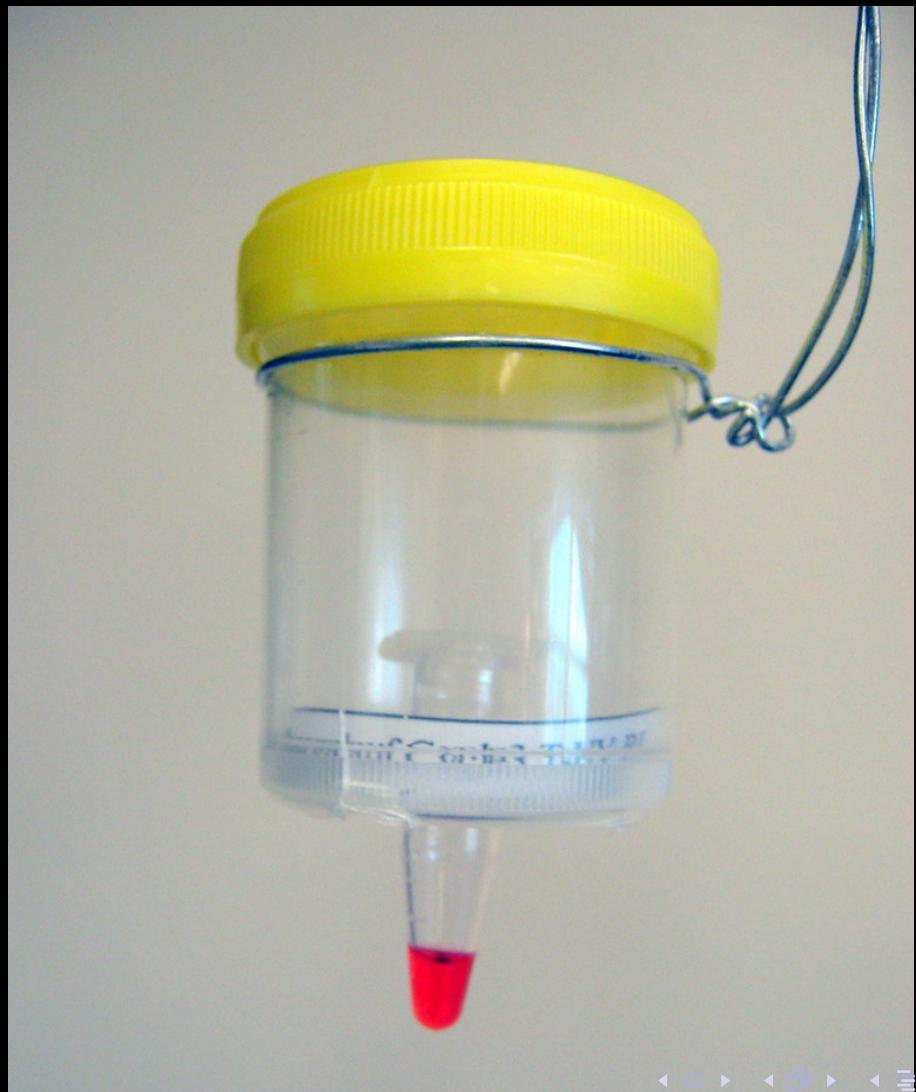
Mean number of beetles caught per trap-day

90 day trapping period ending on 01 Jan 2014



Mean number of beetles caught per trap-day

Reduced Release Rate



Ultraviolet Light Emmitting Diodes (UVLED)



Development of the Hotel California Trap



“Beetles Check In But Can Never Leave”







Enhanced Pheromone Trap: >3X Standard Trap Catch



Barrel Trap V2: >10X Standard Trap Catch



Sanitation









**GRUBS – 296
PUPAE – 41
ADULTS - 15**





DETECTOR DOGS



CHEMICAL CONTROL



Insecticides Being Evaluated

- ▶ CYPERMETHRIN: quick knockdown of all stages; not persistent
- ▶ PYRIPROXIFEN (NYGARD®): insect growth regulator; prevents production of adults
- ▶ SPLAT RB® + CYPERMETHRIN: experimental attracticide; adults only

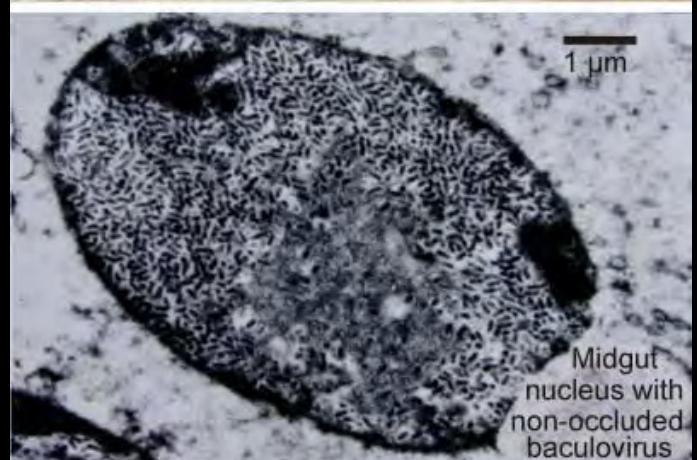
Spraying Crowns with DEMON MAX (Cypermethrin)

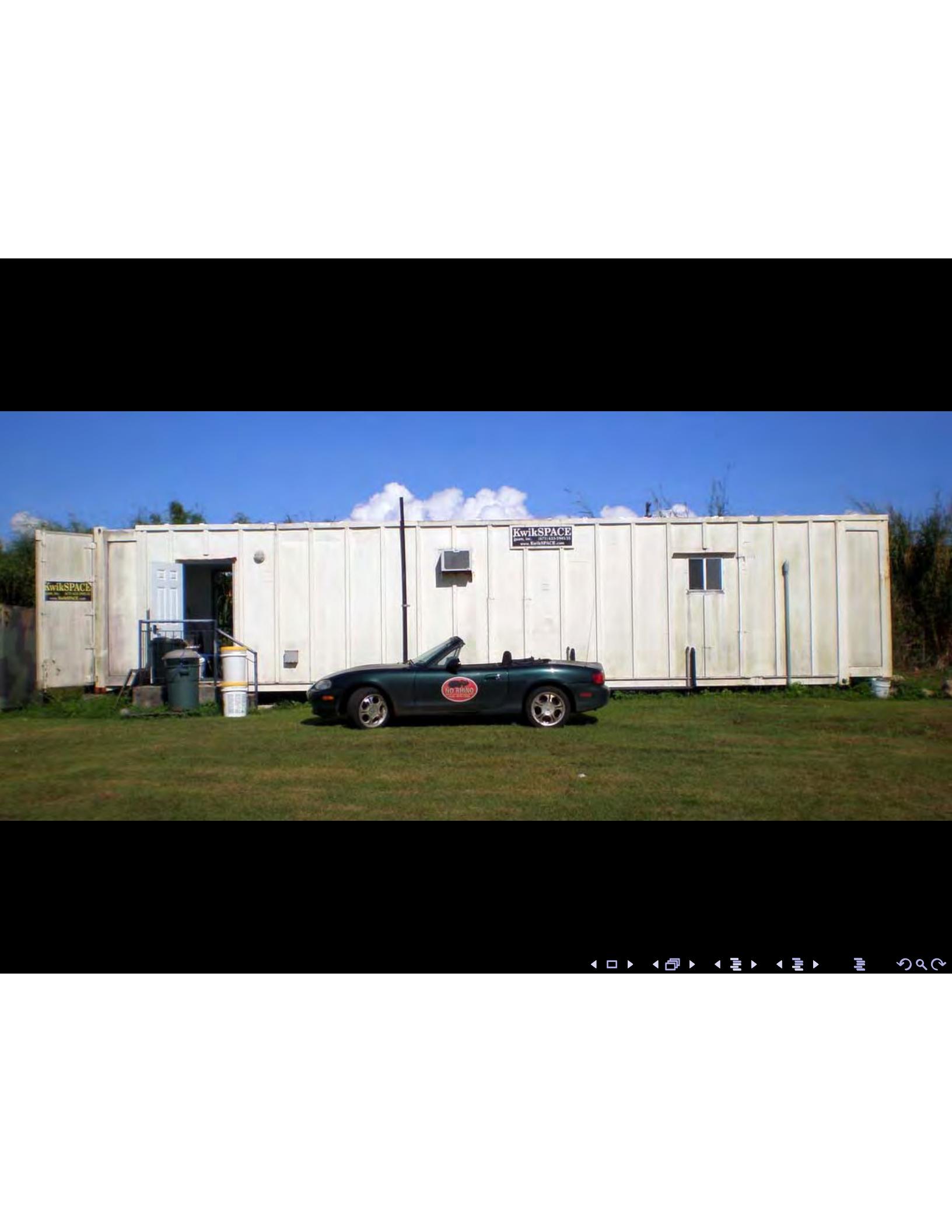


Efficacy of Crown Spraying



BIOCONTROL









Metarhizium for Biological Control

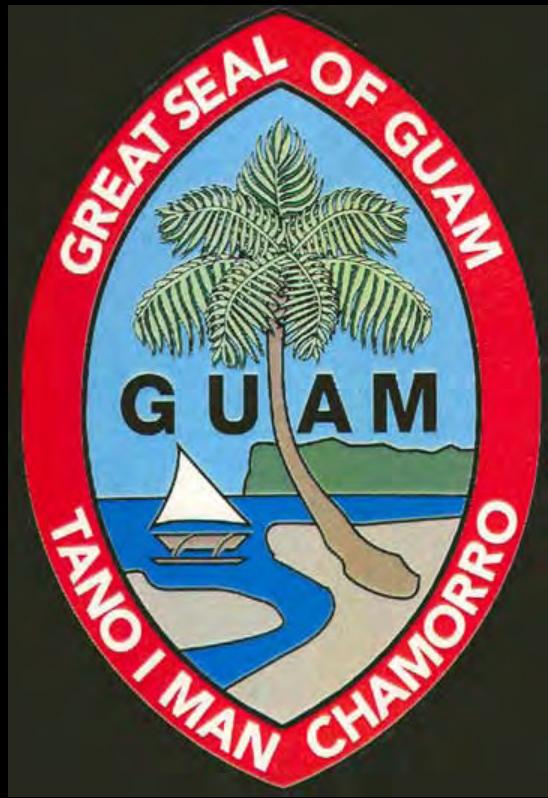
- ▶ a USDA import and release permit was obtained for *Metarhizium* which is being produced for biocontrol of CRB by the Philippines Coconut Authority
- ▶ 15 kg of spores were imported on September 10, 2011 and December 10, 2011
- ▶ following lab bioassays, field releases were started by incorporation into breeding sites and autodissemination by adult males
- ▶ *Metarhizium* appears to be working well: we are finding dead grubs with fungus even in areas where we did not apply spores

Biological Control of the Coconut Rhinoceros Beetle

A







5.5.6 *Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivirus*

Please see next page.



47th ANNUAL MEETING
of the
SOCIETY FOR
INVERTEBRATE
PATHOLOGY
and
INTERNATIONAL CONGRESS ON
INVERTEBRATE PATHOLOGY
AND MICROBIAL CONTROL

Berichte aus dem Julius Kühn-Institut

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Sunday – 3 August		
9:00-17:30	SIP Council Meeting	P 203
10:00-19:00	Registration	P1
18:00-21:00	Mixer	Alte Mensa
Monday – 4 August		
7:30-18:00	Registration	P1
8:30-10:00	Opening Ceremony Johannes Jehle, Organizing Committee Jørgen Eilenberg, President SIP	P1
	Welcome Addresses	
	Student Travel Award Presentation by M. van Oers	
	Founder's Lecture James Becnel, Chair of Founders' Lecture Committee Honoree: Alois M. Huger Lecturer: Trevor A. Jackson	
10:00-10:30	Break	
10:30-12:30	Plenary Symposium Microbial Control - from Bench to Business Potentials for utilizing and controlling insect pathogens <i>Richou Han</i> Story of an African firm: 10 years in the biopesticide business – lessons learned along the way <i>Sean Moore</i> A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods <i>Willem J. Ravensberg</i> BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop <i>Sebastian Bachem</i>	P1
12:30-14:00	Lunch	Mensa
14:00-16:00	Symposium 1 (Nematode Division) Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling Small molecule signals in nematodes - common motifs and species specific modifications <i>Stephan H. von Reuss</i> Olfactory Plasticity in Entomopathogenic Nematodes <i>Elissa Hallem</i> Multiple Consequences of Belowground Herbivore Induced Volatile Signals <i>Jared G. Ali</i> Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals <i>Hans T. Alborn</i>	P4
14:00-16:00	Contributed Papers Bacteria 1 Viruses 1 Fungi 1	P5 P1 P2
16:00-16:30	Break	
16:30-18:30	Symposium 2 (Microsporidia Div.) Microsporidiology: Advances in Europe A new intracellular parasite is a missing link between fungi and microsporidia <i>Karen L. Haag</i> Parasite takes fly - A Drosophila model of Microsporidia infection <i>Sebastian Niehus</i> White Sea metchnikovellids: morphology, life	P3 P2
	cycles; potential ancestral features of microsporidia <i>Yuliya Y. Sokolova</i> Microsporidia: Pathogens of Opportunity <i>James J. Becnel</i>	
Tuesday - 5 August		
16:30-18:30	Contributed Papers Nematodes 1 Viruses 2 Fungi 2	P4 P1 P2
20:00-21:30	Division Business Meetings and Workshops Microbial Control Diseases of Beneficial Invertebrates Nematodes	P3 P5 P4
Tuesday - 5 August		
7:30-13:00	Registration	P1
8:00-10:00	Symposium 3 (Fungi Division) Fatal Attraction: Fungi and Odours in deadly Combinations for Pest Control Conifer - bark beetle - fungus interactions <i>Tao Zhao</i> Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi <i>Stefan Vidal</i> Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system <i>Stine Kramer Jacobsen</i> How Fusarium graminearum influences insect-plant interactions <i>Drakulic Jassy</i> Plant-microorganism interactions that shape host-plant selection in the grapevine moth <i>Marco Tasin</i> Effect of host plant on aphid susceptibility to the fungal pathogen Pandora neoaphidis <i>Cezary Tkaczuk</i>	P2
8:00-10:00	Contributed Papers Nematodes 2 Viruses 3 Bacteria 2	P4 P1 P5
10:00-10:30	Break	
10:30-12:30	Symposium 4 (Virus Division) Small non-coding RNAs as Regulators of Insect Host-Virus Interactions and Immunity Role of cellular and virus-encoded microRNAs in insect host-virus interactions <i>Sassan Asgari</i> Sensing viral RNA in <i>Drosophila melanogaster</i> <i>Carine Meignin</i> Small RNA-directed antiviral immunity in disease-vector mosquitoes <i>Kevin M. Myles</i> Controlling viral infection in insects <i>Raul Andino</i>	P1
10:30-12:30	Contributed Papers Microbial Control 1 Diseases of Beneficial Invertebrates 1 Fungi 3	P3 P4 P2
12:40	Departure of buses for optional excursion + 5K	Univ.
15:00	Departure of buses for BBQ + 5K	Univ.



47th ANNUAL MEETING
of the
SOCIETY FOR
INVERTEBRATE
PATHOLOGY
and
INTERNATIONAL CONGRESS ON
INVERTEBRATE PATHOLOGY
AND MICROBIAL CONTROL

Berichte aus dem Julius Kühn-Institut

174

Kontaktadresse

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47th ANNUAL MEETING

of the

**SOCIETY FOR
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and

**INTERNATIONAL CONGRESS ON
INVERTEBRATE PATHOLOGY
AND MICROBIAL CONTROL**

PROGRAM and ABSTRACTS

3-7 August 2014
University of Mainz, Germany

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PROGRAM

2014

IMPORTANT NOTES:

The abstracts included in this book should not be considered to be publications and should not be cited in print without the author's permission.

Attendants shall not take pictures from projections during the presentations

STU indicates papers being judged for graduate student presentation awards

129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation

SUNDAY - 3 August

9:00–17:30	SIP Council Meeting	P203
10:00–19:00	Registration	P1
18:00–21:00	Mixer	Alte Mensa

MONDAY - 4 August

07:30–18:00	Registration	P1
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Monday, 8:30–10:00. P1

Opening Ceremony and SIP Founders' Memorial Lecture

Opening Ceremonies

Johannes Jehle, Chair, Organizing Committee
Jørgen Eilenberg, President, SIP

Welcome Addresses

Student Travel Award Presentation by M.van Oers

Founders' Memorial Lecture

James Becnel, Chair, Founders' Lecture Committee
Honoree: ALOIS M. HUGER
Lecturer: TREVOR A. JACKSON

10:00–10:30 BREAK

Plenary Symposium Monday, 10:30–12:30. P1

Microbial Control - from Bench to Business

Organizer/Moderator: Ralf-Udo Ehlers

10:30 1 Potentials for utilizing and controlling insect pathogens Richou Han, Xuehong Qiu and Xun Yan, Guangdong Entomological Institute, 105 Xingang Road West, Guangzhou 510260, China

11:00 2 Story of an African firm: 10 years in the biopesticide business – lessons learned along the way Sean Moore, Citrus Research International, Port Elizabeth, South Africa; Rhodes University, Grahamstown, South Africa

11:30 3 A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods Willem J. Ravensberg, Koppert Biological Systems, Berkel en Rodenrijs, the Netherlands

12:00 4 BASF Functional Crop Care, Unlocking Agricultural Potential in Soil, Seed and Crop Sebastian Bachem, BASF – Limburgerhof, Germany

12:30–14:00 LUNCH Mensa

Symposium 1 (Nematodes) Monday, 14:00–16:00. P4

Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling

Organizers/Moderators: R. Campos-Herrera, F. Kaplan and S. Hazir

14:00 5 Small molecule signals in nematodes - common motifs and species specific modifications Stephan H. von Reuss, Max Planck Institute for Chemical Ecology, Department of Bioorganic Chemistry, Jena, Germany

14:30 6 Olfactory Plasticity in Entomopathogenic Nematodes Joon Ha Lee and Elissa Hallem, University of California, Los Angeles, USA

15:00 7 Multiple Consequences of Belowground Herbivore Induced Volatile Signals Jared G. Ali^{1,2}, Raquel Campos-Herrera^{2,3}, Hans T. Alborn⁴, Larry W. Duncan², Lukasz L. Stelinski², ¹Department of Entomology, Michigan State University, USA; ²Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, U.S.A.; ³Departamento de Contaminación Ambiental, Instituto de Ciencias Agrarias, CSIC, Madrid, Spain; ⁴Center for Medical, Agricultural, and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL, U.S.A.

15:30 8 Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semiochemicals Hans T. Alborn¹, Fatma Kaplan², ¹USDA ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville FL, U.S.A.; ²Kaplan Schiller Research LLC and Biology Dept. University of Florida, Gainesville, FL, U.S.A.

Contributed Papers Monday, 14:00–16:00. P5

BACTERIA 1

Moderators: Raffi Aroian and Brian A. Federici

14:00 9 Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species Nasser Yalpani¹; Dan Altier¹, Jennifer Barry¹, Jarred Oral², Ute Schellenberger², Adane Negatu¹, Scott Diehn¹, Virginia Crane¹, Gary Sandahl¹, Joe Zhao¹, Dave Cerf², Claudia Perez Ortega³, Mark Nelson³, Analiza Alves¹, Lu Liu², Gusui Wu¹; ¹DuPont Pioneer, Johnston, IA, U.S.A.; ²DuPont Pioneer, Hayward, CA, U.S.A.; ³DuPont, Wilmington, DE, U.S.A..

14:15 10 Discovery and optimization of hemipteran-active proteins for Lygus control in cotton James A. Baum, Waseem Akbar, Konasale Anil Kumar, David Bowen, Robert S. Brown, Cathy Chay, Thomas Clark, Michael Pleau, Xiaohong Shi, Uma Sukuru, Moritz Von Rechenberg, Halong Vu, Brent Werner, Andrew Wollacott; Monsanto Company, Chesterfield, Missouri U.S.A.

14:30 11 Isolation and identification of potential biological control agent from *Tortrix viridana* L.(Lepidoptera: Tortricidae) pupae Nurcan Albayrak Iskender¹; Yaşar Aksu²; Artvin Coruh University, Faculty of Arts and Sciences, Department of Biology, Artvin, Turkey; ²Artvin Regional Forestry Management, Artvin, Turkey

- 14:45 **12 STU** Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial *Pseudomonas protegens* Peter Kupferschmied¹, Maria Péchy-Tarr¹, Nicola Imperiali¹, Monika Maurhofer², Christoph Keel^{1,2}; ¹Department of Fundamental Microbiology, University of Lausanne, Switzerland; ²Plant Pathology, Institute of Integrative Biology, ETH Zürich, Switzerland
- 15:00 **13 STU** *Paenibacillus larvae*, the etiological agent of American Foulbrood, produces the catechol type siderophore bacillibactin Gillian Hertlein¹; Sebastian Müller²; Eva Garcia-Gonzalez¹; Roderich D. Süßmuth²; Elke Genersch^{1,3}; ¹Institute for Bee Research Hohen Neuendorf, Germany; ²Technische Universität Berlin, Institut für Chemie, Berlin, Germany; ³Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany
- 15:15 **14 Two new *Bacillus thuringiensis* toxins active against Lepidoptera and Coleoptera** Mikel Domínguez¹, Iñigo Ruiz de Escudero^{1,2}, Isabel Matas², Leopoldo Palma^{1,2}, Delia Muñoz², Primitivo Caballero^{1,2}, ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain ²Laboratorio de Entomología Agrícola y Patología de Insectos, Universidad Pública de Navarra, Pamplona, Spain
- 15:30 **15-STU** Entomopathogenic *Bacillus thuringiensis* as PGPR Jiaheling Qi^{1,2}, Daigo Aiuchi²; Shin-ichiro Asano³; Masanori Koike²; ¹The United Graduate School of Agricultural Sciences, Iwate University, Iwate Japan; ²Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Obihiro, Japan; ³ Department of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan
- 15:45 **16 Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?** Carmen Lopez-Joven¹, Jean-Luc Rolland^{1,*}, Eric Abaddie², Mohamed Laabiri¹, Estelle Masseret¹, Audrey Vanhove¹, Audrey Caro¹, Delphine Bonnet¹, Delphine Destoumieux-Garzón¹; ¹Ecology of coastal marine systems, UMR 5119, CNRS, Ifremer, IRD, University of Montpellier, France; ²Laboratoire Environnement Ressource du Languedoc Roussillon, Ifremer, Sète, France.
- 14:30 **19 STU** Bracovirus-derived genes in the genome of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and their role in host susceptibility to pathogens Laila Gasmi, Agata K. Jakubowska, Juan Ferré, Salvador Herrero; Laboratory of Biochemical Genetics and Biotechnology, Department of Genetics, Universitat de València 46100 –Burjassot (Valencia), Spain
- 14:45 **20 Entry of *Bombyx mori* nucleopolyhedrovirus (BmNPV) into BmN Cells by Macropinocytic Endocytosis**, Jinshan Huang^{1,2}, Bifang Hao^{1,2}, Chen Cheng¹, Fei Liang¹, Xingjia Shen^{1,2}; ¹Sericultural Research Institute, Jiangsu University of Science and Technology, ²Sericultural Research Institute, Chinese Academy of Agricultural Science, Zhenjiang, Jiangsu, PRChina
- 15:00 **21 Nuclear translocation of *Autographa californica* nucleopolyhedrovirus ME53** Yang Liu, Jondavid de Jong, Eva Nagy, Peter Krell, University of Guelph, Guelph Ontario, Canada
- 15:15 **22 Nuclear localization and other domains of *Autographa californica* nucleopolyhedrovirus DNA polymerase** Guozhong Feng¹, Peter Krell², ¹State Key Laboratory of Rice Biology, China National Rice Research Institute, Hangzhou, 310006, China; ²University of Guelph, Guelph Ontario, Canada
- 15:30 **23 STU** Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0) and *Trichoplusia ni* kinesin-1 in budded virus nucleocapsid egress Siddhartha Biswas¹; Gary W. Blissard²; David A. Theilmann³, ¹Plant Science, Faculty of Land and Food Systems, University of British Columbia, Vancouver, BC Canada; ²Boyce Thompson Institute at Cornell University, Ithaca, NY, USA; ³Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland BC, Canada
- 15:45 **24 The Twist In Baculoviruses** Loy Volkman, University of California, Berkeley, California, and Expression Systems, LLC, Davis, California, USA

Contributed Papers

Monday, 14:00-15:30. **P2**

FUNGI 1

Moderators: Italo Delalibera and Nina Jenkins

- 14:00 **17 Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays** Jessica Breznik, Nicola Johnson, Mustapha El-Ayoubi and Eric B Carstens, Queen's University, Kingston, Canada
- 14:15 **18 STU Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection** Tyler A. Garretson and Xiao-Wen Cheng, Department of Microbiology, Miami University, Oxford, Ohio, USA
- 14:00 **25 A new mycopesticide developed especially for the control of the citrus greening vector *Diaphorina citri* (Hemiptera: Liviidae)** Italo Delalibera Jr., Celeste P. D'Alessandro, Marcos R. Conceschi, John J. S. Ausique Department of Entomology and Acarology, ESALQ, University of São Paulo, Piracicaba, São Paulo, Brazil
- 14:15 **26 Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa** Cipriano García, Adolfo D. Armenta and Luis A. Gaxiola; Instituto Politécnico Nacional. CIIDIR-IPN Unidad Sinaloa, Guasave, Sinaloa, Mexico
- 14:30 **27 Evaluating *Metarhizium brunneum* F52 Micro-sclerotia Applied in Hydromulch for Control of Asian Longhorned Beetles** Tarryn Anne Goble¹, Ann Hajek¹, Mark Jackson², and Sana Gardescu^{1,2}; ¹Department of Entomology, Cornell University, Ithaca, USA, ²USDA-ARS-NCAUR, Crop Bioprotection Research Unit, Peoria, IL, USA

- 14:45 **28 STU** Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed Sihyeon Kim, Se Jin Lee, Jeong Seon Yu, Yu-Shin Nai and Jae Su Kim; Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju, Korea
- 15:00 **29** Use of *Beauveria bassiana* (Bals) in the management of larger grain borer, *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) on stored maize in Tanzania Daniel Karanja¹, Pierre Grammare², Olivier Potin², Nick Jessop³, Mathew Smith³, Roger Day⁴ and Belinda Luke⁴, ¹CABI Africa, Nairobi, Kenya, ²SylvanBio, Société SOMYCEL SA, Loches, France, ³Exosect Limited, Leylands Business Park, Colden Common, Hampshire, UK, ⁴CABI Europe – UK, Egham, UK
- 15:15 **30** Management of *Frankliniella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi Jae Su Kim¹, Margaret Skinner², Bruce L. Parker², Se Jin Lee¹, Jeong Seon Yu¹ and Si Hyeon Kim¹, Department of Agricultural Biology, College of Agricultural & Life Sciences, Chonbuk National University, Jeonju, Korea. ²Entomology Research Laboratory, University of Vermont, Burlington, USA.
-
- 16:00–16:30 **BREAK**
-
- Symposium 2 (Microsporidia) Monday, 16:30-18:30. **P3**
- Microsporidiology: Advances in Europe**
- Organizers/Moderators: Andreas Linde and Sebastian Gisder
- 16:30 **31** A new intracellular parasite is a missing link between fungi and microsporidia Karen L. Haag¹, Timothy Y. James², Ronny Larsson³, Tobias M. M. Schaefer⁴, Dominik Refardt⁵, Dieter Ebert⁶; ¹Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil; ²University of Michigan, Ann Arbor, MI, USA; ³University of Lund, Lund, Sweden; ⁴Basel University, Basel, Switzerland; ⁵Zurich University of Applied Sciences, Campus Grüental, Wädenswil, Switzerland
- 17:00 **32** Parasite takes fly - A *Drosophila* model of Microsporidia infection Sebastian Niehus¹, Adrien Franchet¹, Frédéric Delbac², Michael Boutros³, Dominique Ferrandon¹; ¹Institut de Biologie Moléculaire et Cellulaire, UPR 9022 du CNRS, Université de Strasbourg, Strasbourg, France; ²Laboratoire Microorganismes: Génome et Environnement, UMR 6023 du CNRS, Université Blaise Pascal, Aubière, France; ³German Cancer Research Center, Division of Signaling and Functional Genomics, and Department for Cell and Molecular Biology, Faculty of Medicine Mannheim, University of Heidelberg, Heidelberg, Germany
- 17:30 **33** White Sea metchnikovellids: morphology, life cycles; potential ancestral features of microsporidia Yuliya Y. Sokolova^{1,2}; ¹Core Microscopy Center, School of Veterinary Medicine, Louisiana State University, Baton Rouge LA, USA; ²Institute of Cytology, St. Petersburg, Russia.
- 18:00 **34** Microsporidia: Pathogens of Opportunity James J. Becnel¹, Louis M. Weiss²; ¹Center for Medical,
- Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL 32608, USA, ²Department of Pathology, Division of Parasitology and Tropical Medicine, and Department of Medicine Division of Infectious Diseases, Albert Einstein College of Medicine, Bronx, NY, USA
- Contributed Papers Monday, 16:30-18:30. **P4**
- NEMATODES 1**
- Moderators: Edwin Lewis and Albrecht Koppenhöfer
- 16:30 **35** Measuring entomopathogenic nematode activity, abundance and soil food web assemblage in Swiss wheat and maize cultivation Raquel Campos-Herrera¹, Geoffrey Jaffuel¹, Xavier Chiriboga¹, Rubén Blanco-Pérez¹, Marie Fesselet², Vladimir Půža³, Fabio Mascher¹, Ted C.J. Turlings¹; ¹Farce Laboratory, University of Neuchâtel, Neuchâtel (Switzerland); ²Département fédéral de l'économie, de la formation et de la recherche DEFR, Agroscope, Institut des Sciences en Production Végétale IPV, Nyon (Switzerland); ³Laboratory of Entomopathogenic Nematodes, Institute of Entomology, Biology Centre, Czech Academy of Sciences, České Budějovice, Czech Republic
- 16:45 **36 STU** Biocontrol and nutrition: understanding the role of environment in the trait deterioration of an entomopathogenic nematode symbiont Dana Blackburn, Burke Crawford, and Byron Adams, Brigham Young University, Provo, UT, USA
- 17:00 **37** Insect-killing nematodes also kill competitors: lethal male-male fighting in *Steinerinema* Annetje Zenner, Kathryn O'Callaghan and Christine Griffin, Department of Biology, National University of Ireland Maynooth, Ireland
- 17:15 **38 STU** Comparison of Life History Traits of the Entomopathogenic Nematodes *Steinerinema feltiae* and *Steinerinema riobrave* Temesgen Addis^{1,3}, Asmamaw Teshome², Olaf Strauch³ and Ralf-Udo Ehlers³; ¹Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University, Kiel, Germany, ²Department of Biology, Ghent University, Ghent, Belgium, ³e-nema, GmbH, Schwentinental, Germany
- 17:30 **39 STU** How does plant domestication influence entomopathogenic nematodes as potential biological control agents? Monique Rivera¹, Cesare Rodriguez-Saona¹, Hans T. Alborn², and Albrecht M. Koppenhöfer¹; ¹Department of Entomology, Rutgers University, New Brunswick, NJ 08901, USA, ²USDA ARS CMAVE, Gainesville, FL, USA
- 17:45 **40** Analysis of intraspecific variability in *Steinerinema kraussei* populations using PCA, M. Clausi¹, G. Rappazzo¹, E. Tarasco², D. Leone¹, M. T. Vinciguerra¹; ¹Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania, Catania (Italy), ²Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "Aldo Moro", Bari, Italy
- 18:00 **41** Population genetic structure of entomopathogenic nematode *Steinerinema affine* (Steinerinematidae: Nematoda) inferred using microsatellite markers Vladimír Půža¹, Martina Žurovcová¹, Jiří Nermut¹, Daniela Chundelová^{1,2}, Zdeněk Mráček¹; ¹Institute of Entomology, Biology Centre of the AS CR, České Budějovice, Czech Republic; ²Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic

18:15 **42 STU Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey** E. Erin Morris¹ and Ann E. Hajek², ¹Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg 1871, Denmark; ²Department of Entomology, Cornell University, Ithaca, New York 14853-2601, USA

Contributed Papers

Monday, 16:30-18:30.

P1

VIRUSES 2

Moderators: Jenny Cory and Agata Jakubowska

16:30 **43 Insect feeding induces transgenerational resistance to NPV in Lepidoptera** Grant L. Olson¹, Judith H. Myers², Jenny S. Cory¹, ¹Dept. of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada; ²Biodiversity Centre, Dept. of Zoology, University of British Columbia, Vancouver, British Columbia, Canada

16:45 **44 The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early *pe38* gene of *Cydia pomonella* granulovirus** Manuela Gebhardt, Karolin E. Eberle, Johannes A. Jehle, Institute for Biological Control, Julius Kühn Institute (JKI), Federal Research Center on Cultivated Plants, Darmstadt, Germany

17:00 **45 CpGV-R5 allows replication of CpGV-M in resistant host insect larvae** Benoit Graillot^{1,2}, Sandrine Bayle¹, Christine Blachere-Lopez^{1,3}, Samantha Besse², Myriam Siegwart⁴, Miguel Lopez-Ferber¹, ¹LGEI, Ecole des Mines d'Alès, Institut Mines-Telecom et Université de Montpellier Sud de France, Alès, France. ²Natural Plant Protection, Arysta LifeScience group, Pau, France. ³INRA, Alès, France. ⁴INRA, unité PSH, AVIGNON, France

17:15 **46 Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*** Agata K. Jakubowska¹, Melania D'Angiolo¹; Rosa M. González Martínez¹; Anabel Millán Leiva¹; Arkaitz Carballo²; Rosa Murillo²; Primitivo Caballero²; Salvador Herrero¹, ¹Department of Genetics, Universitat de València, Burjassot, Spain; ²Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Navarra, Spain

17:30 **47 Mixed SeMNPV genotypes comprised transmission capacities and insecticidal properties** Cristina Virtó¹, David Navarro^{1,2}, M.ª del Mar Tellez², Trevor Williams³, Rosa Murillo^{1,4}, Primitivo Caballero^{1,4}, ¹Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Ctra. de Mutilva s/n 31192, Mutilva Baja, Spain; ²IFAPA, La Mojónera, 04745, Almería, Spain; ³Instituto de Ecología AC, Xalapa 91070, Mexico; ⁴Departamento Producción Agraria, Universidad Pública de Navarra, Pamplona 31006, Spain

17:45 **48-STU A novel mode of resistance of codling moth against *Cydia pomonella* granulovirus** Annette J. Sauer, Eva Fritsch, Karin Undorf-Spahn, Johannes A. Jehle, Julius Kühn-Institut, Darmstadt, Germany

18:00 **49 The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrLeGV-SA) in mortality rates of false codling moth larvae *Thaumatotibia leucotreta*** Devon Brits, Jaryd Ridgeway & Alicia Timm, Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa

18:15 **50 Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by coinfection with granulovirus** Paola Cuartas, Laura Villamizar, Centro de Biotecnología y Bioindustria (CBB), Corpica, Bogotá, Colombia

Contributed Papers

Monday, 16:30-18:30.

P2

FUNGI 2

Moderator: Drauzio Rangel

16:30 **51 Rapid and simple method for overnight development of strain-specific markers: A case study with the commercial *Beauveria bassiana* strain, GHA** George Kyei-Poku, Shajahan Johny, Agathe Roucou and Debbie Gauthier, Canadian Forestry Service, Great Lakes Forestry Centre, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

16:45 **52-STU The functions of two Cu/Zn-superoxide dismutases and a Fe-superoxide dismutase in regulating the growth, antioxidation, UV tolerance and virulence of *Beauveria bassiana* Fang Li** Zheng-Liang Wang², Han-Qing Shi¹, Sheng-Hua Ying¹, Ming-Guang Feng¹, ¹Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China; ²College of Life Sciences, China Jiliang University, Hangzhou, Zhejiang, P. R. China.

17:00 **53 STU Effect of temperature, water activity and UV-B radiation on conidia germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane** María Fernández-Bravo, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, ETSIAM, 14071 Córdoba, Spain

17:15 **54 Non-target aquatic arthropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology** Inmaculada Garrido-Jurado¹, Steffan R. Williams², Ahmed Abdrahman³, Darren L. Oatley-Radcliffe², Enrique Quesada-Moraga¹, Tariq M. Butt³; ¹Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba. Campus de Rabanales. Edificio C4 Celestino Mutis. 14071 Cordoba, Spain, ²Centre for Water Advanced Technologies and Environmental Research (CWATER), College of Engineering, Swansea University, Swansea, UK, ³Department of Biosciences, College of Science, Swansea University, Swansea, UK

17:30 **55 STU Development of analytical methods for the analysis of *Metarhizium brunneum* metabolites in crop matrices** Judith Taibon^{1,2}, Sonja Sturm¹, Christoph Seger^{1,3}, Hermann Stuppner¹, Hermann Strasser², ¹Institute of Pharmacy / Pharmacognosy, Leopold-Franzens University Innsbruck, Austria, ²Institute of Microbiology, Leopold-Franzens University Innsbruck, Austria, ³ZIMCL, University Hospital Innsbruck, Austria.

17:45 **56 STU α-1, 2-mannosyltransferase ktr1, ktr4 and ktr2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in *Beauveria bassiana*** Juan-juan Wang, Lei Qiu, Sheng-Hua Ying, Ming-Guang Feng¹, Institute of Microbiology, College of Life Sciences, Zhejiang Univ., Hangzhou, Zhejiang, People's Republic of China

SIP Division Business Meetings: Monday, 20:00-21:30		
Microbial Control	P3	
DBI	P5	
Nematode Division Workshop	Monday, 20:00-21:30	P4
Invertebrate Pathogens in the Classroom: Current Status and Future Challenges		
Organizers: Glen Stevens and Patricia Stock		
TUESDAY - 5 August		
07:30-13:00 Registration	P1	
Symposium 3 (Fungi)	Tuesday, 8:00-10:00.	P2
Fatal Attraction: Fungi and Odours in deadly Combinations for Pest Control		
Organizer/Moderator: Ingeborg Klingen		
8:00 58 Conifer - bark beetle - fungus interactions <u>Tao Zhao</u> ¹ , Paal Krokene ² , Anna-Karin Borg-Karlson ^{1,3} ; ¹ The Royal Institute of Technology, Department of Chemistry, Ecological Chemistry Group, Stockholm, Sweden; ² Norwegian Forest and Landscape Institute, Ås, Norway		
8:20 59 Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi Mario Schumann ¹ ; Anant Patel ² ; Miriam Hanitzsch ² ; Stefan Vidal ^{1,3} ; Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany; ² Fachhochschule Bielefeld, University of Applied Sciences, Department of Engineering and Mathematics, Bielefeld, Germany		
8:40 60 Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system <u>Stine Kramer Jacobsen</u> ¹ , Jørgen Eilenberg ¹ , Ingeborg Klingen ² , Lene Sigsgaard ¹ ; ¹ Department of Plant and Environmental Sciences, University of Copenhagen, Denmark; ² Norwegian Institute for Agricultural and Environmental Research (Bioforsk) Plant Health and Plant Protection Division, Norway.		
9:00 61-STU How Fusarium graminearum influences insect-plant interactions <u>Drakulic Jassy</u> ^{1,2} , Bruce Taby ² , Ray Rumiana ¹ ; ¹ Division of Plant and Crop Sciences, University of Nottingham, UK; ² Rothamsted Research, Department of Biological Chemistry and Crop Protection, Harpenden, UK		
9:20 62 Plant-microorganism interactions that shape host-plant selection in the grapevine moth <u>Geir K. Knudsen</u> ¹ , Ilaria Pertot ² , <u>Marco Tasin</u> ^{1,3} ; ¹ Bioforsk, Norwegian Institute for Agricultural and Environmental Research, Plant Health and Plant Protection Division, Høgskoleveien 7, 1432 Ås, Norway; ² Edmund Mach Foundation, 38010 San Michele all'Adige, Italy;		
9:40 63 Effect of host plant on aphid susceptibility to the fungal pathogen <i>Pandora neoaphidis</i> <u>Cezary Tkaczuk</u> ¹ ; ¹ Integrated Plant Protection, Dep. of Crop Protection Biology, Swedish University of Agricultural Sciences, Sweden		
Paresh A. Shah ² , Judith K. Pell ^{2,3} , ¹ Department of Plant Protection, Siedlce University, Siedlce, Poland; ² Plant and Invertebrate Ecology Department (now AgroEcology Department), Rothamsted Research, Harpenden, UK; ³ Current Address: J.K. Pell Consulting, Luton, UK		
Contributed Papers	Tuesday, 8:00-10:00.	P4
NEMATODES 2		
Moderators: Patricia Stock and Christine Griffin		
8:00 64 Entomopathogenic nematode behavioral responses to chemical cues from cadavers <u>Paige Redifer</u> , Brittany Gale, Alison McLain, <u>Glen Stevens</u> , Laura Grochowski, School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA		
8:15 65 The Wolbachia Endosymbiont as a Nematode Drug Target for Control of Human Filariasis, a Neglected Tropical Disease and Other insect Borne Pathogens <u>Barton E. Slatko</u> , Molecular Parasitology Group, Genome Biology Division, New England Biolabs, Inc., Ipswich MA USA		
8:30 66 Differential PirAB expression of the entomopathogenic bacterium <i>Photobacterium</i> <i>Photobacterium luminescens</i> (Enterobacteriaceae) based on tissue association and portal of entry to the insect host <u>Anaïs Castagnola</u> ^{1,2} , Nathaniel Davis ³ , Belen Molina ⁴ ; S. Patricia Stock ¹ ; John G. McMullen II ¹ ; ¹ Department of Entomology, University of Arizona; ² Center for Insect Science, University of Arizona; ³ Pima Community College; ⁴ Department of Ecology and Evolutionary Biology, University of Arizona, USA		
8:45 67-STU Candidate Virulence Loci in Pan-Genome of the Entomopathogenic Bacterium, <i>Xenorhabdus bovienii</i> (Gamma-Proteobacteria: Enterobacteriaceae) , <u>John G McMullen II</u> ¹ ; Gaelle Bisch ² , Jean-Claude Ogier ² , Sylvie Pagès ² , Sophie Gaudriault ² ; S. Patricia Stock ³ , ¹ University of Arizona, School of Animal and Comparative Biomedical Sciences, Tucson, AZ; ² Université Montpellier II/INRA, UMR 1333 Laboratoire DGIMI, Montpellier, France; ³ University of Arizona, Department of Entomology, Tucson, AZ, USA		
9:00 68 Molecular mechanism of the nematicidal activity of <i>Photobacterium luminescens</i> LN2 against <i>Heterorhabditis bacteriophora</i> H06 nematodes <u>Xuehong Qiu</u> and Richou Han Guangdong, Entomological Institute, Guangzhou 510260, China		
9:15 70 Natural products from entomopathogenic bacteria: Understanding the interaction of bacteria, insects and nematodes <u>Helge B. Bode</u> , Merck Stiftungsprofessor für Molekulare Biotechnologie, Fachbereich Biowissenschaften, Goethe Universität Frankfurt, Germany		

Contributed PapersTuesday, 8:00-10:00. **P1****VIRUSES 3**

Moderators: Zhihong Hu and Trevor Williams

- 8:00 **71 Characterization and formulation of a Colombian isolate of *Erinnyis ello* granulovirus (L.) (Lepidoptera: Sphingidae)** Juliana Gómez¹, Gloria Barrera¹, Paola Cuartas¹, Carolina Ruiz¹, Adriana Santos¹, Liz Uribe¹, Guillermo León², Laura Villamizar¹, ¹Centro de Biotecnología y Bioindustria (CBB), Corpocica, Bogotá, Colombia, ²Centro de Investigación "La Libertad" Corpocica, Puerto López, Colombia
- 8:15 **72 PRODUCTION OF the *Cydia pomonella* granulovirus (CpGV) IN A HETEROLOGOUS HOST** C.B. Chambers¹, S.D. Moore^{2,3}, M.P. Hill³ & C. Knox⁴, ¹River Bioscience, PO Box 20388, Humewood 6013, Port Elizabeth, South Africa, ²Citrus Research International, PO Box 20285, Humewood 6013, Port Elizabeth, South Africa, ³Department of Zoology and Entomology, Rhodes University, PO Box 64, Grahamstown, South Africa, ⁴Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa
- 8:30 **73 Post-translational cleavage of P74 of the *Helicoverpa armigera* single nucleopolyhedrovirus facilitates per os infection** Huachao Huang¹, Manli Wang¹, Xin Luo¹, Xi Wang¹, Basil M. Arif², Fei Deng¹, Hualin Wang¹, Zhihong Hu¹, ¹State Key Laboratory of Virology and Joint Laboratory of Invertebrate Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, PR China; ²Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada
- 8:45 **74-STU Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (PhopGV)** Michael D. Jukes¹, Caroline M. Knox¹, Sean D. Moore² & Martin P. Hill³, ¹Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140 South Africa, ²Citrus Research International, Humewood, Port Elizabeth, 6013 South Africa, ³Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa
- 9:00 **75 Genetic and biological characterisation of a novel South African *Plutella xylostella* granulovirus, PlxyGV-SA** Fatima Abdulkadir¹, Caroline Knox¹, Tamryn Marsberg², Martin P. Hill³ & Sean D. Moore^{2,3}, ¹Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa; ²Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; ³Citrus Research International, Humewood, Port Elizabeth, South Africa
- 9:15 **76-STU Comparative transcriptome analysis of CpGV-M in susceptible and resistant codling moth *Cydia pomonella*** Diana Schneider, Johannes A. Jehle, Julius Kühn-Institut, Institute for Biological Control, Darmstadt, Germany
- 9:30 **77 Transmission of mixtures of insect pathogenic viruses in a single virion: towards the development of custom designed virus insecticides** Inés Beperet¹, Oihane Simón¹, Trevor Williams², Miguel López-Ferber³, Primitivo Caballero¹, ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilla Baja, Navarra, Spain; ²Instituto de Ecología AC, Xalapa, Mexico; ³LGEI, École des Mines d'Alès, Alès France; ⁴Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

- 9:45 **78 Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope** Jin Li, Yin Zhou, Chengfeng Lei, Xulian Sun, Key Laboratory of Agricultural and Environmental Microbiology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China

Contributed PapersTuesday, 8:00-10:00. **P5****BACTERIA 2**

Moderators: Jean-Louis Schwartz and Juan Ferré

- 8:00 **79 *Yersina entomophaga* MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex** Sean D.G. Marshall¹, Jason N. Busby², J. Shaun Lott², Sandra A. Jones¹, Julie E. Dalziel³, Femke Schepers³, Mark Hurst¹; ¹Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch 8140, New Zealand; ²School of Biological Sciences, University of Auckland, New Zealand; ³Food & Bio-based Products, AgResearch, Grasslands Research Centre, Palmerston North 4442, New Zealand
- 8:15 **80 Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures** Diego Segond^{1,2}, Agnès Rejasse¹, Christophe Buisson¹, Shuyuan Guo^{1,3}, Karine Adel-Patient^{2,4}, Hervé Bernard^{2,4}, Didier Lereclus¹, Christina Nielsen-LeRoux¹; ¹INRA UMR1319-Micalis, team GME, 78352 Jouy en Josas France, ²INRA, UR496 Unité d'Immuno-Allergie Alimentaire, France, ³School of Life Science, Beijing Institute of Technology, Beijing, China, ⁴CEA, IBI-TecS, Service de Pharmacologie et d'Immunoanalyse, Gif-sur-Yvette, France
- 8:30 **81-STU Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins** Shiro Tanaka¹; Ami Iizuka¹; Kazuhisa Miyamoto²; Hiroaki Noda²; Shingo Kikuta¹; Ryoichi Sato¹; ¹Tokyo University of Agriculture and Technology, Koganei, Tokyo, Japan; ²National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan
- 8:45 **82 A necessary step in the mode of action of the Cry8 toxin: the elimination of DNA from the Cry toxin-DNA complex**, Shuyuan Guo, Bingjie Ai, Jie Li, Dongmei Feng, Feng Li, School of Life Science, Beijing Institute of Technology, Beijing, China
- 9:00 **83-STU How does the Bt Cry41Aa toxin kill human cancer cells?** Barbara Domanska, Vidisha Krishnan, Gizem Altun, Michelle West and Neil Crickmore; Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, UK
- 9:15 **84-STU Which regions of the Bt Cry41Aa toxin are responsible for its activity against human cancer cells?** Alicia Elhigazi, Vidisha Krishnan, Fatai Afolabi, Barbara Domanska, Lisa Muhibib, Michelle West, Neil Crickmore. Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton, UK
- 9:30 **85 Parasporin PS1Aa2 induces ionic channels in lipid bilayer membranes and calcium oscillations in sensitive cells** Gabriel Narvaez¹, Vincent Vachon¹, Dong Xu², Jean-Charles Côté², Jean-Louis Schwartz^{1,3}, ¹Groupe d'étude des protéines membranaires, Université de Montréal, Montreal, Quebec, Canada; ²Research Center, Agriculture and Agri-Food Canada, St-Jean-sur-Richelieu, Quebec, Canada; ³Centre Sèvre, Université de Sherbrooke, Sherbrooke, Quebec, Canada

9:45 **86-STU** *Caenorhabditis elegans – Bacillus thuringiensis* interactions: new insights into mechanisms of host resistance and pathogen virulence Igor Iatsenko, Iulia Boichenko, Ralf J. Sommer; Max Planck Institute for Developmental Biology, Department for Evolutionary Biology, Tuebingen, Germany

10:00–10:30 BREAK

Symposium 4 (Viruses) Tuesday, 10:30-12:30. **P1**

Small non-coding RNAs as Regulators of Insect Host-Virus Interactions and Immunity

Organizer/Moderator: Sasan Asgari

10:30 **87** Role of cellular and virus-encoded microRNAs in insect host-virus interactions Sasan Asgari, School of Biological Sciences, The University of Queensland, Brisbane QLD 4072, Australia

11:00 **88** Sensing viral RNA in *Drosophila melanogaster* Simona Paro¹, Eric Aguiar², Bill Claydon¹, Joao Trindade Marques², Jean-Luc Immler^{1,2} and Carine Meignin^{1,2}; ¹IBMC, CNRS-UPR9022, Strasbourg, France; ²Laboratory of RNA Interference, Biochemistry and Immunology, Universidade Federal de Minas Gerais Belo Horizonte, Brazil; ³University of Strasbourg, Strasbourg, France

11:30 **89** Small RNA-directed antiviral immunity in disease-vector mosquitoes Kevin M. Myles, Virginia Tech, Fralin Life Science Institute, Department of Entomology, Blacksburg, Virginia, USA

12:00 **90** Controlling viral infection in insects Mark Kunitomi, Michel Tassetto, Arabinda Nayak, and Raul Andino, Department of Microbiology and Immunology, University of California, San Francisco, California 94143-2280, USA

Contributed Papers Tuesday, 10:30-12:15. **P3**

MICROBIAL CONTROL 1

Moderator: Michael Brownbridge

10:30 **91** Double trouble for thrips: Effective biopesticide combinations to control soil-dwelling stages in chrysanthemums Michael Brownbridge, Taro Saito and Paul Côté, Vineland Research and Innovation Centre, Vineland Station, Ontario, Canada

10:45 **92-STU** Lethal and sub-lethal impacts of fungal biopesticides on house fly populations in simulated field settings of biocosms, Naworaj Acharya¹, Simon Blanford^{1,2}, Edwin G. Rajotte¹, Nina E. Jenkins¹, Mathew B. Thomas^{1,2}, ¹Department of Entomology, Penn State University, 501 Agricultural Sciences and Industries Building, PA 16802, USA, ²Center for Infectious Diseases Dynamics, Penn State University, Merkle Lab, PA 16801, USA

11:00 **93-STU** Management of *Prostephanus truncatus* (Horn.) on stored maize using *Beauveria bassiana* (Bals.) Mavis A. Acheampong¹, Eric W. Cornelius¹, Vincent Y. Eziah¹, Ken O. Fening¹, Clare Storm², Dave Moore³, Nick Jessops², Matthew Smith², Olivier Potin⁴, Pierre Grammare⁴ and Belinda Luke³. ¹Department of

Crop Science, University of Ghana, Legon; ²Exosect Ltd, UK; ³CABI, UK; ⁴SylvanBio, France

11:15 **94-STU** Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae* Peter Cheong¹, Travis R. Glare¹, Michael Rostas¹, Stephen Haines², Jolon Dyer², Stefan Clerens², Jenny Brookes¹ and Stephen Ford³; ¹Bio-Protection Research Centre, P O Box 85084, Lincoln University, Lincoln 7647, Christchurch, New Zealand, ²AgResearch, Lincoln Research Centre, Private Bag 4749, Christchurch 8140, New Zealand, ³Biotelliga Limited, Pukekohe 2120, New Zealand

11:30 **95-STU** Entomopathogenic fungi for control of false codling moth in South African citrus orchards Candice A. Coombes¹, Martin P. Hill¹; Sean D. Moore^{1,2}, Joanna F. Dames³, ¹Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140, South Africa; ²Citrus Research International, Humewood, 6013, Port Elizabeth, South Africa; ³Department of Biochemistry and Microbiology, Rhodes University, Grahamstown, 6140, South Africa.

11:45 **97-STU** Wireworm control with entomopathogenic fungi and plant extracts Sonja Eckard¹, Sven Bacher², Jürg Enkerli¹, Giselher Grabenweger¹, ¹Agroscope, Institute for Sustainability Sciences, Reckenholzstrasse 191, Zürich, Switzerland, ²University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

12:00 **98-STU** Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol Johanna Mayerhofer^{1,2}, Jürg Enkerli², Roland Zelger³ & Hermann Strasser¹; ¹Institute of Microbiology, Leopold-Franzens University Innsbruck, AUT, ²Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Zürich, CH, ³Research Centre for Agriculture and Forestry Laimburg, Ora/Auer, Italy

Contributed Papers

Tuesday, 10:30-12:30. **P4**

DIS. OF BENEFICIAL INVERTEBRATES 1

Moderators: Kelly Bateman and Spencer Greenwood

10:30 **99** The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters Donald C. Behringer^{1,2}, Mark J. Butler^{IV}³, Jessica Moss⁴, Jeffrey D. Shields⁴, ¹University of Florida, Program in Fisheries and Aquatic Sciences, Gainesville, Florida 32653 (USA); ²University of Florida, Emerging Pathogens Institute, Gainesville, Florida 32611 (USA); ³Old Dominion University, Department of Biological Sciences, Norfolk, Virginia 23529 (USA); ⁴Virginia Institute of Marine Science, Gloucester Point, Virginia 23062 USA

10:45 **100** Defining lobster-pathogen interactions via high-throughput gene expression studies: The discovery and description of the interplay between the American Lobster (*Homarus americanus*) and the ciliated parasite *Anophryoides haemophila*, Spencer J. Greenwood^{1,2}; K. Fraser Clark^{1,2,3}, ¹Atlantic Veterinary College Lobster Science Centre, ²Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; ³Department of Plant and Animal Sciences, Dalhousie University, Truro, Nova Scotia, Canada

11:00 **101-STU** Metabolomic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*) Melanie

- Buote¹, Russ Kerr², Rick Cawthorn¹, Spencer Greenwood², Glenda Wright²; ¹Department of Pathology and Microbiology, Atlantic Veterinary College at UPEI, Charlottetown, PEI; ²Department of Biomedical Sciences, Atlantic Veterinary College at UPEI, Charlottetown, PEI
- 11:15 **102-STU** Assessment of immunocompetence in the shore crab, *Carcinus maenas*, to natural exposure of pathogens Lauren Hall¹, Chris Hauton¹, Grant Stentiford², ¹National Oceanography Centre Southampton, University of Southampton, European Way, Southampton, SO14 3ZH, UK, ²CEFAS, The Nothe, Barrack Road, Weymouth, Dorset, DT4 8UB, UK
- 11:30 **103-STU** Effects of artificial infection of juvenile edible crabs, *Cancer pagurus* with the parasitic dinoflagellate, *Hematodinium* sp. Amanda Smith, Andrew Rowley; Department of Biosciences, College of Science, Swansea University, Swansea, SA2 8PP, Wales, U.K.
- 11:45 **104** A role of polychaetes in transmission of white spot syndrome virus in shrimp ponds? H. Desrina^{1,2,3}, Marc C.J. Verdegem², Johan A.J. Verreth², Slamet B. Prayitno³ and Just M. Vlak¹, Laboratories of ¹Virology and ²Aquaculture and Fisheries, Wageningen University, Wageningen, The Netherlands, and ³Department of Fisheries, Faculty of Fisheries and Marine Sciences, Diponegoro University, Jl. Prof Sudharto, Tembalang, Semarang, Indonesia.
- 12:00 **105** Novel Pattern Recognition Receptor Protects Shrimp from *Vibrio* Infection by Binding Flagellin and LPS through Different Recognition Modules, Xian-Wei Wang; Jin-Xing Wang, School of Life Sciences, Shandong University, Jinan, China
- 12:15 **106** Observations on *Agmasoma penaei* and *Perezia nelsoni* in White shrimp *Litopenaeus setiferus* from the Gulf of Mexico Yuliya Sokolova^{1,3}, John Hawke², ¹Core Microscopy Center, ²Dept. Pathobiol.Sci., School Vet. Medicine, Louisiana State University, Baton Rouge LA, USA; ³Institute of Cytology, St. Petersburg, Russia

Contributed Papers

Tuesday, 10:30-12:15. **P2**

FUNGI 3

Moderators: Helen Hesketh and Ann Hajek

- 10:30 **107** Comparison of ecological traits of co-existing *Metarhizium*: What does it take to dominate an agricultural field? Bernhardt M. Steinwender¹, Miriam Stock², Kasper Brink - Jensen³, Jørgen Eilenberg¹, Nicolai V. Meyling¹, ¹Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; ²IST Austria (Institute of Science and Technology Austria), Klosterneuburg, Austria; ³Department of Biostatistics, University of Copenhagen, Denmark
- 10:45 **108-STU** Effect of entomopathogenic fungal strains on non-target arthropods in sour cherry orchard Emese Balog, Zoltán István Tímár, Judit Papp-Komáromi, György Turóczki; Szent István University, Plant Protection Institute, Gödöllő, Hungary
- 11:00 **109-STU** Potential of endophytic *Beauveria bassiana* in grapevine against insects Yvonne Rondot, Annette Reineke, Hochschule Geisenheim University, Center of Applied Biology, Institute of Phytotherapy, Geisenheim, Germany

- 11:15 **111** Horizontal transmission of entomopathogenic fungi by ectoparasitoid *Habrobracon hebetor* Vadim Kryukov, Natalia Kryukova, Olga Yaroslavtseva, Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia

- 11:30 **112 N** Fast spread of the parasitic *Laboulbenia formicarum* in a supercolony of the invasive garden ant *Lasius neglectus* Simon Tragust¹, Heike Feldhaar¹, Jes Søe Pedersen², ¹Animal Ecology I, University of Bayreuth, Germany, ²Centre for Social Evolution, Department of Biology, University of Copenhagen, Denmark

- 11:45 **113** The dietary preference of a beneficial predator in apple orchards reveals an undocumented spore dispersal mechanism for entomopathogenic fungi Anja Amtoft Wynns¹, Annette Bruun Jensen¹, Celeste d'Allesandro², Jørgen Eilenberg¹; ¹Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; ²Department of Entomology and Acarology, ESALQ, University of São Paulo, Brazil

- 12:00 **114** Effects of entomopathogenic fungi on the "Trialeurodes vaporariorum – *Encarsia formosa*" system: preliminary results Monica Oreste, Eustachio Tarasco, Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari, Bari, Italy

12:40-16:30 Optional Excursion

16:30-18:00 5K Race

17:00-21:30 BBQ

WEDNESDAY - 6 August

7:30-18:00 **REGISTRATION**

P1

Symposium 5 (Microbial Control) Wednesday, 8:00–10:00. **P3**

Developments/Issues in the Regulation of Microbial Products: Harmonization across Jurisdictions

Organizers/Moderators: Roma Gwynn and David Grzywacz

- 8:00 **115** The authorisation and regulation of microbial biopesticides: why bother? David Chandler¹, Liam Harvey & Wyn Grant², ¹Warwick Crop Centre, School of Life Sciences, University of Warwick, UK, ²Department of Politics and International Studies, University of Warwick, UK

- 8:24 **116** Registration of Biopesticides in the EU: a company perspective Philip Kessler, Andermatt Biocontrol AG, Grossdietwil, Switzerland

- 8:48 **117** Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North America Jarrod Leland, Novozymes Biologicals, Inc., 5400 Corporate Circle, Salem, United States
- 9:12 **118** Registration of biopesticides: how research can be structured to suit microbial registration needs and promote the commercial development of new biopesticides Roma Gwynn, Biorationale Limited, Duns, UK
- 9:36 **119** Current developments and issues on regulation of biopesticides- Lessons from REBECA project, comparison of EU and USA systems Sabine Asser-Kaiser, Jacqueline Süß, Rüdiger Hauschild; GAB Consulting GmbH, Heidelberg/Lamstedt, Germany
- Contributed Papers** Wednesday, 8:00-9:45. **P5**
- BACTERIA 3**
- Moderators: Juan Luis Jurat-Fuentes and David Heckel
- 8:00 **120** Resistance alleles to *Lysinibacillus sphaericus* are co-select in a *Culex quinquefasciatus* colony and display distinct features Maria Helena N. L. Silva-Filha¹, Karlos D. M. Chalégre¹, Tatiany P. Romão¹, Daniella A. Tavares¹, Hervely S. G. Menezes¹, Cláudia M. F. de Oliveira¹, Osvaldo P. de-Melo-Neto², ¹Department of Entomology, ²Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife, Brazil
- 8:15 **121-STU** Untangling insect pathogenicity in plant-beneficial pseudomonads by a combination of comparative genomics, bioassays and histopathology Pascale Flury¹, Beat Ruffner¹, Shakira Fataar¹, Maria Péchy-Tarr², Regina G. Kleespies³, Cornelia Ullrich³, Johannes A. Jehle³, Theo H. M. Smits⁴, Christoph Keel², Monika Maurhofer¹, ¹Institute of Plant Pathology, Swiss Federal Institute of Technology, Zürich, Switzerland; ²Department of Fundamental Microbiology, University of Lausanne, Lausanne, Switzerland; ³Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; ⁴Research Group for Environmental Genomics and Systems Biology, Institute for Natural Resources Sciences, Zurich University of Applied Sciences ZHAW, Wädenswil, Switzerland
- 8:30 **122** Comparative analysis of the Cqm1 and Aam1 ortholog proteins from mosquitoes that have a differential capacity to bind to the Binary toxin from *Lysinibacillus sphaericus* Lígia M. Ferreira¹, Nathaly A. do Nascimento¹, Tatiany P. Romão¹, Antônio M. Rezende², Osvaldo P. de-Melo-Neto², Maria Helena N. L. Silva-Filha¹, ¹Department of Entomology, ²Department of Microbiology, Centro de Pesquisas Aggeu Magalhães-FIOCRUZ, Recife, Brazil
- 8:45 **123** Resilience of the intestinal epithelium to the action of a bacterial pore-forming toxin and to xenobiotics in *Drosophila* Kwang-Zin Lee, Matthieu Lestrade, Stephanie Limmer, Samuel Liégeois and Dominique Ferrandon; University of Strasbourg Institute for Advanced Study, IBMC, Strasbourg, France
- 9:00 **124** Cadherin mutations and Bt resistance: Field screening and fitness costs Linda Gahan¹; Fred Gould², David G. Heckel³; ¹Clemson University, Clemson, South Carolina, USA; ²North Carolina State University, Raleigh, North Carolina, USA; ³Max Planck Institute for Chemical Ecology, Jena, Germany
- 9:15 **125** Down regulation and mutation of cadherin gene associated with Cry1Ac resistance in Asian corn borer Tingting Jin¹, Xue Chang¹, Angharad M. R. Gatehouse², Zhenying Wang¹, Martin E. Edward², Kanglai He¹, ¹The State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China; ²Newcastle Institute for Research on Environment and Sustainability, School of Biology, University of Newcastle, UK
- 9:30 **126** ABCC transporters mediate insect resistance to multiple Bt toxins revealed by BSA analysis Youngjin Park¹, Rosa M González-Martínez², Gloria Navarro-Cerrillo², Maissa Chakroun², Yonggyun Kim¹, Peio Ziarsolo³, Jose Blanca³, Joaquín Cañizares³, Juan Ferre², Salvador Herrero², ¹Department of Bioscience Sciences, Andong National University, Korea, ²Department of Genetics, Universitat de València, Spain, ³Institute for Conservation & Improvement of Valencian Agrodiversity (COMAV).Polytechnic University of Valencia, Spain
- Contributed Papers** Wednesday, 8:15-9:45. **P4**
- DIS. OF BENEFICIAL INVERTEBRATES 2**
- Moderator: Lena Poppinga
- 8:15 **128** *Nosema ceranae* News: Update on Species Competition and Host-Pathogen Interaction Studies Leellen Sotter¹, Zachary Huang², Wei-Fone Huang¹ and Meghan Milbrath²; ¹Illinois Natural History Survey, University of Illinois; ²Michigan State University
- 8:30 **129** Influence of temperature on the development of *Nosema apis* and *Nosema ceranae* Sebastian Gisder; Elke Genersch; Institute for Bee Research, Hohen Neuendorf, Germany
- 8:45 **130-STU** The involvement of bumblebee small interfering RNA pathway against two different bee viruses Jinzhi Niu, Ivan Meeus, Guy Smagghe; Department of Crop Protection, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium
- 9:00 **131** Impact of Wolbachia endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare* Sébastien Leclercq, Julien Thézé, Isabelle Giraud, Lise Ernenwein, Bouziane Moumen, Pierre Grève, Clément Gilbert, Richard Cordaux, Université de Poitiers, UMR CNRS 7267 Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France
- 9:15 **132** First characterization of a mollusk beta pore forming toxin David Duval^{1,2}, Richard Galinier^{1,2}, Guillaume Mitta^{1,2}, Benjamin Gourbal^{1,2}, ¹CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France, ²Université de Perpignan, Perpignan, France
- 9:30 **133-STU** A first report of an immune-associated cytosolic PLA₂ in insects: Gene structure and function Jiyeong Park and Yonggyun Kim; Department of Bioscience Sciences, Andong National University, Andong, Korea

Contributed PapersWednesday, 8:00-9:30 **P2****FUNGI 4**

Moderator: Richard Humber and Annette Brunn Jensen

- 8:00 **134 Fungal dimorphism in the entomopathogenic fungus *Nomuraea rileyi*: A search for *in vivo* produced quorum-sensing molecules** Boucias, D. L., Liu, Shouzou² and Baniszewski, Julie¹, ¹Entomology and Nematology Department, University of Florida, Gainesville FL, USA, ²Agricultural College, Liaocheng University, Liaocheng, Shandong, China
- 8:15 **135 Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US** Louela A. Castrillo¹, Ann E. Hajek¹, Ryan M. Kepler¹, Juan A. Pajares², Iben M. Thomsen³, György Csóka⁴, Paula Zamora⁵, and Sergio P. Angelis⁶, ¹Department of Entomology, Cornell University, Ithaca, USA, ²Sustainable Forest Management Research Institute, University of Valladolid, Palencia, Spain, ³Department of Geosciences and Natural Resource Management, University of Copenhagen, Copenhagen, Denmark, ⁴Department of Forest Protection, Forest Research Institute, Mátrafüred, Hungary, ⁵Calabazanos Forest Health Center, Castile and Leon, Palencia, Spain, ⁶Faculty of Science and Technology, University of Bolzano, Italy
- 8:30 **136 Preliminary analysis of the genome sequence of *Beauveria caledonica*** Travis R. Glare¹, Aimee C. McKinnon¹ and Murray P. Cox², ¹Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand, ²Massey University, Palmerston North, New Zealand
- 8:45 **137 MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens** Richard A. Humber¹, Rogério Biaggioni Lopes², Marcos Faria², ¹USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA; ²Embrapa Genetic Resources and Biotechnology, Brasília, Brazil
- 9:00 **138 Transcriptomic study reveals *Pandora formicae* expressing pathogenicity related genes in final stages of host infection** Joanna Malagocka¹; Morten N. Grøll¹, Lene Lange², Jørgen Eilenberg¹, Annette Brunn Jensen¹, ¹Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark; ²Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Copenhagen, Denmark
- 9:15 **139 Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens** Paula F. Quiroz Velasquez, Sumayah Abiff, Quincy B. Conway, Norma C. Salazar, Ana Paula Delgado, Jhanelle K. Dawes, Lauren G. Douma, Aurélien Tatar, Nova Southeastern University, Fort Lauderdale, FL, USA

10:00–10:30

BREAK**Symposium 6 (Bacteria)**

Wednesday, 10:30-12:30.

P5**Structure and Function of Novel Insecticidal Toxins**

Organizers/Moderators: Ken Narva and Colin Berry

- 10:30 **140 Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1** Matthew S. Kelker¹, Colin Berry², Matthew D. Baker², Steven L. Evans³, Reetal Pai¹, David McCaskill¹, Joshua C. Russell^{1‡}, Nick X. Wang¹, J.W. Pflugrath³, Cheng Yang³, Matthew Wade⁴, Tim J. Wess^{4#}, Kenneth E. Narva¹, ¹Dow AgroSciences, LLC, Indianapolis, Indiana, USA; ²Cardiff School of Biosciences, Cardiff University, Cardiff, Wales, UK; ³Rigaku Americas Corporation, The Woodlands, Texas, USA; ⁴School of Optometry & Vision Sciences, Cardiff University, Cardiff, Wales, UK, [‡]Current address: Department of Biochemistry, University of Washington, Seattle, Washington, USA; [#]Current address: Office of the Dean of Science, Charles Sturt University, New South Wales, Victoria, Australia

- 10:50 **141 Structure/function studies of Cry5B via alanine-scanning mutagenesis** Jillian Sesar¹, Melanie Miller¹, Yan Hu^{1,2}, Raffi V. Aroian^{1,2}, ¹Division of Biological Sciences, University of California, San Diego, CA, USA; ²Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, MA, USA

- 11:10 **142 Insights into the structures of non-3-domain toxins through structural modelling** Colin Berry, Cardiff School of Biosciences, Cardiff Univ., Cardiff, UK

- 11:30 **143 Novel MTX Toxins for Insect Control** Yong Yin, Monsanto Company, St. Louis, MO, USA

- 11:50 **144 Insecticidal toxins from *Photorhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins** Thomas Jank, Alexander E. Lang and Klaus Aktories, Institute of Experimental and Clinical Pharmacology and Toxicology, University of Freiburg, Freiburg, Germany

- 12:10 **145 Molecular basis of parasporin-2 action toward cancer cells** Sakae Kitada, Yusuke Yoshida, Yoshimi Ozaki, Hironoyasu Shimada, Kyushu Institute of Technology, Izuka

Contributed Papers

Wednesday, 10:30-12:30.

P3**MICROBIAL CONTROL 2**

Moderator: Surrendra Dara

- 10:30 **146 Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms** Irene Ketseoglou, Gustav Bouwer, School of Molecular and Cell Biology, University of the Witwatersrand, Johannesburg, South Africa

- 10:45 **147 *Bacillus thuringiensis* 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests** Cheng Bai¹, Haibo Long¹, Liping Liu¹, Yanling Yang², Jianjun Yue¹, ¹Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan, China; ²North University of China, Taiyuan, China

- 11:00 **148 Investigations on residues of *Bacillus thuringiensis* on tomato** Dietrich Stephan¹, Heike Scholz-Döblin², Hans Kessler², Theo Reintges², ¹Julius Kühn-Institute, Darmstadt, Germany, ²Landwirtschaftskammer Nordrhein-Westfalen, Germany

- 11:15 **149** Biological control of western corn rootworm larvae (*Diabrotica virgifera virgifera*) with Dianem® (*Heterorhabditis bacteriophora*) Ralf-Udo Ehlers, e~nema, GmbH, Schwentinental, Germany
- 11:30 **150** Evaluation of Ten Plant Extracts as Ultraviolet Protectants for *Spodoptera littoralis* nucleopolyhedrovirus Koko Dwi Sutanto, Said El Salamouny, Martin Shapiro, Merle Shepard, Sukirno Miharjo, Muhammad Tufail, Khawaja Ghulam Rasool and Abdulrahman S. Aldawood, Plant Protection Department, College of Food Sciences and Agriculture, King Saud University, Riyadh, Saudi Arabia; CREC, Clemson University, Charleston South Carolina, USA
- 11:45 **151** Interactions among Fungal and Viral Pathogens and Parasitoids Ann E. Hajek¹; Saskya van Nouhuys², ¹Department of Entomology, Cornell University, Ithaca New York, USA; ²Department of Biosciences, University of Helsinki, Helsinki, Finland
- 12:00 **152** *Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivorus* Sean D.G. Marshall¹, Aubrey Moore², Russell K. Campbell³, Roland J. Quitugua², Trevor A. Jackson¹, Innovative Farming Systems, AgResearch, Lincoln Research Centre, Christchurch, New Zealand; ²College of Natural and Applied Science, University of Guam, USA; ³Biosecurity Division, Guam Department of Agriculture, Guam, USA
- 12:15 **153** The Control of Fungi Using with Liposomal Formulation of Essential Oil of *Satureja hortensis* and its cell viability assay Müge Yazıcı¹, Gülgül Duman², Ismail Aslan², Burçin Asutay¹, Tuğçe Palamut¹, Sıdika Tapşın¹, Fikrettin Şahin¹, ¹Department of Genetics and Bioengineering, Yeditepe University, Istanbul, Turkey, ²Faculty of Pharmacy, Yeditepe University, Istanbul, Turkey
- 11:15 **157** Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal Diane Bigot¹, Marion Ballenghién², Vincent Cahais², Nicolas Galtier², Elisabeth Herniou¹, Philippe Gayral¹, ¹Institut de Recherches sur la Biologie de l'Insecte, CNRS UMR 7261, Université François-Rabelais, Tours, France, ²Université Montpellier 2, Institut des Sciences de l'Evolution de Montpellier, Montpellier, France
- 11:30 **158** Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons Clément Gilbert¹, Aurélien Chateigner², Lise Ermenwein¹, Valérie Barbe³, Annie Bézier², Elisabeth A. Herniou^{2,*} & Richard Cordaux¹, ¹Université de Poitiers, Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France; ²Université François-Rabelais de Tours, Tours, France, ³Laboratoire de Finition, CEA/IG/Genoscope, Evry, France
- 11:45 **159** Genomic analysis of five *Lymantria dispar* multiple nucleopolyhedrovirus isolates and biological activity against different host strains of *Lymantria dispar* Robert L. Harrison¹; Daniel L. Rowley¹; Melody Keena², ¹Invasive Insect Biocontrol and Behavior Laboratory, Beltsville Agricultural Research Center, USDA Agricultural Research Service, Beltsville, Maryland, USA; ²Northern Research Station, USDA Forest Service, Hamden, CT, USA
- 12:00 **160** Phylogenomics reveals ecological factors that lead to speciation in *Baculoviridae* Julien Thézé¹; Carlos Lopez Vaamonde²; Jennifer S. Cory³; Elisabeth A. Herniou¹, ¹Université François-Rabelais, UFR Sciences, Tours, France; ²INRA, Zoologie Forestière, Orléans, France; ³Dept of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, Canada

Contributed Papers

Wednesday, 10:30-12:15.

P1

VIRUSES 4

Moderators: Martin Erlandson and Robert Harrison

- 10:30 **154** *Mamestra configurata* nucleopolyhedrovirus-A transcriptome from infected host midgut Martin A. Erlandson¹, B. Cameron Donly², David A. Theilmann³, Dwayne D. Hegedus¹, Cathy Couto¹ and Douglas Baldwin¹, ¹Saskatoon Research Centre, AAFC, Saskatoon, Canada; ²Southern Crop Protection & Food Research Centre, AAFC, London, Canada; ³Pacific Agri-Food Research Centre, AAFC, Summerland, BC, Canada
- 10:45 **155-STU** Genomic adaptation to different hosts – Impact of genetic diversity on viral fitness Aurélien Chateigner; Cindy Pontlevé; Carole Labrousse; Elisabeth Herniou, Institut de Recherche sur la Biologie de l'Insecte, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Tours, France
- 11:00 **156-STU** Transcriptomic analysis of a host-parasitoid interaction between a Hymenoptera *Cotesia congregata*, a Lepidoptera *Manduca sexta* and a Polydnaviridae (Gérard Chevignon; Sébastien Cambier; Jean-Michel Drezen; Elisabeth Huguet; Sébastien Moreau; Institut de Recherche sur la Biologie de l'Insecte, Université François Rabelais de Tours, Faculté des Sciences et Techniques, Tours, France

Contributed Papers

Wednesday, 10:30-12:15.

P2

FUNGI 5

Moderators: Travis Glare and Jürg Enkerli

- 10:30 **162** An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus Barker* Yulin Gao¹, Shengyong Wu¹, Zhongren Lei¹, Xuenong Xu¹, ¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China
- 10:45 **163-STU** Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea* Chad A. Keyser, Birgit Jensen, and Nicolai V. Meyling, Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen, Denmark
- 11:00 **164** Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin Natalia González-Mas, Lola Ortega-García, Carlos Campos-Porcuna, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga; University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

- 11:15 **165-STU** Recovery and detection of an entomopathogenic endophyte: overcoming the challenges involved Aimee McKinnon¹; Travis Glare¹, Hayley Ridgway², Andrew Holyoake¹, Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand; ²Faculty of Agriculture and Life Sciences, Lincoln University, Christchurch, New Zealand
- 11:30 **166-STU** Intense spatio temporal pattern in pathogen-host interaction between *Pandora formicae* and *Formica rufa* Joanna Malagocka; Jørgen Eilenberg, Annette Bruun Jensen; Centre for Social Evolution, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark
- 11:45 **167 Patterns of host adaptation in fly infecting Entomophthora species** Henrik H. De Fine Licht; Annette Bruun Jensen, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
- 12:00 **168-STU** Plant volatile organic compound manipulation by endophytic entomopathogenic fungi Aragón, Sandra^{1,2}, Cotes, Alba Marina², Vidal, Stefan¹, ¹Georg-August-Universität Göttingen, Department of Crop Sciences, Göttingen, Germany. ²Biotechnology and Bioindustry Center, Colombian Corporation for Agricultural Research Corpoica, Mosquera, Colombia

12:30–14:00	LUNCH	Mensa
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Contributed Papers	Wednesday, 13:15-14:00. P203
JIP EDITORIAL BOARD	

Student Workshop	Wednesday, 12:30-14:00. P2
HOW TO WRITE A PAPER	

Moderators: Rich Humber, Mark Goettel and Yukino Inoue

Contributed Papers	Wednesday, 14:00-16:00. P4
MICROSPORIDIA 1	

Moderator: Susan Bjørnson

- 14:00 **169 Effects of the microsporidium *Nosema adaliae* on the multicoloured Asian lady beetle, *Harmonia axyridis*** Bryan Ellis, Susan Bjørnson, Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada
- 14:15 **170-STU Effects of two microsporidia from lady beetles on the green lacewing, *Chrysoperla carnea*** Jackline Sirisio, Susan Bjørnson, Department of Biology, Saint Mary's University, Halifax, Nova Scotia, Canada
- 14:30 **171 Features of the genomes of microsporidia in mosquitoes: status and preliminary findings** James J. Becnel¹, Christopher Desjardins², Neil Sancristante¹, and Christina Cuomo², ¹Center for Medical, Agricultural and Veterinary Entomology, USDA/ARS, Gainesville, FL, USA, ²Genome Sequencing Center for Infectious Disease, Broad Institute of MIT and Harvard, Cambridge, MA, USA

- 14:45 **172 Multi-gene phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts** K.S. Bateman¹, R. Kerr¹, D. Wiredu-Boakye², B. Williams², G.D. Stentiford¹, ¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset, UK, ²Biosciences, University of Exeter, Devon, UK

- 15:00 **173-STU Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidians** Dominic Wiredu Boakye¹, Bryony Williams¹; Grant Stentiford¹, and Thomas Williams³, ¹College of Life and Environmental Sciences, University of Exeter, Exeter; UK, ²Centre of Environment, Fisheries and Aquaculture Science, CEFAS, Weymouth, UK; ³Institute for Cell and Molecular Biosciences, University of Newcastle, Newcastle upon Tyne, Tyne and Wear, UK

- 15:15 **174-STU Temporal trends and the effect of seasonal temperature on the prevalence of *Nosema* spp. in *Apis mellifera* in north-east Germany** Anto Raia Dominic^{1,3}, Sebastian Gisder², Elke Genersch², Andreas Linde¹, Hochschule für nachhaltige Entwicklung Eberswalde, Dept. of Forest and Environment, Eberswalde, Germany, ²Länderinstitut für Bienenkunde Hohen Neuendorf e.V., Hohen Neuendorf, Germany, ³Freie University, Berlin, Germany

- 15:30 **175 STU Characterising putative virulence factors of the bee pathogen *Nosema ceranae*** Graham Thomas, Ken Haynes; University of Exeter, UK

- 15:45 **176 Detection of Microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia)** Yuri Tokarev¹, Vladimir Voronin², Egor Rusakovich³, Irma Issi¹, ¹All-Russian Institute of Plant Protection, St. Petersburg, Russia; ²St. Petersburg Veterinary Medical Academy, St. Petersburg, Russia; ³Herzen State Pedagogical University of Russia, St. Petersburg, Russia

Contributed Papers	Wednesday, 14:15-15:45. P3
MICROBIAL CONTROL 3	

Moderator: Stefan Jaroski

- 14:15 **178-STU Synthesis and Characterization of fungus mediated silver nanoparticle for the toxicity on filarial Vector, *Culex quinquefasciatus*** Siva Kamalakanan¹, Chandrasekaran Gobinath², Sivapunya Ananth³, Kadarkarai Murugan¹, ¹Division of Entomology, Department of Zoology, Bharathiar University, Coimbatore, Tamil Nadu, India; ²Bio control laboratory, Department of Biotechnology and Genetic Engineering, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India; ³Insect control division, Department of Biotechnology, Anna Arts and Science College, Kumbakonam, Tamil Nadu, India

- 14:30 **179-STU Entomopathogenic fungi as endophytes: interaction with phytohormones** Dalia Muftah Alkhayat, Katharina Döll, Petr Karlovsky, Stefan Vidal; Institute for Plant Protection and Plant Pathology, Georg-August University, Göttingen, Germany

- 14:45 **180 Pathogenicity of three entomopathogenic fungi on larvae and adults of the sisal weevil: The less the better?** Vasiliki Gkounti¹, Markogiannaki Dimitra², Dimitris Kontodimas², ¹SLU, Sweden, ²Benaki Phytopathological Institute, Greece

- 15:00 **181** Understanding *Beauveria bassiana* infection within its host *Triatoma infestans*: time course expression of genes encoding fungal toxic nonribosomal peptides and insect humoral immune proteins Luciana S. Lobo^{1,2}, Éverton K. K. Fernandes², Christian Luz², M. Patricia Juárez¹, Nicolás Pedrini¹. ¹Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La Plata, Argentina; ²Instituto de Patología Tropical e Saúde Pública (IPTSP), Universidade Federal de Goiás, Goiânia, Brazil
- 15:15 **182** Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of the olive fly preimaginalis in the soil Enrique Quesada-Moraga, Inmaculada Garrido-Jurado, Meelad Yousef, University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain
- 15:30 **183** The Seed Corn Maggot and *Metarhizium* are Related to Maize Yield in an Organic, Cover Crop-Based Farming Systems Experiment Mary Barbercheck; Christina Mullen, Department of Entomology, Penn State University, University Park, USA
- 15:15 **189** Iteraviruses (Densovirinae) from monarch and black swallowtail butterflies and slug caterpillar moths and characterization of their expression strategies Qian Yu, Max Bergoin, and Peter Tijssen, INRS-Institut Armand-Frappier, Laval, QC, Canada
- 15:30 **190** Remarkable genetic diversity of single-stranded DNA viruses in cultured shrimps and crickets Hanh T. Pham, Qian Yu, Max Bergoin, Peter Tijssen, INRS-Institut Armand-Frappier, Université du Québec, Laval, QC, Canada
- 15:45 **191** How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level? Alicia Eva Timm¹ & Annette Reineke², ¹Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa, ²Institut für Phytomedizin, Geisenheim Hochschule, Geisenheim, Germany

Contributed Papers Wednesday, 14:00-15:45 **P5**
BACTERIA 4
Moderators: Yulin Gao and Neil Crickmore

- Contributed Papers Wednesday, 14:00-16:00. **P1**
VIRUSES 5
Moderators: Bryony Bronning and Alicia Timm
- 14:00 **184** Soybean aphid viruses exploit contrasting transmission strategies Diveena Vijayendran, Sijun Liu, Bryony C. Bonning, Department of Entomology, Iowa State University, Ames, USA
- 14:15 **185** Characterization of mechanisms involved in the transmission of a lepidopteran densovirus Cécilia Multea¹, Doriane Mutual², Manuela Rakotomanga², Anne Kenaghan², Clément Bousquet², Rémy Froissart^{3,4}, Nathalie Volkoff² and Mylène Ogliastro²; ¹InVivo AgroSolutions, Valbonne, France; ²INRA, UMR 1333 DGIMI, INRA, Montpellier, France; ³CNRS, UMR 5290 MIVEGEC, Montpellier, France; ⁴CIRAD-SupAgro, UMR 385 BGPI, Montpellier, France
- 14:30 **186** Discovery of circular single-stranded DNA viruses in top insect predators Karyna Rosario¹, Anisha Dayaram², Jessica Ware³, Milen Marinov², Mya Breitbart¹, Arvind Varsani², ¹College of Marine Science, University of South Florida, Florida, USA; ²School of Biological Sciences, University of Canterbury, Christchurch, New Zealand; ³School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA
- 14:45 **187-STU** Single-stranded DNA viruses in marine crustaceans Ryan Schenck¹, Karyna Rosario¹; Rachel Harbeitner¹; John Cannon²; Mya Breitbart¹, ¹University of South Florida College of Marine Science, Tampa, Florida, USA; ²University of South Florida College of Medicine Department of Pediatrics, USA
- 15:00 **188** Remarkable diversity of endogenous viruses in the genome of an isopod crustacean Julien Thézé, Sébastien Leclercq, Bouziane Moumen, Richard Cordaux, Clément Gilbert; Université de Poitiers, Laboratoire Ecologie et Biologie des Interactions - UMR CNRS 7267, Equipe Ecologie Evolution Symbiose, Poitiers Cedex, France
- 14:00 **192** Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle Zane Metla^{1,2,3}, Monika Maurhofer², Liga Jankevica^{1,3}, ¹Plant Pathology, Institute of Integrative Biology (IBZ), Swiss Federal Institute of Technology, Switzerland; ²Laboratory of Experimental Entomology, Institute of Biology, Univ. Latvia, Latvia; ³Univ. of Daugavpils, Latvia
- 14:15 **193** Contacting microbe induce grooming behaviour in *Drosophila* Aya Yanagawa^{1,2}, Tsuyoshi Yoshimura¹, Hata Toshimitsu¹ and Frédéric Marion-Poll^{2,3}, ¹Kyoto University, Uji, Japan; ²CNRS, Laboratoire Evolution, Génomes et Spéciation, Gif-sur-Yvette, France; ³AgroParisTech, Département Sciences de la Vie et Santé, Paris, France
- 14:30 **194** Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication Yueming Shan^{1,2}, Changlong Shu², Neil Crickmore³, Chunqin Liu⁴, Wensheng Xiang¹, Fuping Song², Jie Zhang², ¹School of Life Science, Northeast Agricultural University, Harbin, P.R. China; ²State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P.R. China; ³School of Life Sciences, University of Sussex, Falmer, Brighton, UK; ⁴Cangzhou Academy of Agricultural and Forestry Sciences, Cangzhou, P.R. China
- 14:45 **195** Interactions between the Med fly *Ceratitis capitata* (Wied.) and a new *Bacillus cereus* sensu lato strain Luca Ruiu^{1,2}, Giovanni Falchi², Ignazio Floris¹, Maria G. Marche^{1,2}, Maria E. Mura², Alberto Satta¹, ¹Dipartimento di Agraria, University of Sassari, Italy, ²Bioecopest Srl. Technology Park of Sardinia, Italy
- 15:00 **196** Long-term effect of *Bacillus thuringiensis* subsp. *israelensis* application on *B. cereus* group populations in Swedish riparian wetland soils Salome Schneider¹, Tania Tajrin¹, Niels B. Hendriksen², Jan O. Lundström³, Petter Melin¹, Ingvar Sundh¹, ¹Department of Microbiology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, ²Department of Environmental Science, Aarhus University, Roskilde, Denmark, ³ Mosquito and Environment Group, Program for Population and Conservation Biology, Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden

- 15:15 **197 Proteomics of *Brevibacillus laterosporus* and its insecticidal action against noxious Diptera**
 Maria G. Marche^{1,2}, Maria E. Mura¹, Giovanni Falchi¹, Luca Ruiu^{1,2}, ¹Dipartimento di Agraria, University of Sassari, Italy, ²Bioecopest Srl. Technology Park of Sardinia, Italy
- 15:30 **198-STU Outer membrane vesicles are vehicles for the delivery of *Vibrio* virulence factors to oyster immune cells** Audrey S. Vanhove¹, Marylise Duperthuy^{1,2}, Guillaume M. Charrière¹, Frédérique Le Roux³, David Goudénèg³, Benjamin Gourbal⁴, Sylvie Kieffer-Jaquinod⁵, Yohann Couté⁵, Sun N. Wai² and Delphine Destoumieux-Garzón¹, ¹Ecology of coastal marine systems, CNRS, Ifremer, IRD, University of Montpellier, France; ²Umeå University, Department of Molecular Biology, The Laboratory for Molecular Infection Medicine Sweden (MIMS), Sweden; ³Integrative Biology of Marine Models, CNRS, Ifremer, Université Pierre et Marie Curie, Station Biologique de Roscoff, France; ⁴Université de Perpignan, Ecology and Evolution of Interactions, France; ⁵Université Grenoble-Alpes, CEA, iRTSV, Biologie à Grande Echelle; INSERM, France

16:00–16:30 **BREAK**

Wednesday, 16:30–18:30. **Philosophicum**
POSTERS
**Posters should be displayed from Monday
 UNTIL NOT LATER THAN 18:00 THURSDAY**

BACTERIA

- BA-1 A New Local Bio-Insecticide: Developing, Optimization, Toxicity and Determination of Activity**
 Kazım Sezen, Remziye Nalcacioglu, Ismail Demir, Hüseyin Tepe, Islam Yildiz, Ardahan Eski, Zihni Demirbag, Karadeniz Technical University, Faculty of Science, Department of Biology, Trabzon, Turkey
- BA-2 'Candidatus Rickettsiella isopodorum', a new lineage of intracellular bacteria infecting woodlice**
Regina G. Kleespies¹; Andreas Leclerque^{1,2}; ¹Institute for Biological Control, Julius Kühn Institute (JKI), Germany, ²Geisenheim University, Institute for Microbiology and Biochemistry, Geisenheim, Germany
- BA-3-STU Analysis and characterization of binary AB toxins in the honey bee pathogen**
Paenibacillus larvae Julia Ebeling, Lena Poppinga, Anne Fünfhaus, Elke Genersch Institute for Bee Research, Hohen Neuendorf, Brandenburg, Germany
- BA-4 Interplay of Regulators Controlling Fit Insect Toxin Expression in the Biocontrol Bacterium**
Pseudomonas protegens Nicola Imperiali¹, Flavia Büchler¹, Maria Péchy-Tarr¹, Peter Kupferschmid¹, Monika Maurhofer², and Christoph Keel¹; ¹Department of Fundamental Microbiology, University of Lausanne, Switzerland, ²Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland

- BA-5-STU Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity** Luis A. Verduzco-Rosas and Jorge E. Ibarra. CINVESTAV IPN, Irapuato, Mexico
- BA-6 Evaluation of Culture media for maximal growth, Cry toxin production and insecticidal toxicity of *Bacillus thuringiensis*** M. Tripathi¹, A. Kumari², L. Saravanan³ G.T. Gujar⁴, ^{1,4}Division of Entomology, Indian Agricultural Research Institute, New Delhi, ²TERI, India Habitat Centre, New Delhi, ³Directorate of Medicinal and Aromatic Plants Research, Anand
- BA-7 Gene organization of large plasmids of novel mosquitocidal *Bacillus thuringiensis* TK-E6** Mayu Noda, Naruhiko Okamoto, Kimie Hayasaki, Yoshinao Azuma, and So Takebe; Faculty of Biology-Oriented Science and Technology, Kinki University, Wakayama, Japan
- BA-8-STU Testing of Vip3 proteins for the control of caterpillar pests** Iñigo Ruiz de Escudero^{1,2}, Núria Banyuls³, Yolanda Bel³, Mireya Maeztu¹, Baltasar Escrivé³, Delia Muñoz², Primitivo Caballero^{1,2}, Juan Ferré³, ¹Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, Campus Arrosadía, Mutilva, Navarra, Spain. ²Laboratorio de Entomología Agrícola y Patología de Insectos, Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain. ³Departamento de Genética, Facultad de CC. Biológicas, Universitat de València, Valencia, Spain
- BA-9 Interactions between Cry and Vip proteins from *Bacillus thuringiensis* against different lepidopteran pests** Ana Rita Nunes Lemos¹, Camila Chiaradia Davolos¹, Paula Cristina Brunini Crialesi Legori¹, Odair Aparecido Fernandes², Juan Ferré³, Manoel Victor Franco Lemos¹, Janete Apparecida Desiderio¹; ¹Dpto de Biología Aplicada à Agropecuária, UNESP/Campus de Jaboticabal, Brazil, ²Dpto de Fitossanidade, UNESP/Campus de Jaboticabal, Brazil, ³Dpto de Genética, Universidade de Valéncia, Spain
- BA-10 Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chrysodeixis (=Pseudoplusia) includens*.** Yolanda Bel 1, Ken Narva 2, Joel Sheets 2, Baltasar Escrivé¹, ¹Dept. Genetics, ERI BioTecMed, Universitat de València, Dr. Moliner, Burjassot, Valencia, SPAIN; ²Dept. Biochemistry/Mol. Biology, Dow AgroSciences, Zionsville Rd. Indianapolis, USA
- BA-11-STU In vivo and in vitro binding of Vip3Aa to *Spodoptera frugiperda* midgut and characterization of binding sites using ¹²⁵I-radiolabeling** Maissa Chakroun and Juan Ferré, Department of Genetics, University of Valencia, 46100-Burjassot (Valencia), Spain
- BA-12 Comparative histopathology of two novel bacterial insecticidal proteins in *Tenebrio molitor* and *Diabrotica virgifera virgifera* larvae** Heba Abdelgaffar¹; Cris Oppert², Jayme Williams², Deepa Balasubramanian¹, Juan Luis Jurat-Fuentes¹, ¹Department of Entomology and Plant Pathology, University of Tennessee, Knoxville (TN), USA; ²Bayer CropScience, Morrisville (NC), USA
- BA-13-STU Role of ABC-C2 in the interactions of *Heliothis virescens* with its host plants and Bt toxins** Anne Karpinski, Yannick Pauchet, Heiko Vogel and David Heckel, Department of Entomology, Max Planck Institute for Chemical Ecology, Jena Germany

- BA-14-STU** AminomethylidaseN in *Popillia japonica*
Newman larvae is putative *Bacillus thuringiensis* Cry8Da toxin receptor Yuu Taniguchi, Takuya Yamaguchi, Hisanori Bando, Shin-ichiro Asano, Graduate School of Agriculture, Hokkaido University, Sapporo, Japan
- BA-15** A Whole Genome Approach to Determine Cadherins associated with Bt toxicity in the Diamondback Moth, *Plutella xylostella* Youngjin Park and Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, South Korea
- BA-16** RNA Interference of Integrin subunit β1 Impairs Development and Immune Responses of the Oriental tobacco budworm, *Helicoverpa assulta* against Bacteria Youngjin Park and Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, South Korea
- BA-17** A natural hybrid of a *B. thuringiensis* Cry2A toxin implicates domain I in specificity determination.
Guihua Chen^{1,3}, Changlong Shu¹, Jacob Evans², Fuping Song¹, Guoxun Li³, Neil Crickmore², Jie Zhang¹; ¹State Key Laboratory of Biology for Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, P. R. China; ²School of Life Sciences, University of Sussex, Falmer, Brighton, UK; ³College of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao, China
- BA-18** *Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection Yulin Gao¹, Zhongren Lei¹, Xuenong Xu¹, ¹State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, PR. China
- BA-19** InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria Christina Schuster¹, Katharina Saar¹, Regina G. Kleespies¹, Andreas Leclercque^{1,2}, ¹Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; ²Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany
- BA-20** Type IV Secretion System (T4SS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria Andreas Leclercque, Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany
- BA-21** Unbalanced Polyphosphate Levels Impair Insect Pathogenicity in Plant-Beneficial *Pseudomonas protegens* Maria Péchy-Tarr¹, Nicolas Wenner¹, Peter Kupferschmied¹, Romane Keller¹, Monika Maurhofer², Christoph Keel¹; ¹Department of Fundamental Microbiology, University of Lausanne, Switzerland; ²Plant Pathology, Institute of Integrative Biology, ETH Zurich, Switzerland
- BA-22-STU** *Paenibacillus* larvae and the virulence factor SpIA- an ERIC II specific S-layer Protein Henriette Knispel, Lena Poppinga, Anne Fünfhaus, Elke Genersch*, Institute for Bee Research, Hohen Neuendorf; Division of Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany

- BA-23** Influence of (varying) population size on host-parasite coevolution: an experimental approach Andrei Papkou, Rebecca Schalkowski, Mike-Christoph Barg, Ines Braker, Hinrich Schulenburg, Evolutionary Ecology Genetics, Zoological Institute, CAU Kiel. Address for Correspondence: apapkou@zoologie.uni.kiel.de
- BA-24** An *in vivo* experimental evolution system for analyzing bacterial adaptation and evolution of *Bacillus cereus sensu lato* in an insect model Rafael Patiño Navarrete^{1,2}, Isabelle Jéhanno^{1,2}, Christina Nielsen-Leroux^{1,2} and Vincent Sanchis^{1,2}, ¹INRA, UMR1319 Micalis, F-78350 Jouy-en-Josas, France, ²AgroParisTech, UMR Micalis, F-78350 Jouy-en-Josas, France

DISEASES OF BENEFICIAL INVERTEBRATES

- DB-1-STU** Identification and Characterization of Immune Inhibitor A Metalloprotease of the Honey Bee Pathogen *Paenibacillus larvae* Birte Arit^{1,2}, Gillian Hertlein¹, Lena Poppinga¹, Eva Garcia-Gonzalez¹, Elke Genersch^{1,3}, ¹Institute for Bee Research Hohen Neuendorf, Hohen Neuendorf, Germany; ²Technische Universität Berlin, Institute of Biotechnology, Berlin, Germany; ³Freie Universität Berlin, Institute of Microbiology and Epizootics, Berlin, Germany
- DB-2** Awareness and Concept of Insects in a Korean Population Sung Min Bae, Tae Young Shin, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo, Department of Agricultural Biology, Chungbuk National University, Chungju, Korea
- DB-3** Virus Epizootiology in Managed and Native Bee Populations John P. Burand¹, Matthew Boucher², Anne Averill³, Departments of ¹Microbiology, ²Biology and ³Environmental Conservation, University of Massachusetts - Amherst, Amherst, USA
- DB-4** Honeybee Virus Epizootiology in Bee Populations in Connecticut, USA John P. Burand¹, Shuning Zheng², Kimberly Stoner³, ¹Department of Microbiology, ²Graduate Program in Molecular and Cellular Biology, University of Massachusetts - Amherst, Amherst, USA and ³Connecticut Agricultural Experiment Station, New Haven, USA
- DB-5** High-throughput sequence analysis of the change in expression profile of Ig2-, Ig3- and Ig7- variant domains in *Carcinus maenas* Down Syndrome Cell Adhesion (*Cmdscam*) mRNAs in response to pathogenic infection Chris Hauton¹, John A. Hammond², ¹School of Ocean and Earth Sciences, University of Southampton, National Oceanography Centre, Southampton, Hants, UK; ²Immunogenetics Group, The Pirbright Institute, Pirbright, Woking, UK
- DB-6** A novel pathogenic *Paenibacillus* strain of *Biomphalaria glabrata*, an intermediate host for schistosomiasis David Duval^{1,2}, Richard Galinier^{1,2}, Gabriel Mouahid^{1,2}, Eve Toulza^{1,2}, Anne Rognon^{1,2}, Nathalie Arancibia^{1,2}, Jean Francois Allienne^{1,2}, Guillaume Mitta^{1,2}, André Thérón^{1,2}, Benjamin Gourbal^{1,2}, ¹CNRS, UMR 5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France, ²Université de Perpignan Via Domitia, Perpignan, France

DB-7 Venom from the ectoparasitic wasp *Habrobracon hebetor* activates calcium-dependent processes of haemocytic degradation in *Galleria mellonella* larvae
Natalia A. Kryukova¹, Ekaterina A. Chertkova¹, Alexandra D. Semenova², Yuri I. Glazachev², Irina A. Slepneva², Victor V. Glupov¹, ¹Institute of Systematics and Ecology of Animals, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia; ²Institute of Chemical Kinetics and Combustion, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia

DB-8 Histopathological analyses of different tissues of diseased honey bees (*Apis mellifera*) Lena
Poppinga¹, Heike Aupperle², Elke Genersch¹, ¹Institute for Bee Research, Molecular Microbiology and Bee Pathology, Hohen Neuendorf, Germany; ²Laboklin GmbH & Co KG, Bad Kissingen, Germany

DB-9 New findings in genome of *Apis mellifera* filamentous virus Lukasz Rabalski¹, Urszula Grzeda², Grazyna Topolska²; Martyna Krejmer¹; Boguslaw Szewczyk¹, ¹Department of Recombinant Vaccines, Intercollegiate Faculty of Biotechnology of the University of Gdansk and Medical University of Gdansk, Gdansk, Poland; ²Laboratory of Bee Diseases, Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Warsaw, Poland

DB-10 Development of prototypes of rapid molecular diagnostic tests for pathogens of honeybees (*Apis mellifera* L.) on chromatographic NALF platform (Nucleic Acid Lateral Flow) Adriano Ragni¹, Francesca Tabarrini¹, Mario Carucci¹, Claudio E. Lorenzetti¹, Antonella Cersini², Silvia Puccica²; Valeria Antognetti², Marcella Milito²; Alessandra Giacomelli²; Giovanni Formato²; Francesco Panara³, ¹RAPID BIOTECH, Perugia; ²Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma; ³ENEA - Centro Ricerche Trisaia S.S. 106 Ionica, Rotondella Matera, Italy

DB-11 What Kind of Insects Do You Like? Tae Young Shin, Sung Min Bae, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo, Department of Agricultural Biology, Chungbuk National University, Chungju, Korea

DB-12 A muscle-infecting microsporidium infecting pink shrimp (*Pandalus montagui*) from Europe: closing in on the type species of *Theholania*? Stentiford, G.D., Ross, S., Kerr, R., Bateman, K.S., European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, Dorset DT4 8UB, UK

FUNGI

FU-1-STU Monitoring of entomopathogenic fungi in *Metarhizium* and *Beauveria* treated fields
Emese Balog, Do Van Hung, Zoltán Mayer, György TuróczsiSzent István University, Plant Protection Institute, Gödöllő, Hungary

FU-2 Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia
Medea Burjanadze¹, Richard Humber², Mariam Arjevanidze¹, Tea Abramishvili¹, Giuli Tsereteli¹, Manana Lortkipanidze³, ¹Agricultural University of Georgia, Department of Forest protection, Georgia;

²USDA-ARS BioIPM Resarch, RW Holley Center for Agriculture and Health, Ithaca, NY., USA; ³Ilis State, University Institute of Zoology, Georgia.

FU-3 Diversity of Entomopathogenic fungi in different citrus cropping systems in Brazil

Celeste P. D'Alessandro, Vanessa da Silveira Duarte, Elisa S. Dominguez, Ana C. Oliveira dos Santos, Italo Delalibera Jr. Department of Entomology and Acarology, ESALQ, University of São Paulo, Av. Pádua Dias 11, CP. 9, Piracicaba, São Paulo, Brazil.

FU-4 The Entomopathogenic Fungus *Isaria* for Pest

Insect Control in Vegetables Katharina Saar¹; Andreas Leclerque²; Dietrich Stephan¹, ¹Institute for Biological Control, Julius Kühn-Institut (JKI), Darmstadt, Germany; ² Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany

FU-5 Prevalence of *Beauveria pseudobassiana* among tick-associated fungal isolates from the Republic of Moldova

Natalia V. Munteanu¹; Polina V. Mitkovets²; Galina V. Mitina²; Alexandru Movila¹; Yuri S. Tokarev²; Andreas Leclerque^{3,4}, ¹Institute of Zoology, Academy of Sciences of Moldova, Chișinău, Republic of Moldova; ²All-Russian Institute for Plant Protection, Saint-Petersburg, Russia; ³Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; ⁴Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany.

FU-6 Diversity and abundance of entomopathogenic

fungi on strawberry crops in Brazil Thiago Rodrigues de Castro^{1,2}, Lívia Maria Alves Porto¹, Jørgen Eilenberg¹, Italo Delalibera Junior¹, University of São Paulo (ESALQ), Brazil; ²Department of Plant and Environmental Sciences, University of Copenhagen, Denmark.

FU-7 Abundance and diversity of *Metarhizium* spp. in an agricultural landscape in Sweden Salome Schneider¹,

Stefan Stranne¹, Hanna Friberg², Ingvar Sundh¹, ¹Department of Microbiology and ²Department of Forest Mycology and Plant Pathology, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden.

FU-8 Diversity and distribution of entomopathogenic

fungi in Czech Republic soils Kateřina Šimáčková^{1,2}, Jana Kročáková², Andrea Bohatá², Noemi Herrero¹, ¹Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic; ²University of South Bohemia, Faculty of Agriculture, České Budějovice, Czech Republic

FU-9 Entomopathogenic fungi as plant growth enhancers

Surendra K. Dara¹, Sumanth S. Dara², Suchitra S. Dara³, ¹Division of Agriculture and Natural Resources, University of California; ²Stockdale High School, Bakersfield, USA; ³Warren Junior High School, Bakersfield, USA.

FU-10 The entomopathogenic fungus *Beauveria bassiana* improves the growth of *Triticum aestivum* and *Triticum durum* Antonio Rafael Sánchez-Rodríguez¹,

María del Carmen del Campillo², Inmaculada Garrido-Jurado¹, Enrique Quesada-Moraga¹, ¹Departamento de Ciencias y Recursos Agrícolas y Forestales, Universidad de Córdoba, España, ²Departamento de Agronomía, Universidad de Córdoba, España

FU-11-STU Interactions between cowpea plants vs. *Metarhizium* spp. entomopathogenic fungi Patrícia S. Golo¹; Walquíria Arruda²; Flávia R. S. Paixão²; Fabricio

- M. Alves²; Éverton K. K. Fernandes²; Donald W. Roberts³; Vânia R. E. P. Bittencourt¹; ¹Universidade Federal Rural do Rio de Janeiro, Seropédica, Brazil; ²Universidade Federal de Goiás, Goiânia, Brazil; ³Utah State University, Logan, USA.
- FU-12 Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus *Beauveria bassiana* as an endophyte in oilseed rape plants** Cornelia Ullrich¹; Saoussene Talbi¹; Andreas Leclerque^{1,2}; Frank Rabenstein³; Regina G. Kleespies¹; ¹Institute for Biological Control, Julius Kühn Institute (JKI), Germany; ²Hochschule Geisenheim, University, Geisenheim, Germany; ³Julius Kühn Institute, Quedlinburg, Germany
- FU-13 Azygo- and zygospore formation of *Neozygites floridana* in the two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions** Karin Westrum¹; Vanessa S. Duarte²; Richard A. Humber³; Italo Delalibera Jr²; Ingeborg Klingen¹; ¹Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Ås, Norway; ²ESALQ – University of São Paulo, Piracicaba, Brazil; ³USDA-ARS BiolPM Research, Ithaca, NY, USA.
- FU-14 Susceptibility of *Biomphalaria glabrata* egg masses to fungal infection** Glennyha F. Duarte, Juscelino Rodrigues, Éverton K. K. Fernandes, Christian Luz, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, GO, Brazil
- FU-15 Antimicrobial, Antioxidant and Anticancer Activity of Culture Filtrates from Entomopathogenic Fungi** Tae Young Shin, Sung Min Bae, Jae Bang Choi, Won Seok Kwak, Yong Oh Ahn, See Nae Lee, In Hui Kim, Ra Mi Woo, Dong Jun Kim and Soo Dong Woo; Department of Agricultural Biology, Chungbuk National University, Chungju, Korea
- FU-16 Evolutionary-ecological strategies of *Metarhizium robertsii*** Olga Yaroslavtseva, Vadim Kryukov, Ivan Dubovskiy, Maxim Tyurin, Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia
- FU-17 Mycelial and conidial thermotolerance of *Metarhizium anisopliae* s.l. IP 46 and *Metarhizium robertsii* ARSEF 2575** Flávia R. S. Paixão¹; Elen R. Muniz¹; Cíntia C. Bernardo¹; Gabriel M. Mascarin²; Christian Luz¹; Éverton K. K. Fernandes¹; ¹Universidade Federal de Goiás, Goiânia, Brazil; ²Embrapa Arroz e Feijão, Goiânia, Brazil.
- FU-18 Delayed germination of heat-stressed conidia of *Metarhizium anisopliae* on tick cuticle** Lucas P. Barreto¹; Fabrício M. Alves¹; Christian Luz¹; Gabriel M. Mascarin²; Donald Roberts³; Walquíria Arruda¹; Éverton K. K. Fernandes¹; ¹Universidade Federal de Goiás, Goiânia, Brazil; ²Embrapa Arroz e Feijão, Goiânia, Brazil; ³Utah State University, Logan, USA.
- FU-19 Influence of environmental factors on insects resistance to anamorphic fungi** Vadim Kryukov; Ivan Dubovskiy, Olga Yaroslavtseva, Maxim Tyurin, Natalia Kryukova, Victor Glupov; Institute of Systematics and Ecology of Animals, Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia
- FU-20 Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi** Claudineia A. S. Araújo¹, Clara C. Oliveira¹, Marília A. Rodrigues¹, Breno Pupin¹, Luciana P. Dias¹, John E. Hallsworth², and Drauzio E. N. Rangel¹. ¹Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil. ²School of Biological Sciences, MBC, Queen's University Belfast, UK
- FU-21 Different intensities of visible light during mycelial growth induce differently the conidial tolerance to menadione in *Metarhizium robertsii*** Luciana P. Dias^{1,2}; Drauzio E. N. Rangel¹, ¹Instituto de Pesquisa e Desenvolvimento, Universidade do Vale do Paraíba, São José dos Campos, Brazil.
- FU-22 Effect of *Metarhizium* spp. growth media on the accumulation of destruxins in a 10-L stirred tank reactor** Martin Parth¹, Judith Taibon^{1,2}, Hermann Strasser¹, ¹Institute of Microbiology, Leopold-Franzens University Innsbruck, Austria; ²Institute of Pharmacy / Pharmacognosy, Leopold-Franzens University Innsbruck, Austria
- FU-23 Evaluation of destruxin A production in four strains of *Metarhizium* by capillary electrophoresis** Alex Ríos-Moreno¹, Azahara Carpio¹, Inmaculada Garrido-Jurado¹, Lourdes Arce², Miguel Valcárce², Enrique Quesada-Moraga¹; ¹Department of Agricultural and Forestry Sciences, ETSIAM, University of Cordoba. Campus de Rabanales. Edificio C4 Celestino Mutis. Cordoba, Spain, ²Department of Analytical Chemistry, University of Cordoba, Annex C3 Building, Nanochemistry and Fine Chemistry Research Institute (IUIQFN), Campus of Rabanales, Cordoba, Spain
- FU-24 Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge** Ryan M. Kepler¹, Stephen A. Rehne¹, Richard A. Humber², ¹USDA-ARS Systematic Mycology and Microbiology Laboratory, Beltsville, Maryland, USA; ²USDA-ARS Biological IPM Research, RW Holley Center, Ithaca, New York, USA
- FU-25 Genotyping of Georgian isolates of entomopathogenic fungi *Beauveria* spp. Nana Kunelauri¹, Vladimir Baramidze¹, Medea Burjanadze¹, Ekaterine Shubladze¹, Eka Mikkeladze¹, ¹Agricultural University of Georgia, Tbilisi, Georgia, G. Tevzadze Laboratory of Microbial Genomics, ²Agricultural University of Georgia, Deapartment of Forest Protection, Tbilisi, Georgia**
- FU-26 Genetic characterization, fungicide sensitivity, and aphicidal potential of *Lecanicillium* fungi from Argentina** Romina Manfrino^{1,2}, Christina Schuster³, Julieta Tornesello Galván¹; Katharina Saar³, Juan J. García¹, Claudia C. López Lastra¹; Andreas Leclerque^{3,4}, ¹Centro de Estudios Parasitológicos y de Vectores (CEPAVE), La Plata (BsAs), Argentina; ²Instituto Nacional de Tecnología Agropecuaria (INTA), Rafaela (Santa Fe), Argentina; ³Institute for Biological Control, Julius Kühn Institute (JKI), Darmstadt, Germany; ⁴Institute for Microbiology and Biochemistry, Geisenheim University, Geisenheim, Germany
- FU-27 Species-specific PCR assay to identify and discriminate *M. pingshaense*, *M. anisopliae*, *M. brunneum*, and *M. robertsii*** Johanna Mayerhofer¹, Andy Lutz¹, Franco Widmer¹, Stephen A. Rehner², Ryan M. Kepler³, Adrian Leuchtmann³, Jürg Enkerli¹, ¹Molecular Ecology, Institute for Sustainability Sciences, Agroscope, Reckenholzstrasse Zurich, Switzerland; ²Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville, Maryland, USA; ³Plant Ecological Genetics, Institute of Integrative Biology, ETH Zurich, Switzerland

FU-28 Species identification of entomopathogenic fungi of the genus *Lecanicillium* (=*Verticillium lecanii* s.l.) by mitochondrial gene sequences Galina V. Mitina, Yuri S. Tokarev, Igor A. Kazartsev, All-Russian Institute for Plant Protection, Saint-Petersburg, Russia

FU-29 The genomic basis for evolved resistance to *Beauveria bassiana* in *Drosophila melanogaster* Parvin Shahrestani¹, John Vandenberg², Michael Griggs², Stephen Wright², Yonathan Estrella¹, Susan Rottschaefer¹, Andrew Clark³, Brian Lazzaro¹, ¹Department of Entomology, Cornell University, Ithaca NY, USA; ²USDA Agricultural Research Service, Ithaca NY, USA; ³Department of Molecular Biology and Genetics, Cornell University, Ithaca NY, USA

FU-30-STU Behavioral control of malarial mosquito by entomopathogenic fungi: Death as the vector Minehiro Ishii¹; Masanori Koike²; Daigo Aiuchi², ¹The United Graduate School of Agricultural Sciences, Iwate University, Japan; ² Department of Agro-environmental Science, Obihiro University of Agriculture & Veterinary Medicine, Japan.

FU-31 Effect of *Metarhizium brunneum* strain LRC112 and *M. anisopliae* F52 on non-target Carabid Beetles Alida F. Janmaat¹, Chera Rempel¹, Rita Quik¹, Todd Kabaluk², Manon Peyre², Remi Thomasset², ¹Biology Department, University of the Fraser Valley, Abbotsford, BC, Canada; ² Agriculture and Agri-Food Canada, Agassiz, BC, Canada

FU-32 Effect of a local strain of the fungus against *Corythucha ciliata* (Say) and *Glyphodes pyralalis* (Walker) in Georgia Manana Kereselidze, Mzia Beruashvili, Mzagho Lobzhanidze, Agricultural University of Georgia, Tbilisi, Georgia

FU-33 The effect of pesticides used in strawberry and soybean on the mite pathogenic fungus *Neozygites floridana* Thiago Rodrigues de Castro¹; Samuel Roggia^{1,4}, Vitalis Wafula Wekesa², Ingeborg Klingen³, Italo Delalibera Júnior¹, ¹University of São Paulo (ESALQ), Brazil; ²The Kenya Polytechnic University College, Kenya; ³Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Norway, ⁴The Brazilian Agricultural Research Corporation – Embrapa Soybean, Brazil.

FU-34 Development of a granular formulation of *Metarhizium brunneum* based on mycelial fragments Christopher Seib; Johannes Schäfer; Dietrich Stephan, Julius Kühn Institute, Darmstadt Germany

FU-35 Innovative biological products for soil pest control: Outline of an EU project Stefan Vidal¹; Anant Patel²; Hermann Strasser³; Tariq Butt⁴; Joergen Eilenberg⁵; Juerg Enkerli⁶; Enrique Quesada-Moraga⁷; Justus Wesseler⁸; Francesca Tencalla⁹; Arne Peters¹⁰; Miloslav Nesrsta¹¹; Andrew Shearer¹²; Hermann Limbers¹³; Erik Hansen¹⁴; Athanasios Koukoutsakis¹⁵; ¹Georg-August-Universität Göttingen, Germany; ²University of Applied Sciences, Germany; ³University of Innsbruck, Austria; ⁴Swansea University, United Kingdom; ⁵University of Copenhagen, Denmark; ⁶Agroscope Reckenholz Tänikon, Switzerland; ⁷University of Córdoba, Spain; ⁸Technische Universität München, Germany; ⁹Toximinds, Belgium; ¹⁰e-nema GmbH, Germany; ¹¹Fytovita, Czech Republic; ¹²Neem Biotech Ltd, United Kingdom; ¹³Klasmann-Deilmann GmbH, Germany; ¹⁴EWH BioProduction Aps, Denmark; ¹⁵Torux Software Ltd, UK

FU-36 Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons Carla Huarte-Bonnet, Nicolás Pedrini, Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, Calles 60 y 120, La Plata, Argentina

MICROBIAL CONTROL

MC-1-STU Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables Wakuma Bayissa^{1,2}, Sunday Ekesi¹; Godwin P. Kaaya²; Samira Mohamed¹; John M. Wagacha²; and Nguya K. Maniania¹, ¹International Centre of Insect Physiology and Ecology (icipe), Nairobi, Kenya, ²School of Biological Sciences, University of Nairobi, Nairobi, Kenya

MC-2 Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum* Eduardo Abreo & Nora Altier; Bio-production Lab, INIA Las Brujas, Canelones, Uruguay

MC-3-STU Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency? Thomas Bawin¹, Frank Delvigne², Frédéric Francis¹, ¹Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liege, Belgium, ²Bio-industries, Gembloux Agro-Bio Tech, University of Liege, Belgium

MC-4 The basis for rootstock resilient to *Capnodis* species: screening for genes encoding delta-endotoxins from *Bacillus thuringiensis* Eitan Ben-Dov¹; Galina Gindin², Zvi Mendel², Arieh Zaritsky³; Ariel Kushmaro⁴, ¹Department of Life Sciences, Achva Academic College, Israel; ²Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel; ³Faculty of Natural Sciences, Ben-Gurion University of the Negev, Be'er-Sheva, Israel; ⁴Department of Biotechnology Engineering, Ben-Gurion University of the Negev, Be'er-Sheva, Israel

MC-5 Selection of entomopathogenic fungi for the control of *Aegorhynus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions Ernesto Cisternas¹, Andrés France² and Irina Urtubia², ¹Instituto de Investigaciones Agropecuarias (INIA), La Cruz, Chile. ²INIA Quilamapu, Chillán, Chile

MC-6 Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1 Caroline Placidi De Bortoli¹, Ricardo Antonio Polanczyk¹, Neil Crickmore², Rafael Ferreira dos Santos¹, Alessandra Marieli Vacari¹ and Sergio Antonio De Bortoli¹, ¹Department of Plant Protection, São Paulo State University, Jaboticabal, São Paulo, Brazil, ²Department of Biochemistry, University of Sussex, Brighton, UK

MC-7 Sublethal effects of the Cry1Ac toxin of *Bacillus thuringiensis* Berliner in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations Sergio Antonio De Bortoli¹, Caroline Placidi De Bortoli¹, Ricardo Antonio Polanczyk¹, Neil Crickmore², Rafael Ferreira dos Santos¹ and Alessandra Marieli Vacari¹, ¹Department of Plant Protection, São Paulo State University, Jaboticabal, São Paulo, Brazil, ²Department of Biochemistry, University of Sussex, Brighton, UK

MC-8 Effect of *Bacillus thuringiensis* Berliner on biological characteristics of *Orius insidiosus* Say (Hemiptera: Anthocoridae) fed with eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) Sergio Antonio De Bortoli, Ricardo Antonio Polanczyk, Alessandra Marieli Vacari, Roberto Marchi Goulart and Caroline Placidi De Bortoli, Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil

MC-9-STU Evaluating microbial biocontrol agents: effects of *Metarhizium brunneum* on a non-target arthropod Martina Falagiarda, Chad Alton Keyser, Bernhardt M. Steinwender, Lene Sigsgaard, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

MC-10-STU An experimental autoinoculation device to control an invasive Asiatic pest, *Drosophila suzukii* María Fernández-Bravo, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, ETSIAM, Córdoba, Spain

MC-11 Use of a commercial *Metarhizium anisopliae* s.l. formulation to control *Rhipicephalus microplus* ticks in pen study Mariana G. Camargo¹; Allan F. Marciano¹; Filipe A. Sá¹; Wendell M. S. Perinotto¹; Simone Quinelato¹; Patricia S. Golo¹; Isabele C. Angelo¹; Márcia C. A. Prata²; Vânia R. E. P. Bittencourt¹, ¹Departamento de Parasitologia Animal, Instituto de Veterinária, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, Brazil; ²Embrapa Gado de Leite, Juiz de Fora, MG, Brazil

MC-12 Two Colombian entomopathogenic fungi are highly efficient on *Cerotoma tingomariana* Erika Grijalba¹; Adriana Santos¹; Carlos Espinel, Center of Biotechnology and Bioindustry CBB; Colombian Corporation for Agriculture Research, CORPOICA. Mosquera, Colombia

MC-13-STU Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana* Deborah Kaiser¹, Sven Bacher² and Giseler Grabenweger¹, Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland, ²University of Fribourg, Department of Biology, Unit of Ecology and Evolution, Fribourg, Switzerland

MC-14 Pathogenicity and virulence of *Beauveria* spp. against mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytidae) George Kyei-Poku¹, Shahajahan Johnny¹, William Fick¹, and Katherine Bleiker², ¹Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada, ²Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, British Columbia, Canada

MC-15 The Use of Microbial Plant Protection Agents for Insect Control in Germany Johannes A. Jehle, Annette Herz, Brigitte Keller, Regina G. Kleespies, Eckhard Koch, Andreas Larem, Annetret Schmitt, Dietrich Stephan, Julius Kühn Institute, Darmstadt, Germany

MC-16-STU Synthesis and secretion of volatile organic compounds by *Triatoma infestans* infected with *Beauveria bassiana* Luciana S. Lobo^{1,2}, Sergio J. Mijailosky¹, M. Patricia Juárez¹, Christian Luz², Éverton K. K. Fernandes² and Nicolás Pedrini¹, ¹Instituto de Investigaciones Bioquímicas de La Plata (CCT La Plata CONICET-UNLP), Facultad de Ciencias Médicas, La

Plata, Argentina; ²Instituto de Patología Tropical e Saúde Pública, Universidade Federal de Goiás, Goiânia, Brasil

MC-17 Preliminary studies of entomopathogenic microorganisms present in Latvian population of horse-chestnut leaf miner *Cameraria ohridella* Zane Metla^{1,2}, Rita Seskena¹, Santa Voitkane¹, Monika Maurhofer Bringolf², Liga Jankevica¹, ¹Laboratory of Experimental Entomology, Institute of Biology, University of Latvia, Latvia, ²Plant Pathology, Institute of Integrative Biology (IBZ). Swiss Federal Institute of Technology, Switzerland

MC-18 Toxicity of *Bacillus thuringiensis* BERLINER Cry toxins in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations Ricardo Antonio Polanczyk¹, Caroline Placidi De Bortoli¹, Neil Crickmore², Rafael Ferreira dos Santos¹, Alessandra Marieli Vacari¹ and Sergio Antonio De Bortoli¹, ¹Department of Plant Protection, Sao Paulo State University, Jaboticabal, Sao Paulo, Brazil, ²Department of Biochemistry, University of Sussex, Brighton, UK

MC-19 *Bacillus thuringiensis* isolation from Brazilian soil samples: molecular characterization and biological activity against *Plutella xylostella* (Lepidoptera: Plutellidae) Ricardo Antonio Polanczyk¹, Thiago Trevisoli Agostini¹, Lais Fernanda Moreira¹, Rogério Teixeira Duarte¹, Fernando Hercos Valicente², ¹Microbial Control of Pests Lab, Plant Protection Department, Universidade Estadual Paulista, Jaboticabal, Brazil, ²EMBRAPA Milho e Sorgo, Sete Lagoas, Brazil

MC-20 STU Effect of endophytic *Beauveria bassiana* on herbivore defence in *Arabidopsis thaliana* Maya Raad, Travis Glare, Michael Rostás, Bio-Protection Research Centre, Lincoln University, Lincoln, New Zealand

MC-21-STU Pathogenicity of *Beauveria* and *Metarhizium* to the two stink bug species *Nesara viridula* and *Piezodorus guildinii* (Hemiptera: Pentatomidae) in laboratory and semi-field *Yordanys Ramos González*¹, Ingeborg Klingenberg², Jorge R. Gómez Sousa³, ¹Universidad Central "Marta Abreu de Las Villas" (UCLV), Faculty of Agricultural and Animal Science, Villa Clara, Cuba; ²Norwegian Institute for Agricultural and Environmental Research (Bioforsk), Plant Health and Plant Protection Division, Aas, Norway

MC-22 STU Evidence for synergies between *Heterorhabditis bacteriophora* (Nematoda: Heterorhabditidae) and *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) in western corn rootworm control Hannes Rauch^{1,2}, Hermann Strasser¹, Roland Zelger², ¹Institute of Microbiology, Leopold-Franzens University Innsbruck, Innsbruck, Austria; ²Research Centre for Agriculture and Forestry Laimburg, Laimburg Auer/Ora, Italy

MC-23 Evaluation of the effectiveness of the entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat Gadi V.P. Reddy¹, Khanobporn Tangtrakulwanich¹, Shaohui Wu¹, John H. Miller¹, Victoria L. Ophus¹, Stefan T. Jaronski², ¹Western Triangle Agricultural Research Center, Montana State University, Conrad, USA; ²United States Department of Agriculture, Agricultural Research Service, Northern Plains Agricultural Research Laboratory, Sidney, USA

MC-24 STU Using the combination of entomopathogenic

fungi and extracts improves control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) Gloria Resquín-Romero, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga; University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain

MC-25 STU **Wireworm control with fungus colonized barley kernels in cover-crops** Sina Rogge; Giselher Grabenweger, Agroscope, Institute for Sustainability Sciences, Zurich, Switzerland

MC-26 A resource efficient method to test non target effects of new biocontrol agents in vitro Bernhardt M. Steinwender, Jørgen Eilenberg, Elina Panahi, Kiri M. Fløistrup, Marta M. Cáceres, Gabriela M. Vergara, Lene Sigsgaard; Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg C, Denmark

MC-27 Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) after consumption of prey with the *Bacillus thuringiensis* strain HD1 Alessandra Marieli Vacari, Vanessa Fabiola Pereira de Carvalho, Caroline Placié De Bortoli, Ricardo Antonio Polanczyk and Sergio Antonio De Bortoli, Department of Plant Protection, São Paulo State University, Jaboticabal, São Paulo, Brazil

MC-28 Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae* Inajá M. Wenzel^{1,2}, Antonio Batista Filho²; Moacir R. Forim¹; Isabella B. Giordano¹; Bárbara E. Denadai¹; ¹Federal University of São Carlos/Chemistry Departament/ Natural Products Laboratory/São Carlos city, São Paulo state, Brazil, ²Biological Institute/Biological Control Laboratory/ Campinas city, São Paulo state, Brazil

MC-29-STU Identification and functional analysis of two ABCC family genes in *Helicoverpa armigera* Yutao Xiao, Kongming Wu, The State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

MICROSPORIDIA

MI-1 Decline of native bumblebees (*Bombus*) and *Nosema* (Microsporidia: Nosematidae) infections associated with introduction of the European bumblebee in Northern Japan Maki N. Inoue, Takahiro Yanagisawa, Madoka Nakai, Yasuhisa Kunimi, Institute of Agriculture, Tokyo University of Agriculture and Technology, Japan

MI-2 Development and application of a loop-mediated isothermal amplification method for rapid detection of *Nosema ceranae* George Kyei-Poku, Debbie Gauthier, Shahzahan Johnny, Great Lakes Forestry Centre, Canadian Forestry Service, Natural Resources Canada, Sault Ste. Marie, Ontario, Canada

MI-3 Permanent level of pathogens within ten bark beetles generations Karolina Lukášová, Jaroslav Holuša; Jiří Trombík, Department of Forest Protection and Entomology, Faculty of Forestry and Wood Science, Czech University of Life Sciences, Prague, Czech Republic

MI-4 Microsporidia in beet webworm *Loxostege sticticalis*

(Pyraloidea: Crambidae): a survey of 2013 Julia Malysh, Yuri Tokarev, Andrei Frolov, Anastasia Ignatieva, Irma Issi, All-Russian Institute of Plant Protection, St. Petersburg, Russia

MI-5 Microsporidia from larvae of different lepidopteran species in Bulgaria Daniela Pilarska¹, Danail Takov¹, Miroslav Hylis², Renate Radek³, Leellen Solter⁴, Andreas Linde⁵, ¹Institute of Biodiversity and Ecosystem Research, Sofia, Bulgaria; ²Faculty of Science, Charles University, Prague, Czech Republic, ³Free University of Berlin, Berlin, Germany, ⁴Illinois Natural History Survey, University of Illinois, USA; ⁵University of Applied Sciences, Eberswalde, Germany

MI-6 Ultrastructural characterization of a new microsporidium (Opisthokonta: Chytridiopsida) from the pigeon feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea) Renate Radek¹, Madlen Kariton¹, Jacek Dabert², Gerd Alberti³, ¹Free University of Berlin, Berlin, Germany, ²Adam Mickiewicz University, Poznań, Poland; ³Ernst-Moritz-Arndt-Universität Greifswald, Greifswald, Germany

MI-7 Infectivity of a *Thelohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm, *Bombyx mori* Liangen Shi, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang Province, China

NEMATODES

NE-1 First release of the mermithid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina Evangelina Muttis¹, María F. Achinelly²; María V. Miceli³; ¹Fellowship CONICET Centro de Estudios Parasitológicos y de Vectores, CEPAVE, La Plata, Argentina; ^{2,3}Researcher CONICET, Centro de Estudios Parasitológicos y de Vectores, CEPAVE, La Plata, Argentina

NE-2 Increased infectivity in *Steinerinema websteri* IJ after development in desiccation-stressed hosts Andrea Binnebose and Susan M. Bornstein-Forst; Marian University, Fond du Lac, WI 54935 USA

NE-4-STU Characterization of symbiotic bacteria *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis bacteriophora* isolated from Turkey Harun Cimen; Selçuk Hazır, Adnan Menderes University Faculty of Arts and Science Department of Biology, Turkey

NE-5 Pathogenicity of nematobacterial complexes and its development Pavel Dobes; Jakub Berka; Jana Hurychová; Libor Vojtek; Pavel Hyrszl, Department of Animal Physiology and Immunology, Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

NE-6 Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards Andrés France¹, Ernesto Cisternas², Irina Urtubia¹, ¹Instituto de Investigaciones Agropecuarias (INIA), Quilamapu, Chillán, Chile, ²INIA La Cruz, La Cruz, Chile

NE-7 Nematodes of large larch bark beetle *Ips cembrae*

(Coleoptera: Scolytinae) Sarka Grucmanová¹; Václav Čermák²; Jaroslav Holuša¹, ¹Czech University of Life Sciences Prague; Czech Republic; ²Central Institute for Supervising and Testing in Agriculture, Olomouc, Czech Republic

NE-8 Natural Occurrence of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in the Aydin district of Turkey
Barış Gulcu¹, Canan Hazır², Mehmet Karagoz³, M. Alper Kesici³, Düzce University, Faculty of Arts and Science, Department of Biology, Düzce, Turkey; ²Aydın Vocational School of Health Services, Adnan Menderes University, Aydin, Turkey; ³Adnan Menderes University, Faculty of Agriculture, Department of Plant Protection, Aydin, Turkey

NE-9 Detection of dsRNA virus-like molecules in entomopathogenic nematodes Noemí Herrero; Jiří Nermut¹; Vladimír Půža; Zdeněk Mráček, Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i. Institute of Entomology, České Budějovice, Czech Republic

NE-10 Cellular and humoral interactions between the white grub, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes
Jamilah Avandi¹, Javad Karimi¹, Mohammad Ghadamyan² & Ahmad Asoode³, ¹Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; ²Department of Plant Protection, College of Agriculture Science, University of Guilan, Rasht, Iran, ³Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Iran

NE-11 *Oscheius rugaoensis*, new genus and species of insect parasitic nematodes from Iran
Reyhaneh Darsouei & Javad Karimi, Biocontrol and Insect Pathology Laboratory, Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

NE-12 Reproduction status of *Tribolium castaneum* affects its response to infection by *Steinerinema feltiae* Dariusz Małek¹, Joanna Homa², Maria Gaweł¹, Paulina Kramarz¹, ¹Institute of Environmental Sciences, Jagiellonian University, 30-387 Krakow, Poland, ²Institute of Zoology, Jagiellonian University, 30-387 Krakow, Poland

NE-13 Effect of culture type, container type, and temperature on a Korean strain of the entomopathogenic nematode, *Steinerinema carpocapsae* DongWoon Lee¹; Ho Yul Choo², ¹Major of Applied Biology, School of Ecological Environment and Tourism, Kyungpook National University, Sangju, Republic of Korea; ²Department of Applied Biology, College & Institute of Agriculture & Life Sciences, Gyeongsang national University, Jinju, Republic of Korea

NE-14 *Steinerinema feltiae* (Nematoda: Steinernematidae) to control fungus gnat, *Bradysia mabiusi* (Diptera: Sciaridae): effect of dosage and application time*
Patricia Ballone¹; Luis G. Leite¹; Fabio S. Schmidt²; Victória R. Campos¹; Roselaine N. S. Bueno¹; ¹Instituto Biológico, CEIB, CP70, Campinas, Brazil, ²Bio Controle, Indaiatuba, SP 13347-630, Brazil

(Tylenchida: Neotylenchidae) and its development on different strains of *Amylostereum* (Basidiomycota: Russulales) Isis A. L. Caetano, Ann E. Hajek, Department of Entomology, Cornell University, Ithaca, New York, USA

NE-16 Use of entomopathogenic nematodes in the biological control of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae) Manana Lortkipanidze, Oleg Gorgadze, Madona Kuchava, Nana Gratiashvili, Mzia Kokhia, Nino Gabroshvili, Institute of Zoology, Ilia State University, Tbilisi, Georgia

NE-17 The susceptibility of Colorado potato beetle *Leptinotarsa decemlineata*, and mulberry moth *Glyphodes pyloalis* to entomopathogenic nematodes, *Steinerinema carpocapsae* and *Steinerinema feltiae* in Georgia Nona Mikhaia, Sokhumi State University, Tbilisi, Georgia

NE-18 Co-infection interactions between entomopathogenic fungi and *Steinerinema feltiae* using *Tenebrio molitor* as a model system
E. Erin Morris, Annette B. Jensen, Anja A. Wynns, Jørgen Eilenberg, Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

NE-19 Some observation on morphology and ecology of mollusc-parasitic nematode *Alloionema appendiculatum* Jiří Nermut¹, Vladimír Půža, Zdeněk Mráček; Biology Centre ASCR v.v.i., Institute of Entomology, Branišovská 1160/31, 370 05 České Budějovice, Czech Republic

NE-20 Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments Jaime Ruiz-Vega¹, Teodulfo Aquino-Bolaños¹, Juan R. Delgado-Gamboa² and Carlos I. Cortés-Martínez², Becarios ¹COFAA-IPN y ²PIFI-IPN, Laboratory of Biological Control, CIIIDIR U. OAXACA, IPN, Santa Cruz Xoxocotlan, Oax., México

NE-21 Assessing entomopathogenic nematode population genetics: a research and teaching approach Abigail Lewis, Logan Jefferson, Glen Stevens, Michaela Gazdik, School of Natural Sciences and Mathematics, Ferrum College, Ferrum, VA, USA

NE-15 The non-sterilizing strain of *Deladenus siricidicola*

VIRUSES

- VI-1 High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System** Sung Min Bae¹; Tae Young Shin¹; Jae Bang Choi¹; Yeon Ho Je²; Byung Rae Jin³; Soo Dong Woo¹, ¹Department of Agricultural Biology, Chungbuk National University, Chungju, Korea; ²Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul, Korea; ³College of Natural Resources and Life Science, Dong-A University, Busan, Korea
- VI-2 A natural recombinant between *S. frugiperda* MNPV and *S. litura* NPV** Gloria Barrera¹, Laura Villamizar¹; Manuel Alfonso Patarroyo², Oihane Simón³, Primitivo Caballero³, Mariano Belaich⁴, Daniel Ghiringhelli⁴; ¹Centro de Biotecnología y Bioindustria (CBB), Corpica, Bogotá, Colombia, ²Fundación Instituto de Inmunología de Colombia (FIDIC), Bogotá, Colombia, ³Instituto de Agrobiotecnología, CSIC-Gobierno de Navarra, Navarra, España, ⁴Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Área Virosis de Insectos, Universidad Nacional de Quilmes, Argentina
- VI-3 Host specificity and PIFs based phylogeny of Betabaculovirus isolates from Gelechiidae family** Juliana Gómez¹, Laura Villamizar¹; Gloria Barrera¹; Cecilia Turco², Mariano Belaich², Daniel Ghiringhelli², ¹Centro de Biotecnología y Bioindustria (CBB), Corpica, Bogotá, Colombia ²Laboratorio de Ingeniería Genética y Biología Celular y Molecular – Área Virosis de Insectos, Universidad Nacional de Quilmes, Argentina
- VI-4 Diagnosing the unknown – advancing the taxonomy of aquatic invertebrate viruses** Kelly S. Bateman¹, Grant D. Stentiford¹ and Monique M. van Oers², ¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Dorset, UK, ²Laboratory of Virology, Wageningen UR, Wageningen, Netherlands
- VI-5 Proteomic analysis of the occluded *Tipula oleracea* nudivirus (ToNV)** Annie Bézier¹, Grégoire Harichaux², Julien Gaillard³, Karine Musset¹, Valérie Labas², Elisabeth A. Herniou¹, ¹Institut de Recherche sur la Biologie de l'Insecte, CNRS UMR 7261, Université François Rabelais, France; ²Laboratoire de Spectrométrie de masse, Plateforme d'Analyse Intégrative des Biomolécules et des Phénomènes des Animaux d'Intérêt Bio-agronomique. UMR INRA 0085-CNRS 7247-UFR-IFCE, Nouzilly, France; ³Laboratoire de Biologie Cellulaire, Microscopie Electronique, Faculté de Médecine, Université François Rabelais, Tours, France
- VI-6 Nucleopolyhedrovirus and Microsporidia in Winter Moth (*Operophtera brumata*, L.) and Bruce Spanworm (*O. bruceata*, Hurst) populations in the Northeast US** Hannah J. Broadley^{1,2}, Joseph S. Elkinton^{1,2}, John P. Burand³, Lina Tian³, Leellen F. Solter⁴; ¹Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts Amherst, USA; ²Department of Environmental Conservation, University of Massachusetts Amherst, USA; ³Department of Microbiology, University of Massachusetts Amherst, USA; ⁴ Department of Entomology, University of Illinois, USA

- VI-7 Regulation and activation of two effector caspases that affect Sindbis virus replication in *Aedes aegypti* mosquitoes** Ning Huang, A. Lorena Passarelli, and Rollie J. Clem, Division of Biology, Kansas State University, Manhattan, KS
- VI-8 Proteomic analysis and *in vivo* differential gene expression of *Trichoplusia ni* granulovirus (TnGV)** Angeles Bivián Hernández; Ingrid Zanella-Sainz; Paloma Dávila-Alvarez, J. Eleazar Barboza-Corona; Fabiola León-Galván; M. Cristina Del Rincón-Castro, Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México
- VI-9 Recombinant Iridovirus IIv-6 expresing the Cn-10 neurotoxin from *Centruroides noxius* scorpion** Flor C. Arellano-Villagómez¹; Jorge E. Ibarra²; M. Cristina Del Rincón-Castro¹, ¹Food Department, Division of Life Sciences, University of Guanajuato, Irapuato, Gto. México, ²CINVESTAV-IPN Unidad Irapuato, Irapuato, Gto. México
- VI-10 Genomic sequencing and analysis of *Sucra jujuba* nucleopolyhedrovirus** Xiaoping Liu, Feifei Yin, Zheng Zhu, Dianhai Hou, Jun Wang, Lei Zhang, Hualin Wang, Zhihong Hu, Fei Deng, State Key Laboratory of Virology, Virus Resource and Bioinformatics Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, P.R. China
- VI-11 Functional analysis of exonuclease gene (012L) of *Chilo* iridescent virus** Yesim Aktürk Dizman^{1,2}, Cemal Sandallı², Zihni Demirbağ¹ and Remziye Nalçacıoğlu¹, ¹Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey, ²Recep Tayyip Erdoğan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey
- VI-12 Identification of a new multiple nucleopolyhedrovirus isolated from the Jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt** Regina G. Kleespies¹, Yongjie Wang², Said El Salamouny³, Mona Awad³, Essam Agamy³, Ramadan Salama³ and Johannes A. Jehle^{1,2}; ¹Institute for Biological Control, Julius Kühn Institute, Darmstadt, Germany; ²Agricultural Service Station Palatinate, Neustadt/Weinstr., Germany; ³Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, Egypt.
- VI-13 A single baculovirus for the production of recombinant Adeno-Associated Virus 8 vectors** Lionel Galibert; Aurélien Jacob; Bérangère Bertin; Marjorie Boutin Fontaine; Delphine Bonnin; Christophe Lecomte; Christel Rivière; Otto-Wilhelm Merten Genethon, 1bis, rue de l'Internationale, Evry, France
- VI-14 Determining the role of P10 during baculovirus infection through the development of novel mutants in *Autographa californica* multicapsid Nucleopolyhedrovirus** Leo Graves¹, Farheen Raza¹, Sarah L. Irons¹; Robert D Possee^{1,2} & Linda A King¹, ¹Department of Biological and Medical Sciences, Oxford Brookes University, Oxford UK, ²Oxford Expression Technologies Ltd, Oxford, UK
- VI-15 Evaluation of the transcriptional transactivation of betabaculovirus regulatory elements in transformed cell lines by alphabaculovirus transcription factors** Santiago Haase¹, M. Leticia Ferrelli¹; Matías L. Pidre¹, Alicia Sciocco-Cap², Víctor Romanowski¹, ¹IBBM-UNLP-CONICET, La Plata, AR; ²IMYZA-INTA, Castelar, AR

- VI-16 Enhancin Genes of *Lymantria dispar* NPV Do Not Increase Potency Via Metalloprotease Activity** Kelli Hoover¹, James Slavicek², Algimantas P. Valaitis^{2,3}, Nancy Hayes-Plazolles², and Elizabeth McCarthy¹.
¹Department of Entomology, Penn State University, University Park, PA USA; ²USDA Forest Service, Delaware, OH USA; ³Retired
- VI-17 A Cypovirus VP5 Displays the RNA Chaperone-like Activity that Destabilizes RNA Helices and Accelerates Strand Annealing** Jie Yang, Jiamin Zhang, Yuehua Kuang and Yuanyang Hu, State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China
- VI-18 A recombinant *Autographa californica* nucleopolyhedrosis virus expressing a Cyt1A/GFP chimera in *Trichoplusia ni* larvae** Miguel A. Salas-Marina¹, Cristina Del Rincón-Castro² and Jorge E. Ibarra¹; ¹CINVESTAV-Irapuato, Irapuato, GTO, Mexico; ²División de Ciencias de la Vida, Universidad de Guanajuato, Irapuato, GTO, Mexico
- VI-19 iLOV baculovirus: Using a novel small fluorescent protein for imaging virus proteins during infection** Farheen Raza¹, Sarah Irons¹, Leo Graves¹, Stan Botchway², Robert Possee^{1,3}, Linda King¹; ¹Department of Biological and Medical Sciences, Oxford Brookes University, Oxford, UK; ²Central Laser Facility, STFC, Harwell, UK; ³Oxford Expression Technologies, Oxford, UK
- VI-20 Expression analysis of the nsd-2 gene encoding the putative densovirus receptor in the midgut** Katsuhiko Ito¹, Hiroko Tabunoki¹, Takeshi Yokoyama¹, Keiko Kadono-Okuda², ¹Tokyo University of Agriculture and Technology, Tokyo, Japan; ²National Institute of Agrobiological Sciences, Ibaraki, Japan
- VI-21 Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*** Agata K. Jakubowska¹; Melania D'Angiolo¹; Rosa M. González Martínez¹; Anabel Millán Leiva¹; Arkaitz Carballo²; Rosa Murillo²; Primitivo Caballero²; Salvador Herrero¹; ¹Department of Genetics, Universitat de València, Dr Moliner 50, 46100 Burjassot, Spain; ²Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, CSIC-UPNA, Gobierno de Navarra, 31192 Mutilva Baja, Navarra, Spain
- VI-22-STU A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System** María Martínez-Solis¹; Silvia Gomez-Sebastian²; Jose M Escribano³; Agata K. Jakubowska¹; Salvador Herrero¹; ¹Department of Genetics, Universitat de Valencia, Burjassot, Spain; ²Alternative Gene Expression S.L. (ALGENEX), Centro Empresarial, Parque Científico y Tecnológico de la Universidad Politécnica de Madrid, Campus de Montegancedo, Madrid, Spain; ³Departamento de Biotecnología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain
- VI-23 Construction and Characterization of a Recombinant Invertebrate Iridovirus** Arzu Ozgen¹, Hacer Muratoglu², Zihni Demirbag¹, Just M. Vlak³, Monique M. van Oers³, Remziye Nalcacioglu¹, Karadeniz Technical University; Faculty of Science, Department of Biology, Trabzon, Turkey; ²Karadeniz Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Trabzon, Turkey; ³Laboratory of Virology, Wageningen University, Wageningen, The Netherlands

- VI-24 RNA interference and insect-virus interactions** David Neunemann, David G. Heckel, Heiko Vogel; Max Planck Institute for chemical ecology, Jena ,Germany
- VI-25 Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa** John K. Opoku-Debrah^{1,4}; Martin Hill¹; Sean Moore^{1,2}; Caroline Knox³; ¹Department of Zoology and Entomology, Rhodes University, Grahamstown, South Africa; ²Citrus Research International, Humewood, Port Elizabeth, South Africa.; ³Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown, South Africa; ⁴River Bioscience (Pty) Ltd, Humewood, Port Elizabeth, South Africa
- VI-26 Effects of the baculovirus fibroblast growth factor on Sindbis virus replication** Wenbi Wu, Rollie J. Clem, and A. Lorena Passarelli, Division of Biology, Kansas State University, Manhattan, USA
- VI-27 Sensitivity and vertical transmission of nucleopolyhedrovirus in various populations of gypsy moth *Lymantria dispar*** Olga Polenogova¹, Alexander Ilyinsky¹, Dmitry Kurenschikov², Philipp Ilyinsky³, Elena Imranova², Alexandr Baburin²; ¹Institute of Systematics and Ecology of Animals Siberian Branch of Russian Academy of Sciences, Novosibirsk, RUSSIA; ²Institute of Water and Ecological Problems Far Eastern Branch of Russian Academy of Sciences Kim-Yu-Chena, Khabarovsk, RUSSIA; ³State Research Center of Virology and Biotechnology "Vector", Novosibirsk, RUSSIA
- VI-28 Establishment of SeMNPV Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus** Weng Qingbei¹, Li Min¹, Yang Kai², Pang Yi²; ¹School of Life Sciences, Guizhou Normal University, Guiyang, China; ²State Key Laboratory of Biocontrol and Institute of Entomology, Sun Yat Sen University, Guangzhou, China
- VI-29-STU Larvical activity of an ascovirus from *Spodoptera litura* against parasitoid wasps** Shiori Sagawa, Eiko Arai, Maki Inoue, Yasuhisa Kunimi, Madoka Nakai; Graduate School of Agriculture, Tokyo University of Agriculture and Technology
- VI-30 "11K" genes family sf68, sf95 and sf138 modulate transmissibility and insecticidal properties of *Spodoptera frugiperda* multiple nucleopolyhedrovirus** Inés Beperet¹; Oihane Simón¹; Trevor Williams²; Miguel López-Ferber³; Primitivo Caballero^{1,4}; ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; ²Instituto de Ecología AC, Xalapa, Mexico; ³LGEI, Ecole de Mines d' Alès, Alès, France; ⁴Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain
- VI-31 Characterization of two ORFs undergoing positive selection in a genotype of *Chrysodeixis chalcites* single nucleopolyhedrovirus from the Canary Islands** Oihane Simón¹; Leopoldo Palma¹; Alexandra Bernal¹; Delia Muñoz²; Trevor Williams³; Primitivo Caballero^{1,2}; ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; ²Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain; ³Instituto de Ecología AC, Xalapa, Mexico

VI-32 Genome sequence and organization of a *Betabaculovirus* pathogenicto cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)
Daniel M. P. Ardisson-Araújo¹; Fernando Lucas Melo¹; Miguel S. Andrade¹; William Sihler²; Sonia N. Bão¹; Bergmann M. Ribeiro¹; Marlinda L.Souza²; ¹Laboratory of Baculovirus, Cell Biology Department, University of Brasília, 70910-900, Brasília, DF;Brazil. ²Embrapa Genetic Resources and Biotechnology, Biological Station Park, 70770-917, Brasília, DF, Brazil.

VI-33-STU Analysis of genetic interactions among four non-essential genes of BmNPV Hitomi Taka¹, Chikako Ono², Masanao Sato³, Shin-ichiro Asano¹, Hisanori Bando¹; ¹Graduate School of Agriculture, Hokkaido University, Sapporo, Japan; ²Research Institute for Microbial Diseases, Osaka University, Suita, Japan; ³Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, Okazaki, Japan

VI-34-STU Comparative fitness of a granulovirus mutant possessing larger occlusion bodies than wild type *Adoxophyes orana* granulovirus Haruaki Uchida, Yasuhisa Kunimi, Maki Inoue, Madoka Nakai; Graduate School of Agriculture, Tokyo University of Agriculture and Technology

VI-35 Granulovirus detection in larvae of sugarcane borers *Diatraea* spp. (Lepidoptera: Pyralidae) in Colombia Cristian Guzmán, Diana Pinzón, Carolina Ruiz, Juliana Gómez, Carlos Espinel, Gloria Barrera, Laura Villamizar; Centro de Biotecnología y Bioindustria (CBB), Corpoica, Bogotá, Colombia

VI-36 Earthworm-mediated dispersal of baculovirus occlusion bodies in soil: a laboratory study Dennis A. Infante-Rodríguez¹; Delia Muñoz²; Jorge Valenzuela¹; Trevor Williams¹; ¹Instituto de Ecología AC, Xalapa, Mexico; ²Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain

VI-37-STU Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) to *A. honmai* nucleopolyhedrovirus Takeshi Yamaga, Madoka Nakai, Maki Inoue, Yasuhisa Kunimi, Laboratory of biological control, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu city, Tokyo, Japan

VI-38 Characterization of Nodaviral Protein A Revealed RNA Synthesis and Terminal Nucleotidyl Transferase Activity Zhaowei Wang, Xi Zhou, Dong Li and Congyi Zheng; State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China

SIP Division Business Meeting: Wednesday evening
BACTERIA + Workshop 20:00-21:30. **P5**
Non-Target Effects on Biological Pesticides Transgenic Crops
Moderator: Ken Narva

199 The impact of herbicide tolerant crops on non-target organisms Ramon Albajes; Marina S. Lee; and Agnès Ardanuy, Universitat de Lleida, Agrotecnio Center, Lleida, Catalonia, Spain

200 Your Right to Know What You Eat: On the Occurrence of Viable *Bacillus thuringiensis* in Commercial Food Products Brian Federici, Department of Entomology and Interdepartmental Graduate Programs in Microbiology & Cell, Molecular and Developmental Biology, University of California, Riverside, Riverside, California USA

201 Environmental risk assessment of genetically engineered crops for spiders Michael Meissle, Jörg Romeis, Agroscope, Institute for Sustainability Sciences, Zürich, Switzerland

202 Conclusions from 10 years of accumulated evidence from publicly funded field trials research with Bt-maize in Germany Stefan Rauschen, Forschungszentrum Jülich GmbH, Projektträger Jülich, Jülich, Germany

SIP Division Business Meeting: Wednesday evening
MICROSPORIDIA + Workshop 20:00-21:30. **P4**

SIP Division Business Meeting: Wednesday evening
FUNGI 20:00-21:30. **P2**

SIP Division Business Meeting: Wednesday evening
VIRUSES 20:00-21:30. **P3**

THURSDAY - 7 August

7:30-16:30 REGISTRATION

P1

Symposium 7 (Dis. of Ben. Invertebr.) Thursday, 8:00 -10:00. P2
Emerging Tools for Aquatic Pathogen Discovery and Description

Organizers/Moderators: Spencer Greenwood and Grant Stentiford

- 8:00 **203 Early mortality syndrome is an infectious disease with a bacterial etiology** Loc Tran^{1,2,3}, Kevin Fitzsimmons² and Donald V. Lightner¹, ¹Aquaculture Pathology Laboratory, School of Animal and Comparative Biomedical Sciences, University of Arizona, Tucson, AZ 85721, USA, ²Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721, USA, ³Department of Aquaculture Pathology, Nong Lam University at Ho Chi Minh, Vietnam
- 8:30 **204 Policy, phylogeny, and the parasite**
Grant D. Stentiford^{1,2}, Stephen W. Feist², David M. Stone², Edmund J. Peeler² and David Bass³, ¹European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, UK, ²Aquatic Pests and Pathogens Group, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK, ³Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, Cromwell Road, London, UK
- 9:00 **205 The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics**
K. Fraser Clark^{1,2,3}, Spencer J. Greenwood^{1,2,4}, ¹Atlantic Veterinary College Lobster Science Centre, ²Department of Biomedical Sciences, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada; ³Department of Plant and Animal Sciences, Dalhousie University, Truro, Nova Scotia, Canada
- 9:30 **206 Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns** Hanna Hartikainen^{1,5}, Grant D. Stentiford^{2,3}, Kelly Bateman^{2,3}, Stephen W. Feist^{1,3}, David M. Stone³, Matt Longshaw^{3,4}, Georgia Ward¹, Charlotte Wood¹, Beth Okamura¹ and David Bass¹, ¹Department of Life Sciences, The Natural History Museum, London, UK; ²European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; ³Aquatic Pests and Pathogens Group, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, Dorset, UK; ⁴Fish Vet Group, Inverness, ⁵ETH Zürich and Eawag, Duebendorf, Switzerland

Contributed Papers

Thursday, 8:00-10:00.

P4

Nematodes 3

Organizer/Moderator: Luis Leite and Glen Stevens

- 8:00 **207 The Role of biocontrol agents within IPM of *Tuta absoluta* on tomato in Egypt** Mahfouz Abd-Elgawad, Phytopathology Department, National Research Center, Giza, Egypt.
- 8:15 **208 Insecticidal activity of *Heterorhabditis bacteriophora* Shandong toward *Brontispa longissima* and *Cryptothelae variegata*** Cheng Bai*, Liping Liu, Haibo Long, Qian Jin and Zhengjiang Peng; Key Laboratory of Pests Comprehensive Governance for Tropical crops, Ministry of Agriculture, Hainan Key Laboratory for Monitoring and Control of Tropical Agricultural Pests, Hainan Engineering Research Center for Biological Control of Tropical Crops Diseases and Insect Pests, Environment and Plant Protection Institute, Chinese Academy of Tropical Agricultural Sciences, Haikou, Hainan China.
- 8:30 **209 Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, *Planococcus ficus*, in South African Vineyards**
Patrique D. Le Vieux, Antoinette P. Malan; Department of Conservation Ecology and Entomology, Stellenbosch University, Stellenbosch, Matieland, South Africa.
- 8:45 **210 New data on *Steinernema ichnusae* distribution in the Mediterranean Area** E.Tarasco¹, M. Clausi², G. Rappazzo², M. Oreste¹, L. Rubino², D. Leone², M. T.Vinciguerra², ¹Departement of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "Aldo Moro", Bari (Italy), ²Department of Biological, Geological and Environmental Sciences, Section of Animal Biology "M. La Greca", University of Catania, Italy
- 9:00 **211-STU Evaluation of entomopathogenic nematodes for control of the diapausing overwintering codling moth population**
Odendaal Deidré, Addison F. Matthew, Malan P. Antoinette; Department of Conservation Ecology and Entomology, Faculty of AgriSciences, University of Stellenbosch, South Africa
- 9:15 **212-STU A new entomopathogenic *Oscheius* (Nematoda: Rhabditidae) from Italian cave** Giulia Torrini¹, Beatrice Carletti¹, Giuseppe Mazza¹, Pio Federico Roversi¹, Elena Fanelli², Francesca De Luca², Alberto Trocchi², Eustachio Tarasco³, Agricultural Research Council - Agrobiology and Pedology Research Centre (CRA-ABP), Firenze (Italy); ²Istitute of Plant Protection (IPP)-CNR, Bari (Italy); ³Department of Soil, Plant and Food Sciences, Section of Entomology and Zoology, University of Bari "A.Moro", Bari, Italy
- 9:30 **213 Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora*** Ralf-Udo Ehlers, e~nema, GmbH, Schwentinental, Germany
- 9:45 **214-STU Perspectives of new nematode formulation technology for biological control to pest insects in Georgia** Mariam Chubinashvili, Tsisia Chkhubianishvili, Manana Kakhadze, Iatamze Malania, Kanchaveli Institute of Plant Protection, Agricultural University of Georgia, Tbilisi, Georgia

Contributed PapersThursday, 8:00-10:00. **P1****Viruses 6**

Moderator: Adly Abd-Alla and Madoka Nakai

- 8:00 **215** **Interactions between salivary gland hypertrophy virus and tsetse microbiota** Güler Demirbaş Uzel¹, Vangelis Doudoumis², Antonios Augustinos¹, Gisele Ouedroogo¹, Andrew Parker¹, Dion Boucias³, Kostas Bourtzis¹, Adly Abd-Alla¹, ¹Insect Pest Control Laboratory, Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, Vienna, Austria; ²Department of Environmental and Natural Resources Management, University of Patras, Agrinio, Greece; ³Entomology and Nematology Department, University of Florida, Gainesville, Florida, USA
- 8:15 **216 STU** **Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV** Yue Han, Stineke van Houte, Vera I.D. Ros, Just M. Vlak and Monique M. van Oers, ¹ Laboratory of Virology, Wageningen University, Netherlands
- 8:30 **217** **Temporal proteomics to study virus infection and function in the host cell** Ikbal Agha Ince¹, Sjef Boeren², Just Vlak³, Monique van Oers³; ¹Department of Medical Microbiology, Acibadem University, School of Medicine, Istanbul, Turkey; ²Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands; ³Laboratory of Virology, Wageningen University, Wageningen, The Netherlands
- 8:45 **218** **Characterization of an atypical fast-killing ascovirus: *Spodoptera frigiperda* ascovirus 1d (SfAV-1d)** Eiko Arai¹, Shiori Sagawa¹, Yasumasa Saito¹, Xiao-Wen Cheng², Dennis Bideshi^{3,4}, Maki Inoue¹, Yasuhisa Kunimi¹, Brian Federici³, Madoka Nakai¹, ¹Institute of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; ²Department of Microbiology, Miami University, Oxford, Ohio, USA; ³Department of Entomology, University of California, Riverside, USA; ⁴California Baptist University, Riverside California, USA
- 9:00 **219-STU** **Two nucleopolyhedroviruses isolated from the genus *Adoxophyes* inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity** Yasumasa Saito^{1,2}, Shizuo G. Kamita², Bruce D. Hammock², Yasuhisa Kunimi¹, Maki N. Inoue¹, Madoka Nakai¹, ¹Laboratory of Biological Control, United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, Fuchu, Tokyo, Japan; ²Laboratory of Pesticide Biotechnology, Department of Entomology and Nematology, University of California, Davis, USA
- 9:15 **220** **Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase** Stineke van Houte, Carmen Embregts, Esther van Andel, Vera I.D. Ros, Just M. Vlak and Monique M. van Oers. Laboratory of Virology, Wageningen University, Wageningen, Netherlands
- 9:30 **221-STU** **The genome of a baculovirus isolated from *Lonomia obliqua* (Lepidoptera: Saturniidae) reveals a new transcription terminator factor possible acquired from the host** Clara Wandenkolk Silva Araújo¹, Bergmann Moraes Ribeiro¹, Fernando Lucas Melo¹, ¹University of Brasília- UnB- Brazil

- 9:45 **222** **The essential baculovirus protein VP1054 is a hijacked cellular PURα, a nucleic-acid-binding protein specific for GGN repeats** Martin Marek¹, Christophe Romier¹, Lionel Galibert², Otto-Wilhelm Merten² and Monique M. van Oers³, ^{1,2,3}Biologie Structurale Intégrative, Institut de Génétique et Biologie Moléculaire et Cellulaire (IGBMC), UDS, CNRS, INSERM, Illkirch, France; ²Laboratory of Applied Vectoriology, Généthon, Évry, France; ³Laboratory of Virology, Wageningen University, Netherlands

Symposium (Special)

Thursday, 8:00-10:00. **P5****DFG Priority Program
Host Parasite Coevolution**

Organizer/Moderator: Joachim Kurtz

- 8:00 **223** **Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*** Christoph Kurze¹, Oleg Lewkowski¹, Yves Le Conte², Claudia Dussaubat², Thomas Müller³, Silvio Erler¹, Per Kryger⁴, and Robin F.A. Moritz¹; ¹Institute of Biology, MLU Halle-Wittenberg, Germany; ²Abeilles et Environnement, INRA Avignon, France; ³Department of Internal Medicine IV, MLU Halle-Wittenberg, Germany; ⁴Department of Agroecology, Aarhus University, Denmark.
- 8:15 **224** **Overcoming external immunity: An increase in virulence as a result of host-parasite coevolution in *Beauveria bassiana*** Charlotte Rafaluk¹, Wentao Yang¹, Philip Rosenstiel², Hinrich Schulenburg¹ and Gerrit Joop^{1,3}; ¹Evolutionary Ecology Genetics, Zoological Institute, Christian-Albrechts-Universität zu Kiel, Germany, ²Institut für Klinische Molekularbiologie, Christian-Albrechts-Universität zu Kiel, Universitätsklinikum Schleswig-Holstein, Campus Kiel, Germany, ³Institute for Phytopathology and Applied Zoology, University of Giessen, Gießen, Germany
- 8:30 **225** **Rapid adaptation of *Bacillus thuringiensis* to its nematode host *Caneorhabditis elegans*** Leila Masri^{1,2}, Antoine Branca³, Anna Sheppard^{1,4}, Hinrich Schulenburg¹, ¹Dept. Evolutionary Ecology and Genetics, University of Kiel, Germany; ²Present address: IST Austria, Austria; ³CNRS-Université Paris-Sud, Orsay, France; ⁴Present address: Nuffield Department of Medicine, University of Oxford, Oxford, UK
- 8:45 **226** **Intra-host parasite interactions between co-infecting *Bacillus thuringiensis* strains** Michaela H. Klössener, Joy Bose, Rebecca D. Schulte, Department of Behavioural Biology, University of Osnabrück, Germany
- 9:00 **227** **Experimental evolution *in silico*: host-parasite coevolution versus parasite adaptation** Jakob Strauß¹, Philip Crain², Sultan Beshir¹, Joachim Kurtz¹, Hinrich Schulenburg¹, Arndt Telschow¹; ¹Westfälische Wilhelms Universität, Institute of Evolution and Biodiversity, Münster Germany; ²DuPont Pioneer, Delaware USA; ³Christian-Albrechts-Universität zu Kiel, Department of Evolutionary Ecology and Genetics, Kiel Germany
- 9:15 **228** **Immune priming with *Bacillus thuringiensis* in *Tribolium castaneum*** Joachim Kurtz, Barbara Milutinovic, Robert Peuss, Kevin Knoblich, Hendrik Eggert, Sarah Behrens, Jenny Greenwood, Westfälische Wilhelms Universität, Institute of Evolution and Biodiversity, Münster, Germany

- 9:30 **229** Rapid reciprocal adaptation between the red flour beetle and *Bacillus thuringiensis* bacteria during experimental coevolution Barbara Milutinovic & Joachim Kurtz, Institute for Evolution and Biodiversity, Münster, Germany
- 9:45 **230** Means of fast virulence adaption: the plasmid and prophage equipment of selected *Bacillus thuringiensis* strains Jacqueline Hollensteiner¹; Joachim Kurtz²; Hinrich Schulenburg³; Heiko Liesegang¹; Georg-August University Göttingen, Institute für Mikrobiologie und Genetik, Germany; ¹Westfälische Wilhelms-Universität Münster, Germany; ²Christian-Albrechts-Universität Kiel, Zoological Institute, Germany

10:00–10:30 **BREAK**

Thursday, 10:30-12:30. **P1**
SOCIETY FOR INVERTEBRATE PATHOLOGY
Annual Business Meeting

Presiding: Jørgen Eilenberg

12:30–14:00 **LUNCH** Mensa

Symposium 8 (Cross-Divisional) Thursday, 14:00-16:00. **P2**
Host – Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing

Organizers/Moderators:
Christina Nielsen-LeRoux and Elke Genersch

- 14:00 **231** The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host Didier Lereclus, INRA, UMR1319 - Micalis, La Minière, 78280 Guyancourt, France.
- 14:30 **232** The interplay of *Paenibacillus* larvae with honey larvae during infection Elke Genersch; Anne Fünfhaus; Eva Garcia-Gonzalez; Gillian Hertlein; Lena Poppinga, Institute for Bee Research, Hohen Neuendorf, Germany
- 15:00 **233** Antimicrobial defense and persistent infection in insects revisited Jens Rolff, Evolutionary Biology, Fachbereich Biologie, Chemie, Pharmazie , Freie Universität Berlin, Berlin, Germany
- 15:30 **234** *Vibrio* and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes Audrey Vanhove¹, Annick Jacq², Frédérique Le Roux³, Tristan Rubio¹, Alexandra Calteau⁴, Evelyn Bachère¹, Julie Nicod¹, Agnès Vergnes¹, Astrid Lemire³, Guillaume Charrière¹ and Delphine Destoumieux-Garzón¹; ¹Ecology of coastal marine systems, University of Montpellier, France; ²Institut de Génétique et Microbiologie, Université de Paris Sud, France; ³Integrative Biology of Marine Models, Ifremer, Université Pierre et Marie Curie. Station Biologique de Roscoff, France; ⁴Laboratory of Bioinformatics Analyses for Genomics and Metabolism, Genoscope, Evry, France

Contributed Papers Thursday, 14:00-15:45. **P3**
MICROBIAL CONTROL 4

Moderator: Trevor Jackson

- 14:00 **235** Establishing the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV) Lara R. Jaber & Nida' Salem, Department of Plant Protection, Faculty of Agricultural Sciences, The Univ. of Jordan, Amman, Jordan
- 14:15 **236** Bean plant *Phaseolus vulgaris* endophytically colonized by *Beauveria bassiana* and *Hypocrea lixii* acquires protection against *Liriomyza huidobrensis* (Diptera: Agromyzidae) in the field Jane W. Gathage, Komivi S. Akutse, Komi K.M. Fiaboe, Sunday Ekesi and Nguya K. Maniania, International Centre of Insect Physiology and Ecology, Nairobi, Kenya
- 14:30 **237** Colonized plants with entomopathogenic fungi produce mortality in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae Gloria Resquín-Romero, Cristina Delso, Carlos Campos, Lola Ortega, Inmaculada Garrido-Jurado, Enrique Quesada-Moraga, University of Córdoba, Department of Agricultural and Forestry Sciences, Córdoba, Spain
- 14:45 **238** *Beauveria bassiana* and California strawberries: endophytic, mycorrhizal, and entomopathogenic interactions, Surendra K. Dara, Division of Agriculture and Natural Resources, University of California, USA
- 15:00 **239** Perceptions, trust, terminology and influence: What do consumers think about biological control? Michael Brownbridge and Alexandra Grygorczyk, Vineland Research and Innovation Centre, Vineland Station, Ontario, Canada
- 15:15 **240** A phylogenetic survey of protistan parasites David Bass¹, Hanna Hartikainen², Cedric Berney¹, Sigrid Neuhauser¹, Georgia Ward¹, Grant Stentiford³; ¹Division of Genomics and Microbial Diversity, Department of Life Sciences, Natural History Museum, UK ; ²ETH Zürich and Eawag, Duebendorf, Switzerland; ³European Union Reference Laboratory for Crustacean Diseases, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth Laboratory, UK
- 15:30 **241** *Bacillus thuringiensis* toxins vs baculovirus: differential induction of immune system related genes in *Spodoptera exigua* Cristina M. Crava, Agata Jakubowska, Salvador Herrero, Baltasar Escriva, Yolanda Bel, Department of Genetics, ERI BIOTECMED, Universitat de Valencia, Burjassot, Spain

Contributed PapersThursday, 14:00-16:00. **P1****VIRUSES 7**

Moderator: Zihni Demirbag and Mehin Yuan

- 14:00 **242 Lysine Residues in N-terminal Tail of a Viral Histone H4 are Crucial in Controlling Host Gene Expression** Rahul Hepat, Yonggyun Kim, Department of Bioresource Sciences, Andong National University, Andong, Korea
- 14:15 **243 Heat-shock protein 90 is a broadly active regulator for baculovirus infection** Shufen Li; Dianhai Hou; Fei Deng; Hualin Wang; Manli Wang; Zhihong Hu, Wuhan Institute of Virology, Chinese Academy of Sciences, P. R. China
- 14:30 **244 Development and immunity-related microRNAs of the lepidopteran model host *Galleria mellonella*** Krishnendu Mukherjee and Andreas Vilcinskas, Fraunhofer Institute of Molecular Biology and Applied Ecology, Department of Bioresources, Giessen, Germany
- 14:45 **245 The *sf122* gene of *Spodoptera frugiperda* nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction** Inés Beperet¹; Oihane Simón¹; Trevor Williams²; Sarah L. Irons³; Leopoldo Palma¹; Miguel López-Ferber⁴; Linda A. King³; Primitivo Caballero^{1,5}, ¹Bioinsecticidas Microbianos, Instituto de Agrobiotecnología, Mutilva, Spain; ²Instituto de Ecología AC, Xalapa, Mexico; ³Department of Biological and Medical Sciences, University of Oxford, United Kingdom; ⁴LGEI, Ecole de Mines d' Alès, Alès, France; ⁵Departamento de Producción Agraria, Universidad Pública de Navarra, Pamplona, Spain
- 15:00 **246 Effect of a Viral Encoded Protein Kinase on Gene Expression in *Amsacta moorei* Entomopoxvirus Infected Cells** Hacer Muratoglu¹, Remziye Nalcacioglu², Basil Arif³, Zihni Demirbag², ¹Karadeniz Technical University, Faculty of Sciences, Department of Molecular Biology and Genetic, Trabzon, Turkey; ²Karadeniz Technical University, Faculty of Sciences, Department of Biology, Trabzon, Turkey; ³Laboratory for Molecular Virology, Great Lakes Forestry Centre, Sault Ste. Marie, Ontario, Canada
- 15:15 **247 FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses** Shufen Li, Manli Wang, Zhihong Hu, Fei Deng, Hualin Wang, State Key Laboratory of Virology, Virus Resource and Bioinformatics Center, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, P.R. China.

- 15:30 **248 The leucines in the transmembrane domain of *Autographa californica nucleopolyhedrovirus* Ac76 are important for intranuclear microvesicle formation** Denghui Wei, Yan Wang, Xiaomei Zhang, Meijin Yuan, Kai Yang, State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou, China

- 15:45 **249 High-throughput purification of dsRNA against sacbrood virus disease in honey bees *Apis cerana* (Hymenoptera: Apidae)** Jianging Zhang, Yi Zhang and Richou Han^{*}, Guangdong Entomological Institute, Guangzhou China

16:00-16:30 **Student Business Meeting** **P4**

18:30 **Bus transfer to SIP Banquet** Alte Lokhalle

IMPORTANT NOTE: Remove all posters before 18:00

**19:00-1:00 RECEPTION
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2014

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129 indicates abstract number for ORAL presentation

B-11 indicates abstract number for POSTER presentation

MONDAY - 4 August

PLENARY SYMPOSIUM Monday, 10:30–12:30

Microbial Control - from Bench to Business

PLENARY SESSION. Monday, 10:30. **1**

Potentials for utilizing and controlling insect pathogens

Richou Han, Xuehong Qiu and Xun Yan
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Insects are attacked by different invertebrate pathogens. Diverse strategies are used to deal with these pathogens. In this presentation, three examples are presented to show the utilization and control of insect pathogens in Guangdong Entomological Institute, China: (1) ***Ophiocordyceps sinensis* fungus as health food.** *O. sinensis* (Clavicipitaceae) (best known as *Cordyceps sinensis*) is one of the entomopathogenic fungi endemic on above 3000 m Tibetan Plateau. The fungus parasitizes larvae of moths (Lepidoptera) and fruiting bodies grow from the infected larvae. Regarded as "Himalayan Viagra", the fungus-insect complex is used to treat a variety of ailments including fatigue, impotence and cancer, and costs \$60000–\$75000 per kilogram. The growing worldwide demand and resource limitation drive the research to artificial cultivation of this fungus for commercial trade. (2) ***Photobacterium* bacteria for insect control.** *Photobacterium* bacteria associated with entomopathogenic *Heterorhabditis* nematodes produce oral protein toxins for killing insects. For sustainable termite control, the toxic genes are transformed into *Enterobacter cloacae*, one of the indigenous gut bacteria of the Formosan subterranean termite (*Coptotermes formosanus*), and the termites are fed with these genetically modified bacteria. (3) **Control of Chinese sacbrood virus (CSBV) by RNAi-mediated technology.** CSBV is the most serious virus of oriental honey bees *Apis cerana*. To protect the honey bees, RNAi technology is successfully used to control this harmful virus, by feeding second instar larvae of *A. cerana* with specific sequences of CSBV double-stranded RNA (dsRNA). The results from these examples show the research strategies in invertebrate pathology and potentials for implementing the research results in commercial purpose.

Key words: Invertebrate pathogens, *Ophiocordyceps sinensis*, *Photobacterium* bacteria, CSBV

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PLENARY SESSION. Monday, 11:00. **2**

Story of an African firm: 10 years in the biopesticide business – lessons learned along the way

Sean Moore

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In 2003, River Bioscience in South Africa became the first African company to successfully produce and commercialise an insect virus as a biopesticide. This was the *Cryptophlebia leucotreta granulovirus* (CrleGV). The outcome was instant success, mainly due to the good fortune of perfect timing. The target pest, the false codling moth (*Thaumatotibia leucotreta*), was a very serious one and there was a dearth of alternative products. River Bioscience originated as a spin-off company from grower-funded citrus research and for the first few years of existence, served that single agricultural sector as a one-product company: a high risk situation. Subsequently, the company expanded its product range into other viruses, including the *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and the *Cydia pomonella* granulovirus (CpGV), entomopathogenic nematodes and a range of biorational products, such as Attract and Kill products for a range of fruit fly species. The success of the commercial venture can be attributed to a number of factors, including product quality and competitiveness, being market driven rather than product driven, starting small (hence not over capitalizing) and growing organically, a close association with research organisations and being owned by its major market – the citrus growers. However, all has not been moonlight and roses: many hard lessons have been learned. For example, simply having a good product is not sufficient – it is the way in which the product is marketed that determines how it sells relative to the competition, which has increased dramatically since the emergence of the company.

PLENARY SESSION. Monday, 11:30. **3**

A Roadmap to the Successful Development and Commercialization of Microbial Pest Control Products for Control of Arthropods

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Microbial pesticides have been developed for a hundred years, but many of these crop protection products have not been successful in the market. Therefore, there is a need for a model that facilitates the development and commercialization of these products. A model has been developed for a biocontrol product based on entomopathogenic bacteria, fungi, viruses and nematodes. The model aims to develop a rational and structured approach that will increase the chances of achieving success with microbial pest control products. The building blocks of the entire process are identified and essential aspects highlighted. This systematic roadmap with a strong focus on economics and market introduction will assist academic researchers and industrial developers of biopesticides in accomplishing their goal: the development of successful cost-effective biopesticides.

PLENARY SESSION. Monday, 12:00. **4**

BASF Functional Crop Care. Unlocking Agricultural Potential in Soil, Seed and Crop

Sebastian Bachem
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For many years BASF has been active in the area of biological pest control with its pheromone based mating disruption solutions and in 2012 it acquired the leading biologicals company Becker Underwood. With its broad range of seed,

soil and foliar products Becker Underwood was an excellent fit and has now been fully integrated into the company.

The presentation will outline the different key segments BASF is focusing on in the areas of soil, seed and foliar treatments. Furthermore it will focus on the main opportunities BASF sees in developing an integrated portfolio of biological and chemical products that are able to reliably cover a broad spectrum of farmer's needs. Beyond this we will look forward and outline how we expect the crop protection market to develop and what motivates BASF to invest into finding best possible solutions to meet these changing market demands.

SYMPORIUM 1 (Nematodes) Monday, 14:00-16:00

Above and Belowground Interaction, Root-Shoot Interaction, Chemical Signaling

Symposium. Monday, 14:00. 5

Small molecule signals in nematodes - common motifs and species specific modifications

Stephan H. von Reuss

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Chemical communication in nematodes via small molecule signals has been known since the 1960s. However, despite considerable efforts chemical structures have remained elusive for several decades. Recent research focusing on the model organism *Caenorhabditis elegans* has revealed a modular library of small molecule signals, the ascarosides, glycolipids of the dideoxysugar ascarylose linked to fatty acid derived side chains, that modulate nematode development and behavior. Furthermore, we have shown that production of ascaroside components is highly conserved among nematodes from different clades, life-styles and ecological niches.

Our ongoing research aims to comprehensively characterize ascaroside signaling in selected nematode species including bacteriovorus and entomopathogenic species. Identification of putative ascaroside signals is accomplished using our recently developed highly sensitive HPLC-MS/MS precursor ion screen that facilitates the detection of known and novel ascaroside components in crude nematode metabolome extracts. Novel ascarosides are subsequently isolated by SPE and HPLC and identified using a combination of HR-MS/MS and NMR techniques. We found that diverse nematode species share a large variety of common ascarosides and in addition also produce several highly species-specific derivatives. Chemical synthesis and subsequent functional characterization of these putative small molecule signals in different nematodes will reveal their importance in intra- and interspecific communication and help to decipher the evolution of ascaroside signaling in nematodes.

Symposium. Monday, 14:30. 6

Olfactory Plasticity in Entomopathogenic Nematodes

Joon Ha Lee and Elissa Hallem

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Many parasites, including entomopathogenic nematodes (EPNs), use host-emitted olfactory cues to locate hosts. However, how parasitic nematodes respond to host-emitted odors remains poorly understood. In particular, little is known about how parasitic nematodes integrate host odor cues with environmental cues such as temperature and intrinsic cues

such as age to mediate context-appropriate host-seeking behaviors. To address this question, we are investigating the olfactory behavior of EPNs from the genera *Steinerinema* and *Heterorhabditis*. We find that EPNs are attracted to the general host cue carbon dioxide under all conditions tested. However, responses to many odorants exhibit extreme olfactory plasticity as a function of IJ cultivation temperature and/or age. For example, in *Steinerinema carpocapsae*, many odorants that are strongly attractive at lower temperatures are strongly repulsive at higher temperatures and vice versa. This temperature-dependent olfactory plasticity occurs in individual IJs and is reversible, since temperature-swapping IJs reverses their olfactory preferences. By contrast, other species appear to show primarily age-dependent changes in olfactory preferences, while still other species show little or no olfactory plasticity. Thus, the type and extent of olfactory plasticity varies among EPNs. In addition, we find that foraging strategy can also vary with temperature. For example, *Steinerinema carpocapsae* behaves more like an ambusher at higher temperatures and more like a cruiser at lower temperatures. Some EPNs are found in geographical regions that undergo substantial seasonal temperature variation, and we hypothesize that plasticity of olfactory behavior and foraging strategy may enable EPNs to optimize host seeking under changing environmental conditions.

Symposium. Monday, 15:00. 7

Multiple Consequences of Belowground Herbivore Induced Volatile Signals

Jared G. Ali^{1,2}, Raquel Campos-Herrera^{2,3}, Hans T. Alborn⁴, Larry W. Duncan², Lukasz L. Stelinski²

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Plants can influence the behavior of and modify community composition of soil dwelling organisms through the exudation of organic molecules. Given the chemical complexity of the soil matrix, soil-dwelling organisms have evolved the ability to detect and respond to these cues for successful foraging. A key question is how specific these responses are and how they may evolve. Soil nematodes are a group of diverse functional and taxonomic types, which may reveal a variety of responses. Herbivore-induced volatile emissions benefit plant hosts by recruiting natural enemies of herbivorous insects. Such tritrophic interactions have been examined thoroughly in aboveground terrestrial environments. Recently, similar signals have been described in the subterranean environment, which may be of equal importance for indirect plant defense. Our work has shown that plant roots of citrus defend themselves against root herbivores by releasing an herbivore-induced plant volatile (HIPV), pregeijerene (1,5-dimethylcyclohexa-1,5,7-triene), that attracts naturally occurring entomopathogenic nematodes (EPNs) to larvae when applied in the field. However, the soil community is complex, containing a diversity of interspecies relationships that modulate food web assemblages. In a series of experiments we examine the specificity of this HIPV in the complex nematode community, including beneficial entomopathogenic nematodes, plant-parasitic nematodes, as well as, hyper-parasitic nematodes and nematophagous fungi. We provide the first evidence showing subterranean HIPVs behave much the

same as those aboveground, attracting not only parasitoids, but also hyperparasites and other food web members.

Symposium. Monday, 15:30. **8**

Root Zone Chemical Ecology; New Techniques for Below Ground Sampling and Analyses of Volatile Semochemicals

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The ban of the fumigant methyl bromide has led to a need for new methods to control soil-dwelling plant pests. The use of semiochemicals is one such avenue of research since studies of plants above ground release of volatile organic compounds (VOCs) in response to herbivory have resulted in effective control methods for insect pests and also plant roots might release induced VOCs that attract organisms such as entomopathogenic nematodes. However, studies of such below ground interactions lags because of the complexity of the system. For example, in addition to plants roots, potentially important VOCs can be produced also by microorganisms, insects and nematodes and in soil VOCs are released into a virtually static airspace where they disperse solely by diffusion. To bypass this complexity root-related VOCs have been sampled by transferring roots from a pot to an artificial environment where most of the air surrounding the roots is drawn through an adsorption filter that trap VOCs, or by maceration and solvent extraction. This creates an artificial VOC profile with little relevance to the system intended to be studied. To address the need for more sensitive and less intrusive *in vivo* studies of below-ground VOC governed interactions probes were designed for direct in-soil sampling. In combination with improved thermal desorption GC/MS analyses the probes allowed short sampling times and required removal of minimal air volumes. This technique makes it possible to continuously monitor and follow the dynamics of root zoon VOCs in response to insect or nematode infestations.

discovery of alternative actives that can complement or substitute for Cry toxins. A screen of bacterial collections led to the discovery of several insecticidal protein genes with great potential for developing insect resistant crops. Two examples representing actives from non-Bacillus sources will be presented: PIP-1A is a 30 kD protein isolated form a *Pseudomonas* strain showing strong activity against hemipteran and certain lepidopteran pests. AfIP-1A and AfIP-1B is a pair of binary proteins isolated from an *Alcaligenes* strain demonstrating potent corn rootworm killing activity. Corn plants expressing this pair of proteins display high resistance to WCRW. Preliminary studies on AfIP-1A and AfIP-1B in terms of protein biochemical characteristics, insecticidal activity spectrum and insect mid-gut binding properties indicate this pair of binary proteins may function in ways similar to some Cry proteins from Bacillus sources. Our work demonstrates that bacteria that are not Bacillus can be valuable sources of insecticidal proteins.

Contributed paper. Monday, 14:15. **10**

Discovery and optimization of hemipteran-active proteins for Lygus control in cotton

James A. Baum, Waseem Akbar, Konasale Anilkumar, David Bowen, Robert S. Brown, Cathy Chay, Thomas Clark, Michael Pleau, Xiaohong Shi, Uma Sukuru, Moritz Von Rechenberg, Halong Vu, Brent Werner, Andrew Wollacott

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The plant bugs *Lygus hesperus* and *Lygus lineolaris* have emerged as economic pests of cotton in the United States. These hemipteran species are not controlled by the lepidopteran-specific insect control traits (*Bacillus thuringiensis* Cry proteins) found in genetically-modified commercial varieties of cotton. We have identified several novel Bt Cry proteins that are toxic to Lygus nymphs in artificial diet bioassays. Several of these proteins have been further modified to exhibit improved toxicity towards both Lygus species while retaining the insecticidal specificity of the parent protein. Cotton plants expressing modified Cry proteins show enhanced protection from Lygus feeding damage in the field.

Contributed paper. Monday, 14:30. **11**

Isolation and identification of potential biological control agent from *Tortrix viridana* L.(Lepidoptera: Tortricidae) pupae

Nurcan Albayrak Iskender¹; Yaşar Aksu²

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Tortrix viridana is one of the most important pest in the oak fields in Turkey. The aim of this study is to find a more effective and safe biological control agent against *Tortrix viridana*. For this purpose, pupae of *T. viridana* were collected from Artvin province, Turkey in 2013. According to the morphological, biochemical tests, API20E and API50CH panel test system and 16S rRNA gene sequence analysis, the bacterial isolates were identified as *Serratia liquefaciens* (Tv1), *Enterococcus* sp. (Tv2), *Rhodococcus erytropolis* (Tv3), *Rahnella aquatilis* (Tv4), *Curtobacterium flaccumfaciens* (Tv5), *Pseudomonas* sp. (Tv6). Future research will be tested insecticidal effects of these bacterial isolates against *T. viridana*.

CONTRIBUTED PAPERS Monday, 14:00-16:00 BACTERIA 1

Contributed paper. Monday, 14:00. **9**

Discovery of Insecticidal Proteins from Non-Bacillus Bacterial Species

Nasser Yalpani¹; Dan Altier¹, Jennifer Barry¹, Jarred Oral², Ute Schellenberger², Adane Negatu¹, Scott Diehn¹, Virginia Crane¹, Gary Sandahl¹, Joe Zhao¹, Dave Cerf², Claudia Perez Ortega³, Mark Nelson³, Analiza Alves¹, Lu Liu², Gusui Wu¹

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Crops expressing various *Bacillus thuringiensis*-derived insecticidal Cry protein genes have been on the market for over 15 years and have provided significant value to growers. Such products also provide a significant positive impact on the environment due to the reduced need for chemical insecticides. However, there remains the need for the

Contributed paper. Monday, 14:45. **12 STU**

Evolution of a Sensor Protein Controlling Production of an Insecticidal Toxin in Plant-Beneficial *Pseudomonas protegens*

Peter Kupferschmied¹, Maria Péchy-Tarr¹, Nicola Imperiali¹, Monika Maurhofer², Christoph Keel¹

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Pseudomonas protegens is a plant-associated bacterium that is not only able to promote plant growth by efficiently protecting roots from attack by fungal phytopathogens but also can turn into an insect pathogen. The microorganism is capable of killing certain pest insects upon oral infection. The major goal of our work is to understand the molecular mechanisms that allow *P. protegens* and related bacteria to detect, to survive within and to kill the insect host. The entomopathogenic activity of *P. protegens* relies in part on the production of an insecticidal toxin termed Fit. We found that the pseudomonad produces the Fit toxin in the insect host, but not on plant roots, demonstrating that the bacterium is capable of distinguishing between these two environments. An array of sensor proteins makes bacteria able to sense the environment they live in and to adapt their behavior accordingly. Here we provide evidence that the sensor histidine kinase FitF is a key regulator of insecticidal toxin production. Our experimental data and bioinformatic analyses indicate that FitF shares a sensing domain with DctB, a histidine kinase regulating carbon uptake in Proteobacteria. This suggests that FitF has acquired its specificity through domain shuffling from a common ancestor. This particular event appeared to be crucial for host-dependent activation of toxin production and thus contributed to the evolution of insect pathogenicity in these bacteria. We propose that inhibition of the FitF sensor during root colonization is the underlying mechanism by which *P. protegens* differentiates between the plant and insect host..

Contributed paper. Monday, 15:00. **13 STU**

***Paenibacillus larvae*, the etiological agent of American Foulbrood, produces the catechol type siderophore bacillibactin**

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The Gram positive, spore forming bacteria *Paenibacillus larvae* is the causative agent of American Foulbrood, a fatal disease affecting the brood of honey bees. The whole vegetative life cycle of *P. larvae* takes place inside the larvae and all micronutrition must be obtained from the host-including iron-a scarce atom essential for growth of host and pathogen likewise. Bacteria often answer this iron deficiency with the production of siderophores, small molecules which act as powerful iron chelators. Such siderophores are often synthesized by multienzyme complexes through non-ribosomal peptide-synthetases (NRPS). The genes of these multienzyme complexes are arranged in giant gene clusters. Here we present data on the identification of an NRPS gene cluster in *P. larvae* encoding the biosynthetic machinery for

the production of a siderophore, which was identified as bacillibactin by MS/MS. Exposure bioassays with mutant *P. larvae* strains lacking bacillibactin production showed that neither total mortality nor disease progression in infected larvae was significantly changed compared to larvae infected with the corresponding wild-type strain. These results are in line with results published on the role of bacillibactin in other pathogenic bacteria like *Bacillus thuringiensis* and *B. anthracis*.

Contributed paper. Monday, 15:15. **14**

Two new *Bacillus thuringiensis* toxins active against Lepidoptera and Coleoptera.

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The entomopathogenic spore-forming bacteria, *Bacillus thuringiensis* (Bt), is widely distributed around the world and is able to produce toxins with insecticidal activity during the vegetative and sporulation phase. The great genetic variety of *B. thuringiensis* strains represents a huge diversity of potential insecticidal proteins. The host range of these proteins is highly variable, but includes a large number of species of the most damaging lepidopteran insect pests and also, other harmful species of the orders Diptera, Coleoptera and Hymenoptera. In order to extend the number of Bt proteins active against important coleopteran and lepidopteran pests, total DNA of a strain from a Spanish collection was completely sequenced. Two ORFs of ~900 bp were selected due to their low identity with other Bt proteins and were cloned in a Bt expression plasmid. Proteins were produced and their insecticidal activity was determined. Bm_47 protein was toxic against *Leptinotarsa decemlineata*, with an LC₅₀ of 54 µg/ml, while Bm_1711 protein was active against the lepidopterans *Helicoverpa armigera* and *Ostrinia nubilalis*, with an LC₅₀ of 164 and 34 ng/cm², respectively. We discuss the importance of this protein to combat species of coleopteran and lepidopteran pests, including species that have developed resistance to other Bt toxins..

Contributed paper. Monday, 15:30. **15-STU**

Entomopathogenic *Bacillus thuringiensis* as PGPR
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Bacillus thuringiensis has been used as an effective bio-insecticide because it produces the proteins Cry and Cyt, which are highly toxic to insects in certain situations. But, recently *B. thuringiensis* was treated as a biological control agent which could control the plant disease. We already demonstrated that the antagonistic activity of *B. thuringiensis* AS17 japonensis, AS18 kurstaki against *Fusarium oxysporum* f.sp. *lycopersici* race2 (FOL) was examined by dual culture technique(Qi et al. 2013). In this study, *B. thuringiensis* strains could control the development of wilt symptoms caused by FOL in tomato plants was confirmed. Inoculate six

strains of *B. thuringiensis* suspension to the tomato seedlings in pot, and transplanted the treated tomato seedlings to FOL infested soil, after 4 weeks the development of wilt symptoms and wilting score become less than control, especially *B. thuringiensis* AS17 *japonensis* and AS20 CR371-H. Also, this study proved that *B. thuringiensis* strains are PGPR. PGPR (Plant growth promoting rhizobacteria) are beneficial bacteria which have the ability to colonize the plant roots and either promotes plant growth through direct action or via biological control of plant diseases. Six strain of Insect Pathogenic *Bacillus thuringiensis* were tested for PGPR effect. Culture filtrates of six strains had remarkable plant growth promotion activity in tomato and alfalfa plants; in each plant after treatment of culture filtrates, both of seed germination rates and the fresh weight were increased compared with control treatment.

Contributed paper. Monday, 15:45. **16**

Vibrios pathogenic for oysters are found associated to plankton species. What possible consequences on pathogen transmission to oysters?

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Vibrios cause major losses in shellfish farming and are associated to recurrent mortalities of oysters. However, to date, the role of plankton species in the transmission of pathogenic vibrios in oyster *Crassostrea gigas* is largely unknown. The main objective of the present study was to identify *in situ* and *in vitro* the interactions of pathogenic Vibrios with local species of planktons from different sites of Thau lagoon, an important region for shellfish farming in south of France. Quantitative-PCR was used to monitor *Vibrio splendidus* and *Vibrio aestuarianus* over the year 2013 at two sites of the Thau lagoon. Out of the oyster farm area, *V. splendidus* was found from May to July and from June to August associated to 5-180 µm and >180µm plankton fractions, respectively. *V. aestuarianus* was also detected in fraction 5-180 µm in May and >180µm in August, before and after the warmer months of the year. For the farm oysters point, *V. splendidus* was found in January and June associated with the 5-180 µm plankton and with the >180 µm fraction in spring and winter. *V. aestuarianus* was not detected. In laboratory controlled conditions, by using a GFP-expressing *V. splendidus* LGP32 and epifluorescence microscopy, we showed that *V. splendidus* LGP32 exhibits strong interactions with copepods of the *Acartia* and *Paracartia* genus as well as with microalgae of the *Alexandrium* genus. Altogether, our data show that vibrios pathogenic for oysters can establish close associations with plankton species, which may enhance the transmission of pathogenic vibrios to oysters.

CONTRIBUTED PAPERS Monday, 14:00-16:00

VIRUSES 1

Contributed paper. Monday, 14:00. **17**

Investigation of Baculovirus RNA Polymerase Subunit Protein-Protein Interactions with *in vivo* Bimolecular Fluorescence Complementation Assays

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Baculovirus transcription utilizes two different DNA-directed RNA polymerases (RNAPs): the insect host RNAP transcribes early genes while a virus RNAP transcribes late and very late genes. The virus RNAP consists of four proteins: P47, LEF-4, LEF-8 and LEF-9. Conserved motifs in LEF-8 and LEF-9 suggest that the interface of these subunits forms the catalytic site of the RNAP, while LEF-4 has RNA capping-associated enzymatic activities. No specific function has yet been demonstrated for P47. To investigate the *in vivo* intracellular localization and interactions of these proteins, two individually non-fluorescent fragments (V1 and V2) of the Venus yellow fluorescent protein were fused with the N-termini of each RNAP subunit in plasmid expression vectors. We also constructed similar fusions with two components of the virus replisome complex, LEF-3 and P143, and of the host *Spodoptera frugiperda* TATA binding protein. Bacmids, expressing each of these fusion proteins, were constructed and used to generate recombinant viruses expressing each of the V1- or V2-tagged protein subunits. Protein-protein interactions of these subunits were investigated using bimolecular fluorescence complementation assays. Co-infections were used to investigate the interactions of these subunits in the presence of the full complement of virus proteins. Reciprocal co-transfections of the original plasmid constructs were performed to investigate the potential for these proteins to form homo-oligomers, as well as their ability to interact with heterologous partners in the absence of any other viral proteins. The results of co-transfection and co-infection assays will be presented.

Contributed paper. Monday, 14:15. **18-STU**

Characterization and Quantitative Analysis of *Autographa californica* Multiple Nucleopolyhedrovirus (AcMNPV) FP25K Localization and Aggregate Formation During Cell Infection

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Localization of AcMNPV FP25K was previously studied by western blot using fractionation. This study, however, was not quantitative. By inactivating the endogenous *fp25k* gene through passage of the AcBacmid in Sf21 cells and subsequent insertion of an *fp25k-egfp* fusion gene at the *polyhedrin* locus, we investigated FP25K localization during infection. Western blot confirmed the 53-kDa FP25K-EGFP fusion protein from infected cells. By using a nuclear stain, we were able to assess and quantify the nuclear to cytoplasmic localization of FP25K-EGFP during Hi5 and Sf9 cell infection through confocal microscopy. During late phase of infection, small aggregates were formed and FP25K-EGFP was found exclusively in the cytoplasm. However, during very late phase of infection, larger aggregates were observed in both the

cytoplasm and nucleus and about 1% of FP25K-EGFP localized to the nucleus. In addition, bioinformatic analysis of FP25K predicts a highly conserved coiled-coil domain at the N-terminus. We hypothesize that this coiled-coil domain may be responsible for the formation of these amorphous aggregates in the cytoplasm and nucleus. Therefore, disruption of the coiled-coil domain will disorder aggregate formation. Quantifying FP25K localization and studying aggregate formation may help to understand the role of FP25K aggregates in infection and polyhedrin promoter activities.

Contributed paper. Monday, 14:30. **19 STU**

Bracovirus-derived genes in the genome of *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae) and their role in host susceptibility to pathogens

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The association between parasitic wasp, its polydnavirus and the lepidopteran host can represent an interesting model to study the horizontal transfer of genes since three different genomes are simultaneously in contact. In this context, the transcriptome of *Spodoptera exigua* revealed the presence of eight unigenes with high homology to bracovirus genes. All of them encoded for lectin-like proteins except one coding for a protein with homology to proteins of unknown function, which we named *gasmin*. Sequence analysis of the genomic region of *gasmin* and of one of the bracovirus lectin-like proteins (*Se-BLL2*) confirmed their integration into the *S. exigua* genome. *Gasmin* as well as the lectins were mainly expressed in the hemocytes which indicate their possible role in the interaction with the parasitic wasp and insect's immune response. Functional analysis of *gasmin* revealed that this protein interacts with the cellular actin inhibiting its polymerization. This inhibition leads to a drastic reduction in the capacity of hemocytes to phagocytise bacteria. Moreover, high expression of *gasmin* reduces the multiplication and the production of baculovirus particles in cell culture experiments. Analysis of the bracovirus-derived lectins revealed that they respond to gram-positive and gram-negative bacteria in addition to baculovirus infection. Remarkably, *Se-BLL2* responds to all tested pathogens. Further characterization of *Se-BLL2* showed that it recognizes and agglutinates gram-negative as well as gram-positive bacteria. The results obtained suggest that the insect has domesticated the viral genes to cope with infections by pathogens.

Contributed paper. Monday, 14:45. **20**

Entry of *Bombyx mori* nucleopolyhedroviruse (BmNPV) into BmN Cells by Macropinocytic Endocytosis

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Abstract: *Bombyx mori* nucleopolyhedroviruse (BmNPV) is a serious virus pathogen of silkworm, *Bombyx mori*, and no drugs or specific protection is available presently, whereas knowledge on BmNPV entry, a remarkable target for the

development of protection target, is still limited. Here we used BmNPV virus combined with different drugs and subcellular analysis to investigate BmNPV entry mechanism. Results indicated that BmNPV entry into BmN cells was clathrin- and caveolar/lipid raft-independent endocytosis pathway, but actin-, microtubule-, PKC-, Rac1- and PI(3)K-dependent, and virus entry mediated by cholesterol in a dose dependent manner, these results suggested that BmNPV entry into BmN cells by macropinocytic endocytosis, which was further confirmed by TEM and live image analysis. Our study suggested that BmNPV take a different mechanism to invade host cell that was different with that of AcMNPV..

Contributed paper. Monday, 15:00. **21**

Nuclear translocation of *Autographa californica* nucleopolyhedrovirus ME53

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The baculovirus early/late *me53* is conserved in all sequenced lepidopteran baculovirus genomes. If AcMNPV *me53* is deleted, DNA replication is normal but virus replication and spread is severely compromised. The 449 amino acid AcMNPV ME53 is a nucleocapsid-associated protein colocalizing with the major envelope glycoprotein GP64 at putative "budding" foci on the cell membrane. However, ME53 also localizes to the nucleus. In the absence of an easily identifiable nuclear localization signal we wished to identify ME53 sequences responsible for its nuclear translocation. To that end we generated a series of HA- or GFP-tagged N and C-terminal and internal deletions of ME53 as well as internal ME53 peptides through a baculovirus bacmid intermediate. Localization of the tagged ME53 variants following bacmid transfection, was monitored by confocal fluorescence microscopy. An HA-tagged ME53 lacking aa83-152 was excluded from the nucleus while an internal HA-tagged aa83-152 peptide showed nuclear localization. Further N-terminal deletions up to aa107 (or carboxy terminal deletions up to aa250) showed nuclear localization of GFP-tagged ME53, while N-terminal deletions up to aa121 did not. Among several internal deletions tested, the aa107-121 deletion lacked nuclear localization. Overlapping that region was an alpha helical domain aa107-133. However alanine mutagenesis of some of the basic residues (E121A, R122A, K126A and even double E121A/R122A) predicted to destroy the alpha helix, failed to prevent nuclear localization. As the aa83 to 152 peptide on its own showed nuclear localization we predict the ME53 nuclear localization domain to begin between aa107 and 121 and end upstream of aa152.

Contributed paper. Monday, 15:15. **22**

Nuclear localization and other domains of *Autographa californica* nucleopolyhedrovirus DNA polymerase

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The baculovirus *dnapol* is a core gene essential for viral DNA replication. The 984 aa AcMNPV DNAPol has polymerase and 3'-5' exonuclease domains spanning aa189-750. To determine if these domains were sufficient for viral DNA replication and virus production we generated a series of bacmids with DNAPol C-terminal deletions. Virus spread and DNA replication were monitored following transfections using GFP

fluorescence. Deletion of the C-terminal 184 aas was detrimental to virus production, and even deletion of the C-terminal 36 aas severely compromised virus spread. Thus almost the entire C-terminus beyond the polymerase domain was required for normal virus replication. Confocal fluorescence microscopy showed this might be due to failure of DNAPol nuclear localization. Of several expression plasmids with C-terminal DNAPol truncations fused to EGFP, only pBC949, expressing DNAPol aa1-949 translocated to the nucleus; shorter truncations remained cytoplasmic, mimicking the results for the same truncations in bacmid constructs. AA sequences in aa804-827 and aa939-948 were consistent with a bipartite and monopartite NLS, respectively. Peptides with either NLS fused to EGFP, independently allowed for strong nuclear localization. However, deletion of either NLS in DNAPol:EGFP fusions resulted in only cytoplasmic DNAPol:EGFP. A highly conserved C-terminal sequence at aa972-981 was found in all group I alphabaculoviruses. For bacmid constructs with alanine mutagenesis in this region, there was limited spread of GFP fluorescence but only by 144 hpt. Thus DNAPol requires both NLSs and even the C-terminal 10 aas for nuclear translocation, viral DNA replication and virus production.

Contributed paper. Monday, 15:30. **23-STU**

Investigations into the role of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) AC141 (EXON0 and *Trichoplusia ni* kinesin-1 in budded virus nucleocapsid egress

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The nucleocapsids (NC) of alphabaculoviruses budded virus (BV) virions are assembled in the nuclei of infected cells, transported from the nucleus, through the cytoplasm and bud from the plasma membrane enabling systemic spread of infection. The AcMNPV viral protein AC141 (EXON0) is required for efficient BV production and has been shown to associate with β -tubulin and potentially directly interact with a Drosophila kinesin-1 TPR domain. The objective of this study was to determine if AC141 can associate with the host lepidopteran kinesin-1. To enable these studies the sequence of *T. ni* kinesin-1 heavy (KHC) and light (KLC) chains were identified from a transcriptome analysis of *T. ni* Tnms42 cells. *T. ni* KLC and KHC cDNAs were subsequently generated and cloned into plasmid expression vectors, and tagged at the 5' and 3' ends with Myc or HA epitope tags, or EGFP. These constructs were used to generate stably transformed High Five (BTI-Tn5B1-4) cell lines. Initial experiments showed that both N- and C-terminal HA-tagged KLC expressed in stable cell lines co-immunoprecipitates AC141 and β -tubulin. In addition, HA-tagged AC141 co-immunoprecipitates with WT KLC. Sequential confocal laser scanning microscopy shows that Myc-KLC in stable cell lines co-localizes with HA-AC141 in regions adjacent to the plasma membrane at 20, 24 and 48 hpi. This technique was also used to examine co-localization of AC141, microtubules and tagged KLC molecules. These studies provide additional support to a model in which the association of AC141 with microtubules plays an important role in anterograde trafficking of BV NCs

Contributed paper. Monday, 15:45. **24**

The Twist In Baculoviruses

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It has been known for over forty years that baculovirus genomes are supercoiled ds DNA molecules, yet the implications of this fact has had little effect on current explanations of baculovirus replication and hyperexpression. It is now known that negatively supercoiled ds DNA is spontaneously bound by nucleosomes upon entering the nucleus, which is what happens to baculovirus genomes on nuclear entry. Because both replication and transcription require that nucleosomes be slid or removed for these processes to occur, baculoviruses also must be able to regulate chromosome remodeling. Two of the four major classes of chromosome remodelers, INO80 and SWI/SNF, contain actin as an essential subunit. If either or both were necessary for transitioning from late to very late gene expression, the observed transient dependence on polymerizable actin for the period of transition would be explained. Moreover, it is now known that replication of covalently-closed circular DNA in eukaryotic systems requires topoisomerase II (topo 2). Topo 2 makes double-strand breaks (DSBs) and DSBs are considered to be among the most deleterious of DNA lesions. Their occurrence could explain the induction of the DNA damage response during baculovirus replication. SWI/SNF complexes facilitate topo 2 positioning for dsDNA cleavage, hence polymerizable actin is also required. An SV40-based model of baculovirus replication will be presented.

CONTRIBUTED PAPERS Monday, 14:00-15:30

FUNGI 1

Contributed paper. Monday, 14:00. **25**

A new mycopesticide developed especially for the control of the citrus greening vector *Diaphorina citri* (Hemiptera: Liviidae)

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The citrus greening also known as Huanglongbing or yellow dragon disease is one of the most serious citrus diseases in the world. This disease has devastated millions of hectares of citrus crops throughout Brazil and the United States. Considering that once infected the plant has no cure, the primary control strategies currently employed requires intensive use of chemical insecticides against the vector, *Diaphorina citri*. We have developed a new suspension concentrate formulation based on *Isaria fumosorosea* for controlling this pest. The product is effective against adults and nymphs of *D. citri* but it can also contribute to the management of other citrus pests such as the black citrus aphid, *Toxopterna citricida*, the citrus blackfly, *Aleurocanthus woglumi*, and the snow scale, *Unaspis citri*. The *I. fumosorosea* isolate used presented UV tolerance up to two times higher than other fungal isolates tested, and it is compatible and can be tank mixed with most chemicals sprayed in citrus

(pesticides, foliar fertilizers, adjuvants) except for the fungicides. Field sprays ($n = >15$) on adults confined in voile bags on commercial citrus groves using 60mL of suspensions ($2.5-5.0 \times 10^6$ conidia/mL) per m^3 of leaf area in the citrus canopy caused total mortality ranging from 60-96%. Transmission of the fungus from *D. citri* and *T. citricida* cadavers to uninfected *D. citri* were effectively demonstrated in laboratory and semi-field conditions. The mycotoxicide is currently in preparation for commercial registration.

Contributed paper. Monday, 14:15. **26**

Effectiveness of biorationals and *B. bassiana* against tomato fruitworm in Sinaloa

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During autumn-winter 2012 was conducted a field trial with applications of biorational products *B. thuringiensis* (Versa™), Pyrethrins (Abatec™), *S. carpocapsae* (Capsanem™) and native strains of *B. bassiana* and water as control, against neonate larvae of tomato fruitworm *Heliothis virescens* (Fabricius) in a tomato crop cultivated in Guasave Sinaloa, México. The variables evaluated were larvae mortality (LM), fruit damage and yield. The better treatments were: *B. thuringiensis* 39.6%, Pyrethrins 32.3 % and *S. carpocapsae* 23.33%, while the native *B. bassiana* strains (2.1×10^6 spores/ml) had 6.3 to 6.6%, and the control 2.66% of LM after 72h. Not statistical differences were found in fruit damage between Versa and Abatec, but they were found in the control and Bb1 strain; in the yield, neither were founded differences between biorational products, these also showed the highest fruit yields, followed by Bb strains. These results indicated a lower field efficacy of fresh native Bb strains at this spore concentration, respect to the other products against *H. virescens*.

Contributed paper. Monday, 14:30. **27**

Evaluating *Metarhizium brunneum* F52 Microsclerotia Applied in Hydromulch for Control of Asian Longhorned Beetles

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The entomopathogenic fungus *Metarhizium brunneum* F52 (Hypocreales: Clavicipitaceae) is able to produce environmentally persistent microsclerotia. Incorporating these desiccation-tolerant microsclerotia (Mb MS) granules into hydromulch [a mixture of water + wheat straw mulch + psyllium tackifier], represents a novel, easy-to-use and environmentally-friendly mycoinsecticide that can be sprayed onto the trunks of forest or orchard trees to control insect pests. Hydromulch holds moisture that allows microsclerotia to germinate, and the production of conidia in turn, causes lethal infections in Asian longhorned beetles, *Anoplophora glabripennis* Motschulsky (Coleoptera: Cerambycidae). To test how quickly beetles could be killed, moist and dry bark pieces and filter paper were sprayed with a low dose (~9 Mb MS/cm²) of microsclerotia in hydromulch. Median survival times of beetles exposed to moist bark and filter paper were 17.5 d and 19.5 d, respectively. Beetles exposed to dry bark died

significantly slower. In an attempt to kill beetles faster, moist bark pieces were sprayed with three doses of microsclerotia in hydromulch: low (6-9 Mb MS/cm²); medium (10-19 Mb MS/cm²) and high (20-30 Mb MS/cm²). At high doses, 50% of beetles died in 12.5 d but at lower doses it took significantly longer to kill beetles (16.5 d-17.5 d). In a two week oviposition period, total beetle fecundity was highest in high-humidity controls, females produced 18.5 viable offspring compared to high-humidity hydromulch treatments that significantly reduced fecundity to 7.9 viable offspring. This however was not significantly different from the low-humidity hydromulch (8.1 viable offspring) or that associated control (9.1 viable offspring). Outdoor spore production by microsclerotia, using moist bark pieces, sprayed with a high dose of hydromulch (20-30 Mb MS/cm²) and attached to trees in the woods was quantified. There was a significant increase in spore production over 24 days and in the second replicate (another 24 days) spore production was significantly higher than in the first replicate ($P \leq .0001$). Importantly, rainfall was significantly correlated ($P \leq .0042$) to this increase in spore production. Environmental moisture plays a big role in the spore production by microsclerotia and will subsequently affect the level of insect mortality.

Contributed paper. Monday, 14:45. **28-STU**

Management of entomopathogenic fungal disease in rearing mealworms, *Tenebrio molitor* as animal feed

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Mealworm, *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) has high and safe protein contents, which enables it to be animal feed. However, occurrence of entomopathogenic fungi in mealworms is one of the limitations for mass production. In this work, we investigated relationships between abiotic conditions and occurrence of fungal pathogens and established an effective control method using fungicides. In virulence assay, third instar mealworm larvae were sprayed by six entomopathogenic *Beauveria bassiana* isolates and kept under high relative humidity; *B. bassiana* ERL1575 isolate had highest virulence. Under normal humidity, ERL1575 conidial showed different virulence between spray (~0% virulence) and digestion (~80% virulence) method. Furthermore, mealworms, which digested conidia, were exposed to various temperature (20-35°C) and humidity (1-3 ml distilled water spray/35 mm diam. dish) conditions for 5 days. All the treatments showed ~90% virulence except 35°C incubations (~20% virulence), but irrespective to the humidity conditions. Forty chemical fungicides were assayed against conidial germination and hyphal growth of ERL1575. Fluazinam and mancozeb showed strong inhibition of conidial germination at standard application dose (SD), 1/2 SD and 1/5 SD; besides, fluazinam showed strong inhibition of hyphal growth. When fluazinam and mancozeb were applied to the fungal conidia-inoculated wheat bran, most of mealworms were alive after 3 days post application. However, high mortality rate (~100%) were observed in the conidia-inoculated wheat bran without any fungicides. In conclusion, this work suggests that *B. bassiana* isolates could be pathogens at <30°C when they were digested by mealworms, and fluazinam and mancozeb would be used as effective control agents against the pathogen.

Contributed paper. Monday, 15:00. **29**

Use of *Beauveria bassiana* (Bals) in the management of larger grain borer, *Prostephanus truncatus* (Horn.) (Coleoptera: Bostrichidae) on stored maize in Tanzania

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Maize (*Zea mays* L.) is important for livelihoods in sub-Saharan Africa as it is the major staple food for the majority of people. In Tanzania 82 % of all farms, 4.5 million farmers in total, produce maize. The greater proportion of the maize (98 %) is produced by resource poor farmers, on an average of 0.8 hectares, in remote villages with poor road networks and post-harvest storage facilities which often make them incur high post-harvest losses. Grain loss in Africa due to insect pests' damage in storage systems is estimated at 20 to 30 %. The larger grain borer (LGB), *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae), a native to meso-America, is known to cause considerable economic losses of up to 48% dry weight. While satisfactory control of LGB has been obtained by use of synthetic pesticides in Tanzania, since its accidental introduction in the late 1970s, their adverse effects on environment, possible development of resistance and residues in food have motivated the search for safer alternative methods. One such strategy is the use of biological control using entomopathogenic fungi such as *Beauveria bassiana* (Bals.-Criv.) Vuill. The current paper presents the findings of an ongoing laboratory study to evaluate the efficacy of a formulation (8.65×10^8 CFU g⁻¹ spore powder) of *B. bassiana*, isolate IMI 389521 against unsexed adult LGB and the maize weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) in Tanzania.

Contributed paper. Monday, 15:15. **30**

Management of *Franklinella occidentalis* (Thysanoptera: Thripidae) with granular formulations of entomopathogenic fungi

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Western flower thrips (WFT), *Franklinella occidentalis*, is a major pest of ornamentals. Mycotized millet grains with entomopathogenic fungi applied to soil of potted marigold plants was tested to target pupating thrips. Two experimental fungal isolates, (*Beauveria bassiana* [ARS7060] and *Metarhizium anisopliae* [ERL1171]), were compared with the registered *B. bassiana* strain GHA [commercialized as BotaniGard®] and untreated controls in greenhouse caged trials. Mycotized millet grains were mixed into the upper surface of the potting soil in pots of flowering 'Hero Yellow' marigolds (4 g/pot). One week after application five mated WFT females were released onto each plant (four plants per cage). At 8 wks post-infestation, the mean total number of thrips per plant was 81 and 90% less in the ERL1171 and ARS 7060 treatments,

respectively, than in the controls. The mean numbers of thrips per plant for the control and GHA treatments were not significantly different. Plant damage was 60% less on plants treated with the experimental fungi than the control and GHA treatments. At 10 wks post-application, 75-90% of WFT collected from the treatments were infected with the experimental isolates. These results demonstrate that soil applications of entomopathogenic fungi can reduce WFT populations significantly and prevent damage.

SYMPORIUM 2 (Microsporidia) Monday, 16:30-18:30

Microsporidiology: Advances in Europe

Symposium. Monday, 16:30. **31**

A new intracellular parasite is a missing link between fungi and microsporidia

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Intracellular obligate parasitism results often in extreme adaptations, whose evolutionary history are difficult to understand, because intermediate forms are hardly ever found. Microsporidia belong to an early-diverging clade of fungi, which evolved extreme physiologic and genomic simplification as well as exceptionally high rates of molecular evolution. They possess the smallest eukaryotic genomes with very few introns, short intergenic regions and bacterial-sized ribosomal genes. As observed in other eukaryotic intracellular parasites, mitochondria in microsporidia have degenerated into small double-layered organelles called mitosomes, which have lost the genome and cannot produce ATP anymore. Instead, they steal it from their hosts. We describe the evolutionary history of a gut parasite of the crustacean *Daphnia* with remarkable morphological similarity to the microsporidia, but genomic features of ancient fungi. This parasite, which we formally name *Mitosporidium daphniae* gen. et sp. nov., possesses mitochondria, genes for oxidative phosphorylation and an infection apparatus typical for microsporidia. Phylogenomics places *M. daphniae* together with the microsporidia in a clade that also includes the most ancient fungi, the Cryptomycota. Comparative genomics further supports the missing link status of *M. daphniae* highlighting both its microsporidian and fungal like characteristics, and reveals the intermediate evolutionary steps that led to extreme metabolic simplification. The new species demonstrates that the extreme reduction in energy metabolism genes as well as the loss of introns in microsporidia was preceded by a reduction in the machinery controlling cell cycle, DNA recombination, repair and gene expression that may have contributed to the characteristically accelerated rate of microsporidia evolution..

Symposium. Monday, 17:00. **32**

Parasite takes fly - A *Drosophila* model of Microsporidia infection

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More than 150 years of Microsporidia research led to a basic understanding of many aspects of microsporidial biology, yet little is known about the genetic basis and molecular mechanisms of the intimate host-parasite relationship that govern Microsporidia infections. Genetic model organisms such as *Drosophila melanogaster* are relevant to the study of human infectious disease as most disease-associated genes have homologues in the fly genome. The knowledge about *D. melanogaster* host defense against obligate intracellular parasites remained, however, particularly patchy for lack of good infection models. A few years ago, a strain of *Tubulinosema ratisbonensis* infested our laboratory fly cultures and led us to model Microsporidia infections in *Drosophila*. Thus, we developed the first infection model of parasitism by a eukaryotic intracellular parasite of *Drosophila*, *T. ratisbonensis*. A unique feature of the *Drosophila* model is that we have developed infection models both in permissive cell lines and in adults. In addition, we have identified several nonpermissive cell lines that will allow us to identify some host defense genes. The ease to move from insights gained at the cellular level from *Drosophila* cell cultures to the whole-organism level using transgenic techniques will allow gaining an in-depth understanding of the biology of Microsporidia in flies, especially when combined with multi-'omics' and functional genomic approaches that we have started to implement. This infection system provides thus novel opportunities to understand the mechanisms underlying microsporidiosis in other invertebrates such as bees and vertebrates hosts and may hopefully lead to novel concepts relevant to parasitology.

Symposium. Monday, 17:30. **33**

White Sea metchnikovellids: morphology, life cycles; potential ancestral features of microsporidia

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Family Metchnikovellidae (Class Rudimicrosporea Sprague 1977) seemingly a basal taxon of Microsporidia, remains understudied. We present data on ultrastructure of two species of metchnikovellids infecting lecudinid gregarines from polychaetes *Pygospio elegans* sampled in the White Sea silt littoral zone. The first species, *Metchnikovella incurvata*, Caullery and Mesnil was described in 1914, the second, *M. spiralis* -- only recently (Sokolova et al., in press). The two species have similar structure of free spores, vary in intracellular development, and produce dissimilar spore sacs (cysts). The cysts of the latter species exhibit unusual morphology: they are limited by a thick electron dense wall, externally ornamented with spirally wound cords of dense material. Basing on comparison of fine morphology and life cycles of metchnikovellids and other microsporidia, I believe

that the following traits could be treated as plesiomorphic among microsporidia: paired nuclei, meiosis, division by internal budding (endopolygeny), short or anisofilar polar filaments, and sequence producing thick-walled environmental cysts. Metchnikovellidean spores possess short polar filaments (manubria) and likely do exploit the mechanism of dispersion via evertting the polar tube with the attached sporoplasm, the major synapomorphy of Microsporidia. At the same time metchnikovellidan spores are devoid of most elements of the extrusion apparatus: a polaroplast, posterior vacuole, rigid spore wall, and long polar filament connected with a polar disc. The minimal apparatus of metchnikovellids may allow dissemination only within one cell (autoinvasion), whereas production of thick-walled cysts enables horizontal transmission of spores among hosts. .

Symposium. Monday, 18:00. **34**

Microsporidia: Pathogens of Opportunity

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Kudo published the first comprehensive treatise on the microsporidia "A Biologic and Taxonomic Study of the Microsporidia" in 1924 which was a critical review and systematic treatment of all literature on the microsporidia at the time. It would be more than 50 years before another treatise would be produced, "Biology of Microsporidia" in 1976 and Systematics of the Microsporidia" in 1977 by Vavra and Sprague. This would remain the "go to" authority on the microsporidia until 1999 when "The Microsporidia and Microsporidiosis" was published containing chapters on microsporidia in vertebrate and invertebrate hosts and the first comprehensive review of the growing field of molecular biology and phylogeny of the microsporidia. With the field rapidly advancing in many aspects of basic and molecular biology of the microsporidia it was apparent that a revision and expansion of the previous volume was needed. This effort has resulted in the Microsporidia: Pathogens of Opportunity L.M. Weiss and J., J. Becnel (Eds.) John Wiley & Sons, Oxford, UK with 25 chapters compiled by experts including evolutionary and molecular biologists, veterinarians, entomologists, ichthyologists and physicians who study microsporidia. This is intended as a resource for those students and young researchers with an interest in the study of microsporidia as well as expanding the knowledge base of microsporidiologists from different disciplines within the field. An overview of the various chapters will be presented and topics of current relevance highlighted.

CONTRIBUTED PAPERS Monday, 16:30-18:30

NEMATODES 1

Contributed paper. Monday, 16:30. **35**

Measuring entomopathogenic nematode activity, abundance and soil food web assemblage in Swiss wheat and maize cultivation

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Wheat and maize are major crops in Switzerland. As part of a research consortium that explores ways to improve soil health, we study how entomopathogenic nematodes (EPNs) can be better exploited for the biological control of soil-dwelling insect pests. We evaluated the impact of different agricultural management practices on native EPN populations in two 30-years Swiss field trials. One experiment compared tillage versus no-tillage and monoculture (wheat) versus crop rotation (maize), whereas the second studied four levels of tillage in two soil types planted with wheat. Soil samples were taken in April and in October 2013 ($n = 88$). Total nematode activity, as recorded with the *Galleria*-bait technique was <5%, with no significant effect of the treatments. Real time qPCR revealed that >95% of infected cadaver contained a mix of EPN with the competing *Acroboloides* -group and/or *Oscheius* sp. The available molecular probes identified and quantified 13 organisms from soil, comprising six nematophagous fungi (NF), ectoparasitic bacterium, two free-living nematodes (FLN), and four EPNs (the evaluation of an additional ten EPN species is ongoing). In general, only trace levels of EPN were detected in all soils. *Heterorhabditis* spp. were the dominant EPN, with *H. bacteriophora* being significantly reduced by tillage ($P < 0.001$). Monoculture favored the competitors of EPN ($P < 0.01$). The abundance of EPN, NF and FLN was positively correlated ($P < 0.05$). Since only low numbers of EPN are naturally present in Swiss agricultural soils, an augmentation strategy may help to improve the control of root pests of wheat and maize.

Contributed paper. Monday, 16:45. **36-STU**

Biocontrol and nutrition: understanding the role of environment in the trait deterioration of an entomopathogenic nematode symbiont

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Entomopathogenic nematodes (EPNs; genera *Heterorhabditis* and *Steinernema*) kill their invertebrate hosts with the aid of a mutualistic bacterium. The bacteria (*Xenorhabdus* spp. for steinerinemats and *Photorhabdus* spp. for heterorhabditids) are primarily responsible for killing the host and providing the nematodes with nutrition and defense against secondary invaders. EPNs are amenable to laboratory rearing and mass production for biocontrol applications against insects; however,

EPNs and their symbiotic bacteria exhibit trait deterioration or changes due to laboratory rearing. The overall goal of this project is to understand how virulence in the nematode-symbiont *Photorhabdus* has evolved in an *in vitro* environment and the role nutrition plays in this process. Nutritional effects in trait deterioration were determined using monoxenic cultures of a freshly isolated strain of *P. luminescens* subsp. *luminescens* where base populations were compared with bacteria that were sub-cultured repeatedly to determine fitness loss. Trait stability was monitored in three different liquid media that are frequently used in laboratory culture: Liquid Lipid Medium, nutrient broth, and tryptic soy both + 0.5% yeast extract. Subpopulations were compared to base populations for inclusion bodies after 5, 15, and 20 liquid growth cycles (of 48 hours each). Additionally, growth curves and LT_{50} values were determined for the base and deteriorated populations. Differences were observed among the media types and the base/deteriorated populations. Understanding nutritional effects on important biocontrol traits may aid in more efficient methods of mass production.

Contributed paper. Monday, 17:00. **37**

Insect-killing nematodes also kill competitors: lethal male-male fighting in *Steinernema*

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Steinernema spp. are well-known as entomopathogenic nematodes. We have found that males of certain species fight and kill each other, and that killing is influenced by the developmental pathway followed. The transmission stage of *Steinernema* is an infective juvenile (IJ) analogous to the dauer juvenile of *Caenorhabditis elegans*. IJs seek out and enter living insects in soil. Inside the insect they release symbiotic bacteria (*Xenorhabdus* spp.) which proliferate and digest the host tissues. This provides a rich nutritive medium for the developing nematodes, which reproduce in the host cadaver. A large host may support several generations of nematodes, and thus represents a valuable resource, worth competing for. In *Steinernema longicaudum*, males of the founding generation (those developing from IJ) fight by wrapping their tail ends around each others' bodies and squeezing. Victims may appear paralysed within minutes of such an encounter, and frequently die. Worms that develop within the host cadaver in second or later generations develop directly, without passing through the IJ stage. For such worms, the benefits of fighting (the quality of the resource) is diminishing, while the large number of rivals present means that the benefits of killing do not necessarily accrue to males that kill. We have found that males that develop directly, without passage through the IJ stage, are much less likely to fight than those that do, and that this appears to be a developmental effect rather than a response to conditions at the time of fighting. .

Contributed paper. Monday, 17:15. **38-STU**

Comparison of Life History Traits of the Entomopathogenic Nematodes *Steinernema feltiae* and *Steinernema riobrave*

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Life history traits (LHTs) of *Steinernema feltiae* and *S. riobrave* were assessed at 25°C using a hanging drop technique. The LHTs were studied with 5 x, 10x and 20x 10⁹ cells ml⁻¹ of *Xenorhabdus bovieri* and *X. cabanillasii* for *S. feltiae* and *S. riobrave*, respectively, in semi-fluid nematode growth gelrite. The results indicated that increased food density had a significant positive influence on offspring production and net reproductive rate (R_0) on both, *S. feltiae* and *S. riobrave*. Highest offspring production was recorded at bacterial food densities of 20 x 10⁹ cells ml⁻¹ with 813/female for *S. feltiae* and 1,913 offspring/female for *S. riobrave*. Higher R_0 values of 707 and 1,903 were recorded for *S. feltiae* and *S. riobrave*, respectively. A significant positive correlation between bacterial density and body volume that contributed to an increased offspring production was found in both species. The lowest intrinsic rate of natural increase (r_m) (1.1 days) was recorded for *S. feltiae* and the highest (1.4) for *S. riobrave*. A population doubling time of PDT = 0.6 days was recorded for *S. feltiae* and 0.5 days for *S. riobrave*. The life span of female nematodes was not significantly different among the bacterial food densities tested. Significant differences in offspring production and population growth rate were recorded between the two species. The result can be used to further investigate the optimal bacterial food density for mass production in bioreactors for maximum DJ recovery in liquid bacterial suspension, synchronised population development and DJ yields of *S. feltiae* and *riobrave*.

Contributed paper. Monday, 17:30. **39 STU**

How does plant domestication influence entomopathogenic nematodes as potential biological control agents?

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We are studying the effects of plant domestication on belowground plant induced responses to herbivore feeding and how this affects entomopathogenic nematode (EPN) populations. In the New Jersey Pinelands, natural populations of highbush blueberries (*Vaccinium corymbosum*) are commonly found adjacent to commercial fields of domesticated highbush blueberries. In a 3-year field study, we found that EPN were more prevalent but less diverse in cultivated fields than in natural stands. The dominant species in both habitats was *Steinernema glaseri* (Sg); numerous isolates of two distinct Sg strains were identified. In laboratory studies with oriental beetle (*Anomala orientalis*) larvae, the dominant root-feeder in cultivated fields, Sg blueberry isolates were less virulent than the Sg NC strain, and strains from cultivated fields tended to be more virulent than those from natural stands. We are using the same Sg strains in laboratory and field studies on EPN attraction to blueberry roots as affected by oriental beetle feeding. Ongoing studies suggest that Sg is attracted more strongly by damaged roots. We have yet to identify any herbivore induced plant volatile (HIPV) responsible for enhanced attraction. A comparison of 2 known HIPVs emitted from roots in other systems suggests that (E)-β-caryophyllene is more attractive than pregeijerene.

Contributed paper. Monday, 17:15. **40**

Analysis of intraspecific variability in *Steinernema kraussei* populations using PCA

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Species determination in Entomopathogenic Nematodes of the genera *Steinernema* and *Heterorhabditis* is a very complex task, given the broad variability of both morphological and morphometric traits within a single species. To accomplish that, molecular techniques have been adopted which however require additional knowledge. Particularly relevant would be the possibility of testing in a reliable way the variability between different populations of the same species, which might represent different strains with different biological properties. Aim of our work was to determine if morphometric analysis, performed using the "Principal Component Analysis" approach, was able to get evidences of characters with significant diagnostic value, allowing to make reliable distinctions among strains. Four strains of *Steinernema kraussei* were found in Italy, three from Sicily and one from Alps (Tarasco et al., 2014; doi:10.1017/S0022149X14000194). Morphometric analysis of morphological traits commonly used in nematode taxonomy (referred to as variables) was done on 20 juveniles, males and females of first and second generations (or observations) belonging to three strains: 3D and PL (Sicily) and BT (Alps). Statistics was done by SIMCA package v.13. Up to three components were routinely computed; score plots, loading plots, X/Y overview and contribution plots were obtained. Our results showed that some of the morphometric variables employed could reliably be used to discriminate both juvenile and adult forms of PL strain, whereas an insufficient distinction could be made between BT and 3D.

Contributed paper. Monday, 17:30. **41**

Population genetic structure of entomopathogenic nematode *Steinernema affine* (Steinerematidae: Nematoda) inferred using microsatellite markers

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Population genetic structure of entomopathogenic nematodes is still poorly understood even though such knowledge could help us to assess stability and vulnerability of natural EPN populations. Molecular markers used in EPN taxonomy and phylogeny (ITS and LSU regions of rDNA, NAD4, COII) are too conservative to be used to assess within species variability. So far only few studies attempted to use AFLP method to investigate EPN intra-population variability. In present study, microsatellite markers for *Steinernema affine* were developed. In total 218 bioinformatically validated pairs of primers for various oligonucleotides were obtained. Thirty most promising oligonucleotides were selected and tested for the use in the study of the species' population genetic structure. Markers showing variability were identified and examined in various populations of *S. affine*, collected mainly in the area of South Bohemia..

Contributed paper. Monday, 17:45. **42 STU**

Eat or Be Eaten: Fungus and Nematode Switch off as Predator and Prey

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The parasitic nematode *Deladenus siricidicola* is widely used for the biological control of the invasive pine-killing woodwasp, *Sirex noctilio*. The nematode has a unique life cycle where it lives in pine trees, feeding on the symbiotic fungus of *S. noctilio*, the basidiomycete white rot fungus *Amylostereum areolatum*. In the presence of *S. noctilio* larvae, however, the nematode develops into a parasitic form which invades the woodwasp larvae, ultimately leading to sterilization of the host. The fungal-feeding stage of the nematode is used to commercially mass produce it for biological control programs. Previous studies investigating the effect of *A. areolatum* strain on *D. siricidicola* reproduction suggested the possibility of a role reversal where the fungus could eat the nematode. The present study examined the relationships between three species of *Deladenus* nematodes and their associated *Amylostereum* fungi. For *D. siricidicola* and *A. areolatum*, we hypothesized that significantly fewer nematode eggs placed in petri dishes containing potato dextrose agar medium would hatch in the presence of *A. areolatum* fungus than in control petri dishes with no fungus. Results supported this hypothesis. Additionally, light microscopy, fluorescence microscopy, and cryogenic scanning electron microscopy were used to show the ability of both *A. areolatum* and a second species, *A. chailletii*, to penetrate nematode eggs and adult living females of three species of *Deladenus* nematodes.

CONTRIBUTED PAPERS Monday, 16:30-18:30

VIRUSES 2

Contributed paper. Monday, 16:30. **43**

Insect feeding induces transgenerational resistance to NPV in Lepidoptera

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When population density increases, insects experience a number of stresses as a result of crowding including alteration in food quality and quantity. These changes have been shown to alter the resistance of insects to pathogens. Previous studies have tended to investigate the impact of these factors individually; however, in nature density-related factors are likely to interact simultaneously. While changes that occur within a single generation have been well documented, we know less about the transgenerational impact of changes in food quality on disease resistance. We tested the impact and interaction of three factors that are likely to occur in insect populations when density rises using the western tent caterpillar, *Malacosoma californicum pluviale*. Western tent caterpillars exhibit population cycles every 8-11 years, which

are characterized by NPV epizootics at high density. We manipulated food quality, food quantity and the presence of phylloplane bacteria in the parental generation and measured the impact on immunity and resistance to NPV in the offspring. The treatments, particularly the foliar treatments, had clear impacts on the disease resistance of the offspring generation; however, not necessarily in the direction predicted. We discuss these data in relation to how changing levels of susceptibility could influence population cycles in these forest insects.

Contributed paper. Monday, 16:45. **44**

The resistance of *Cydia pomonella* against baculoviruses is provoked by a mutation of the immediate-early pe38 gene of *Cydia pomonella* granulovirus

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The *Cydia pomonella* granulovirus (CpGV) (*Baculoviridae*, genus *Betabaculovirus*) is a worldwide used biological agent to control the infestation of pome fruits by codling moth (*C. pomonella* L.). In 2005, first CM field populations resistant to commercial CpGV products containing the isolate CpGV-M (so-called Mexican isolate) were discovered in Europe. These resistant CM populations showed 1,000 – 100,000fold reduced susceptibility to CpGV-M when compared to normally susceptible CM populations. Infection experiments with isolates from different geographical origins showed that various CpGV isolates were able to overcome CM resistance in the genetically homogenous resistant laboratory CM strain. Molecular analysis of these resistance overcoming isolates (-I12, -I07, -S, and -E2) showed that the only genomic difference, which all resistance overcoming isolates have in common, is a single common 24 nucleotide indel mutation coding for eight amino acids within the immediate-early gene pe38. Phylogenetic analyses presume that this mutation is an insertion within the genome of CpGV-M.

Therefore, the role of pe38 in overcoming the resistance of CM was analyzed by constructing knockout and rescue pseudoviral mutants based on a CpGV-M bacmid. According to the source of pe38, we could show that the pseudoviruses are infective against susceptible larvae only - in the case of pe38 from CpGV-M - or against both susceptible and resistant larvae - in the case of pe38 from CpGV-S. Therefore, we conclude that pe38 is not only an essential factor for the infectivity of CpGV but also the key factor in overcoming CpGV resistance in CM.

Contributed paper. Monday, 17:00. **45**

CpGV-R5 allows replication of CpGV-M in resistant host insect larvae

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In resistant codling moth larvae, CpGV-M replication is blocked at an early step in all tissues. Among others, the CpGV-R5 isolate is able to overcome this resistance. A genetically heterogeneous virus population, containing 1% CpGV-R5, and

99% CpGV-M has been used to infect non permissive host populations. Unexpectedly, this mixture, and the OBs recovered from killed larvae performed 100 times and 2000 times better than CpGV-M used alone, respectively. qPCR analysis using specific markers for each viral isolate was performed. The viral mixture CpGV-R5, 1%, and 99% of CpGV-M was amplified on permissive (Cp_{NPP}) and non-permissive (R_{GV}) populations and their offspring was tested for their respective proportion of each kind of marker. On permissive host, the R/M markers ratio raised to 15/85. On resistant host, a similar R/M ratio (12/88) was obtained, indicating that CpGV-M has been able to perform a complete replication cycle in a non-permissive host. These results suggest that in the presence of a small proportion of CpGV-R5, CpGV-M is able to replicate in resistant hosts. Accordingly, CpGV-R5 seems to act as a helper for CpGV-M genomes. Understanding the mechanism involved in the unlocking of the replication process opens the possibilities of innovative control strategies.

Contributed paper. Monday, 17:15. **46**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

Agata K. Jakubowska¹; Melania D'Angiolo¹; Rosa M. González Martínez¹; Anabel Millán Leiva¹; Arkaitz Carballo²; Rosa Murillo²; Primitivo Caballero²; Salvador Herrero¹
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Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Contributed paper. Monday, 17:30. **47**

Mixed SeMNPV genotypes comprised transmission capacities and insecticidal properties

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Recent studies have demonstrated that transmission of *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) parents to offspring (vertical transmission) is frequent and could contribute to biological control of this pest by causing viral mortality in the pest population in successive cropping cycles. The aim of this work was to study the efficacy of using mixtures of two SeMNPV genotypes that had either high insecticidal properties (SeG25) or the capability to be transmitted through host generations (SeAl1). Mixed populations containing 25 and 75% of SeG25 resulted in increased pathogenicity (LC_{50}) compared to the SeAl1 genotype. However in terms of virulence (mean time to death) and productivity (OBs/larva), no differences were observed between the individual genotypes or their mixtures. The capacity to induce persistent infections by each genotype and their mixtures was evaluated using qPCR (*DNA-polymerase* gene) in adult survivors of a sublethal dose of the virus. The prevalence of covert infection varied between 70 and 100% in adults that survived inoculation with the vertically transmitted genotype Se-Al1. The adult survivors to the mixtures and the SeG25 genotype alone are currently being analyzed to determine covert infection. Finally, field trials were carried out to evaluate the capacity of mixed virus populations to establish covert infections in greenhouse conditions. Adults developed from larvae collected in experimental plots sprayed with either single genotypes or one of the mixtures 75%Al1+ 25%G25 (75:25) and 25%Al1 +75%G25 (25:75) are being processed currently. The F₁ offspring from adult survivors of SeAl1, 75:25, 25:75, SeG25 and control treatment did not show differential susceptibility to a 25:75 mixture of OBs. The implications of these findings will be discussed.

Contributed paper. Monday, 17:45. **48-STU**

A novel mode of resistance of codling moth against *Cydia pomonella granulovirus*

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The codling moth (CM, *Cydia pomonella*) is one of the most devastating pests in nearly all pome fruit growing regions. An alternative to the application of chemical insecticides is the application of *Cydia pomonella granulovirus* (CpGV) (family *Baculoviridae*), which is registered as biological control agents in 34 countries worldwide. Since 2005, CM populations with a reduced susceptibility to CpGV products have been reported from about 40 plantations in seven European countries. For many of these CM populations, the resistance could be traced back to a single, dominant allele that is linked to the sex chromosome Z. CpGV-M, the so-called Mexican isolate, was the common agent used in all commercial CpGV products registered in Europe. Currently, resistance management strategies are based on the application of improved CpGV products, containing resistance-overcoming isolates. However, a CM field population, termed NRW-WE showed even resistance to most resistance overcoming CpGV isolates, suggesting a second mode of CpGV resistance.

In order to elucidate the inheritance of this type of resistance and after failure of single crossing experiments, successive mass crossings under virus pressure were carried out to establish a genetically homogenous resistant strain of the CM population NRW-WE. Subsequent reciprocal crossing experiments with the resulting CM strain and a susceptible laboratory CM strain (CpS) followed by bioassays fitted to a dominant but autosomal inheritance model. Further analyses of the mode of resistance are under way. .

Contributed paper. Monday, 18:00. **49**

The effects of temperature on *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) in mortality rates of false codling moth larvae *Thaumatotibia leucotreta*

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False codling moth (FCM), *Thaumatotibia leucotreta* is a major citrus pest in South Africa. *Cryptophlebia leucotreta* granulovirus (GrleGV-SA) has been found to be a successful biological control agent for FCM. South Africa grows citrus in many different geographical areas throughout the country that experience different temperature differences; this in turn could affect the efficiency of the virus upon the larvae. The aim of this study was to determine the effectiveness of the virus on larvae at temperatures ranging between 15–35°C. Unpaired T-tests, one-way ANOVA tests and post-Hoc Tukey's HSD tests were conducted on both virus and control treatments to test for significant differences among different temperatures as well as between the virus and control treatments. The number of deaths between infected and control treatments were significantly different at all temperatures. The differences between treatment mortality times were significantly different for all infection stages except the final death stage (5th stage). The virus was found to be most efficient at higher temperatures since the larvae grow faster at higher temperatures. The virus was found to have very little effect at 15°C. These results should assist with the control of FCM in citrus orchards, and in particular would affect the timing of applications, to ensure that the virus is used at its maximum efficiency.

Contributed paper. Monday, 18:15. **50**

Enhancement of insecticidal activity of a nucleopolyhedrovirus isolated from *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) by coinfection with granulovirus

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Spodoptera frugiperda is a polyphagous pest with wide geographical distribution. Biological control of this pest has included the use of its nucleopolyhedrovirus SfMNPV, which has shown high potential as biopesticide with efficacies higher than 80% but with some disadvantages related with cost production and time of action. In this sense, other viruses as betabaculovirus (GV) may act as synergists, increasing the insecticidal activity of NPVs. In this work, a Colombian granulovirus isolated from *S. frugiperda* larvae (VG008) was mixed with two different NPVs samples, one corresponding to a wild virus NPV003 and other corresponding to a pure genotype variant obtained from NPV003 (NPV003-A). Each mixture was evaluated in different proportions and in five different concentrations since 1×10^4 OB/mL to 1×10^9 OB/mL. For each mixture, the median lethal concentration (LC_{50}) and mean time of mortality (MTM) were determined by laboratory bioassay in second instar larvae of *S. frugiperda*. Majority of mixtures between the VG008 and NPV003 showed a higher biological activity compared with each individual isolate, confirming the coinfection enhancement effect. The mixture corresponding to 2.5% of VG008 and 97.5% of NPV003, showed the highest enhancement of the NPV insecticidal activity with a decrease of 9.92 times in the LC_{50} and 4 days (96 hours) in the MTM. This virus mixture was selected and will be used as an active ingredient for the development of a new biopesticide based on both viruses in order to improve NPV efficacy for controlling the pest in the field.

CONTRIBUTED PAPERS Monday, 16:30-18:00

FUNGI 2

Contributed paper. Monday, 16:30. **51**

Rapid and simple method for overnight development of strain-specific markers: A case study with the commercial *Beauveria bassiana* strain, GHA.

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Genetic markers have proved useful for assessing taxonomy and identifying specific-strains of entomopathogenic fungi. We targeted *Beauveria bassiana* commercial strain, GHA to develop a new reliable, simple, specific, sensitive and cost effective method that allows specific detection and discrimination of GHA from other *Beauveria* strains. We applied a combination of software with intrinsic manipulations to design GHA strain-specific primers by exploiting available *Bloc* nuclear intergenic sequences of GHA and other *Beauveria* strains. The generated primers were used in PCR assays to probe strains of *B. bassiana* (50), *Beauveria pseudobassiana* (13), *Beauveria brongniartii* (3), *Beauveria amorpha* (2), *Beauveria vermicronia* (2), *Beauveria asiatica*, *Beauveria australis*, *Beauveria kipukae*, *Beauveria malawiensis*, *Beauveria sungii* and *Beauveria varroae*. In the specificity test, we amplified the expected target gene and ~300-bp-fragment from *B. bassiana*, GHA DNA. All other tested strains/isolates reacted negatively with the exception of four out of fifty *B. bassiana* strains that produced positive signals. In addition, the designed primers were highly sensitive; capable of detecting ~20 pg/ μ l of GHA genomic DNA. For operational feasibility, the newly designed marker would be used for studying the ecology, persistence and monitoring autodissemination of post-released GHA in the environment. To date, our methodology and associate protocol could be considered the simplest with high sensitivity and specificity, and most cost effective strategy for strain-specific marker design in the highly heterogeneous *Beauveria* species complex. Our approach provides a general framework that can be readily or easily adapted for designing strain-specific markers targeting any organism of choice.

Contributed paper. Monday, 16:45. **52-STU**

The functions of two Cu/Zn-superoxide dismutases and a Fe-superoxide dismutase in regulating the growth, antioxidation, UV tolerance and virulence of *Beauveria bassiana*

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The biocontrol potential of filamentous entomopathogenic fungi, such as *Beauveria bassiana*, depends not only on the virulence of a candidate strain to target pests but also on its tolerance to high temperature and solar UV irradiation often encountered in the field. The stress of UV, heat, drought, or

chemical may induce the production of cellular reactive oxygen species (ROS), which cause damages to most biomolecules such as DNA, protein, and lipids. Fungal superoxide dismutases (SODs) that detoxify superoxide anions could be putative virulence factors for entomo-pathogenic fungi. Three genes encoding SODs have been identified in the *Beauveria bassiana*: a cytoplasmic Cu/ZnSOD (BbSod1), a mitochondrial FeSOD (BbSod4) and a cell-wall Cu/ZnSOD (BbSod5). During growth, *BbSod4* was weakly expressed compared with other SODs and the deletion of *BbSod4* was lethal. To probe their effects on the biocontrol potential of *B. bassiana*, $\Delta BbSod1$, $\Delta BbSod5$ and three hairpin RNA-interfered (RNAi) mutants were constructed and assayed for various phenotypic parameters in conjunction with $\Delta BbSod1$ /*BbSod1*, $\Delta BbSod5$ /*BbSod5* and wild-type (control strains). The knockout mutants showed phenotypic alterations, including delayed sporulation and impaired conidial quality, but little change in RNAi mutants. Their mycelia or conidia became more sensitive to menadione or H_2O_2 induced oxidative stress but had little change in resistance to hyperosmolarity and wet-heat stress. Their UV tolerance and virulence was also impaired. Transcriptional changes of five *Sod* genes and other relative genes described try to explain the phenotypic changes among the mutants. Our finding highlight that these three *Sods* regulate the oxidative resistance in different method, thereby exerting profound effects on the fungal biocontrol potential.

Contributed paper. Monday, 17:00. **53-STU**

Effect of temperature, water activity and UV-B radiation on conidium germination and colony growth of *Beauveria bassiana* isolates from soil and phylloplane

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Have entomopathogenic fungi phylloplane isolates any advantage over soil isolates in terms of environmental competence and virulence? To address this question, 20 *Beauveria bassiana* isolates from soil and phylloplane of two holm oak ecosystems in Southern Spain pathogenic to medfly *Ceratitis capitata* adults and belonging to different type sequences and genotypes as interfered from EF-1 α , Bloc and microsatellites were selected and their comparative response to temperature, water activity and UV-B investigated. Effect of temperature on germination and colony growth rate was monitored in the range of 15–35°C, with optimum temperature ranging from 23.8–28.7 °C. All isolates showed maximum germination values between 1 and 0.996 water activity (a_w). Germination at a_w values lower than 0.928 were not observed for any isolate. Moreover, conidia were exposed to different irradiances (920 and 1200 mWm $^{-2}$) during 2, 4 and 6 hours, and germination, culturability and mycelia growth were evaluated. These results show that a "recovery" of the fungal propagules could occur after being exposed to UV-B, even if such recovery is lower for longer exposure times (6h) and irradiance (1200 mWm $^{-2}$). Therefore, the answer may be now addressed: the fungus isolation habitat does not always provide advantage in terms of environmental competence.

Contributed paper. Monday, 17:15. **54**

Non-target aquatic arthropods testing of *Metarhizium* strains and their crude extracts produced by solvent extraction and nanofiltration technology

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Strains of insect pathogenic fungi within the genus *Metarhizium* have increasingly been developed for the control of pest species. Risk assessment studies are a prerequisite before the fungus can be registered as a plant protection product. In this work we determined the risks posed by preparations of secreted metabolites and viable conidia to two aquatic, ecological toxicity indicator species, *Artemia salina* and *Daphnia pulex*. Organic solvents (OS) are normally used to extract the metabolites but they pose a risk to human health and the environment. Nanofiltration (NF) is an environmentally responsible technology that can be used to extract the metabolites as an alternative to the OS. Since risk assessment of each secondary metabolite produced by EPF could be a long and expensive process, the RAFBCA-REBECA decision scheme proposes evaluation of the risks posed by crude extracts. Therefore, three fungal strains (BIPESCO5, ARSEF 4556, and ARSEF 3297) were produced in three different culture media [Czapek-dox + peptone, Czapek-dox + yeast, and 10:1 (C:N ratio)], and their metabolites extracted by OS and NF methods. The chromatographic profiling of all the products was determined and their toxicity tested against *A. salina* and *D. pulex*. Concomitantly, the pathogenicity of the strains was tested against these non-target arthropods. At a relatively high dose (10^8 conidia ml $^{-1}$), the conidia could cause 69% and 75% mortality in *A. salina* and *D. pulex* respectively. Both arthropods were sensitive to metabolites. Mortality depended on the fungal strain, extraction method, and test organism. Our study showed that *A. salina* and *D. pulex* mortality was due to the combination of *Metarhizium* conidia induced stress as well as secreted metabolites.

Contributed paper. Monday, 17:30. **56-STU**

Development of analytical methods for the analysis of *Metarhizium brunneum* metabolites in crop matrices

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The main secondary metabolites produced by the entomopathogenic fungus *Metarhizium brunneum* are destruxins (dtxs), cyclic hexadepsipeptides, which exhibit a wide variety of biological activities. Overall they are best known for their insecticidal and phytotoxic activities. Since the fungus is used for biological control of insect pests there are some concerns regarding whether the produced secondary metabolites entail risks to humans and the environment. To assess if the major secondary metabolites secreted by *M. brunneum* enter the food chain a two-step sample preparation protocol, consisting of the sample extraction by the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method followed by the sample purification by offline solid phase extraction on a reversed phase material was established. For the analysis and quantification of dtx congeners a fast and selective UHPLC-DAD/TOF-MS method based on a previously developed method was optimized. It

turned out that the QuEChERS-method is an efficient way to extract dtxs from different crop matrices. Using offline SPE for the clean-up of the samples analytes can be separated from disturbing matrix compounds and quantified by the UHPLC-DAD/TOF-MS method.

Contributed paper. Monday, 17:45. **57-STU**

α-1, 2-mannosyltransferase ktr1, ktr4 and kre2 regulate positively growth, conidiation, viability, virulence, and multi-stress tolerances in Beauveria bassiana

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Entomopathogenic fungus *Beauveria bassiana* is a mycoinsecticide against arthropod pests. Three α -1, 2-mannosyltransferase proteins (Ktrp) named Bbktr1, Bbktr4 and Bbkre2 are responsible for extension of the second and third mannose residues on secretory protein. Here, we characterized the role of three Ktrp in *B. bassiana* and found that they were positive, but differential, regulators of the growth, conidiation, multi-stress tolerance and virulence of the entomopathogenic fungus. The three disruptions accompanied with their corresponding complement $\Delta Bbktr1/ktr1$, $\Delta Bbktr4/ktr4$, $\Delta Bbkre2/kre2$ and wild-type were constructed. $\Delta Bbktr4$ and $\Delta Bbkre2$ grew 50–83% slower on nutrition-rich and limited media while $\Delta Bbktr1$ show similar colony sizes on all the tested media. Their conidial yields on a standard medium were reduced by 31–96%, accompanied with abnormal germination. All the mutants became significantly less tolerant to most stresses of cell wall perturbation, high osmolarity, oxidation, wet heat and UV-B irradiation during colony growth. Furthermore, the Ktrp mutants were altered in cell wall structure and composition, which contributed to the thickness of cell wall, increased sensitivity to lyase, the low conidial hydrophobicity and cell surface carbohydrate epitopes. Coincidentally, the attenuated cell wall in Ktrp mutants also brought out the more protoplast to release. Remarkably, insect bioassays revealed decreased virulence in $\Delta Bbktr4$, $\Delta Bbkre2$ for 18% and 1.2-fold with topical application, and 31% and 26% with intrahemocoel injection. Our findings revealed that Ktrp plays a central regulatory role in *B. bassiana*.

typographus) have huge economic and ecological impacts in conifer forests worldwide. Just in the last 25 years the spruce bark beetle has killed millions of cubic meters of Norway spruce (*Picea abies*) in Europe. Trees are killed by a combination of pheromone-mediated mass-attacks and infection with phytopathogenic bluestain fungi vectored by the beetles. *Ceratocystis polonica*, the most virulent fungal associate of the spruce bark beetle, can kill healthy trees in the absence of beetle attack if it is experimentally inoculated into the bark at high densities. Norway spruce protects itself against combined beetle-fungus attacks by multiple preformed and inducible defense mechanisms. Structurally diverse mixtures of mono-, sesqui- and diterpenes are central components of these defenses. Preformed terpenes stored in resin ducts in the bark and sapwood may repel or inhibit initial attacks. Terpene levels increase tremendously following induction by e.g. fungal infection or application of methyl jasmonate (a defense-inducing plant hormone). This induced terpene response reduces pheromone emission by the spruce bark beetle and inhibits tree colonization in a dose-dependent manner. However, fungal associates of the spruce bark beetle can greatly reduce monoterpane levels in the tree by biotransforming them to oxygenated monoterpenes. In addition, the fungi also produce different metabolites which may play multiple roles in bark beetle host finding and colonization. These observations demonstrate the complicated interactions between conifer-bark beetle-fungi.

Symposium. Tuesday, 8:20. **59**

Carbon dioxide as an orientation cue for western corn rootworm and wireworm larvae - implications for an attract and kill approach using entomopathogenic fungi

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The larvae of soil dwelling insects use carbon dioxide gradients, established by growing roots, to orientate towards their host plants. This long distance orientation cue is complemented by other volatile cues to finally accept a host plant for feeding. Previous application strategies using entomopathogenic fungi for soil pest control were using high concentrations of spores per m², set against competing microorganisms in the rhizosphere. In the attract and kill approach the strategy is turned upside down: larvae voluntarily make their way to the spores, contained in capsules emitting CO₂. When near to these capsules, probability of larval infestation with spores is higher. However, to make this strategy work, the capsules need to fulfill several prerequisites, such as building up a gradient significantly higher than the background CO₂ concentration in the soil, maintained for at least several weeks, and the larvae need to be attracted to the capsules to feed on them. In lab experiments we assessed the larval behavior of corn rootworms and wireworms towards these artificial CO₂-capsules. Both pest larvae were attracted by the capsules, but only stayed for short periods at these sites. Thus, additional compounds need to be incorporated into these capsules to increase their attractiveness for the larvae. In German field experiments these capsules, combined with *M. brunneum*, were used in potato fields for wireworm control. Treatments resulted in significantly lower tuber damage in some, but not all fields. Necessary improvements of the attract and kill strategy for anapplication in the field are discussed.

TUESDAY - 5 August

SYMPORIUM 3 (Fungi) Tuesday, 8:00-10:00

Fatal attraction: Fungi and Odours in Deadly Combinations for Pest Control

Symposium. Tuesday, 8:00. **58**

Conifer - bark beetle - fungus interactions

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Tree-killing bark beetles such as the spruce bark beetle (*Ips*

Symposium. Tuesday, 8:40. **60**

Different behavioral responses in specialist and generalist natural enemy interactions (predators and fungi) in a strawberry-mite pest system

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Natural enemies like arthropods and entomopathogenic fungi both contribute to the natural regulation of pests in many crops. As arthropod natural enemies have evolved, they have become a part of a complex multitrophic system and they exist alongside species of entomopathogenic fungi. Some of these entomopathogenic fungi may actually also be a potential threat to arthropod natural enemies. Both arthropod predators and entomopathogenic fungi are important biological control agents of the two spotted spider mite, *Tetranychus urticae* in strawberry. Previous studies on the interactions between these two types of natural enemies show variable results in regards to synergistic/antagonistic effects. We speculated if the degree of specialization of the predator or the fungus could play a significant role. Therefor a behavioral study was conducted to investigate the searching and feeding time of predators (two species tested) in the presence of entomopathogenic fungal spores (two species tested). The predator species used in this study were the generalist predatory bug, *Orius majusculus* and the specialist predatory mite, *Phytoseiulus persimilis*. The entomopathogenic fungal species used was the generalist *Metarrhizium brunneum* and the specialist *Neozygorites floridana*. Predator behavior was recorded by observations in an experimental setup where the predator was given a choice between two strawberry leaf discs; one with entomopathogenic fungal spores and one without, and both with healthy *T. urticae*. Results suggest that searching and feeding times of both predator species was lower on leaf discs with presence of *M. brunneum* spores compared to no fungal spores. On leaf discs with *N. floridana* spores the searching time of both predators was higher compared to no fungal spores. *O. majusculus* spent more time feeding on prey on the leaf disc with spores of *N. floridana* than on leaf discs without spores, while *P. persimilis* spent less time feeding on the leaf discs with *N. floridana* spores, compared to leaf discs with no fungal spores. Results indicate that the degree of specialization of the beneficial organisms plays a role in the interaction between arthropods and entomopathogenic fungi. Such interactions are important to consider when biological control using several biological control agents is developed.

Symposium. Tuesday, 9:00. **61-STU**

How *Fusarium graminearum* influences insect-plant interactions

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Fusarium Head Blight (FHB) disease affects cereals globally, and is caused by a range of toxicogenic fungi from the genus *Fusarium*. Wheat (*Triticum aestivum*) is most susceptible to FHB during flowering. The role of insect pests in FHB epidemiology is poorly understood, so the objective of this work was to determine the interactions between the most dominant FHB pathogen, *Fusarium graminearum*, and insect pests that

would co-localise on host plants. Grain aphids, *Sitobion avenae*, were used as they are known to colonise wheat ears during flowering. Wheat ears were treated with combinations of fungal inoculum and grain aphids transferred from either healthy or infected previous hosts. Ears treated simultaneously with *F. graminearum* inoculum and aphids incurred significantly higher disease severity, pathogen DNA and accumulation of the mycotoxin deoxynivalenol than ears treated with *F. graminearum* inoculum alone. Olfactometer assays using headspace samples of volatiles from wheat ears inoculated with the pathogen showed that *F. graminearum*-induced volatiles were repellent to aphids. Chemicals responsible for repellency were identified via GC-linked electroantennography and GC-MS followed by olfactometer assays of the electrophysiologically active components. Furthermore, decreased fecundity and survival was observed for aphids fed with *F. graminearum* symptomatic ears. Aphid feeding increased disease progression, therefore benefitted the colonising pathogen, possibly by altering plant defence responses. However, disease induction negatively impacted on aphid survival and reproductive success. Exhibition of a repellent response by aphids to volatiles from diseased plants can be interpreted as an adaptation by aphids to evade the inhospitable environment created by the pathogen.

Symposium. Tuesday, 9:20. **62**

Plant-microorganism interactions that shape host-plant selection in the grapevine moth

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Plant-micro-organisms associations may play a role in shaping plant-herbivore interactions. Here, we tested whether the inoculation of a host-plant with a variety of microorganisms would be able to affect the attraction to the plant, the oviposition preference and the fitness of an herbivorous insect. We worked on the system of a phytophagous species (grapevine moth *Lobesia botrana*), its host plant (grapevine *Vitis vinifera*) and the microorganisms associated with the plant. In vineyards, *L. botrana* use a volatile signal to locate the host-plant from a distance and to oviposit on grape. In our experiments, the attraction from a distance and the oviposition preference of the moth were influenced by the microbial activity on the plant. In addition, the quality of the host plant as larval food was importantly changed by the presence of pathogenic or opportunistic microorganisms on the plant. Taken together our results indicated a major role of endemic microorganisms on *L. botrana* host-selection and life-trait. Microbial volatiles appear to be a major cue mediating this kind of interaction.

Symposium. Tuesday, 9:40. **63**

Effect of host plant on aphid susceptibility to the fungal pathogen *Pandora neoaphidis*.

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Virulence of the aphid-specific fungus *Pandora neoaphidis*, as

measured in dose-response assays, was compared against the pea aphid, *Acyrtosiphon pisum*, that had been reared on different host plant species. *A. pisum* were reared on dwarf bean then inoculated with *P. neoaphidis* and returned to dwarf bean or inoculated and transferred to field bean, pea or lucerne. The smallest estimated median lethal concentration (LC_{50}) was 7.7 conidia mm^{-2} for aphids returned to dwarf bean, with LC_{50} s of 13.0 and 14.6 conidia mm^{-2} for aphids transferred to field bean or pea, respectively. The largest LC_{50} was achieved when aphids were transferred to lucerne: 2941.0 conidia mm^{-2} . In a subsequent experiment, *A. pisum* were reared on either pea or dwarf bean for four generations before bioassays. The LC_{50} for aphids reared and incubated on dwarf bean was 7.3 conidia mm^{-2} , compared to 13.3 and 15.3 conidia mm^{-2} when aphids were transferred between dwarf bean and pea, or pea and dwarf bean, respectively. The LC_{50} for aphids reared then incubated on pea plants was 27.9 conidia mm^{-2} . Overall, the virulence of *P. neoaphidis* was greatest when *A. pisum* was reared and maintained on dwarf bean, the plant used for long-term routine culturing of the aphid. In conclusion, virulence of *P. neoaphidis* was influenced by host plant and particularly by the plant species to which the host aphid had become adapted. Plant resources may affect the population dynamics of *P. neoaphidis* and could result in a greater impact on aphid herbivores that are not suffering physiological stress related to a change in host plant.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00 NEMATODES 2

Contributed paper. Tuesday, 8:00. **64**

Entomopathogenic nematode behavioral responses to chemical cues from cadavers.

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Entomopathogenic nematodes (EPN) are exposed to a range of cues in the soil. To the extent these cues are positively associated with the presence of insect hosts, one might hypothesize that EPN would respond positively to such cues. Decomposing animals release many different chemical compounds into the soil, attract large numbers of foraging insects, and produce large numbers of insect larvae. Thus, these chemical compounds may serve as an important cue for foraging EPN. We hypothesized the *Steinernema feltiae* and *Steinernema glaseri* IJs would respond generally positively to two particular compounds (putrescine and cadaverine) produced during animal cadaver decomposition. We further hypothesized that *S. feltiae* would respond more strongly to putrescine, and that *S. glaseri* would respond more strongly to cadaverine. We initially used standard agar-based "bulls-eye" attraction assays, and assessed *S. feltiae* and *S. glaseri* responses to diffusion discs soaked in 5 μ l of 50, 100, 500, and 1000 μ mol concentrations of each of the two compounds. We followed those agar trials with more realistic small sand column assays, assessing responses to the compounds when they were presented with additional stimuli such as host presence. On agar, responses differed between the different EPN species, chemical compounds, and concentrations, but the chemicals were never attractive and often strongly repellent. Responses were more complex in the sand columns; in particular, the compounds seem to attract more IJs to areas that also contained hosts.

Contributed paper. Tuesday, 8:15. **65**

The *Wolbachia* Endosymbiont as a Nematode Drug Target for Control of Human Filarisis, a Neglected Tropical Disease and Other Insect Borne Pathogens

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Most human filarial nematode parasites and arthropods are hosts for a bacterial endosymbiont, *Wolbachia*. In filaria, *Wolbachia* are required for normal development, fertility and survival, whereas in arthropods, they are largely parasitic and can influence development and reproduction, but are generally not required for host survival. Due to their obligate nature in filarial parasites, *Wolbachia* have been a target for drug discovery initiatives using several approaches including diversity and focused library screening and genomic sequence analysis. *In vitro* and *in vivo* anti-*Wolbachia* antibiotic treatments have been shown to have adulticidal activity, a long sought goal of filarial parasite drug discovery. In mosquitoes, it has been shown that the presence of *Wolbachia* can inhibit the replication of certain viruses, such as Dengue, Chikungunya, Yellow Fever West Nile, and the infectivity of the malaria-causing protozoan, *Plasmodium* and filarial nematodes. Furthermore, *Wolbachia* can cause a form of conditional sterility that can be used to suppress populations of mosquitoes and additional medically important insects. Thus *Wolbachia*, a pandemic endosymbiont offers great potential for elimination of a wide-variety of devastating human diseases.

Contributed paper. Tuesday, 8:30. **66**

Differential PirAB expression of the entomopathogenic bacterium *Photobrhabdus luminescens* (Enterobacteriaceae) based on tissue association and portal of entry to the insect host

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Photobrhabdus bacteria gain access to an insect host by their association with the free-living infective juvenile stage (IJ) of *Heterorhabditis* nematodes. Penetration of the insect can be achieved through three different portals of entry: a) digestive (mouth, anus), b) tracheal (spiracles) and c) integument. Studies have shown that *Photobrhabdus* may colonize other tissues before they establish in the insect's hemocoel, the final destination for full release of bacterial symbionts and completion of their life cycle. It is likely that *Photobrhabdus* employs effectors related to virulence factors in pathogens for adhesion, invasion, and intracellular growth in its host's cells. In this study we investigated tissue aggregations and virulence factors by measuring PirAB toxin expression of *Photobrhabdus luminescens* (TT01) in different insect tissues and concurrent to different portals of entry used by their nematode hosts.

Contributed paper. Tuesday, 8:45. **67 STU**

Candidate Virulence Loci in Pan-Genome of the Entomopathogenic Bacterium, *Xenorhabdus bovienii* (Gamma-Proteobacteria: Enterobacteriaceae)

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Xenorhabdus spp. has dual life styles: they are pathogenic to insects and mutualistic with *Steinernema* nematodes. The nematodes vector the bacteria from one insect to another. In return, bacteria provide a suitable environment in the insect cadaver for the nematodes to mature and reproduce. Each *Steinernema* spp. carries one *Xenorhabdus* sp. Contrarily, a *Xenorhabdus* spp. may associate with more than one nematode host. The most promiscuous bacterium is *X. bovienii*, which associates with nine *Steinernema* spp. In this study, we performed a comparative genomic analysis of nine *X. bovienii* strains to depict novel virulence factors. Furthermore, virulence assays were performed considering three different lepidopteran hosts. Results revealed that four *X. bovienii* strains were attenuated, whereas the other five were virulent. The genomic platform MicroScope was used to identify known and candidate genes that contribute to their pathogenicity. Additionally, loci involved in their association with the nematodes were investigated. Two loci were identified as novel candidates involved in the bacterium's ability to interact with both nematode and insect hosts. The first region appears to be specific to interactions with nematode partners. The second region contains a type six secretion system (T6SS), which is known to contribute to bacterial pathogenicity. We hypothesize T6SS may contribute to the bacterium's ability to cause death in a wide range of insect hosts. Further molecular studies are undergoing to expand our understanding on the role of these loci and their mode of action in the dual lifestyle of this bacterium.

Contributed paper. Tuesday, 9:00. **69**

Molecular mechanism of the nematicidal activity of *Photobacterium luminescens* LN2 against *Heterorhabditis bacteriophora* H06 nematodes

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Photobacterium luminescens subsp. *akhurstii* LN2 (Enterobacteriaceae) is a symbiont of entomopathogenic nematodes *Heterorhabditis indica* LN2 and showed nematicidal activity against *H. bacteriophora* H06 infective juveniles (IJs). The LN2 bacteria may secrete unidentified toxic factors lethal for the H06 nematodes. The trans-specific nematicidal activity of the bacteria against the non-symbiotic nematode may have an impact on competitive interactions when one insect host is co-infected by different nematode species. To explore the molecular mechanism of the trans-specific nematicidal activity of *P. luminescens* LN2 against *H. bacteriophora* H06, the complete genome of *P. luminescens* LN2 was sequenced; two mutagenesis libraries of *P. luminescens* LN2 were constructed using Tn5 transposon and rifampicin antibiotic respectively; the mutants from the libraries were tested for nematicidal activity and mutants negative for nematicidal activity were genetically and proteomically characterized. At least 9 putative proteins including DsbA, HipA, RhIE, RplC, RpOB, NamA, NamB (a protein from T3SS), and 2 hypothetical proteins (similar to unknown protein YgdH and YggE of *Escherichia coli* respectively) were involved in the nematicidal activity of LN2 bacteria against H06 nematodes. This hypothesis was further confirmed by creating insertion-deletion mutants of corresponding genes. It seems that a big network system is involved in this nematicidal activity.

Contributed paper. Tuesday, 9:15. **70**

Natural products from entomopathogenic bacteria: Understanding the interaction of bacteria, insects and nematodes

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Entomopathogenic bacteria of the genera *Xenorhabdus* and *Photobacterium* live in symbiosis with nematodes of the genera *Steinernema* and *Heterorhabditis*, respectively, and together they are able to infect and kill several different insect larvae. We have shown recently by chemical analysis and genome sequencing that these bacteria are able to produce a huge variety of different low molecular weight natural products. These compounds show insecticidal but also antibiotic and anticancer activity and novel bacterial signalling compounds have also been identified.

Recent work indicates that several of the bacterial natural products are addressing different parts of the insect immune system in order to make sure that the bacteria can evade it and kill the insect host. As the nematode immune system shows the same basic principles, it is of high interest how the natural products can differentiate between insect prey and nematode host. We will present our recent finding on natural products and their natural targets as well as ways to improve the production of these – probably also pharmaceutically useful – compounds.

CONTRIBUTED PAPERS Tuesday, 8:00-10:00

VIRUSES 3

Contributed paper. Tuesday, 8:00. **71**

Characterization and formulation of a Colombian isolate of *Erinnyis ello* granulovirus (L.) (Lepidoptera: Sphingidae)

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Erinnyis ello (L.) is a polyphagous lepidopteran pest that may cause serious annual losses in the rubber industry. The use of granulovirus represents an interesting alternative as a biological control agent for this insect. One Colombian granulovirus isolate (VG010) was obtained from *E. ello* larvae in the field and was characterized at morphological, biological and molecular level. Occlusion bodies showed an oval morphology with a unique nucleocapsid, and a size of $302.9 \pm 22 \times 181.5 \pm 16$ nm. The VG010 viral genome size was estimated to be approximately 88.7 kb. Phylogenetic relationships based on selected gene sequences *lef-8*, *lef-9* and *gran* showed a close relationship between VG010 and another isolate from *E. ello* previously reported (M34-4), suggesting that these isolates are genotypic variants of the same viral species. The mean lethal dose of VG010 against second instar *E. ello* larvae was 4.3×10^3 OBs/mL and the viral productivity ranged between 2.1×10^9 and 3.8×10^9 OBs/g of larval tissue. With this virus, a wettable powder formulation was

developed which photostabilized viral OBs against UVB radiation and improved shelf life. This product presented an efficacy of 99% for controlling the pest in laboratory and quality control limits for the product were established. This biopesticide constitutes a new tool with high quality and efficacy that needs to be scaled up and evaluated under field conditions in order to confirm its potential for controlling this important pest in rubber crops.

Contributed paper. Tuesday, 8:15. **72**

Production of the *Cydia pomonella* granulovirus (CpGV) in a heterologous host

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The codling moth (CM), *Cydia pomonella* (Linnaeus) (Lepidoptera: Tortricidae), is considered one of the most significant pests of apples and pears in the Western Cape, South Africa. Traditionally, control measures have relied heavily on the use of broad spectrum insecticides. *Cydia pomonella* granulovirus (CpGV) has proved to be an effective alternative to chemical application. The main objectives of this study were to identify a novel South African isolate of CpGV and to ascertain the viability and shortcomings of producing CpGV in the heterologous host, false codling moth (FCM), *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Initially four field collected isolates were compared genetically to two commercially available products. PCR amplification and sequencing of CpGV *granulin* and *egt* genes as well as single restriction endonuclease digestion of genomic DNA isolated from purified occlusion bodies indicated that the South African isolates were genetically similar to the Mexican strain. A further two isolates have been collected from the Langkloof (Eastern Cape) and Harrismith (Free State) areas in which there is no previous record of commercial virus application. Genetic comparisons are currently being conducted. Initial results indicate genetic variation in the Harrismith isolate when compared to the Mexican strain. Rearing parameters for CM and FCM, including fecundity, percentage hatch, larval developmental times and percentage mortality, were compared. The quantity of CpGV per larval unit was calculated for both FCM and CM. Mortality and virus yields were assessed by inoculating early 4th and 5th instar larvae with eight concentrations of purified CpGV. The mortality data obtained from the virus yield trials were used to establish the concentrations required to conduct surface dose bioassays against both FCM larval larvae. Dose and time response values for 4th and 5th instar FCM larvae were determined and used in establishing a virus production technique. Effective quality control parameters have been established to ensure the integrity of virus being produced, namely bioassay, RE analysis using *Hind* III as there is no recognition site for this enzyme in CpGV DNA and, lastly, development of a set of standards for a qPCR reaction, which can be used to calculate the proportion of CpGV in a mixed virus solution. If this production technique was to be successfully implemented into a mass production programme the cost of producing CpGV could be significantly reduced.

Contributed paper. Tuesday, 8:30. **73**

Post-translational cleavage of P74 of the *Helicoverpa armigera* single nucleopolyhedrovirus facilitates *per os* infection

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Baculovirus oral infection is mediated by binding and fusing of occlusion derived virus (ODV) with the microvilli of midgut epithelium under alkaline condition. Previous studies showed that ODV attachment protein, P74, of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) undergoes two sequential cleavage events, the primary one being conducted by the endogenous alkaline protease at an unidentified site and the secondary one by host midgut trypsin at amino acids R195/R196/R199. Here we report that *Helicoverpa armigera* single nucleopolyhedrovirus (HearNPV) P74 was first cleaved after translation in the host cell and was not dependent on the endogenous protease during ODVs release. The cleavage produces two subunits which were not associated by disulfide bonding. Judging from the molecular mass of the subunits, the cleavage was predicted at an arginine and lysine (R/K) rich region in the middle of HearNPV P74. A series of site-directed mutants in this region were generated. Feeding experiments showed that the single or multiple mutations significantly impaired *per os* infectivity and mutagenesis of R334Q/R339Q/R344Q/R347Q eliminated the specific cleavage of HearNPV P74. A mutant of the proposed second cleavage site R220Q/R221Q/R224Q was also generated and bioassays showed that the region was essential for oral infection. The results suggested that although there are some differences during the first cleavage, P74 of both AcMNPV and HearNPV undergo two steps cleavage, and the cleavage sites are likely to be conserved in the two viruses. An integrated model of P74 cleavage is provided which sheds lights on the molecular mechanism of ODV entry.

Contributed paper. Tuesday, 8:45. **74 STU**

Isolation, genetic characterisation and evaluation of biological activity of a novel South African *Phthorimaea operculella* granulovirus (PhopGV)

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The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a major pest of solanaceous crops in sub-tropical and tropical regions worldwide. This pest has developed resistance to many traditional pesticides, thus alternate means of control are required to protect the R2.5 billion (€168 million) potato industry in South Africa. The *Phthorimaea operculella* granulovirus (PhopGV) is considered a promising biopesticide that can be incorporated into integrated pest management programmes. Several PhopGV isolates recovered from geographically different insect populations have been genetically characterised and the full

genome of the Tunisian PhopGV-1346 isolate has been sequenced, providing a reference strain for comparison with novel isolates. This study reports the identification and genetic characterisation of a South African PhopGV isolate recovered from a *P. operculella* colony reared in the laboratory. Sequencing of the *lef-8*, *granulin* and *egt* genes confirmed the identity of the virus as PhopGV. Phylogenetic analysis of *egt* sequences grouped PhopGV-SA together with the Kenyan and South American isolates. Virulence evaluation against *P. operculella* larvae using surface dose and egg dip methods are currently underway and the preliminary data indicate that the virus has potential for development as a biopesticide for control of the pest in both the field and storage.

Contributed paper. Tuesday, 9:00. **75**

Genetic and biological characterisation of a novel South

African *Plutella xylostella* granulovirus, PlxyGV-SA

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The diamondback moth, *Plutella xylostella* (L.) (Lep, Plutellidae), is a serious world-wide pest of cruciferous crops, with a global estimated cost of control and damage amounting to approximately US\$4–5 billion annually. The *P. xylostella* granulovirus (PlxyGV) is considered a promising alternative to synthetic chemical insecticides and *Bt* insecticidal proteins for control due to the development of resistance in pest populations. Several PlxyGV isolates have been genetically and biologically characterised although many of these have not been commercialised as bio-pesticides. This is the first study to describe a novel South African PlxyGV in terms of genotype and biological activity. PlxyGV was recovered from an overcrowded laboratory *P. xylostella* colony established using field-collected insects. Occlusion bodies (OBs) were extracted from diseased larvae and purified by glycerol gradient centrifugation. PlxyGV-SA was genetically characterised by restriction endonuclease (REN) analysis of genomic DNA, and PCR amplification and sequencing of *granulin*, *ecdysteroid UDP-glucosyltransferase* (*egt*), *late expression factor 8* (*lef-8*) and *late expression factor 9* (*lef-9*) genes. Comparison of PlxyGV-SA REN profiles with those of PlxyGV-Japan (GenBank accession No. AF 270937.1) and other documented PlxyGV isolates together with sequence and alignment data showed that PlxyGV-SA is genetically unique. Neonate larvae were more susceptible to PlxyGV-SA infection than fourth instars at the same virus concentration. Biological activity determined by surface dose bioassays was estimated to be 3.56×10^5 OBs/ml (LC₅₀), which is comparable with values obtained in similar studies. These results suggest that PlxyGV-SA has significant potential for development as an effective biopesticide for the control of *P. xylostella* in the field.

Contributed paper. Tuesday, 9:15. **76-STU**

Comparative transcriptome analysis of CpGV-M in susceptible and resistant codling moth *Cydia pomonella*

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The *Cydia pomonella* granulovirus (CpGV) is commercially widely used and a cornerstone in the control of codling moth,

C. pomonella L. (CM), in both organic and integrated pome fruit production. Recently, nearly 40 CM populations resistant to products based on the Mexican isolate CpGV-M have been located in Europe. So far, new CpGV isolates overcoming this resistance were identified and are applied in orchards with resistant CM populations. However, only limited information on the infection process of CpGV is available. To gain a better understanding of the interaction between CpGV-M and its host microarray analyses of the transcription of CpGV-M genes in the midgut of susceptible and resistant CM individuals was performed. Therefore, CM larvae were infected with CpGV-M and RNA samples were taken from midguts between 0 and 120 h post infection. Microarray analysis of the susceptible CM strain resulted in a detailed overview of the temporal transcription of all 143 CpGV-M genes. Four representative gene clusters were identified by performing a k-means clustering. Some correlation between the promoter motif and the course of the infection pattern could be observed. Thereby, it was also possible to group uncharacterized CpGV-M genes according to their transcriptional profile. In contrast, a delayed and limited transcriptional activity of CpGV-M genes was observed in midguts of CM strains resistant to CpGV-M. This indicated that CpGV-M is able to enter the midgut in resistant CM and start the viral transcription. This truncated infection does not result in a permissive infection of the host. In addition, the transcription of the resistant CM strain infected with the resistance overcoming isolate CpGV-I12 was followed by qPCR to proof if a successful infection of a resistant CM strain leads to the same course of infection as seen as in susceptible CMs. Six representative genes (*ie-1*, *lef-8*, *mcp*, *pe38*, *f-protein* and *granulin*) were chosen for this analysis. All of them showed the same course of infection in the resistant CM strain as seen in the susceptible CM strain.

Contributed paper. Tuesday, 9:30. **77**

Transmission of mixtures of insect pathogenic viruses in a single virion: towards the development of custom designed virus insecticides

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Alphabaculoviruses (lepidopteran nucleopolyhedroviruses) have a characteristic physical structure that facilitates the transmission of genetic diversity. We demonstrate that coinfection of *Spodoptera exigua* larvae by SeMNPV and a deletion genotype of SfMNPV resulted in the production of mixed virus occlusion bodies (OBs) containing both the parental viruses. This also occurred when phylogenetically more distant viruses were used: SfMNPV and AcMNPV coinfections in *S. frugiperda* larvae also resulted in mixed virus species OBs. Approximately half the virions present in OBs produced following coinfection with mixtures of different alphabaculoviruses contained both viruses, indicating that the viruses coinfect and replicated in a single cell, and were enveloped within the same virion. Serial passage experiments revealed that both viruses persisted in the mixed-virus population by coinfection of insects during several rounds of insect-to-insect transmission. These results have dramatic implications in alphabaculovirus evolution and ecology. This mixed virus production technology is the subject of a PCT (patent) and opens the way to the development of custom-designed insecticides for control of different species of caterpillar pests on crops.

Contributed paper. Tuesday, 9:45. **78**

Improvement of UV-resistance of Baculovirus by displaying the Nano-material binding peptides on the Polyhedron Envelope

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Baculoviruses are sensitive to UV radiation and this characteristic causes the control efficacy of viral insecticides unsteady in the fields. The polyhedron envelope of baculoviruses, which is composed of carbohydrate and phosphorylated protein (PEP), is the first barrier against the disadvantageous environment. We found that orthologs of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) PEP, such as *Helicoverpa armigera* nucleopolyhedrovirus PEP, *Cydia pomonella* granulovirus Cp20 or Cp22 might not repair the absence of polyhedron envelope in the *pep*-knocked-out AcMNPV construct. The C-terminal (168~252aa) of AcMNPV PEP might deliver GFP to be expressed on the surface of polyhedron. Consequently, we had constructed the AcMNPV recombinants in which the C-terminal of PEP was fused with the peptides which might specifically bind melanin or nano-scale ZnO. These results may lay a foundation for developing intensive UV-resistant viral insecticides.

functions. The current model proposes that the TcA component binds to the cell surface and forms a pH-triggered channel that allows translocation of the TcBC subcomplex into the cytoplasm. Once in the cytoplasm the carboxy-terminus of the TcC subunit dissociates and becomes active, which causes toxicity in both insect and mammalian cells. A major component of this model is the requirement of an intact toxin complex in allowing TcBC to be transported into the cell. Based on our investigations of Yen-Tc, the YenBC subcomplex and the YenC subunit do not necessarily require full complex assembly to trigger cell toxicity. We will present and discuss our findings in relation to the current model.

Contributed paper. Tuesday, 8:15. **80**

Interaction of *Bacillus thuringiensis* Cry1Ab toxin with Mucus-rich structures

Diego Segond^{1,2}, Agnès Rejasse¹, Christophe Buisson¹, Shuyuan Guo^{1,3}, Karine Adel-Patient^{2,4}, Hervé Bernard^{2,4}, Didier Lereclus¹, Christina Nielsen-LeRoux¹

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Bacillus thuringiensis larvicidal Cry toxins are currently known for their strong host specificity; which is mainly due the presence of specific toxin binding sites on midguts of susceptible insect larvae. Meanwhile Cry toxins can also bind to compounds in the peritrophic matrix (PM) of several insects (*Rees *et al.* 2009; Valaitis and Podgwaite 2013). In *G. mellonella* infected with toxin alone, we observed structural modification of the peritrophic matrix but no evidence for the biochemical explanation for this modification is found so far. Knowing that "mucus" is along with chitin the main components of PM and that mucus is commonly found in several organisms, we aim to investigate the capacity of Cry1Ab to bind to several mucus rich structures. Indeed, our hypothesis is that the heavily glycosylated proteins (peritrophins and mucins) and proteoglycans shared by both vertebrate and invertebrate mucus may bind Cry toxins, therefore questioning on the "specificity" of these toxins used in GMO crops. Using, commercial pork stomach mucins, mice intestinal mucus, vertebrate cell-culture mucus and PM and peritrophins from *G. mellonella*, we then deeply analyzed Cry1Ab-mucus interactions. The presentation will deal with results from far western blot studies, ELISA binding experiments, inhibition ELISA with sugars, lectins or anti-Cry1Ab monoclonal antibodies. Identification of the interacting structure by LC/Ms/Ms analysis and resulting toxicity using insect and cellular models will be also shown.

**J Invertebr Pathol.* 2009 Mar; 100(3):139-46; *J Invertebr Pathol.* 2013 Jan; 112(1):1-8.

Contributed paper. Tuesday, 8:30. **81-STU**

Pore formation helping ability and binding affinity of BmABCC2 and BtR175 against Cry1A toxins.

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By *in vitro* toxicity assay using Sf9/Baculovirus expression system, we previously provided a novel evidence that *Bombyx*

CONTRIBUTED PAPERS Tuesday, 8:00-10:00 BACTERIA 2

Contributed paper. Tuesday, 8:00. **79**

Yersinia entomophaga MH96 (Enterobacteriaceae) BC subcomplex of the Yen-Tc ABC toxin is able to induce toxicity independent of the A subcomplex

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A novel gram-negative, rod-shaped, non-spore-forming bacterium, *Yersinia entomophaga* MH96 (Enterobacteriaceae), was isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* (Coleoptera: Scarabaeidae). *Y. entomophaga* produces a proteinaceous toxin complex (Yen-Tc) that is responsible for mortality in a range of insect species, mainly within the Coleoptera and Lepidoptera. The Yen-Tc is made up of two chitinase subunits (Chi1 and 2) and five Yen subunits (A1, A2, B, C1, and C2). The TcA, B, and C subunits are related to members of the Toxin complex (Tc) toxin family, with orthologs identified from several other bacterial species including *Serratia entomophila* and *Photorhabdus luminescens*. Characterization of Yen-Tc pathology has revealed a progressive deterioration of the midgut epithelium of susceptible insects. Although the specific mechanism of Yen-Tc remains unknown, cellular and molecular work has begun shedding light on how the Tc family

mori ABC transporter C2 (BmABCC2) functions as a receptor for Cry1A toxins. We also demonstrated that BmABCC2 can confer approximately 10-1000 times higher susceptibility to the cells than cadherin-like receptor (BtR175) and BmABCC2 and BtR175 co-expression exerts synergistic effect in susceptibility conferring ability. This synergistic effect suggested that these two receptors have different roles in the mode of action of Cry1A toxins in Sf9. Thus, we addressed to find the difference in the roles of the two receptors. First, we evaluated pore formation helping ability of the receptors using xenopus oocyte expression system and the two-microelectrode voltage clamp technique. When Cry1Aa or Cry1Ab toxin was administrated to BmABCC2 expressing oocytes, current continuously increased during toxin incubation, indicating that pores were continuously assembled on the cell membrane. However, when BtR175 expressing oocytes were administrated with toxins current increment speed was lower than in BmABCC2 expressing oocytes, indicating that BtR175 has lower function than BmABCC2 in pore formation helping. In contrast, BmABCC2 and BtR175 co-expressing oocytes showed at least 4 times higher current increment speed than BmABCC2 single expressing oocytes. This indicates that synergism occurs at least in part in the pore formation process, although synergistic effect is very low in comparison to that seen in Sf9 expression system. We also compared the binding affinity to Cry1A toxins of BmABCC2 and BtR175 using Biacore systems. We will discuss these results, too.

Contributed paper. Tuesday, 9:00. **82**

A necessary step in the mode of action of the Cry8 toxin: the elimination of DNA from the Cry toxin-DNA complex

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Several crystal (Cry) proteins are known to occur as DNA-protein complexes. However, the role of the DNA associated with the activated toxin in the mechanism of action of the Cry toxin has long been ignored. Here, we focused on the DNA-activated Cry toxin complex. Both forms of the Cry8Ca2 and Cry8Ea1 toxins, i.e., with or without bound DNA, were separately obtained. Size-exclusion chromatography analysis indicated that the Cry8Ca2 toxin-DNA complex has a tight or compact structure. The Cry8 toxin-DNA complex is more likely to move toward the air/water interface and is more hydrophobic than the toxin without DNA. Competitive binding assays indicated that the Cry8Ca2 and Cry8Ea1 toxins without DNA specifically bind to the midgut of *Anomala copulenta* and *Holotrichia parallela* larvae, respectively. In contrast, the association of DNA with each toxin might result in the nonspecific recognition of the Cry toxin and its target receptor in the insect midgut. The association of the DNA fragment with the Cry8 toxin was shown to protect the Cry protein from digestion by proteases. Based on our results, we propose an additional step in the mechanism of action of the Cry8 toxin and elucidate the function of the associated DNA as well as the importance of the removal of this DNA for the insecticidal activity of the toxin.

Contributed paper. Tuesday, 9:15. **83 STU**

How does the Bt Cry41Aa toxin kill human cancer cells?

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In this study the cytotoxicity associated with the Cry41Aa human cancer cell-active toxin of *Bacillus thuringiensis* (*Bt*), also known as Parasporin-3, was characterized. We investigated the effects of recombinant Cry41Aa on the human hepatic cancer cell line HepG2 to elucidate its mode of action. Cry41Aa shares structural homology with commercially used insecticidal toxins. The fact that some *Bt* toxins are able to kill mammalian cells may threaten the use of *Bt* toxin-based pesticides in the future. Moreover the preferential and narrow cytotoxicity of Cry41Aa has potential for anticancer drug design. Significant uptake of fluorescent dye was observed in susceptible cells as little as 10 minutes post administration, suggesting rapid membrane damage. Microscopic observation revealed cellular and nuclear swelling induced within the first hour of treatment. The activation of apoptosis effectors Caspase 3/7 was not observed within 24 hours, although phosphorylation of p38 MAP kinase was. Our results suggest that Cry41Aa, like its insecticidal homologues - but unlike some other Parasporins, is a pore-forming toxin that rapidly increases membrane permeability in the target cell. Research is on-going to identify whether a specific receptor is present on the surface of susceptible cells.

Contributed paper. Tuesday, 9:30. **84 STU**

Which regions of the Bt Cry41Aa toxin are responsible for its activity against human cancer cells?

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The *Bacillus thuringiensis* human cancer cell-active Cry41Aa toxin (Parasporin3) contains the five conserved sequence blocks found in many insecticidal toxins and is believed to possess the same three domain fold. However, Cry41Aa is predicted to have an extra loop in its domain II as well as an additional "ricin" domain at its C-terminus. Deletion of the "ricin" domain resulted in a stable protein with a toxicity to HepG2 cells not significantly different to the non-modified toxin. Several deletions of the loop region all resulted in an unstable protein that could not be further analyzed. Various bioinformatic procedures were used to identify the loops at the apex of domain II that have previously been implicated in receptor binding in the insecticidal Cry toxins. A range of mutations in the putative loop 1 were made but none affected toxicity to HepG2. In loop 3 the presence of an aromatic residue at position 509 was found to be important for toxicity. In an attempt to further dissect which regions are important for toxicity hybrids have been made between insecticidal and cancer cell-active toxins. Our data to date suggest that Cry41Aa has a mechanism of action similar to the three-domain insecticidal Cry toxins.

Contributed paper. Tuesday, 9:45. **85**

Parasporin PS1Aa2 induces ionic channels in lipid bilayer membranes and calcium oscillations in sensitive cells

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Parasporins are *Bacillus thuringiensis* Cry toxins that are active against tumor cells. Like many Cry toxins, parasporin

PS1Aa2 (Cry31Aa2) formed pores in artificial membranes. These pores had several levels of conductance; the most frequently observed in 150 mM KCl solutions were of 11, 16 and 21 pS. Microspectrofluorometric experiments with the Fura-2 probe showed that the presence of PS1Aa2 can induce changes in intracellular calcium levels, most often in the form of calcium oscillations and sometimes as sustained increases. Such responses were observed in the presence and absence of extracellular calcium, with the tumor cell lines HeLa and HepG2, and with the non-tumorous cell line HEK 293. Calcium oscillations have not been described previously for Cry toxins even though some studies have shown that calcium appears to be involved in their mode of action. Our experiments required the use of much higher concentrations of toxin than suggested from the published cytotoxicity results. Despite the presence of fragments previously identified as active, its low efficacy appears to be related to the presence of DNA in the preparations causing the protein to precipitate. Future work aimed at elucidating the origin of these calcium oscillations and their role in toxicity will be greatly facilitated by an improvement in the method of preparation of this toxin.

Contributed paper, 10:00. **86-STU**

***Caenorhabditis elegans – Bacillus thuringiensis* interactions: new insights into mechanisms of host resistance and pathogen virulence**

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Genetically tractable model nematode *Caenorhabditis elegans* has been successfully used in the host-pathogen interaction studies and helped to uncover conserved virulence factors of clinically relevant pathogens. At the same time interactions of this nematode with its natural pathogens are poorly investigated. Bacteria from the genus *Bacillus* are among potential natural pathogens of the nematodes. Therefore, previously we isolated 768 *Bacillus* strains and tested them for the virulence to nematodes. Although only 3% of tested *Bacillus* strains were pathogenic, one strain called *B. thuringiensis* DB27 exhibited extreme virulence to *C. elegans*. Currently we are trying to tackle both sides of this host-pathogen equation and aiming to identify virulence factors of *B. thuringiensis* DB27 as well as *C. elegans* defense mechanisms. First, combining plasmid-curing and genome sequencing, we discovered that novel nematicidal Cry21 toxins with synergistic activity are the main nematicidal factors of DB27. We expressed these novel toxins in *E. coli* and confirmed their activity against *C. elegans*. Importantly, these toxins are also active against other free-living and animal parasitic nematodes, suggesting their potential application against parasitic nematodes. Our parallel work on the host side led to the discovery of *C. elegans* novel innate immunity pathway involved in the defense against pathogens. Specifically, we identified novel function for Dicer in *C. elegans* antibacterial innate immunity and showed that this function is largely associated with microRNA processing. Taken together, our reciprocal studies uncovered a previously unknown role for DCR-1/Dicer in *C. elegans* antibacterial immunity as well as identified novel nematicidal toxins.

SYMPORIUM 4 (Viruses) Tuesday, 10:30-12:30
Small non-coding RNAs as regulators of insect host-virus

Symposium. Tuesday, 10:30. **87**

Role of cellular and virus-encoded microRNAs in insect host-virus interactions

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MicroRNAs (miRNAs) are small non-coding RNAs of ~22 nucleotides which play significant roles in gene regulation at transcriptional as well as post-transcriptional levels. They are produced in almost all eukaryotes and also encoded by some viruses. Besides cellular miRNAs that may participate in anti-viral responses following infection, virus-encoded miRNAs may target host genes to interfere with host survival, proliferation and immunity. Furthermore, virus-encoded miRNAs may regulate replication of virus to avoid over replication and quick demise of the host or facilitate virus entry into persistent infection. The interaction may become more complicated in the presence of third parties, such as microbiota and endosymbionts, that in turn may affect the host's miRNA profile and indirectly disturb virus replication. In the presentation, the role of miRNAs in mosquito-arbovirus interactions with a reference to the effect of Wolbachia as an endosymbiont on the interactions will be discussed.

Symposium. Tuesday, 11:00. **88**

Sensing viral RNA in *Drosophila melanogaster*

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RNA interference plays a central role in antiviral innate immunity in flies. Indeed, flies mutant for the three key components of the small interfering (si)RNA pathway, namely Dicer-2, R2D2 and Argonaute (AGO) 2 are highly sensitive to a wide range of viruses (1). Dicer-2 produces virus derived-siRNAs from viral RNAs throughout its RNaseIII activity. The Dicer-2/R2D2 heterodimer then loads these siRNAs onto AGO2 in the RNA-induced silencing complex, RISC. The RISC complex is then able to target viral RNAs, thus impairing the ability of the virus to successfully replicate. Although *in vitro* and *in vivo* experiments clearly indicate that Dicer-2 can process long double stranded RNA, the exact nature of the viral RNAs sensed *in vivo* in infected cells remains mysterious. We are interested in understanding how Dicer-2 senses viral RNAs, with a particular focus on the contribution of the N-terminal DEAD/C helicase domain, which is conserved in mammalian RIG-I like receptors. Indeed, *in vitro* experiments have revealed a critical role of this domain in both processivity of the enzyme and discrimination of the extremities of the template RNA (1,2). To address this question, we take advantage of a combination of approaches including *Drosophila* genetics, next-generation sequencing technologies and bioinformatics analysis.

(1) Kemp et al., J. Immunol. 2013

(2) Cenik et al., Mol Cell. 2011

Symposium. Tuesday, 11:30. **89**

Small RNA-directed antiviral immunity in disease-vector mosquitoes

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The natural maintenance cycles of many mosquito-borne pathogens require establishment of persistent non-lethal infections in the invertebrate host. While the mechanisms by which this occurs are not well understood, antiviral responses directed by small RNAs are important in modulating the pathogenesis of viral infections in disease vector mosquitoes. Infection of Aedine vector species with viral pathogens triggers the production of short interfering (siRNAs) and another class of virus-derived small RNAs, ping-pong-dependent piwi-interacting RNAs (piRNAs). Unlike ping-pong-dependent piRNAs that have been described previously, from repetitive elements or piRNA clusters, our work suggests biogenesis in the mosquito soma. Similar to siRNAs, viral piRNAs also appear capable of modulating the pathogenesis of viral infections in mosquito cells. Thus, the non-canonical piRNA pathway present in the soma of Aedine vector species may provide robustness to the primary siRNA-based antiviral response.

Symposium. Tuesday, 12:00. **90**

Controlling viral infection in insects

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The paradigm held for a long time that RNAi response in most metazoans does not undergoes an amplification step and only acted cell-autonomously has prevailed in the model system *Drosophila melanogaster* and it is widely accepted for higher metazoans. The absence of systemic RNAi spread in *Drosophila* was directly tested in one study that used dsRNA-expressing transgenes *in vivo* in flies. We challenged this idea by identifying a dsRNA uptake pathway in *Drosophila* and showing that flies defective in several of the RNAi uptake genes are hypersensitive to virus infection, indicating that RNAi uptake is essential in the process of antiviral defense. In a second area, using a cloning approach to capture small RNAs with a 5' triphosphate group, we show that virus-derived siRNAs (vsRNA) bearing 5' triphosphate group accumulate in Sindbis virus (SINV) infected *Drosophila melanogaster*, suggesting that secondary vsRNA are produced during infection. Finally, we found that Cricket Paralysis virus encoding RNAi suppressor, CrPV-1A specifically interacts with Ago-2 within assembled holo-RISC, without modifying RISC composition, and that this interaction prevents RISC cleavage of target mRNAs. Interestingly, we discovered that CrPV1A recruit an E3 ligase. The implication.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

MICROBIAL CONTROL 1

Contributed paper. Tuesday, 10:30. **91**

Double trouble for thrips: Effective biopesticide combinations to control soil-dwelling stages in chrysanthemums

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Western flower thrips (WFT) are pests of global significance and a constant challenge in greenhouse floriculture. Faced with a lack of conventional control products, Canadian growers have embraced the use of biological control strategies to manage this pest. Soil-dwelling stages of thrips (pro-pupae, pupae) can be targeted with different natural enemies, including biopesticides. *Steinernema feltiae* (e.g., Nemasys®), applied as a drench, is widely used. *Metarhizium brunneum* (formerly *anisopliae*; Met52™) has recently been registered in Canada and the granular biopesticide product is incorporated into potting media. A series of trials were set up to assess compatibility of the two control agents, and the relative efficacy of individual and combined, i.e., nematode plus Met52, treatments against WFT. Fewer *S. feltiae* were recovered from Met52 treated soils over time than from untreated media; this was generally accompanied by a concurrent increase in the number of free-living nematodes recovered. The rice carrier in the biopesticide may have served as a food source for the free-living nematodes, promoting population growth, which may have affected survival of *S. feltiae*. The individual nematode and fungus treatments had a measurable suppressive effect on thrips, but the combined nematode/fungus treatment provided superior control throughout. WFT populations were consistently lower on plants receiving the combined treatment and significantly fewer WFT (< 2 per plant) were found on the plants at the conclusion of the trial (8 weeks). Opportunities therefore exist to enhance the reliability and cost-effectiveness of thrips biocontrol strategies by taking an integrated approach to the deployment of biopesticides.

Contributed paper. Tuesday, 10:45. **92-STU**

Lethal and sub-lethal impacts of fungal biopesticides on house fly populations in simulated field settings of biocosms

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Management strategies for control of house flies in poultry houses include cultural, biological and chemical tactics; however, use of broad-spectrum chemical larvicides and adulticides is the only reliable tool for poultry growers to manage 'fly burst' situations. Our aim was to exploit post-eclosion resting behaviors of teneral flies to evaluate the population control potential (lethal and sub-lethal impacts) of oil formulations of *Beauveria bassiana* and *Metarhizium*

anisopliae under simulated field settings called 'biocosms'. Experimental biocosms were created in plastic boxes where the vertical walls were fitted with sprayed plastic sheeting (blank oil or conidia in oil). A cohort of 300 fly pupae was added to each biocosm; on emergence, the adult flies moved to the vertical surfaces to harden their wings, simulating the exposure likely to occur in the fields. The biocosms were monitored daily for mortality and enumeration of egg laying and egg viability until all adult flies had died. Fungal treated biocosms resulted in 100% mortality within 10-17 days, depending on the fungal species. Treated populations also showed significant reduction in egg viability and life-time fecundity. Furthermore, >20% reduction in basic reproductive rate (B_0) was observed in treated fly populations. Together these results demonstrate that application of oil formulations of entomopathogenic fungi could suppress existing fly populations and substantially reduce population growth rates in poultry houses as part of an IPM program. Further studies will focus on evaluating fungal persistence on typical structural surfaces, optimizing application parameters and validating these strategies under actual field conditions in poultry houses.

Contributed paper. Tuesday, 11:00. **93-STU**

Management of *Prostephanus truncatus* (Horn.) on stored maize using *Beauveria bassiana* (Bals.)

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Control of the larger grain borer (*Prostephanus truncatus* Horn) using chemical insecticides is no longer desirable due to environmental and food safety issues. Classical biological control using the Histerid beetle, *Teretrius nigrescens* has been adapted in several locations in Ghana. However, *P. truncatus* is still causing tremendous losses of stored maize. There is a growing interest in using mycopesticides to complement other integrated pest management measures. Recent research in the UK has identified *Beauveria bassiana*, IMI 389521 as a suitable control agent for grain storage pests in the UK. In this study, the pathogenicity of *B. bassiana* IMI 389521 was evaluated against adults of *P. truncatus*, *Sitophilus zeamais* and *T. nigrescens*. The result obtained from the study indicates that *B. bassiana*, is pathogenic against adults of *P. truncatus* and *S. zeamais*. *Teretrius nigrescens* was less susceptible to the fungus. To determine the most effective concentration of *B. bassiana* for the control of *P. truncatus* in a semi-field trial, a laboratory dose response experiment using four concentrations of *B. bassiana*, (1×10^8 to 10^{11} /kg maize) was studied. Successful control of *P. truncatus* on infested maize was achieved at 1×10^{10} conidia per kg maize. Semi-field trial to evaluate the efficacy of *B. bassiana*, against *P. truncatus* on maize stored on cobs (dehusked) and on shelled kernels is on-going. The availability and safety of maize will be enhanced, through reduction in the use of chemical insecticides if the isolate is proved effective thereby improving the livelihood of smallholder farmers in Ghana.

Contributed paper. Tuesday, 11:15. **94-STU**

Lack of involvement of chitinase in direct toxicity of *Beauveria bassiana* exudates to the aphid *Myzus persicae*

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Insect disease causing *Beauveria bassiana* produces a range of insecticidal metabolites and enzymes such as chitinases and proteases although few have been shown to be toxic simply through contact. Using supernatant from broth cultures of a single strain, *B. bassiana* could cause significant mortality of green peach aphid, *Myzus persicae*, within 24 hours of application. Three day old broth cultures were the most effective, with less insect mortality seen using 6 day old broth. However, aphicidal activity increased again for 7 day old broth. Submerged cultures grew better and produced stronger aphicidal supernatants when cultured in media with start pH above 5.5. Chitinase was produced a day earlier than protease Pr1. The enzymes, however, appeared to have little impact on aphicidal mortality given that their peak production periods do not correlate with the aphicidal activities of 3 or 6 day old cultures. Supernatants treated with EDTA and heat respectively, still killed aphids. High quantities of hydrolytic enzymes produced using insect cuticle medium showed no aphicidal activity. No beauvericin nor bassianolide, two known insecticidal metabolites, were detected in the supernatants. The identities of the key aphicidal components of the *B. bassiana* supernatants thus remain to be resolved.

Keywords: supernatant, *Beauveria bassiana*, chitinase, Pr1, aphicidal, EDTA, beauvericin, bassianolide

Contributed paper. Tuesday, 11:30. **95-STU**

Entomopathogenic fungi for control of false codling moth in South African citrus orchards

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False codling moth, *Thaumatotibia leucotreta* Meyrick (1912) (Lepidoptera: Tortricidae) is a key economic pest of citrus in South Africa causing pre- and post-harvest damage. Soil microbes, such as entomopathogenic fungi, offer an additional means of controlling this pest by targeting the soil-dwelling life stages. Three fungal isolates, two of the species *Metarrhizium anisopliae* s.l. and one of the species *Beauveria bassiana* s.l. caused the highest levels of mortality of *T. leucotreta* fifth instar larvae in laboratory bioassays. In addition, these isolates were capable of persisting in a citrus orchard for six months, in sterile soil, whilst still remaining infective towards *T. leucotreta* fifth instar larvae. Since results may differ substantially under field conditions, further research was undertaken to determine whether these isolates remained effective when applied to non-sterile soil beneath the canopy of citrus trees in an orchard. A field trial consisting of one hectare treatment blocks, and a smaller caged trial were initiated to address this issue. Fungal spores were applied via spraying in an aqueous suspension at a concentration of 1×10^{14} spores per hectare for the field trial and at three different concentrations for the caged trial. Results of the large scale field trial, four months post application, support the

persistence capability of these isolates and, suggest that, although all three isolates were capable of reducing *T. leucotreta* infestation in comparison to the control block, *B. bassiana* performed best with an 81.33% reduction. It is thus suggested that future trials focus on the performance of this isolate.

Contributed paper. Tuesday, 11:45. **97-STU**

Wireworm control with entomopathogenic fungi and plant extracts

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Wireworms, the soil-dwelling larvae of click beetles, cause severe damage in arable crops and vegetable production. Currently, no registered and effective insecticides are available. The development of alternative control approaches including botanicals or insect pathogenic organisms are demanded and favoured by EU legislation (Directive 2009/128/EC). Limited efficacy of already tested entomopathogenic fungi (EPF) could be improved by synergistically acting botanicals. In the present study repellency of botanicals towards the wireworm species *Agriotes lineatus* and potential interactions of the most effective repellent with a wireworm-infesting fungus strain (*Metarhizium brunneum*) was investigated. Behaviour and mortality of wireworms were assessed in two-dimensional terraria (40cm x 50cm x 0.6cm) with a peat-sand substrate in a choice test for up to three weeks. Wireworm location was recorded and locomotion trails were manually traced, photographed and trail length determined on the treated and untreated half of the terrarium. We found that the garlic extract R3 repelled wireworms at rates of 1.2 g/L substrate, while this concentration hardly reduced efficacy of the EPF strain. Thyme oil was comparably repellent, but also strongly antifungal. The EPF strain was not repellent. Potential synergies between EPF and efficacy enhancing botanicals will be discussed for a biological control strategy.

Contributed paper. Tuesday, 12:00. **98-STU**

Long-term persistence of *Beauveria brongniartii* BIPESCO 2 used for cockchafer control in the Euroregion Tyrol

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The fungus *Beauveria brongniartii* (Sacc.) Petch has been used to control the European cockchafer *Melolontha melolontha* L. for more than two decades. The goal of this study was to assess persistence of the applied *B. brongniartii* strain in the soil of 20 cockchafer infested sites in East, North and South Tyrol. The sites have been treated with

Melocont® Pilzgerste (BIPESCO 2) at different frequencies and time points during the last 20 years. At all sites the density of *M. melolontha* larvae decreased from high levels at the start of treatments to levels below the damage threshold at the time point of sampling in 2012. A selective medium was used to determine *B. brongniartii* density and recover *B. brongniartii* isolates from soil samples. Collected isolates were subjected to genetic analyses to discriminate the applied strain from naturally occurring strains. Highest densities of *Beauveria* spp. (up to 6.8×10^5 CFU g⁻¹ soil dry weight) were detected in soils which have been treated with Melocont® Pilzgerste at least three times during the last three years (3 sites) prior to the sampling date. BIPESCO 2 was detected in 7 sites of which one was treated for the last 15 years prior to sampling. *Beauveria* spp. density varied strongly among and within fields and in 71% of the 162 soil samples no *Beauveria* was detected. Results suggest that periodic applications of the *B. brongniartii* biological control agent increase density and persistence of the fungus in soil and support a long-term control of *M. melolontha*.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

DIS. OF BENEFICIAL INVERTEBRATES 1

Contributed paper. Tuesday, 10:30. **99**

The Curious Case of the PaV1 in Adult Caribbean Spiny Lobsters

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The pathogen PaV1 (Panulirus argus Virus 1) exacts a heavy toll from juvenile Caribbean spiny lobsters with an estimated 24% in Florida dying of it before they reach maturity and recruit to the fishery. Prevalence is also high among adult populations, especially in the northern Caribbean (e.g., Puerto Rico – 17%). However, PaV1 manifests differently in adults. They may act as “carriers” because adults rarely develop visible infections and do not seem adversely affected by the pathogen. Infected adults are not avoided by healthy conspecifics, as occurs among juveniles. Moreover, adult females with subclinical PaV1 infections are often captured from the wild with a spermatophore or fertilized eggs, indicating that males are willing to mate with them. Adults with subclinical infections of PaV1 are not infectious to other adults or to the more susceptible juveniles. Although postlarval lobsters infected with PaV1 occur in the nearshore waters of Florida, experiments indicate that vertical transmission of PaV1 from females to embryos is not the mode of transmission. Instead, postlarvae acquire PaV1 shortly after arriving inshore from the oceanic plankton. These recent results suggest that PaV1 may be of little consequence to adult lobsters in contrast to its major effect on juvenile ecology and population dynamics. Just how adult lobsters retain subclinical infections of PaV1 remains a mystery.

Contributed paper. Tuesday, 10:45. **100**

Defining lobster-pathogen interactions via high-throughput gene expression studies: The discovery and description of the interplay between the American Lobster (*Homarus americanus*) and the ciliated parasite *Anophryoides haemophila*

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The American lobster (*Homarus americanus*) fishery is the economic engine for hundreds of coastal communities in Atlantic Canada and represents the last remaining significant wild fishery in Canada. Lobsters appear remarkably resistant to microbes in their natural environment however they are susceptible to the opportunistic ciliated pathogen *Anophryoides haemophila*, the causative agent of bumper car disease, during live holding. We have completed numerous controlled experimental infection studies to define the gross, histopathology, biochemical and molecular responses of lobster to this ciliated parasite. Recently completed high throughput oligonucleotide microarray and RNA-Seq transcriptomics studies have revealed a more comprehensive understanding of the molecular pathogenesis of disease in this unique lobster – parasite interaction. One caveat is interpreting the overwhelming wealth of bioinformatic data generated. This issue will be explored in the context of current annotation limitations for both arthropods and protistan parasites.

Contributed paper. Tuesday, 11:00. **101-STU**

Metabolomic investigation of Bitter Crab Disease in snow crabs (*Chionoecetes opilio*)

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Bitter crab disease (BCD) is a fatal disease of crustaceans caused by parasitic dinoflagellates of the genus *Hematodinium*. This emerging disease has been reported in over forty species of crustaceans world-wide including several commercially important crustacean species. In Atlantic Canada BCD occurs in snow crabs (*Chionoecetes opilio*) off the northern coasts of Newfoundland and Nova Scotia. In the late stages of this disease, the dinoflagellate parasites proliferate within the hemolymph and hemal spaces within the crustacean's organs, with no apparent cellular inflammatory response to the infection. The cause of death in cases of BCD is presumed to be metabolic and osmotic dysregulation. In this study, we use a combination of untargeted and targeted metabolomic approaches to characterize some of the metabolic changes associated with BCD.

Contributed paper. Tuesday, 11:15. **102-STU**

Assessment of immunocompetence in the shore crab, *Carcinus maenas*, to natural exposure of pathogens

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UK populations of the shore crab *Carcinus maenas* host various pathogen assemblages. In particular, two geographically close but distinct populations in Weymouth, (Newton's Cove and Harbour), demonstrated entirely different pathogen profiles. Immune biomarkers were used to assess the immunocompetence of individuals in these populations in relation to their pathogen burden. Selected immune genes included *carcinin*, (antimicrobial peptide), *peroxinectin* (cell adhesion molecule and osponin) and the zymogen *prophenoloxidase*, (cleaved to form active *phenoloxidase*, involved in the melanisation of many invading pathogens). Immune gene expression was quantified using real-time PCR. Histopathology revealed greater pathogen incidence in Newton's Cove (95%) compared with Harbour (37%) and a high dissimilarity in the pathogen profile (82.61% SIMPER) between sites. Host immune expression in relation to the presence and absence of pathogens and number of different infections per crab, revealed significant ($p < 0.01$) differences in transcription between populations, suggesting site-specific factors also influenced immune expression. In addition, host RNA quality was compared between pathogen groups ('viruses', 'bacteria', 'macroparasites' and 'no pathogens' groups). Further analysis may reveal whether RNA degradation is a function of pathogen type within the host. This is the first study to compare immunocompetence and histopathology between different *Carcinus maenas* populations in the wild.

Contributed paper. Tuesday, 11:30. **103-STU**

Effects of artificial infection of juvenile edible crabs, *Cancer pagurus* with the parasitic dinoflagellate, *Hematodinium* sp.

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Parasitic dinoflagellates of the genus, *Hematodinium*, are thought to be significant pathogens of a wide range of crustaceans. Much is known of the ecology and effects of this disease on the sustainability of crustacean populations but significantly less is known about the mode of transmission and fate of infected animals. Attempts have been made to transmit the disease under aquarium conditions to several species of crabs resulting in a great deal of variation in mortality levels and the timescale of disease progression. To determine if *Hematodinium* infections are significant drivers of mortality in juvenile edible crabs (*Cancer pagurus*), crabs were injected with either 1×10^5 *Hematodinium* trophonts from an infected animal or sterile saline. Crabs were bled every four weeks to determine the progression of infection and its effects on the numbers of circulating haemocytes. Thirty three percent of the *Hematodinium*-infected crabs became infected and mortality occurred between 93 and 378 days post-challenge. Infected crabs appeared to moult less frequently than their uninfected counterparts but mortality did not appear to be directly caused by *Hematodinium*, as there was no significant difference in the mean time to death between infected and uninfected crabs. Both *Hematodinium*-infected and uninfected crabs exhibited infections by a number of other disease causing agents including haplosporidium-like parasites, fungi and bacteria. These appeared to be key drivers of the mortality observed. These studies, albeit carried out on small cohorts of edible crabs, imply that *Hematodinium* is not a driver of host mortality at least under aquarium conditions.

Contributed paper. Tuesday, 11:45. **104**

A role of polychaetes in transmission of white spot syndrome virus in shrimp ponds?

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White spot disease (WSD) is caused by white spot syndrome virus (WSSV) (*Nimaviridae*). WSSV emerged in the early-to-mid 1990s in Southeast Asia and became panzootic since. The disease can be mitigated by introducing rigorous sanitation protocols, proper pond management, use of specific pathogen-free shrimp and by early diagnosis followed by eradication. The virus is transmitted horizontally by healthy individuals preying on diseased ones, via feeding on detritus or by intake of WSSV-contaminated water. WSSV can also be transmitted vertically via broodstock. The virus infects a wide range of crustaceans beyond the penaeids such as crabs and crayfish, and these co-inhabitants of ponds form a reservoir of WSSV for disease transmission to penaeids. Much less knowledge is there on the potential of resident benthic organisms as vectors for WSSV. A literature survey indicates that WSSV is present in a number of non-crustacean invertebrates, which sometimes vector the disease to penaeid shrimp. *Dendronereis* spp. is a most ubiquitous resident annelid in shrimp ponds and used as food source for shrimp. We showed that WSSV replicates in *Dendronereis* spp. and can be transmitted from this polychaete to penaeid shrimp. Furthermore there appears to be a positive correlation between the past incidence of WSD in ponds and the occurrence of WSSV in resident *Dendronereis* spp., whereas there is no correlation with other pond parameters. We hypothesize that *Dendronereis* spp., as a replicative host for WSSV, may serve as a reservoir for WSSV and may be associated with the persistence of this virus in pond systems.

Contributed paper. Tuesday, 12:00. **105**

Novel Pattern Recognition Receptor Protects Shrimp from *Vibrio* Infection by Binding Flagellin and LPS through Different Recognition Modules

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Pattern recognition receptors (PRRs) recognize pathogens thorough the pattern recognition modules. For example, Toll like receptors recognize the ligands through leucine-rich repeats (LRRs), and C-type lectins bind to glycans on the surface of pathogens by the C-type carbohydrate recognition domain (CRD, also called C-type lectin like domain CTLD). Many PRRs contain more than one kind of modules. In the present study, we identified a novel PRR, named *Leulectin*, which contains several LRRs and a CTLD. Such unique arrangement has not been found in any other organisms. Recombinant Leulectin and the modules (LRRs and CTLD) were found to protect shrimp from *Vibrio* infection. An ELISA-based screen was performed to identify the potential ligands the two modules may recognize. Results showed that LRRs could recognize the *Vibrio* flagellins, and CTLD could recognize lipopolysaccharides (LPS). The Leulectin-flagellin interaction was determined by the third LRR of Leulectin and

the N-terminus of flagellin, and the Leulectin-LPS interaction was dependent on the long loop region of CTLD in a calcium-independent manner. The ligand-recognition activity of LRRs and CTLD was critical for Leulectin to bind to bacteria, and the binding was the basis for Leulectin to protect shrimp from bacterial infection. This study clearly showed the interesting synergy between distinct modules of a PRR.

Contributed paper. Tuesday, 12:15. **106**

Observations on *Agmasoma penaei* and *Perezia nelsoni* in White shrimp *Litopenaeus setiferus* from the Gulf of Mexico

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In June 2012 a few shrimp from Plaquemines parish LA with the symptoms of microsporidiosis were delivered to the Louisiana Aquatic Diagnostic Laboratory for identification. Light microscopy including examination of Luna-stained paraffin sections, and electron microscopy showed the infection was limited to ovaries and was caused by a microsporidium producing roundish pansporoblasts with 8 spores (3.6 x 2.1μm) and anisofilar (2+6) polar filaments, the features corresponding to the diagnosis of *Agmasoma penaei* (=*Thelohania penaei* Sprague 1950, n.comb Hazrad and Oldacre, 1973). Comparison of the SSUrDNA sequence of the novel isolate to *A.penaei* from Thailand revealed 95% similarity, which suggests these geographical isolates, may be two different species, a conclusion supported by several ultrastructural dissimilarities and different tissue tropism. Phylogenetic analyses places this species as a divergent taxa within the clade IV (microsporidia of terrestrial origin) sensu Vosbrinck, Debruner-Vossbrinck, 2005. In two shrimps infection of ovaries with *A. penaei* was accompanied by heavy infestation of muscles with another microsporidium *Perezia nelsoni*. *P.nelsoni* produces individual spores (2.0 x 1.1μm). Structurally and genetically (SSUrDNA sequence similarity >99%) LA isolate was identical to *Perezia nelsoni* from the Mississippi coast of the Gulf (Canning et al., 2002). Previously reported infection of muscles with *A.penaei* may be due to overlooked double infection with *P.nelsoni*. Supported by Louisiana Department of Wildlife and Fisheries.

CONTRIBUTED PAPERS Tuesday, 10:30-12:30

FUNGI 3

Contributed paper. Tuesday, 10:30. **107**

Comparison of ecological traits of co-existing *Metarhizium*: What does it take to dominate an agricultural field?

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It is expected that particular adaptive ecological traits influence species abundance and distribution within an ecosystem. We evaluated selected traits of different co-existing species and genotypes of the entomopathogenic fungi *Metarhizium* isolated from an agroecosystem in Denmark. Fifteen fungal isolates representing 11 genotypes were tested for: UVB tolerance, *in vitro* growth at 12.5°C and 21.5°C, mycelial growth from the insect cadaver into the surrounding soil, virulence and conidia production on cadavers. The results showed that the relative performance of the most abundant *Metarhizium* genotype was intermediate for mycelial growth in soil and *in vitro* growth at 12.5°C / 21.5°C while it showed high UVB tolerance and conidia production compared to other genotypes. We discuss whether the two latter traits are most important to dominate the *Metarhizium* community in agricultural habitat or whether the "Jack of all trades" performance could be the key to understand the dominance of a particular genotype.

Contributed paper. Tuesday, 10:45. **108-STU**

Effect of entomopathogenic fungal strains on non-target arthropods in sour cherry orchard

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Efficacy of *Metarhizium* and *Beauveria* entomopathogenic fungal strains for the control of cockchafer grubs was evaluated in sour cherry orchards. Safety like possible effect of the inoculum on natural soil microbiota as well as efficacy and fate of these fungi need to be investigated. The applied fungal strains have wide host range, thus we have to determine the risks of their use during repeated long-term applications. Different inoculation methods were compared and the persistence of inoculum was monitored in the soil and on target and non-target organisms. The treatments were applied 2 times (May and July) in the space rows and we used pitfall traps as sampling method. Samples were collected 8 times during the summer of 2013. The samples were processed in laboratory and the numbers of different arthropods (collembolans, mites, thrips, flies, ants, spiders, centipedes, crickets, rove beetles, ground beetles) were recorded in each sample. The comparison of un-treated and treated areas, and the microscopical examination showed no significant differences in the frequency of species. As a conclusion, the effect of these entomopathogens on non-target arthropods is minimal and as such they do not impose any environmental risk.

Contributed paper. Tuesday, 11:00. **109-STU**

Potential of endophytic *Beauveria bassiana* in grapevine against insects

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Fungal entomopathogens are important antagonists of arthropod pests and have attracted increased attention as biocontrol agents in integrated pest management programs. In addition, evidence has accumulated that some entomopathogenic fungi like *Beauveria bassiana* (Bals.) Vuill. can endophytically colonize plants and provide a systemic protection against insect pests. Currently, it is unknown whether *B. bassiana* can exist as an endophyte in grapevine, *Vitis vinifera* (L.) and still maintains its antagonistic potential

against insect pests. In the present study, the antagonistic activity of *B. bassiana* (strain ATCC 74040) after plant inoculation and endophytic establishment in grapevine against the vine mealybug *Planococcus ficus* was assessed using surface sterilized leaves for a bioassay. Possible effects of endophytic *B. bassiana* on the feeding preference of black vine weevil *Otiorrhynchus sulcatus* choosing between control and inoculated plants was examined through choice assays. A significant effect of endophytic *B. bassiana* on growth during the whole observation period and on mortality of mealybugs one week after initial settlement was evident. Adult *O. sulcatus* chose significantly more often the control plants as a host plant compared to grapevine plants with endophytic *B. bassiana*. In addition, a microarray analysis was performed to get insights into genetic mechanisms behind the plant-fungus-interaction. The results indicate an up-regulation of diverse defense related genes in grapevine due to the endophytic establishment of *B. bassiana*. In conclusion, endophytic establishment of the entomopathogenic fungus *B. bassiana* in grapevine might represent an alternative and sustainable plant protection strategy, with the potential for reducing pesticide applications in viticulture.

Contributed paper. Tuesday, 11:30. **111**

Horizontal transmission of entomopathogenic fungi by ectoparasitoid *Habrobracon hebetor*

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Horizontal transmission of entomopathogens by parasitoids is well known for viruses but did not registered for fungi. Our experiments were carried out on the laboratory system *Galleria mellonella* (Lepidoptera, Piraliidae), – *Habrobracon hebetor* (Hymenoptera, Braconidae) – *Beauveria bassiana* (Hypocreales, Cordycipitaceae). We found out that contamination of *H. hebetor* ovipositor with low titers of conidia *B. bassiana* and following envenomation of *G. mellonella* larvae led to mycoses followed by host colonization and conidiation. In addition *H. hebetor* females transmitted fungal conidia from infected (6 hours post inoculation with conidia) to native *G. mellonella* larvae, and this transmission led to successful mycosis of native host larvae. The decreasing of cellular and humoral immune reactions, significant increasing of adhesion and germination of fungus on cuticle of envenomated larvae were registered. As a result susceptibility of envenomated *G. mellonella* larvae to fungal infection was increased in thousands times compared with native control. Thus the paralyzation and strong inhibition of immune reactions of larvae by venom of *H. hebetor* allows to minimize quantity of transmitting with parasitoid fungal inoculum. We assumed that «paralyzing» parasitoids can take part in transmission of entomopathogenic fungi particularly in out-of-the-way places (shelters) as well as disperse of fungal infection under low density of hosts.

Contributed paper. Tuesday, 11:45. **112**

Fast spread of the parasitic *Laboulbenia formicarum* in a supercolony of the invasive garden ant *Lasius neglectus*

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Many ant species are highly successful invaders and can dominate vast areas by forming dense networks of connected nests in contrast to the smaller and discrete, spatially dispersed colonies of most social insects. However, it was recently proposed that such supercolonies are more vulnerable to infection by parasites and diseases as they would serve as large targets with high rates of transmission from nest to nest. Here we studied the invasive garden ant *Lasius neglectus*, a new pest species which is currently spreading throughout Europe where several populations are infected with the ectoparasitic fungus *Laboulbenia formicarum*. In one population (supercolony) we followed the prevalence and intensity of the infection over 10 years, revealing an epizootic spread of the ectoparasite with the mean annual prevalence increasing from 0.126 to 0.997. Distinct body parts of the ants had markedly different infection intensities, and at low intensities antennae and thorax were free from signs of infection. There were no seasonal differences in infection intensity and no other *Lasius* species in the area was found to be infected. These results give the first direct support to the hypothesis that supercolonies of invasive ants potentially face a significantly higher threat from parasites and diseases compared to ants with normal colonies, implying interesting perspectives for biological control of these pest species.

Contributed paper. Tuesday, 12:00. **113**

The dietary preference of a beneficial predator in apple orchards reveals an undocumented spore dispersal mechanism for entomopathogenic fungi.

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In the course of a floristic and ecological study of the entomopathogenic fungi found in apple orchards and strawberry fields (part of the IMBICONT biological control project), we observed resting spores in the frass of the earwig *Forficula auricularia*, a beneficial predator in apple orchards. The presence of resting spores in earwig frass suggests that in addition to being a beneficial predator, earwigs may play a role in the dispersal of Entomophthoromycota—a spore dispersal mechanism not previously documented for this group of fungi. In the lab, we observed that earwigs avidly consumed entomophthoromycotan-infected insects even while the fungus was actively ejecting conidia. We hypothesize that this fungus-insect meal might confer a nutritional benefit but that earwigs avoid foraging on insects infected by generalist entomopathogenic fungi (e.g. *Metarrhizium*, *Beauveria*) because these generalist entomopathogens pose a risk that would potentially outweigh any nutritional benefit. We present the preliminary results from a series of choice-experiments to test these hypotheses.

Contributed paper. Tuesday, 12:15. **114**

Effects of entomopathogenic fungi on the “*Trialeurodes vaporariorum* – *Encarsia formosa*” system: preliminary results

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The effects of a selected group of six entomopathogenic fungal isolates (including the mycoinsecticide Naturalis and the *Beauveria bassiana* ATCC74040 strain contained into the commercial product) on the system “*T. vaporariorum* – *E. formosa*” were evaluated, considering the direct effect on the parasitoid development but also on the *E. formosa* activity and behaviour. The effect of fungal treatments on the parasitoid development was evaluated submitting infested tomato plants to the fungal treatments at different times from the parasitization and recording the parasitization rate and the parasitoids emergence. Then, the effect of fungal isolates on *E. formosa* behaviour and activity was examined in “free-multiplechoice” and “no-choice condition”. Finally, the role of *E. formosa* in transmitting the mycoses from infected to uninfected host population was estimated. Results showed that fungal treatments can affect the *E. formosa* development, particularly when applied before the parasitoids introduction and using the mycoinsecticide Naturalis. *E. formosa* showed no differential tropism in “free - multiplechoice” conditions and it was not able to locate and select the uninfected hosts “at distance” but it was able to detect and avoid infected hosts by direct exploration. Furthermore, *E. formosa* was able in vectoring the fungal propagules from contaminated to uncontaminated hosts through its activity. Results of these laboratory experiments provided important information about the possibility to integrate the entomopathogenic fungal treatments and the *Encarsia formosa* releases and clarified some biological and behavioural aspects of the “host-pathogen-parasitoid” system.

WEDNESDAY - 6 August

SYMPOSIUM 5 (Microbial Control) Wednesday, 8:00–10:00
Developments/Issues in the Regulation of Microbial Products: Harmonization across Jurisdictions

Symposium. Wednesday, 8:00 **115**

The authorisation and regulation of microbial biopesticides: why bother?

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The use of microbial biopesticides and other minimal-risk products is starting to become more widespread as a result of new government legislation that aims to reduce the excessive use of conventional chemical pesticides and increase the use of “alternative” control methods. In the European Union, a paradigm shift in pesticide policy has occurred recently with the enactment of the Sustainable Use Directive on pesticides. This legislation makes IPM mandatory for farmers and growers and gives specific emphasis to biologically based controls including microbial biopesticides. There has been significant recent activity in the biopesticides industrial sector, with multinational agchem / agri-business companies buying up biopesticide

companies. The large financial resources of the global companies should lead to an increase in the effectiveness, sales and availability of microbial biopesticide products, but SMEs will still have a critical role to play through the development of innovative, "next generation" biopesticides. All of these products will need to have authorization for use by government regulatory organisation. Authorization can be slow and expensive, which can be a barrier to product development. The authorities recognize this and have put in place measures designed to improve the system, sometimes with mixed results. We have explored why product authorization is necessary, and in this presentation we will discuss ways in which biopesticide regulation could be improved further.

Symposium. Wednesday, 8:24 **116**

Registration of Biopesticides in the EU: a company perspective

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One characteristic of many biopesticides is their narrow host range. This represents a lot of advantages, e.g. protecting the beneficial fauna, harmlessness towards human health etc., but it significantly reduces the potential market size for such products. The procedures for the registration of a biopesticide are mostly the same as for a chemical plant protection product, even if the characteristics of the active substance and the exigencies for the risk assessment differ in many aspects. The difficulties with registering biopesticides are often unknown or inappropriate data requirements, lack of experience within authorities to assess biopesticides, often resulting in unreasonable delays of the evaluation procedures, and too high registration fees. Under such conditions it is almost impossible for the industry to make the development of biopesticides with small-sized markets cost-effective. Furthermore they jeopardize investments in research for new biopesticides. Although the new EU regulation 1107/2009 provides new criteria for the approval of plant protection products - stricter evaluation timelines, a low risk category and evaluation within distinct zones- the uncertainties and high costs for the registration of biopesticides still exist. As a consequence, the industry will focus its investments in research and development of new biopesticides outside of the EU, where the registration of biopesticides is easier. It will become more difficult for European growers to have access to new, innovative and environmental friendly biopesticides in the future, especially in niche markets.

Symposium. Wednesday, 8:48 **117**

Biopesticide registration, a company perspective and how registration influences biopesticide R&D approach of companies in North America

Jarrod Leland

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When developing a new bioinsecticide active and associated formulations there are a series of stage gates that must be met by R&D to ensure final product registration in North America. At each gate confidence is gained to justify further resourcing. This presentation will discuss in general terms the critical milestones and strategy for prioritizing those milestones for a bioinsecticide. Specific reference will be made to Met52 and R&D's role in generating knowledge for its current registration and label expansion. By presenting this perspective, it may shed light on

the long path from discovering a promising isolate to making it a tool for growers. This may also help improve the dialogue between industry and academia to identify points along this path where collaborations can contribute towards that common goal.

Symposium. Wednesday, 9:12 **118**

Registration of biopesticides: how research can be structured to suit microbial registration needs and promote the commercial development of new biopesticides

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Invertebrate pathology is an applied field, and a major aim of research is to make the technologies discovered available to growers through the development and registration of new biopesticide products. The biopesticide market is growing at over 15% per annum: the global market was valued at \$1.3 billion in 2011 and is predicted to reach \$3.2 billion by 2017. There is a challenge therefore to meet the forecast demand for biopesticide products. Most of the micro-organism based products currently on the market are the 'back catalogue', representing research and technology that has been on the laboratory bench for the last 20 or 30 years. To bring plant protection products to the market they have to be registered, how this happens varies country to country and can take many years. In a biopesticide commercialisation pathway, registration is a significant barrier, demanding considerable investment in time and financial resources. Biopesticide research projects need to be designed and structured so research and industry can work in alignment and so reduce the hurdle of registration. This presentation will explore approaches that have been implemented in biopesticide projects to better align research and industry objectives and build partnerships to facilitate the regulatory process thus reducing commercialisation costs and reducing product development timelines.

Symposium. Wednesday, 9:36 **119**

Current developments and issues on regulation of biopesticides- Lessons from REBECA project, comparison of EU and USA systems

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Microorganisms as active ingredients in plant protection products are gaining more and more importance. This is due to the fact that most of them have little effect (if any at all) on human health, non-target organisms, and the environment. However, registration of Microbial Plant Protection Products is still facing particular problems, which is partly due to the fact that some data requirements which can be easily covered for synthetic chemicals cannot be fulfilled for microorganisms and their products for technical reasons. On the other hand, the major advantage of most microbial products is that the microorganism species are scientifically well known and humans are familiar with them either through direct use or environmental exposure for a long time. Data requirements are similar in different regulatory systems, but acceptance of publicly available data for the risk assessment by authorities varies over time and between different regulatory systems.

BACTERIA 3Contributed paper. Wednesday, 8:00 **120****Resistance alleles to *Lysinibacillus sphaericus* are co-select in a *Culex quinquefasciatus* colony and display distinct features**

Maria Helena N. L. Silva-Filha¹, Karlos D. M. Chalegre¹, Tatiany P. Romão¹, Daniella A. Tavares¹, Hervely S. G. Menezes¹, Cláudia M. F. de Oliveira¹, Osvaldo P. de-Melo-Neto²

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Two alleles of the *cqm1* gene, containing mutations associated to resistance to the Binary (Bin) from *Lysinibacillus sphaericus*, were co-selected in a laboratory resistant colony of *Culex quinquefasciatus* (R2362). The goal of this study was to identify these alleles and to analyze the homozygous larvae for each one, through different approaches. The alleles named *cqm1_{REC}* and *cqm1_{REC-2}* are characterized by distinct mutations, however, they code for transcripts of truncated proteins that are not located in the midgut epithelium and cannot act as receptors for the Bin toxin. Homozygous larvae for each allele show high resistance to the Bin toxin, low specific binding of Bin toxin to midgut microvilli proteins and low transcription level of the both resistance alleles. Their frequency in the R2362 colony showed that the *cqm1_{REC}* has predominated during a long period (> 100 generations), however, it has been replaced by the *cqm1_{REC-2}* that became the most frequent allele. A colony established from the cross of homozygous individuals from each allele (1:1 ratio) showed that *cqm1_{REC}* assumed a higher frequency, compared to *cqm1_{REC-2}*, during a period of 21 generations. An AS-PCR-screening detected the presence of *cqm1_{REC-2}* allele in larvae from field populations and its frequency and distribution was lower than that found for *cqm1_{REC}*, suggesting that this allele has a higher risk to be selected. The fitness cost of individuals homozygous is under study to evaluate the impact on the biological performance of individuals carrying these alleles.

Contributed paper. Wednesday, 8:15 **121-STU****Untangling insect pathogenicity in plant-beneficial pseudomonads by a combination of comparative genomics, bioassays and histopathology**

Pascale Flury¹, Beat Ruffner¹, Shakira Fataar¹, Maria Péchy-Tarr², Regina G. Kleespies³, Cornelia Ullrich³, Johannes A. Jehle³, Theo H. M. Smits⁴, Christoph Keel², Monika Maurhofer¹

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The *Pseudomonas fluorescens* group harbors many root-associated plant-beneficial bacteria that suppress soil-borne

fungal diseases and promote plant growth. Remarkably, two strains, *Pseudomonas protegens* CHA0 and *Pseudomonas chlororaphis* PCL1391, additionally display oral insecticidal activity towards lepidopteran larvae. This ability is associated with the Fit insect toxin and unknown GacA-regulated traits. However, the exact course of infection, the target organs and the virulence factors beyond Fit are yet undiscovered. To tackle these open questions we combined various methods. Fifteen strains of fluorescent pseudomonads, including four new isolates, were characterized for both their plant-beneficial traits and their insecticidal activity. Whereas the former were found throughout the entire *P. fluorescens* group, the latter was restricted to strains of *P. protegens* and *P. chlororaphis*. By next generation sequencing and subsequent comparative genomics we identified a small set of genes common to all insecticidal strains, but absent in non-insecticidal strains. These genes could therefore encode potential virulence factors against insects. Histopathology to detect affected insect tissues and fluorescence microscopy to localize the bacteria during the infection complete this study which reveals intriguing aspects on insect pathogenesis of plant-associated pseudomonads and identifies several strains with potent dual activity against root pathogens and insect pests.

Contributed paper. Wednesday, 8:30 **122****Comparative analysis of the Cqm1 and Aam1 ortholog proteins from mosquitoes that have a differential capacity to bind to the Binary toxin from *Lysinibacillus sphaericus***

Lígia M. Ferreira¹, Nathaly A. do Nascimento¹, Tatiany P. Romão¹, Antônio M. Rezende², Osvaldo P. de-Melo-Neto², Maria Helena N. L. Silva-Filha¹

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The Cqm1 and Aam1 are ortholog proteins from the midgut of *Culex quinquefasciatus* and *Aedes aegypti* larvae, respectively. These related proteins, with 74% of identity, are expressed as membrane-bound alpha-glucosidases and, functionally, Cqm1 also acts as the receptor of the insecticidal Binary (Bin) toxin from *Lysinibacillus sphaericus*, while Aam1 does not. The major goal of this study was to analyze some features of these proteins produced in Sf9 cells. The recombinant proteins obtained in this expression system showed the same molecular weight and kept their differential capacity to bind to the Bin toxin, as the native proteins. The Cqm1 sequence presents three predicted N-glycosylation sites (PGS), however, the analysis of the recombinant protein suggested that it does not have glycans. On the other hand, Aam1 sequence has six PGS and analysis of the recombinant protein showed that four of them contain carbohydrates that can be removed by the glycosidase PNGase F. Site-directed mutagenesis of these PGS prevented the insertion of carbohydrates and these mutant proteins did not bind to the Bin toxin, similarly to the wild Aam1. In terms of their catalytic function, both recombinant proteins displayed alpha-glucosidase activity and Aam1 showed a two-fold increase compared to Cqm1. Analysis of protein sequences showed that one segment of the Cqm1, that is required for Bin toxin binding, is not conserved in the Aam1 and might be an important factor for their differential capacity to interact with the Bin toxin and, thus, for the refractoriness of Ae. aegypti larvae to *L. sphaericus*.

Contributed paper. Wednesday, 8:45 **123**

Resilience of the intestinal epithelium to the action of a bacterial pore-forming toxin and to xenobiotics in *Drosophila*

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The host defense against pathogens encompasses two complementary arms: i) resistance, attacking directly the pathogen, which is mediated by the immune system; ii) resilience, also referred to as tolerance, withstanding and repairing damages inflicted either by the pathogen or by the host's own immune system. We have discovered that the compensatory proliferation of *Drosophila* intestinal stem cells (ISCs) allows the intestinal epithelium to maintain its homeostasis during *Serratia marcescens* infection, and thus constitutes a *bona fide* resilience mechanism. Resilience is not limited to the control of ISC proliferation. Within three hours of ingestion of *S. marcescens*, the epithelium becomes very thin in the absence of cell death. Strikingly, epithelial cells are able to recover their shape and volume in the next 6-9 hours. Attack by *S. marcescens* hemolysin, a 2 nm-wide pore-forming toxin, leads to the controlled extrusion of the cytoplasm of epithelial cells. This may help in purging the cytoplasm from damaged organelles. We have initiated a molecular analysis using both a genetic and a transcriptomics approach and thus identified tens of genes required for the regeneration phase. One of them, a conserved cyclin of previously unknown function, plays a major role noncell-autonomously and is required for the expression of early response genes. Many of these genes are also induced by exposure to xenobiotics such as caffeine. We have found that the cyclin mutants are more susceptible to the ingestion of caffeine. Thus, we may have uncovered a novel stress response pathway that underlies a new resilience mechanism.

Contributed paper. Wednesday, 9:00 **124**

Cadherin mutations and Bt resistance: Field screening and fitness costs

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Insecticidal crystal toxins from *Bacillus thuringiensis* (Bt) of the Cry1A family bind to a 12-cadherin domain protein in the midgut of lepidoptera and eventually form pores in the midgut epithelium, leading to death of the insect. Mutations in this cadherin confer Cry1A resistance to several Lepidoptera. In the course of an F1 screen to estimate the frequency of such mutations in field populations of the tobacco budworm *Heliothis virescens*, a novel mutation was found. Like the first mutation found in this species, it is caused by insertion of a transposable element, but in a different location. Allele frequency changes were recorded over several generations of artificial selection for a homozygous mutant strain, showing a substantial fitness cost to knockout cadherin mutations, even under optimal conditions in the laboratory. Although this type of transposon-induced mutation may be moderately common in field populations, its high fitness cost makes it unlikely to threaten the sustainability of transgenic cotton expressing Cry1A toxins.

Contributed paper. Wednesday, 9:15 **125**

Down regulation and mutation of cadherin gene associated with Cry1Ac resistance in Asian corn borer

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Development of resistance in target insects is a major threat to long-term use of transgenic Bt crops. To delay the evolution of resistance in target insect through the implementation of the effective strategies, it is fundamental to understand the pests' resistance mechanisms. One of the most important mechanisms of insect resistance to Bt crops is the alteration of interaction between Bt toxin and its receptor in the insect midguts. Asian corn borer (ACB), *Ostrinia furnacalis*, is a key pest of maize to be targeted by Bt maize. A Cry1Ac resistant strain of ACB has been established in the laboratory. Compared to the membrane proximal extracellular region (MPR) of cDNA of *ofcad* that encodes a cadherin-like protein in ACB from the susceptible strain, there were three mutant alleles of *ofcad* (MPR-r1, MPR-r2, and MPR-r3) associated with resistance to Cry1Ac toxin. Each of those mutant alleles had 2-3 aa substitution in the putative-toxin binding region (TBR) of the cadherin, especially Thr¹¹¹→Ser¹¹¹ was accurate. In addition, MPR-r2 had a deletion expected to eliminate 26 aa-residues in TBR, which resulted in decline in the binding of MPR to Cry1Ac in the resistant strain compared to the susceptible strain. Furthermore, down regulation of *ofcad* was associated with Cry1Ac resistance, response to the stress of low level Cry1Ac toxin in susceptible strain. These results suggest that Cry1Ac resistance in ACB is primarily associated with the down regulation of *ofcad*. Mutations in *ofcad* resulting in amino-acid substitutions and deletions might mediate higher level of resistance.

Contributed paper. Wednesday, 9:30 **126**

ABCC transporters mediate insect resistance to multiple Bt toxins revealed by BSA analysis

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Insect resistance to *Bacillus thuringiensis* (Bt) is one of the main threats for the long term use of Bt-based products, including Bt crops. Identification of genes conferring resistance to Bt will contribute to delaying the development of resistance as well as to provide additional information about the mode of action of these bacteria and its insecticidal toxins. By using linkage analysis based on high throughput sequencing, we have found a novel type of mutation in the ABCC2 transporter conferring resistance to Bt. In addition we have also found that different members of the ABCC transporters can act as receptors for not only Cry1A toxins but also for the Cry1C type toxins. The identified mutation in the ABCC2 transporter is

localized in a region that does not physically interact with the toxins but in the intracellular ATP-binding domain instead. Our toxin binding studies have revealed that such mutation correlates with a reduction in toxin insertion into the membrane (irreversible binding) and suggests that ABCC activity as transporter is necessary for the proper action of Bt toxins.

CONTRIBUTED PAPERS Wednesday, 8:15-9:45
DIS. OF BENEFICIAL INVERTEBRATES 2

Contributed paper. Wednesday, 8:15 **128**

Nosema ceranae News: Update on Species Competition and Host-Pathogen Interaction Studies

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The apparent recent invasion of *Nosema ceranae* and its dominance over *Nosema apis* in honey bee populations in the USA and elsewhere have presented both an enigma and a treatment problem for apiculturists and scientists. Several studies, including those of our research group, have shown that *N. ceranae* produces more mature spores than *N. apis*, and we demonstrated that reproduction of *N. ceranae* recovers more quickly from fumagillin treatment than does *N. apis*. In addition, *N. ceranae* hyperproliferated in the presence of very low fumagillin concentrations in laboratory bioassays. Proteomic-level studies of fumagillin-*N. ceranae*-honey bee interactions continue and we are investigating the mechanisms of protein regulation in response to infection and fumagillin treatment. In studies of infectivity, we found that *N. ceranae* consistently has a higher IC₅₀ than *N. apis*. The effect is most pronounced at 1 day post eclosion. We investigated the interaction of *N. ceranae* and *N. apis* in individual bees and found that *N. apis* produced more spores than *N. ceranae* in 62% of bees infected with equal dosages of both *Nosema* species. Mixed species infections negatively affected survival time (15-17 days) compared to single species infections (20 and 21 days for *N. ceranae* and *N. apis*, respectively) and uninfected bees (27 days). Midgut spore counts were higher for mixed species infections than for single species infections, but we did not find evidence that *N. ceranae* outcompetes *N. apis* in an individual host..

Contributed paper. Wednesday, 8:30. **129**

Influence of temperature on the development of *Nosema apis* and *Nosema ceranae*

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Nosema apis and *Nosema ceranae* are two fungal pathogens infecting the European honey bee, *Apis mellifera*. These obligate intracellular pathogens, belonging to the phylum Microsporidia, infect epithelia cells of the midgut and elicit nosemosis. Recent studies suggested that *N. ceranae* is more virulent than *N. apis* and can lead to severe colony losses. These colony losses are so far only reported from the Southern parts of Europe. In the Northern parts (e.g., Denmark, Sweden, Finland, and Germany) *N. ceranae* could not be correlated to colony losses so far. While *N. ceranae* seems to have replaced *N. apis* in the bee population in South Europe, this is not the case for the Northern parts of Europe. Both findings suggest a climatic angle for spread, assertiveness, and virulence of *N.*

ceranae. Exact whether parameters as temperature or humidity, which hinder or favor *N. ceranae* infections, are not determined so far. Spanish colleagues recently showed that *N. ceranae* has a better adaptation to complete its endogenous cycle at warmer temperatures. However, the results based on *in vivo*-infections only give a minor hint on different proliferation of both obligate intracellular pathogens exposed to different temperatures. We here present our results on the intracellular development of *N. apis* and *N. ceranae* exposed to different temperatures using our recently established cell culture model for *Nosema* spp.

Contributed paper. Wednesday, 8:45 **130-STU**

The involvement of bumblebee small interfering RNA pathway against two different bee viruses

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Wild pollinators such as bumblebees are in global decline. They share a pathogen network with other pollinators, consisting of multi-hosts pathogens and multi-pathogens hosts. Disturbance of these associations could lead toward the further host decline. Insects have developed certain immune pathways to combat viruses, of which the small interfering RNA pathway (siRNA) is important. By unveiling the interaction of the virus with the host defense pathway we can better understand the virulence of certain viruses in specific hosts. Here we use two viruses, Israeli acute paralysis virus (IAPV) and slow bee paralysis virus (SBPV), representing two infection types after injection in *Bombus terrestris*, i.e. IAPV presents an overt acute infection resulting in mortality, while SBPV results in a covert persistent infection. First, to determine viral replication dynamics by following the negative and positive strands, we developed a new method in combining multiplex ligation-dependent probe amplification and qPCR. The results show both viruses experienced an exponential-plateau phase, and their replication strand were relatively low compared with genome (positive) strand. Second, both viruses increased the expression of Dicer-2 and SID, thereby activating siRNA. Finally we performed small RNAs sequencing to screen if differences in the siRNA production could explain different viral virulence.

Contributed paper. Wednesday, 9:00 **131**

Impact of Wolbachia endosymbionts on the evolution of sex determination in the isopod *Armadillidium vulgare*

Sébastien Leclercq, Julien Thézé, Isabelle Giraud, Lise Ernenwein, Bouziane Moumen, Pierre Grève, Clément Gilbert, Richard Cordaux

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Terrestrial isopods are crustaceans that represent a major component of the litter ecosystem, as they mainly feed on dead plant material and participate in litter decomposition. In the isopod *Armadillidium vulgare*, genetic sex determination follows female heterogamety (ZZ males and ZW females). However, many *A. vulgare* populations harbor maternally-inherited *Wolbachia* bacterial endosymbionts. These bacteria are reproductive parasites that convert genetic males into phenotypic females, leading to populations with female-biased sex ratios. The W sex chromosome has been lost in lines infected by *Wolbachia* and all individuals are ZZ genetic males. The female sex is determined by the inheritance of *Wolbachia* by the *A. vulgare* individual. Surprisingly, some *A. vulgare* lines exhibit

female-biased sex ratios despite the lack of *Wolbachia*. In these lines, female individuals are ZZ genetic males carrying an unknown feminizing factor. To elucidate the genetic basis of female sex determination in these lines, we sequenced the genome of a female by Illumina technology. After *de novo* genome assembly, we identified a large piece of the *Wolbachia* genome transferred into the *A. vulgare* nuclear genome. The transferred genomic fragment co-segregates perfectly with the female sex in pedigrees. These results suggest that sex determination in these *A. vulgare* lines is under the control of nuclear gene(s) of bacterial origin and that bacterial reproductive parasites can drive shifts in sex determination mechanisms in animals. This research is funded by an ERC Starting Grant (EndoSexDet) to RC.

Contributed paper. Wednesday, 9:00 **132**

First characterization of a mollusk beta pore forming toxin

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Aerolysins are virulence factors belonging to the beta pore-forming toxin (b-PFT) superfamily that are abundantly distributed in bacteria. More rarely, b-PFTs have been described in eukaryotic organisms. Recently in our laboratory, a putative cytolytic protein called Biomphalysin have been characterized in the snail, *Biomphalaria glabrata*, who's primary structural features suggest that it could belong to this b-PFT superfamily. We have showed that, despite weak sequence similarities with aerolysins, Biomphalysin shares a common architecture with proteins belonging to this superfamily. A phylogenetic approach revealed that the gene encoding Biomphalysin could have resulted from horizontal transfer. Its expression seems to be restricted to immune-competent cells and is not induced by parasite challenge. Recombinant Biomphalysin showed hemolytic activity that was greatly enhanced by the plasma compartment of *B. glabrata*. We further demonstrated that Biomphalysin is able to bind to parasite and has a plasma dependent anti schistosomal activity. Surprisingly, investigation of *B. glabrata* genome reveals that this family appears to be multi-genic. More than 20 genes were identified suggesting an important role played by Biomphalysin proteins for *B. glabrata*. These results provide the first functional description of a mollusk immune effector protein involved in killing of *S. mansoni*, agent of the second most widespread tropical parasitic disease after malaria.

Contributed paper. Wednesday, 9:30 **133-STU**

A first report of an immune-associated cytosolic PLA₂ in insects: Gene structure and function

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Eicosanoids are a group of C20 polyunsaturated fatty acids most derived from arachidonic acid (AA). A phospholipase A₂ (PLA₂) catalyses AA release from phospholipids at SN-2 position. Among three different groups of PLA₂s (cPLA₂, sPLA₂, iPLA₂), only sPLA₂ (secretory type of PLA₂) has been identified as venom- or immune-associated functions. This study reports the first cPLA₂ (cellular and calcium-dependent PLA₂) in insects. A hemocyte transcriptome of *Spodoptera exigua* possessed 1 for sPLA₂, 2 for iPLA₂, 1 for cPLA₂. Expression of Se-cPLA₂ was

inducible to bacterial challenge in hemocyte and fat body. RNA interference of Se-cPLA₂ expression significantly suppressed cellular immune responses of *S. exigua*. A recombinant of Se-cPLA₂ exhibited a specific enzyme activity influenced by pH, temperature, and calcium. Especially, Se-cPLA₂ was susceptible to a specific cPLA₂ inhibitor, but not to a specific iPLA₂ inhibitor. These results indicate that Se-cPLA₂ is a specific cPLA₂ and associated with immune responses.

Contributed PAPERS Wednesday, 8:00-9:30

FUNGI 4

Contributed paper. Wednesday, 8:00 **134**

Fungal dimorphism in the entomopathogenic fungus *Nomuraea rileyi*: A search for *in vivo* produced quorum-sensing molecules

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Similar to other zoopathogenic fungi, many insect pathogenic hyphomycetes including species within the genera *Metarhizium*, *Beauveria*, *Isaria*, and *Nomuraea* exhibit a defined *in vivo* dimorphic developmental program. This program involves switching between apical to budding growth providing mycopathogens with both tissue-invasive and vegetative growth capabilities. The budding yeast-like vegetative cells absorb nutrients in the hemocoel without apparent damage to tissues allowing the insect to continue to feed and develop. The ability to switch cell phenotypes is crucial for successful *in vivo* development. *N. rileyi* exhibits a defined developmental program that involves the sequential production of cellular phenotypes designed to perform spatially and temporally unique functions. Upon reaching the nutrient-rich hemolymph the penetrant germ tube switches from an apical to a budding growth program leading to the formation of freely circulating hyphal bodies. The yeast-like hyphal bodies grow exponentially in the nutrient-rich haemolymph reaching densities that far outnumber circulating hemocytes. As a critical threshold density is achieved, these hemolymph-borne cells synchronously revert to an apical growth program forming the tissue-invasive cell phenotype. The ensuing mycelial phase produce and secrete a suite of metabolites that can modulate host development, that rapidly kill the host, and that efficiently digests insect tissue leading to the mummification of infected larvae. In this presentation investigation we detail the hyphal body to mycelial transition of *Nomuraea* in the insect host, provide evidence for quorum-sensing that is produced and released into the hemolymph, and detail the extraction and examination of the elicitors that mediate the dimorphic switch.

Contributed paper. Wednesday, 8:15 **135**

Multilocus genotyping of *Amylostereum* spp. associated with *Sirex noctilio* and other woodwasps from Europe reveal clonal lineage introduced to the US

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Sirex noctilio is a woodwasp of Eurasian origin that was inadvertently introduced to the southern hemisphere in the 1900s and to North America over a decade ago. It attacks various *Pinus* species and cause significant mortality in pine plantations. *Sirex noctilio* is associated with a symbiotic white rot fungus, *Amylostereum areolatum*, which females inject into trees when they oviposit and which is required for survival of developing larvae. We examined the genetic diversity of *A. areolatum* isolated from *S. noctilio* and other woodwasps collected from Europe in comparison with samples from northeastern North America to determine origin of introduction(s). Multilocus genotyping of nuclear ribosomal regions and protein genes revealed two widespread multilocus genotypes (MLGs) among the European samples, one of which is present in the US. The other US *S. noctilio*-associated *A. areolatum* represented unique MLGs, although variation was primarily due to the laccase gene, with the other loci having conserved sequences. The closest relative to these US strains is a German strain with identical ITS, mtSSU and tef sequences. These findings indicate multiple introductions of *S. noctilio* to North America from Europe or from Europe via South America. Our results also showed lack of fidelity between wasp hosts and *Amylostereum* species, and we found a North American woodwasp carrying an *A. amylosterum* MLG likely introduced by *S. noctilio*. These results underscore the need to study North American sircids and their fungal symbionts as *S. noctilio* continues to spread in North America.

Contributed paper. Wednesday, 8:45 **137**

MALDI-TOF Mass Spectrometry: A complement to sequence-based identification technologies for major fungal entomopathogens

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Matrix-Assisted Laser Desorption/Ionization Time of Flight mass spectrometry has been tested and proven to be a rapid and inexpensive approach closely replicating the results of gene sequence-based analyses to identify species in such major entomopathogenic fungal genera as *Metarhizium* and *Beauveria*. While MALDI-TOF cannot replace PCR-based approaches for identifications or phylogenetic studies and cannot demonstrate relationships among fungi, it does appear to be extremely valuable for rapidly detecting anomalous isolates that need further detailed PCR-based study. This mass-spectrometric technique may be extremely valuable for ecological and population biology studies, as well as offering significant support for the efficient curation of large culture collections holding hundreds to thousands of isolates for which verified MALDI-TOF profiles are available. In comparison to the results obtained from the more routine analyses of (still) small numbers of individual genes, MALDI-TOF uses large numbers of cell proteins to group samples and, therefore, monitors much larger proportions of a total organismal genome; evidence will be presented that such a more complete coverage of the total genome suggest the existence of biogeographical groupings that may not be easily detected by PCR-based studies.

Contributed paper. Wednesday, 8:30 **136**

Preliminary analysis of the genome sequence of *Beauveria caledonica*

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Beauveria caledonica is a pathogen of a number of insects, especially Coleoptera. Occurrence has probably been under-reported due to the morphological similarity the ubiquitous entomopathogenic fungus *Beauveria bassiana*. Recent phylogenetic studies have shown that *B. bassiana sensu lato* is really a species complex. The genomic differences between species of *Beauveria* can assist understanding of the importance of selected gene in disease and ecology of these fungi. We report on initial comparisons of the genome of *B. caledonica* strain isolated in New Zealand and *B. bassiana*. The genome was sequenced using 3 lanes of a MiSeq by NZGL (New Zealand). 15,890,840 150-bp read pairs were obtained for the 32-Mb *Beauveria* strain (~149 fold coverage). After assembly using the programme ABySS, a total of 10,951 contigs were obtained over 39 bp and an N50 of 21676, with 2827 over 500 bp. Preliminary comparisons were conducted on a range of phylogenetic, secondary metabolite and mitochondrial gene regions. Assembly of the mitochondrial genome was used to assess completeness of the coverage. The genome sequence of *B. caledonica* shows significant divergence from *B. bassiana*.

Contributed paper. Wednesday, 9:00 **138**

Transcriptomic study reveals *Pandora formicae* expressing pathogenicity related genes in final stages of host infection

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Pandora formicae (*Entomophthorales*, Entomophthoro-mycota) is an obligate pathogen of the common red wood ant, *Formica rufa*. The fungus, similarly to other fungi of this group, enters the host body through cuticle, where it exploits nutritional resources within the haemocoel. When the infected ant is close to death, the fungus triggers a change in host behavior, manipulating it to climb a leaf (e.g. grass) or a twig. The fungus attaches the moribund host with rhizoids, the host legs grasp around the leaf or twig and the mandibles bite to vegetation and lock. Then the host dies and soon after the fungus breaks through the cuticle with conidiophores producing asexual spores. This quick transformation requires activity of several enzymes involved in cuticular breakdown, cell wall formation, and other processes. To study this, we have constructed transcriptome libraries of the last two stages: 1) when the ant is just dead with no fungal growth outside except the rhizoids, and 2) when external conidiophores are present. This first *de novo* transcriptome of an entomophthoralean fungus, in interaction with host, provides accurate insight into the plethora of genes expressed during final stages of infection, crucial for fungus transmission and reproductive success.

Contributed paper. Wednesday, 9:15 **139**

Transcriptome analysis of the entomopathogenic oomycete *Lagenidium giganteum* reveals putative virulence factors shared by fungal and oomycete entomopathogens

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The entomopathogenic oomycete *Lagenidium giganteum* is known to infect and kill mosquito larvae and therefore has been seen as a potential biological control agent against disease vector mosquitoes. However, little is known about the pathological process of *L. giganteum* in its mosquito host. In order to detail the molecular basis of entomopathogenicity, a transcriptome analysis was initiated for *L. giganteum*, using various Next Generation Sequencing technologies. Homology searches have led to the annotation of ca. 20,000 transcripts based on significant similarity to known proteins and revealed a full complement of plant pathogenic oomycete effector orthologs. The characterization of full-length transcripts corresponding to Cellulose Binding Elicitor Lectin (CBEL), Crinkler, and elicitin proteins demonstrated that *L. giganteum* is the first described animal pathogenic oomycete to secrete canonical Crinkler and CBEL effectors. In addition, phylogenetic analyses identified a Glycoside Hydrolase 5 (subfamily 27; GH5_27) as a putative virulence factor. Genome mining indicated that GH5_27 orthologs are shared by entomopathogenic oomycetes and fungi, but virtually absent in all other oomycetes and fungi. Using PCR, GH5_27 fragments were amplified and sequenced from additional entomopathogens, suggesting that oomycete and fungi underwent convergent evolution and that GH5_27 proteins may play a crucial role in insect/microbe pathosystems. Detailing the molecular basis of entomopathogenicity may allow for the use of oomycetes and fungi as control agents against insect pests, reducing the use of insecticides that can have negative impacts on the environment and human health.

SYMPORIUM 6 (Bacteria) Wednesday, 10:30–12:30
Structure and Function of Novel Insecticidal Toxins

Symposium. Wednesday, 10:30 **140**

Structural and biophysical characterization of Cry34Ab1 and Cry35Ab1

Matthew S. Kelker¹, Colin Berry², Matthew D. Baker², Steven L. Evans¹, Reetal Pai¹, David McCaskill¹,

Joshua C. Russell^{1†}, Nick X. Wang¹, J.W. Pflugrath³, Cheng Yang³, Matthew Wade⁴, Tim J. Wess^{4#}, Kenneth E. Narva¹

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Bacillus thuringiensis strains are well-known for the production of insecticidal proteins upon sporulation and these proteins are deposited in parasporal crystalline inclusions. The majority of these insect-specific toxins exhibit three domains in the mature

toxin sequence. However, other Cry toxins are structurally and evolutionarily unrelated to this three-domain family and little is known of their three dimensional structures, limiting our understanding of their mechanisms of action and our ability to engineer the proteins to enhance their function. Amongst the non-three domain Cry toxins, the Cry34Ab1 and Cry35Ab1 proteins are required to act together to produce toxicity to the western corn rootworm (WCR) *Diabrotica virgifera virgifera* Le Conte via a pore forming mechanism of action. Cry34Ab1 is a protein of ~14 kDa with features of the Aegerolysin family (Pfam06355) of proteins that have known membrane activity, while Cry35Ab1 is a ~44 kDa member of the Toxin_10 family (Pfam05431) that includes other insecticidal proteins such as the binary toxin BinA/BinB. The Cry34Ab1/Cry35Ab1 proteins are important solutions for control of WCR having been developed as insect resistance traits in commercialized corn hybrids for control of WCR. The structures of Cry34Ab1 and Cry35Ab1 have been elucidated to a resolution of 2.15 Å and 1.80 Å, respectively. The solution structures of the toxins were further studied by small angle X-ray scattering (SAXS) and native electrospray ion mobility mass spectrometry. We present here the first published structures from the Aegerolysin and Toxin_10 protein domain families.

Symposium. Wednesday, 10:50 **141**

Structure/function studies of Cry5B via alanine-scanning mutagenesis

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Cry5B is a three-domain crystal protein that attacks nematodes. In collaboration with the laboratory of Partha Ghosh, the three dimensional structure of Cry5B has been solved. Cry5B shows significant similarities with three-domain insecticidal crystal proteins in domains I and III but significant differences with insecticidal proteins in domain II. To better understand structure function relationships in Cry5B, we performed alanine-scanning mutagenesis of the entire toxin domain in which each point variant was tested in bioactivity assays with the free-living nematode *Caenorhabditis elegans*. Alanine point variants were classified into three classes—those with reduced/no bioactivity, those with relatively normal bioactivity, and those with increased bioactivity against *C. elegans*. More than 400 point variants were successfully tested. Some of those in the latter class (increased bioactivity) have been selected for further study, including fully quantitative analyses and testing their spectrum of increased action against other nematodes. Our results, as well as their implications for crystal protein – nematode interactions, will be presented..

Symposium. Wednesday, 11:10 **142**

Insights into the structures of non-3-domain toxins through structural modeling

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A knowledge of the structure of insecticidal toxins is a major benefit in elucidating mode of action and is, therefore, fundamental for targeted mutagenesis to test mechanistic hypotheses, to alter target range and increase toxicity. Crystal structures of activated 3-domain toxins, Cyt toxins, Vip2, Mtx1 and anthrolysin are available and can be used to model structures for related proteins. There remains a significant

number of potent invertebrate-active toxins that do not fall within these classes. Crystallography is labour-intensive, requiring large quantities of pure, mono-disperse protein and often proves difficult. Recent developments in the field of *in silico*, *ab initio* structural modelling allow the generation of models in the absence of related sequences in the protein structure database. This may allow us to predict protein structures and use these predictions to develop testable hypotheses for the modes of action of the toxins. This procedure has been applied to several non-3-domain toxins and toxin-associated proteins. For one such protein, a structure is proposed, consistent with a pore forming mechanism of action. Analysis of secondary structure content is consistent with this model and evidence of pore formation has been produced. Mutagenesis of a region known to be important in structurally-related toxins was shown to eliminate toxicity. While further study is clearly required, modelling, thus, allows us to predict and test hypotheses related to the mode of action of toxins for which experimental structures are, as yet, unavailable.

Symposium. Wednesday, 10:30 **143**

Novel MTX Toxins for Insect Control

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In addition to the conventional 3-domain Cry proteins, the Gram-positive bacteria *Bacillus thuringiensis* can also harbor other classes of insecticidal toxins with distinct structures, receptors, and modes of action. Among them are a group of proteins that share significant similarities to MTX2/3 toxins at the structural level, but are very divergent at the amino acid sequence level. In this presentation, we will discuss the general features of these MTX toxins, and agriculture applications for the control of insect pests.

Symposium. Wednesday, 11:50 **144**

Insecticidal toxins from *Photobrhabdus luminescens* and *asymbiotica*, targeting the actin cytoskeleton and GTP-binding proteins

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Photobrhabdus luminescens and *asymbiotica* live in the gut of entomopathogenic nematodes, which invade insect larvae, where they release the bacteria. Here, the bacteria produce toxins, which kill the insects. We studied tripartite Tc toxins from *P. luminescens* and a novel toxin (PaTox) from *P. asymbiotica*. Tc toxins consist of three components TcA, TcB and TcC, which occur in several isoforms. TcA is responsible for the binding and up-take of the toxin, B is a linker and C carries the biological activity. Recent crystal structure analysis revealed a novel type of syringe-like injection mechanism, which depends mainly on TcA but needs all components (1). We studied the biological activity of TccC3 and TccC5, which are isoforms of TcC. TccC3 ADP-ribosylates actin at threonine148, thereby actin polymerization is enhanced (2). TccC5 ADP-ribosylates Rho proteins at glutamine61, a modification which persistently activates of Rho GTPases. Both modifications of actin and Rho proteins induce clustering of the actin cytoskeleton (2). The *P. asymbiotica* toxin PaTox glycosylates Rho proteins by attaching GlcNAc at tyrosine32/34 (3). The modification inhibits Rho signaling, because Rho activation and interaction with effectors are blocked. In addition, PaTox harbors a deamidation domain, which activates heterotrimeric G proteins, including Gq/11 and Gi family proteins. Functional consequences of the

actions of *Photobrhabdus* toxins on actin and GTP-binding proteins are discussed.

References

1. Meusch et al. (2014) Nature 508, 61-65.
2. Lang et al. (2010) Science 327, 1139-1142.
3. Jank et al. (2013) Nat. Struct. Mol. Biol. 20, 1273-1280..

Symposium. Wednesday, 12:10 **145**

Molecular basis of parasporin-2 action toward cancer cells

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Parasporin-2 (PS2) is a crystal toxin isolated from parasporal inclusions of *Bacillus thuringiensis* A1547. PS2 has a strong cytotoxic activity in liver and colon cancer cells without showing a typical insecticide. Accumulated molecular, cellular and *in vivo* experimental observations on PS2 indicate that the protein form a pore in membrane with a mega size assembly. The crystal structure of active PS2 monomer reveals that the protein elongates like a short rod, comprising almost β-strands. The polypeptide folding is similar to a class of aerolysin-like β-pore-forming toxins while there is no homology to insecticidal Cry toxins. N-terminal domain of PS2 is rich in aromatic residues and forms a groove which could be capable to grapple the target molecule. Amino acid substitutions of PS2 in the region indicate that the residues could be involved in cell-binding. The C-terminal domain contains β-sandwiches and the surface of the protein has a unique extensive track of exposed side chains of serine and threonine where thought be related to PS2 oligomerization and membrane pore formation. Single-particle EM analysis reveals that PS2 oligomer shows a ring shape with the 24nm length, 8 nm diameter and a 4nm pore while a structure of pore-forming aerolysin is the ring-like mushroom structure with a central pore. We would like to introduce current observations on anti-cancer toxin PS2 *in vitro* and *vivo* in this symposium.

CONTRIBUTED PAPERS Wednesday, 10:30-12:30

MICROBIAL CONTROL 2

Contributed paper. Wednesday, 10:30 **146**

Evaluation of the non-target effects of *Bacillus thuringiensis* subspecies *israelensis* in standardized aquatic microcosms

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Malaria, one of the most deadly vector-borne diseases in the world, is transmitted by the bite of an infected female *Anopheles* mosquito. *Bacillus thuringiensis* subsp. *israelensis* (*Bti*) is a gram-positive, aerobic, spore-forming bacterium that produces crystalline inclusions that contain insecticidal proteins. *Bti* has been shown to be highly insecticidal to larvae of mosquitoes and blackflies, but is considered to have weak insecticidal activity against non-dipteran invertebrates in aquatic environments. Few studies have comprehensively studied the non-target effects of *Bti* under reproducible and standardized conditions. The objective of this study was to evaluate the effects of *Bti* on key non-target invertebrates in a highly reproducible synthetic multi-species system, the

standardized aquatic microcosm (SAM) system. The SAM system is initiated in a chemically defined medium with synthetic sediment and is inoculated with nine different species of photosynthetic microorganisms (PMOs) and different non-target invertebrates. Replicate SAMs were inoculated with a LD₉₀ of *Bti* strain HD-522, whereas the control SAMs were not inoculated with *Bti*. Over a period of 2 months, the abundance of PMOs and invertebrates were determined by biweekly sampling. Differences between *Bti*-treated and control SAMs were assessed by statistical analyses of sampling data and biological diversity indices. The contributions of the SAM experiments to our understanding of the non-target effects of *Bti* are discussed.

Contributed paper. Wednesday, 10:45 **147**

***Bacillus thuringiensis* 00-50-5 strain with high activity against plant-parasitic nematodes and insect pests**

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Plant-parasitic nematodes (PPNs) are serious pathogens of many other crops. Yellow cutworm *Agrotis segetum* is a serious insect pest of vegetable and grains. After liquid fermentation of *Bacillus thuringiensis* (*Bt*) strain 00-50-5, the cell-free supernatant (CFS) and the crystal protein toxins (CPT) have high activities against the root-knot nematode (RKN) *Meloidogyne incognita* and the yellow cutworm *A. segetum*. The mortality for second-stage RKN juveniles (J2) was 100% as early as 5 hrs after exposure to 0.909 µg.mL⁻¹ of dried CFS. The LC₅₀ values were 0.037- and 0.015 µg.mL⁻¹ of partially purified Bt exotoxin at 5 hrs and 24 hrs after exposure, respectively. The mortality of *A. segetum* was 100% for first-instar larvae (L1) after exposure to 10 µg.mL⁻¹ CPT for 72 hrs. The LC₅₀ value for *A. segetum* L1 was 0.417 µg.mL⁻¹. An SDS-PAGE of the purified 00-50-5 CPT resulted in four main proteins with 133-, 60-, 27- and 25 kDa after treatment with 1% SDS, and three proteins with 133-, 60-, and 27-kDa after treatment with 0.1N NaOH. The Bt 00-50-5 has dual nematicidal and insecticidal activities against soil-dwelling pests, such as *M. incognita* and *A. segetum*.

Contributed paper. Wednesday, 11:00 **148**

Investigations on residues of *Bacillus thuringiensis* on tomato

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After an incidence of diarrhea in 2012, high concentrations of presumptive *Bacillus cereus* (including *B. thuringiensis* (*Bti*)) were found in German lettuce samples. Because of this incidence, in Germany a discussion about the risk of *Bti* residues started and is still ongoing.

To proof the degradation of *Bti* spores in glasshouses, experiments were conducted on tomato under laboratory, experimental field station and professional grower conditions. For all experiments the *Bti* product XenTari® was used.

In the glasshouse experiment with five applications of XenTari® applied in a weekly interval the concentration of *Bti*

spores on tomato fruits ranged in all experiments between 4.9x10⁴ und 8.5x10⁴ cfu/g fresh weight. For single application of *Bti* a max. spore concentration of 4.7x10⁴ cfu/g fresh weight was measured corresponding to the laboratory experiments and the experiments at a commercial farm. To proof the degradation *Bti* spores over time samples were taken after the last application over one week. Over all experiments the concentration of *Bti* spores was reduced up to only 46 to 77 % of the initial spore concentration within one week. A distinct reduction of *Bti* spores on fruits was achieved by modifying the application strategy. When only the upper parts of the tomato plant were treated with XenTari, a maximum concentration of *Bti* spores of 3.3 x 10³ cfu/g fresh weight was recorded.

Contributed paper. Wednesday, 11:15 **149**

Biological control of western corn rootworm larvae (*Diabrotica virgifera virgifera*)

with Dianem® (*Heterorhabditis bacteriophora*)

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The biocontrol product dianem® contains entomopathogenic nematodes, *Heterorhabditis bacteriophora*. It is officially registered in Austria as a plant protection product to control larvae of the Western Corn Rootworm (*Diabrotica virgifera virgifera*). Field results from Hungary, Austria and Italy applying 2 x 10⁹ nematodes per ha obtained equally high control like chemical seed dressings with neonicotinoids or application of granular insecticides containing the pyrethroide Tefluthrin. Adapted application technology has been developed to apply nematodes with 200 ltr. of water/ha directly on the seeds. Although the insect larvae occur approximately a month later, the nematodes persist long enough to control the pest. Insects penetrate into the roots where they are not easily reached by insecticides, whereas nematodes follow the insects into the galleries and kill the larvae 2-3 days after infestation. Latest field results, which have used the novel application technology, will be presented. Since product costs reach almost the same level like chemical insecticides and since the seed treatment with neonicotinoids was banned by the European Commission in 2013, the product dianem® is in commercial use for the first time on larger scale against this invasive maize pest.

Contributed paper. Wednesday, 11:30 **150**

Evaluation of Ten Plant Extracts as Ultraviolet Protectants for *Spodoptera littoralis* nucleopolyhedrovirus

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Ten plant extracts were tested as ultraviolet protectants to improve the persistence of *Spodoptera littoralis* multiple embedded nucleopolyhedrovirus (SpilimNPV). In an initial test, the SpilimNPV alone or in combination with 10 plant extracts, each at a concentration of 1% was exposed to ultraviolet B (UV-B) for one hour. Among them, five plant extracts, viz. cloves, henna, green tea, pomegranate and grape showed a high rate of virus protection with original activity remaining

(OAR) at 100 %, 97 %, 91 %, 90.6 %, and 77 %, respectively. However, lemon, kiwi, olive, dates and beetroot extracts provided lower protection with OARs of 71 %, 58.4 %, 53 %, 21 %, and 18 %, respectively. Using the same UVB source, secondary screening was carried out on the five best additives from primary screen, and tested at a concentration of 0.5% and using an exposure timing of 5 hours. Clove and henna showed the highest rate of protection with OAR of 96.6% and 76.5%, respectively. In addition, absorption spectra and the obtained protection rate were correlated. These laboratory findings are very encouraging and that field studies are underway.

Contributed paper. Wednesday, 10:45 **151**

Interactions among Fungal and Viral Pathogens and Parasitoids

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The majority of studies of pathogens are conducted with one pathogen species or even strain and one host species, although in nature numerous pathogens and other parasites form a community attacking a host, and even attacking the same host individual. We conducted studies of natural enemy interactions using *Lymantria dispar* larvae, the fungal pathogen *Entomophaga maimaiga*, the viral pathogen *LdMNPV* and parasitoids, all of which have been introduced to North America. Studies in low density populations were conducted in central New York State over 16 years and high density populations were studied at 59 sites in the mid-Atlantic region in 2009, when an outbreak population was crashing. We found very different interactions at low versus high host population densities. At low host density, *E. maimaiga* and parasitoids were both fairly abundant and *LdMNPV* infections were almost nonexistent. At virtually all higher density sites the emergent *E. maimaiga* was most abundant. Virus infection was positively associated with host density while *E. maimaiga* and parasitoids were both frequency dependent. *E. maimaiga* and parasitoids co-occurred in the same larvae less than expected and *LdMNPV* and parasitoids co-occurred in the same larvae more than expected while the fungus and virus reproduced in the same cadaver as expected, suggesting little interaction. This pattern of co-occurrence suggests that the two semelparous natural enemies (fungus and parasitoid) seldom successfully share a host larva while the iteroparous virus was more successful in co-inhabiting with either *E. maimaiga* or parasitoids.

Contributed paper. Wednesday, 12:00 **152**

Oryctes rhinoceros* population diversity and potential implications for control using *Oryctes nudivirus

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The discovery of *Oryctes nudivirus* (OrNV) in the 1960s by Dr Alois Huger enabled the successful management of coconut

rhinoceros beetle (*Oryctes rhinoceros*) populations through classical biocontrol release in the Pacific, SE Asian, and Indian Ocean regions. OrNV continues to be an important biocontrol agent for the control of *Oryctes rhinoceros* in both coconut and oil palm growing regions. Augmentative release of OrNV is commonly used to enhance the natural spread and ensure its continued presence within palm growing regions and surrounding areas. For over four decades after the distribution of the virus, *O. rhinoceros* was not reported to have established in any new regions. However, in 2007 the beetle was discovered in Guam and the population has now established with a highly damaging outbreak such as those not seen for 40+ years. Initial attempts to introduce OrNV into the Guam population were unexpectedly unsuccessful. This has raised the possibility the *O. rhinoceros* population that invaded Guam is less susceptible to OrNV, or potentially resistant. Furthermore, near the end of 2013, a population of *O. rhinoceros* was detected in Hawaii, although it is not believed to have established yet. The discovery of new *O. rhinoceros* invasions within the Pacific region linked with the possibility of a virus tolerant population suggests the beetle may again be on the move. To assist efforts in identifying the source populations for the Guam outbreak, a simple PCR-RFLP method has been developed to distinguish Guam *O. rhinoceros* from other populations. Analysis of several *O. rhinoceros* populations has demonstrated that the Hawaiian beetles are of the same haplotype as those found in Guam. We will discuss current results in relation to what is known about these new invasions and potential implications for the future.

Contributed paper. Wednesday, 12:15 **153**

The Control of Fungi Using with Liposomal Formulation of Essential Oil of *Satureja hortensis* and its cell viability assay

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Satureja hortensis is an annual herb used as nostrum in Eastern Anatolia region of Turkey for the treatment of different infectious diseases and disorders. It can also be utilized as a biopesticide against greenhouse pests. The aim of the present study was to control *Candida albicans* with liposomal formulation of essential oil of *S. hortensis* incorporated into an ointment. Also toxicity of the liposomal essential oil of *S. hortensis* was investigated by MTS assay analysis on L929 mouse fibroblast cell lines. The liposomal formulations were designed using thin film technique and liposomes were properly incorporated into the ointment. The chemical composition of the essential oil obtained from *S. hortensis* was determined by GC and GC-MS analysis. The liposomal essential oil of *S. hortensis* was tested against *Candida albicans* with disc diffusion assay and micro-well dilution assay. Then the toxicity of liposomal essential oil on mammalian cells was determined with MTS analysis. The results of antifungal tests showed that the essential oil of *S. hortensis* incorporated into the ointment and liposomal essential oil formulation have potential antifungal activity against *Candida albicans*. MTS assay results showed that a concentration of 10^{-7} % liposomal essential oil formulation is the safe dose for L929 mouse fibroblast cells. This liposomal formulation dramatically increases antifungal activity by improving cellular intake without side effects on mammalian cells.

VIRUSES 4Contributed paper. Wednesday, 10:30 **154*****Mamestra configurata* nucleopolyhedrovirus-A transcriptome from infected host midgut**

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 Douglas Baldwin¹

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Infection of an insect by a baculovirus occurs in two distinct phases, an initial infection of host midgut by occlusion-derived virions (ODVs) and subsequent systemic infection of other tissues by budded virions (BV). A vast majority of investigations of the infection process have been restricted to cell culture studies using BV that emulate the systemic phase of infection. In the current study we investigate baculovirus gene expression in ODV infected midgut cells. We have focused on the critical first phase of *in vivo* infection by *Mamestra configurata* nucleopolyhedrovirus-A in *M. configurata* larvae, using qPCR and RNAseq mass sequencing strategies to examine virus gene expression in midgut cells. The earliest genes detected by each method had significant overlap and included known early baculovirus genes as well as genes unique to MacoNPV-A and genes of unknown function. The RNAseq datasets also revealed a large range of expression levels across most ORFs. These datasets provide a whole genome transcriptomic analysis of viral genes required for virus infection *in vivo* and will provide the basis for functionally analyzing specific genes that may be critical elements in baculovirus midgut infectivity and host range.

Contributed paper. Wednesday, 10:45 **155-STU****Genomic adaptation to different hosts – Impact of genetic diversity on viral fitness**

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Ecological and genomic adaptations underpin evolutionary processes. Nucleopolyhedroviruses, enclosing many virions in their occlusion bodies, evolve as populations of genomes, adapting to particular ecological niches. We previously showed that all the possible variation is present in a genome population of the size of baculoviruses. When adapting to a new niche, genome populations should differentiate. We conducted experimental evolution on AcMNPV wild type population by passaging 10 times through 4 different host species, in 10 replicates. We then characterised the genetic make up the original and evolved baculovirus populations by ultra-deep Illumina sequencing and their phenotypes by virulence bioassays. We were able to compare virulence components (time, dose and yield) to population diversity. Our experiment allowed us to follow the evolution of a population of genome and its phenotype in different environments to link

fitness with genomic changes.

From all the evolved populations, different profiles emerge, with different relations between intra-population variation and fitness. Actually, it seems that all the species that have evolved on a host show a reduction of intra-population variation while increasing fitness on this host. But when looking at the generalist potential of the population, a lower diversity doesn't always bring a lower fitness. Of course, there are variations in these results that seem to be modulated with the primary fitness of the virus to the infected host; spectacular fitness increase can emerge when infecting a very resistant host. These results give new indications in the evolution of the relation between fitness and genetic diversity.

Contributed paper. Wednesday, 11:00 **156-STU****Transcriptomic analysis of a host-parasitoid interaction between a Hymenoptera *Cotesia congregata*, a Lepidoptera *Manduca sexta* and a Polydnaviridae**

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Cotesia congregata develops as a gregarious endoparasitoid into larvae of the tobacco hornworm *Manduca sexta*. The parasitoid wasp has evolved virulence strategies using an obligatory viral symbiont from the Polydnavirus (PDV) family named *Cotesia congregata bracovirus* (CcBV). CcBV particles are produced by specialized cells of the wasp ovaries and are injected along with the eggs into the host body and act by manipulating host immune defenses, and development, thereby enabling wasps to survive in a potentially harmful environment.

In the caterpillar host, the expression of only a few selected candidate virulence genes had been studied, and so far we lacked a global vision of viral and host gene expression.

To identify viral and host gene regulation during parasitism we performed a large-scale transcriptomic analysis by 454 sequencing of two distinct immune tissues (fat body and hemocytes) of the host *M. sexta* isolated in four experimental contexts: (i) non-treated *M. sexta*; (ii) parasitism of *M. sexta* by *C. congregata*; (iii) immune stimulation of *M. sexta* by heat-killed bacteria; (iv) parasitism of *M. sexta* by *C. congregata* followed by bacterial challenge. Following this analysis, we were able to identify 76 CcBV genes and 1993 *M. sexta* genes expressed 24hrs after parasitism.

The data obtained allows us to draw for the first time a functional map of the CcBV genome, and to visualize at a global level *M. sexta* genes that are regulated during parasitism. This type of analysis will help us to highlight viral virulence genes that play an essential role in the host-parasitoid interaction.

Contributed paper. Wednesday, 11:15 **157****Expressed viral ORF and new virus discovery from high throughput transcriptomes of non-model animal**

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High-throughput sequencing allows quantifying the viral biodiversity by studying the diversity of endogenous viral ORF and discovering new virus pathogens of impacting host species. A total of 114 non-model wild animal species from 33 taxonomic groups (i.e. 441 individual transcriptomes) were screened. Virus detection pipeline started with de-novo assembly of Illumina reads and prediction of 17 million ORF. Protein annotation was performed by a sequence homology search. Taxonomic assignment of each ORF was finally achieved using the NCBI taxonomy database.

Viral ORF from 8 species of termites, mosquito, ants, crustacean and marine annelid were analyzed thus far. We detected 146 viral ORF, i.e. 10 viral ORF per host species, mostly related to dsDNA viruses. Genomic analysis showed that their (G+C) content was at intermediate level between those from host genes and from exogenous viruses, suggesting a genuine and recent viral origin. Viral ORF were shorter than their exogenous counterparts but still expressed: their function might have thus been retained. This result illustrated potential cases of viral gene domestications by the host's genomes. A dozen of complete viral genomes were identified thus far; mostly RNA viruses. Molecular phylogenies allowed assessing the taxonomic position of the viruses. *Lake sinai virus-like* (LSV; unclassified ssRNA virus) were discovered in ants and solitary bees. LSV was recently discovered in honey-bees associated with colony collapse disorder. LSV-like discovery in non-*Apis* insects suggests that hymenopterans could act as a viral reservoir toward domesticated bees. This work illustrated the great potential of our method for high-throughput virus discovery.

Contributed paper. Wednesday, 11:30 **158**

Population genomics supports baculoviruses as vectors of horizontal transfer of insect transposons

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Horizontal transfer (HT) of DNA is an important factor shaping eukaryote evolution. Although several hundreds of eukaryote-to-eukaryote HTs of transposable elements (TEs) have been reported, the vectors underlying these transfers remain elusive. Here, we show that multiple copies of two TEs from the cabbage looper (*Trichoplusia ni*) transposed *in vivo* into genomes of the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) during caterpillar infection. We further demonstrate that both TEs underwent recent HT between several sympatric moth species (*T. ni*, *Manduca sexta*, *Helicoverpa spp.*) showing different degrees of susceptibility to AcMNPV. Based on two independent population genomics data sets (reaching a total coverage >330,000X), we report a frequency of one moth TE in 8,500 AcMNPV genomes. Together, our results provide strong support for the role of viruses as vectors of TE HT between animals, and they call for a systematic evaluation of the frequency and impact of virus-mediated HT on the evolution of host genomes.

Contributed paper. Wednesday, 11:45 **159**

Genomic analysis of five *Lymantria dispar* multiple nucleopolyhedrovirus isolates and biological activity against different host strains of *Lymantria dispar*

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To evaluate genetic diversity of *Lymantria dispar* multiple nucleopolyhedrovirus (LdMNPV) at the genomic level, five isolates of LdMNPV from North America, Europe, and Asia were selected for complete genome sequence determination. These isolates consist of LdMNPV-2161 from Korea; LdMNPV-3029, a sample of the product Virin-Ensh, from Russia; LdMNPV-3041 from Japan; LdMNPV-3054 from Spain, and LdMNPV-Ab-a624, a plaque isolate from a sample collected in Massachusetts, USA. The genome sequences of these isolates were co-linear with the genome sequence of the reference isolate LdMNPV 5-6, derived from the Gypchek product. LdMNPV 5-6 ORFs Id31, Id66, and Id133 were not found in the other five isolates, while all other ORFs annotated for isolate 5-6 were present in at least one other isolate. The greatest degree of sequence divergence among the isolates was observed among the *bro* genes, especially in the two clusters of *bro* genes between *chitinase* (Id70) and Id76 and between Id111 and *dutpase* (Id116). A 2-nt deletion in the enhancin gene *vef-2* (Id160) of LdMNPV-Ab-a624 resulted in a frameshift and truncation of the *vef-2* ORF, while a deletion in LdMNPV-3041 entirely removed *vef-1* (Id65). Bioassays against the New Jersey Standard Strain of *L. dispar* did not indicate any reduced pathogenicity due to mutation or deletion of *vef* genes in either isolate 3041 or Ab-a624. In bioassays against *L. dispar* from Japan, Russia, Europe, and North America, isolates 2161, 3029, and 3041 exhibited a greater degree of pathogenicity against neonate larvae than a sample of Gypchek at the lower dose range.

Contributed paper. Wednesday, 12:00 **160**

Phylogenomics reveals ecological factors that lead to speciation in Baculoviridae

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The study of drivers of species diversification is complex due to the entanglement of numerous ecological factors defining ecological niches. By their nature, viruses provide a confined system of interactions to study diversification from micro to macroevolution. Virus ecological niches are clearly defined by their hosts, which consequently should primarily drive virus evolution. Baculoviruses (BVs) have been well studied, showing the peculiarity of BV life cycle with the dissemination of viral particles on insect host plants. This strongly suggests that host plants may play an important role in their evolution. Here we study phylogenetic patterns of host use in the large radiation of BVs that attack the insect order Lepidoptera (moths and butterflies). We generated a phylogeny for ~500

BV isolates using four core genes (*polh*, *lef-8*, *lef-9*, *pif-2*) from which we delimited virus species, to obtain a comprehensive timed molecular BV species phylogeny. We then used a combination of phylogenetic (BV and insects) and ecological (host range of BVs, host plant range of lepidopteran hosts) data to address the following hypothesis: BVs are host specialists and show high levels of phylogenetic conservatism, BVs have the same ages as their lepidopteran hosts and the host plants of the insects drive also the evolution of BVs. We found that in general, hosts primarily induced BV species speciation over a short timeframe. But on a larger evolutionary scale, the insect-host co-evolutionary relationship signal is confused. Surprisingly we revealed that insect host plant specificity contributed largely to BV evolutionary history.

CONTRIBUTED PAPERS Wednesday, 10:30-12:15

FUNGI 5

Contributed paper. Wednesday, 10:30 **162**

An entomopathogenic strain of *Beauveria bassiana* against *Frankliniella occidentalis* with no detrimental effect on the predatory mite *Neoseiulus barkeri*

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Among 28 isolates of *Beauveria bassiana* tested for virulence against *F. occidentalis* in laboratory bioassays, we found strain SZ-26 as the most potent, causing 96% mortality in adults at 1×10^7 mL⁻¹ conidia after 4 days. The effect of the strain SZ-26 on survival, longevity and fecundity of the predatory mite *Neoseiulus (Amblyseius) barkeri* Hughes were studied under laboratory conditions. The bioassay results showed that the corrected mortalities were less than 4 and 8% at 10 days following inoculation of the adult and the larvae of the predator, respectively, with 1×10^7 conidia mL⁻¹ of SZ-26. Furthermore, no fungal hyphae were found in dead predators. The oviposition and postoviposition durations, longevity, and fecundity displayed no significant differences after inoculation with SZ-26 using first-instar larvae of *F. occidentalis* as prey in comparison with untreated predator. In contrast, the preoviposition durations were significantly longer. Observations with a scanning electron microscope, revealed that many conidia were attached to the cuticles of *F. occidentalis* at 2 h after treatment with germ tubes oriented toward cuticle at 24 h, penetration of the insect cuticle at 36 h, and finally, fungal colonization of the whole insect body at 60 h. In contrast, we never observed penetration of the predator's cuticle and conidia were shed gradually from the body, further demonstrating that *B. bassiana* strain SZ-26 show high toxicity against *F. occidentalis* but no pathogenicity to predatory mite.

Contributed paper. Wednesday, 10:45 **163-STU**

Interactions between the insect pathogenic fungus *Metarhizium*, the wheat pathogen *Fusarium culmorum* and the mycoparasitic fungus *Clonostachys rosea*

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The current study was conducted to determine if wheat seeds co-inoculated with the insect-pathogenic fungus *Metarhizium* (three species) and the mycoparasitic fungus *Clonostachys rosea* are protected from both insect pests and plant pathogens. The experiment was done in two parts: First, a co-infection bioassay was performed to determine if the virulence of *Metarhizium* was affected by the presence of other fungi by co-treating *Tenebrio molitor* larvae with combinations of *Metarhizium*, *C. rosea*, and the wheat pathogen *Fusarium culmorum*. Second, wheat seeds were co-inoculated with the both beneficial fungi and compared to single inoculations of the effects on *F. culmorum* when allowed to grow for two weeks under controlled laboratory conditions. The resulting root systems were then placed with *T. molitor* larvae which were evaluated daily for mortality. Pathogenicity to insect persisted in all treatments, but *Metarhizium* virulence was affected by co-treatments with other fungi. Root-infection by *F. culmorum* was not reduced directly by the presence of *Metarhizium* while *C. rosea* reduced *F. culmorum* infection and this effect was not diminished in combination with *Metarhizium*. The results of this study suggest that combination of beneficial fungi may effectively protect roots from both pathogens and insects..

Contributed paper. Wednesday, 11:00 **164**

Diversity, ecology and virulence of entomopathogenic fungi isolates naturally infecting the red palm weevil *Rhynchophorus ferrugineus* (Olivier) in the Mediterranean Basin

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae), and the moth *Paysandisia archon* (Burmeister) (Lepidoptera: Castniidae) are considered nowadays the most important palm pest worldwide. Current tactics commonly used to manage the weevil are based on chemical control, although the use of these compounds is hampered by several environmental concerns. In recent years, the *R. ferrugineus* (Rf) microbial control potential of entomopathogenic fungi (EPF) has been highlighted.

In this work, several strains of EPF have been isolated from diverse naturally infected specimens of both species, found in different countries through the Mediterranean Basin.

Firstly, the usefulness of the elongation factor 1-alpha (EF1- α) region, the nuclear intergenic region BLOC and inter simple sequence repeat (ISSR) or microsatellite markers were assessed as *R. ferrugineus* EPFs diagnostic tool, alone or in combination, and relationships among the Mediterranean *Beauveria* and *Metarhizium* isolates obtained from the red palm weevil were inferred.

Secondly, the effect of diverse environmental parameters such as temperature, humidity and UV-B radiation were assessed on germination and colony growth of these EPFs strains as function of their genealogy and geographic origin.

Finally, virulence of selected isolates was tested against both Rf larvae and adults.

Our results show a distribution pattern of *Beauveria bassiana* through the Mediterranean Basin, possibly associated with the host insect dispersion, with the same genetic group presented throughout the European distribution area of phytophagous. Furthermore, several differences were observed between the different genetic groups found, regarding the different factors analyzed: temperature, humidity, UV-B radiation and virulence.

Contributed paper. Wednesday, 11:15 **165-STU**

Recovery and detection of an entomopathogenic endophyte: overcoming the challenges involved

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The prospect of utilising entomopathogenic fungi, such as *Beauveria* spp., as endophytes to enhance their biological control activity is presently a highly topical area of research. However, the endophyte-host relationship is complex and the associated methodology for defining and recovering endophytic strains wrought with problems. A review of the literature on endophyte isolation methods indicated the need for developing a methodology for reliable molecular detection of *Beauveria* spp. *in planta*. The method that was developed included a stringent surface sodium hypochlorite and ethanol based sterilisation with the protocol optimized specifically for propagules of *Beauveria* spp.. This was followed by treatment of plant tissue with propidium monoazide (PMA™) to exclude surface DNA contamination from subsequent PCR. A nested PCR/RT-qPCR protocol capable of detecting as little as 32 fg was developed using novel primer sets designed from the translation elongation factor 1- α gene (TEF). Additionally, epiphytic DNA was isolated separately using a benzyl chloride treatment in order to determine any corresponding occurrence of *Beauveria* with endophyte positive samples. Freshly inoculated samples of *Zea mays* and *Solanum lycopersicum* were surface sterilized using the optimized method and various controls were included for comparison to determine at which stage(s) *B. bassiana* remained viable. Results suggest that *Beauveria* DNA contamination and viability after surface sterilisation is a common and confounding issue associated with endophyte detection and isolation. However, this may be overcome with the improved methodology described here, which delivers reliable detection of endophytic strains.

Contributed paper. Wednesday, 11:30 **166-STU**

Intense spatio temporal pattern in pathogen-host interaction between *Pandora formicae* and *Formica rufa*

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Pandora formicae (*Entomophthorales*, Entomophthoromycota) is a pathogen of the common red wood ant (*Formica rufa*), causing symptoms of "summit disease", where ants attacked by the fungus place themselves on an elevated position before death and sporulation, enabling the pathogen to release infectious spores widely. This facilitates fungal transmission but puts the ant colonies at an enhanced risk of a lethal disease outbreak. Ant workers, on the other hand, respond by removing the cadavers from the nest surroundings, by that lowering the load of conidia, but at the same time putting them at risk while protecting the colony from this hazard. Detailed mapping of the cadavers around an ant nest, twice a day for three subsequent days, three times during one season, shows how ants' behavioral response keeps the fungus prevalence 'at hold'. It also shows the uniqueness of this interaction, the only known example of an entomophthoralean fungus infecting a social insect host, and an evolutionary adjustment of fungal

life strategy to maintain itself in the host population without causing rapid epidemics.

Contributed paper. Wednesday, 11:45 **167**

Patterns of host adaptation in fly infecting *Entomophthora* species

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Insect pathogenic fungi (IPF) differ widely in their capability to infect different hosts. Some are generalists and will, given a sufficient number of infectious spores are present, infect almost any species of insect (e.g. Hypocrealean *Metarhizium* and *Beauveria*). Members of a different main IPF phylum Entomophthoromycota generally have more narrow host-ranges where some species for example only infect aphids or only locusts. Certain species (or strains) are even more host specific and are only known to infect a single or very few taxonomically related insect species under natural conditions (e.g. *Entomophthora*, *Strongwellsea* and *Entomophaga*). Species diversification of the obligate IPF within Entomophthoromycota thus seems to be primarily driven by co-evolutionary host adaptation to specific insect families, genera or species-complexes, but the underlying genetic factors of host adaptation in this fungal order are largely unknown and leave many unanswered questions. For example are the numbers of virulence factors increasing, or decreasing when fungal pathogens adapt to a narrow range of potential hosts? And, are host specialization based on many genetic changes with small effect or few with large effect? Here we examine closely related species within the *Entomophthora muscae* species complex: *E. muscae* s. str. infecting the common housefly *Musca domestica* and *E. muscae* s.l. strains infecting the cabbage fly *Delia radicum*. We use RNA-seq based comparative transcriptomics to unravel genetic differences and similarities in order to detect patterns of host-specific molecular adaptation.

Contributed paper. Wednesday, 12:00 **168-STU**

Plant volatile organic compound manipulation by endophytic entomopathogenic fungi

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The potential of entomopathogenic fungi (EPF) to live as endophytes in various plant tissues has been demonstrated several times in recent years. However, the effects of plant colonization by these endophytes on the metabolism of the colonized plants have been only rarely addressed. We analyzed the volatile organic compound (VOC) profiles of tomato plants (*Solanum lycopersicum*) inoculated with three strains of *Beauveria bassiana* and a plant pathogen biocontrol agent (*Trichoderma koningiopsis*) compared to control plants. We hypothesized that EPF colonized plants should be more attractive for herbivores, mediated by the VOC profiles, but should not exhibit differences when colonized by the plant pathogen antagonist. We found that *B. bassiana* and *T. koningiopsis* inoculated plants had significantly modified VOC profiles, with marked differences between different isolates. Some of the compounds up- or down-regulated are known to play a role in plant-herbivore interactions such as α -pinene, β -

cymene, α -Terpinolen, β -Phellandrene, Caryophyllene and α -Caryophyllene. When aphids (*M. persicae*) were allowed to colonize these plants, VOC profiles again differed with regard to specific compounds and the amount produced. However aphids did not discriminate between tomato plants inoculated with different endophyte isolates compared to control plants. We speculate that the VOC pattern found may play a role for attraction of natural enemies (parasitoids), competing with the EPF for the herbivores.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

MICROSPORIDIA 1

Contributed paper. Wednesday, 14:00 **169**

Effects of the microsporidium *Nosema adaliae* on the multicoloured Asian lady beetle, *Harmonia axyridis*

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Originally imported for use as a biological control agent for pest insects, the multicoloured Asian lady beetle, *Harmonia axyridis* Pallas has itself become a pest in many areas of the world. While it is very effective for biological control, *H. axyridis* tends to displace many native lady beetle species and the alkaloids produced by these beetles may affect the palatability of wine and have adverse effects on human health. The geographic distribution of *H. axyridis* extends throughout North America into Nova Scotia and overlaps with the range of the native two-spotted lady beetle, *Adalia bipunctata* L. The microsporidium *Nosema adaliae* was recently found in a native population of *A. bipunctata* from Nova Scotia and the geographic overlap of *A. bipunctata* with *H. axyridis* provides the opportunity for this microsporidium to be transmitted horizontally to *H. axyridis* in nature. In this study, *H. axyridis* larvae were provided with a combination of uninfected and *N. adaliae*-infected eggs. All of the *H. axyridis* larvae that consumed *N. adaliae*-infected eggs became infected with the pathogen. *H. axyridis* larval development was prolonged significantly, depending on the number of eggs eaten. These results suggest that there is potential for *N. adaliae* to be transmitted to *H. axyridis* in nature if the larvae consume a sufficient number of microsporidia-infected eggs.)

Contributed paper. Wednesday, 14:15 **170-STU**

Effects of two microsporidia from lady beetles on the green lacewing, *Chrysoperla carnea*

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The larvae of green lacewings, *Chrysoperla carnea* (Stephens), are generalist predators that feed on insect eggs, small caterpillars and other, soft-bodied insects. Lacewing larvae are commercially available for aphid control on various agricultural crops. It is common to use several types of biological control agents for controlling aphids at a given time to optimize pest control. Two-spotted lady beetles, *Adalia bipunctata* L., and convergent lady beetles, *Hippodamia convergens* Guerin-Meneville, are often released for aphid control in the same areas that lacewings are used. Two microsporidian pathogens infect these lady beetle species. Because lady beetles and green lacewings are often used

simultaneously for aphid control, it is possible for lacewing larvae to become infected with microsporidia when infected eggs are eaten. The main objective of this study is to determine if microsporidia from lady beetles (*T. hippodamiae* and *N. adaliae*) are transmitted to green lacewings and to examine the effects of these pathogens on lacewing larvae and adults.

Contributed paper. Wednesday, 14:30 **171**

Features of the genomes of microsporidia in mosquitoes: status and preliminary findings

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The status and preliminary findings for full genome sequencing of two distantly related species of microsporidia with mosquitoes as type hosts will be presented. *Vavraia culicis*, the type species of the genus *Vavraia*, was originally described from *Culex pipiens*. Type material was not available and therefore *Vavraia culicis floridensis* isolated from *Aedes albopictus* in Florida was used for sequencing. *V. culicis* has a broad mosquito host range, is infectious for several species of Lepidoptera and characterized by having only uninucleate stages and produces uninucleate spores in multisporous sporophorous vesicles. *Edhazardia aedis* is the type species for the genus and has a limited host range in mosquitoes but can only complete its life cycle in *Ae. aegypti*. *E. aedis* is polymorphic, producing 4 distinctive spore types. It is transmitted both horizontally and vertically and requires 2 generations of the mosquito host to complete the life cycle. Genome and transcriptome sequencing for *E. aedis* and *V. culicis floridensis* is completed. *V. culicis floridensis* has a genome size of approximately 6.1Mb while *E. aedis* is nearly an order of magnitude larger at approximately 51.3Mb, yet the gene content difference is smaller, with 2,773 and 4,190 predicted genes in *V. culicis* and *E. aedis* respectively. RNA-seq data has been analyzed for multiple time points in the life cycle of each species to validate predicted gene structures and to examine gene expression. Preliminary analysis of genome evolution and differential gene expression between life cycle stages will be presented.

Contributed paper. Wednesday, 14:45 **172**

Multi-gene phylogeny applied to the taxonomy of microsporidian parasites of crustacean hosts

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Hepatospora, a recently erected genus, infects epithelial cells of the hepatopancreas of decapod crustaceans. We isolated *Hepatospora* sp. from three different crustacean hosts, inhabiting different habitats and niches; marine edible crab (*Cancer pagurus*), estuarine and freshwater Chinese mitten crab (*Eriocheir sinensis*), and the marine mussel symbiont pea crab, (*Pinnotheres piscum*). Isolates were initially compared using histology and electron microscopy revealing variation in size, polar filament arrangement and nuclear development.

Despite these morphological differences, sequence analysis of the partial SSU rDNA gene did not provide the resolution for distinguishing the isolates (>99% similarity). To investigate relationships between isolates, purified spore samples from the parasite infecting *E. sinensis*, and *C. pagurus*, were prepared for Illumina sequencing. Six additional gene sequences were mined from the resulting genomic data (RNA polymerase, Arginyl tRNA synthetase, Prolyl tRNA synthetase, Chitin synthase, Beta Tubulin and Heat Shock Protein 70). Primers were designed based on the above gene sequences to compare isolates, and to assess corresponding sequences in the genome of the isolate infecting pea crabs. Concatenated phylogenies using sequence data from the six genes revealed that *Hepatospora* isolates from the three different crustacean hosts are likely to be a single species. As such, the concatenated phylogeny supported that derived from analysis of SSU rDNA. Given the host, ecological and morphological distinction of the parasites infecting these three crabs, we provide further support for the concept that morphology is an inappropriate discriminator for even closely related taxa within the phylum, *Hepatospora* may form a widely distributed parasitic 'cline' within the hepatopancreas of aquatic crustacean hosts.

Contributed paper. Wednesday, 15:00 **173-STU**

Understanding the evolutionary loss of glycolysis in intranuclear crab microsporidian

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Enterocytozoon bieneusi is responsible for most cases of microsporidiosis in humans. Interestingly, an intranuclear microsporidian recently isolated from edible crabs appears to be a close relative of this human parasite on SSU based phylogenetic trees. Since microsporidians are known to be devoid of functional mitochondria and rely solely on glycolysis and energy import from their hosts, it was interesting to find that genes coding for glycolytic enzymes were absent from the genome of both parasites. Also, more recent genomic analyses of *Hepatospora spp.*, another crustacean parasite show a similar loss of glycolytic enzymes alluding to a single loss of glycolytic capabilities prior to the divergence of the Enterocytozoonidae lineage.

Absence of glycolysis may be compensated by the increase in host-ATP availability created by the aggregation of host mitochondria around microsporidian meronts, a feature often observed in microsporidian infections. However, *E. cancerii* is an intranuclear parasite and hence, physically walled off from the host mitochondria. This may highlight the presence of a novel host nuclei-dependent metabolic process. To this end, phylogenetic and structural domain analyses on the first enzyme of the glycolytic pathway, hexokinase has revealed that it is severely mutated in deep branching microsporidia hinting to a different substrate-specificity adaptation. Most intriguing is the adjunction of a PTPA domain on the hexokinase of *E. cancerii*. This is the first time severe mutations of hexokinase conserved domains have been documented in eukaryotic cells and our current efforts are directed towards understanding whether these mutations are associated with loss of enzyme function.

Contributed paper. Wednesday, 15:15 **174-STU**

Temporal trends and the effect of seasonal temperature on the prevalence of *Nosema* spp. in *Apis mellifera* in north-east Germany

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Apis mellifera (European honey bee) has long been host to *Nosema apis* and *Apis ceranae* (Asian honey bee) to *Nosema ceranae*. Natural infections of *N. ceranae* were first discovered in *A. mellifera* colonies in 2005. *N. ceranae* has already replaced *N. apis* in the warmer European countries and, as an emergent pathogen, is very competitive. It has been found to be more better adapted to higher temperatures than *N. apis* and is considered more virulent than *N. apis*. Lab experiments have shown the differential effect of temperature on *N. apis* and *N. ceranae* with respect to spore germination and virulence of the pathogen within the host.

Our study is based on a 10-year (2005-2014) cohort study of roughly 20 apiaries from north-east Germany, monitored in autumn and spring. Trend analyses show a significant increase in the prevalence of *N. ceranae* and a decrease in that of *N. apis*, suggesting the gradual replacement of *N. apis* by *N. ceranae*. Weather aggregates from different periods of the season preceding the colony sampling were tested against the proportion of infected colonies. They reveal considerable variability in their effects on *Nosema* prevalence. We are able to confirm, for the first time, the effect of seasonal temperatures on the prevalence of *Nosema* in honey bee colonies. Effect of North Atlantic Oscillation (NAO) indices were also tested as proxies for seasonal weather and were found to be reasonably good predictors of *Nosema* prevalence.

Contributed paper. Wednesday, 15:30 **175-STU**

Characterising putative virulence factors of the bee pathogen *Nosema ceranae*

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Microsporidia are obligate intracellular eukaryotic parasites related to fungi, possessing greatly reduced genomic and cellular components. The microsporidian *Nosema ceranae* threatens the two economically important pollinators, honey (*Apis mellifera*) and bumble (*Bombus* species) bees and has been causally linked to colony collapse disorder. Nosemosis has a complex epidemiology affected by host, pathogen and environmental factors. Although a draft of the *N. ceranae* genome has been published, the molecular basis underpinning pathogenicity is not known. The lack of established culturing techniques and a tractable genetic system necessitates use of model systems for both host and parasite such as *Saccharomyces cerevisiae*. We hypothesis effectors essential to disease progression exist amongst *N. ceranae* secretome genes. In this study we have started characterising these genes using Gateway® cloning technology and identify candidate effectors by their expression in *S. cerevisiae*. We offer experimental data supporting the identities of NcORF-01664 and NcORF-01663 as polar tube proteins (PTP) 1 and 2

respectively and identify a putative PTP4 through their capacity to induce morphological deformities in *S. cerevisiae*. We also show two unknown proteins are targeted to lipid droplets which could function to mobilise resources from this energy-rich organelle. In the future we hope to confirm this function is retained in a system more closely related to the insect host tissue using the *D. melanogaster* Gal4/UAS method. Increased knowledge on virulence factors and disease progression will ultimately lead to disease mitigation.

Contributed paper. Wednesday, 15:45 **176**

Detection of Microsporidia in Gammarids in the Delta of the Kuban River (Azov Sea, Russia)

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Gammarids of the Kuban river basin were surveyed for microsporidia infections at two sites: a) Azov sea coast close to the river Protoka mouth and b) a quarry lake 11 km eastwards from the sea. At the first site, a population of *Dikerogammarus villosus* was abundant in the intertidal zone. In June, 5 out of 100 specimens displayed developed infections with a monomorphic microsporidium. Its ribosomal RNA gene sequence showed high (above 99%) similarity to *Anncalia algerae* with no variability between isolates from individual hosts. In July, the microsporidia were absent in gammarids (N=100). At the second site, in the quarry lake and neighboring ditches, there was an abundant population of *Gammarus* sp. infected with a dimorphic microsporidium at the rates of 100% in May and 50-80% in June. Sequencing of four cloned SSU rRNA gene amplicons (ca 900 bp long) from an individual host sample produced four distinct (97.8-99.4% similarity) haplotypes, suggesting infection with multiple genetically distinct isolates or species of genus *Dyctiocoeila*. The latter taxon unites common and widespread gammarid-infecting microsporidia and revealing a new species of *Dyctiocoeila* in these hosts is quite expected. Conversely, the detection of an *A. algerae*-like parasite in gammarids is somewhat unusual, though logical given the broad host range of *A. algerae* and its ability to develop in amphipods upon injection of spores into the hemocoel. This pathogen has potential risk for human infection and should be taken into account when considering safety of public beaches. Supported by RFBR, 13-04-00284 and 14-04-91176.

CONTRIBUTED PAPERS Wednesday, 14:15-15:45

MICROBIAL CONTROL 3

Contributed paper. Wednesday, 14:15 **178-STU**

Synthesis and Characterization of fungus mediated silver nanoparticle for the toxicity on filarial Vector, *Culex quinquefasciatus*

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Larvicidal activities on myco-synthesized silver nano-particles (AgNPs) against filarial vector, *Cx. quinquefasciatus*. The AgNPs synthesized by filamentous fungus, *Penicillium verrucosum*. Characterized by UV-Vis spectrophotometer, Fourier transform infrared spectroscopy, scanning electron microscopy, and transmission electron microscopy. Furthermore, laboratory evaluation of fungus mediated silver nano-particle against larvae and pupae of *Cx. quinquefasciatus*. The characterization studies confirmed the spherical shape and size (3-24 nm) of silver nanoparticles. The efficacy of fungus AgNPs tested concentrations of 25 and 50 ppm against L1, L2, L3 and L4 instar larvae of *Cx. Quinquefasciatus*. The LC₅₀ (LC₉₀) values are 4.91 (8.13), 5.16 (8.44), 5.95 (7.76) and 7.83 (12.63) in L1 to L4 instar at 25 ppm. Whereas, LC₅₀ (LC₉₀) were 5.24 (8.66), 5.56 (8.85), 6.20 (10.01) and 7.04 (10.92) in L1 to L4 instars treated at 50 ppm. The mortality rates were positively correlated with the concentration of AgNPs. Significant (P<0.05) changes in the larval mortality was also recorded between the period of exposure against all instar of larvae of *Cx. quinquefasciatus*. These finding use of fungus synthesize silver nano-particles is a rapid, eco-friendly, and a single-step approach and potential mosquito larvicidal agents.

Contributed paper. Wednesday, 14:30 **179-STU**

Entomopathogenic fungi as endophytes: interaction with phytohormones

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With increasing interest in entomopathogenic fungi as endophytes (EEPf) in biological control strategies, there is a need for more background information on the interaction of these fungi with their host plant metabolism. Several studies have already reported on changes in the dry weight of plants when endophytically colonized by these EEPFs; however, a more detailed understanding of plant-fungus-interactions is missing. We measured phytohormone levels in plants with regard to the hypotheses that i) EEPFs produce phytohormones as fungal secondary metabolites when growing within plant tissues or ii) that plants react to the presence of EEPFs by increasing/decreasing their phytohormone production. We inoculated the seeds of tomato (*Solanum lycopersicum*) and cotton (*Gossypium hirsutum*) plants with one strain of *Beauveria bassiana* and three different strains of *Metarrhizium anisopliae*, and grew these plants under standardized conditions in the greenhouse. We used LC-MS to analyse several phytohormones (including Salicylic Acid (SA), Abscisic Acid (ABA), Indolic Acetic Acid (IAA), Salicylic Acid Glucoside (SAG), and Jasmonic Acid (JA)) in eight weeks old leaves of these plants. The results will be discussed with regard to induced plant responses as well with regard to potential influences on herbivore-plant-interactions.

Contributed paper. Wednesday, 14:45 **180**

Pathogenicity of three entomopathogenic fungi on larvae and adults of the sisal weevil: The less the better?

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The sisal weevil was first recorded in Greece on May 2010 on ornamental plants of *Agave* sp. As the use of synthetic insecticides is prohibited in urban landscape areas the evaluation of potential biological control agents (BCAs) is essential. Indigenous strains of *Isaria fumosorosea* and *Metarhizium anisopliae* isolated from the soil and a strain of *Beauveria bassiana* obtained from a *Rhynchophorus ferrugineus* cadaver were chosen for evaluation.

Infection of adults and larvae of *S. acupunctatus* was achieved by immersing individuals in aqueous conidial suspensions. Additionally, natural diet of insects was also immersed in conidial suspensions and provided to individuals, in order to assess effectiveness of application through treated surface. *Beauveria bassiana* and *M. anisopliae* were applied in concentrations of 10^7 and 10^6 conidia/ml while *I. fumosorosea* was applied at a concentration of 10^6 conidia/ml. Mortality was recorded daily for up to 11 or 21 days for larvae and adults respectively. The highest adult mortality was achieved by *B. bassiana* through contact application reaching 100%, followed by *M. anisopliae* (48±10% to 28±10%) and *I. fumosorosea* (40±6.3%). In terms of larvae, mortality in all bioassays reached 100% with the exception of the treatment of contaminated diet by *I. fumosorosea* conidia (20±11%). All cadavers produced visible mycelium on their surface within a week. Results indicate a high level of mortality at the most harmful life stage of the pest, even at low concentrations and a lower level of mortality at the mobile adult stage. Benefits of a low concentration application of fungi are discussed.

Contributed paper. Wednesday, 15:00 **181**

Understanding Beauveria bassiana infection within its host *Triatoma infestans*: time course expression of genes encoding fungal toxic nonribosomal peptides and insect humoral immune proteins

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During invasion into insect hemocoel, some entomopathogenic fungi secrete toxins contributing to a successful infection. In response, insect cellular and humoral immune reactions are triggered. In this work, we studied by real-time PCR the expression pattern of *B. bassiana* genes involved in beauvericin, bassianolide, and tenellin biosynthesis throughout the infection process in nymphs of the kissing bug *T. infestans*. We also investigated the expression level of some bug proteins involved in the humoral immune response, i.e. prophenoloxidase, hemolectin and defensin. In conidia-treated insects, the expression of beauvericin synthetase, bassianolide synthetase, and tenellin synthetase peaked 6 days post-inoculation. In blastospore-injected bugs (bypassing the insect cuticle) the expression level peaked 12 hours post-injection. Regarding insect immune response, conidia treatment induced higher expression of defensin and hemolectin, with values of 8.3 ± 1.1 and 2.7 ± 1.4 fold inductions, respectively. In blastospore-treatment, the expression level of all genes tested raised from 12 to 48 hours, reaching 9.3 ± 3.6 (prophenoloxidase) and 26.6 ± 5.4 (defensin) fold induction. These results help to understand at the molecular level the "arm race" taking place in insect hemocoel during fungal invasion.

Contributed paper. Wednesday, 15:15 **182**

Compatibility of herbicides used in olive orchards with a *Metarhizium brunneum* strain used for the control of the olive fly preimaginals in the soil

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In vitro and *in vivo* studies were developed to evaluate the compatibility of the six most common herbicides applied to the soil of olive orchards with *Metarhizium brunneum* EAMa 01/58-Su strain against medfly *Ceratitis capitata* preimaginals. The fungus demonstrated high *in vitro* compatibility with the six active ingredients in malt agar medium, with growth rates (a) ranging between 2.5 mm d^{-1} and (Glyphosate) and 3.3 mm d^{-1} (Oxyfluorfen). This compatibility was also revealed *in vivo* by assaying the fungus towards medfly prepupating larvae in herbicide containing soil (at 1.0×10^8 conidia g soil⁻¹). Even if there was a decrease of the *M. brunneum* level until 10^4 - 10^5 conidia ml⁻¹ in the soil 15 days after inoculation, mortality rates, which were in the range of 70-80%, did not differ significantly to the controls, except the ones observed in soils treated Glyphosate and its herbicide combinations, in which a significant 50% reduction of virulence was detected. These results reveal a general compatibility of *M. brunneum* with the most common herbicides applied to the soil of olive orchards, whereas a mixture of the fungus in the tank of the atomizer for a simultaneous treatment beneath the tree canopy is recommended for all active ingredients except Glyphosate.

Contributed paper. Wednesday, 15:30 **183**

The Seed Corn Maggot and *Metarhizium* are Related to Maize Yield in an Organic, Cover Crop-Based Farming Systems Experiment

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Organic farmers must largely rely on cultural practices and biological processes to prevent crop damage from pests. Many farmers are interested in using cover crop mixtures to gain production and ecosystem benefits, but there has been little research on the effects of cover crop diversity on arthropod pests and their natural enemies. The seed corn maggot, *Delia platura* (Diptera: Anthomyiidae), is an early-season pest of large-seeded crops in conventionally tilled systems. Insect-pathogenic fungi in the genus *Metarhizium* commonly occur in agricultural soils and infect soil-dwelling arthropods. In 2013, we examined the effects of overwintering cover crop diversity, ranging from one to 7 species, on seed corn maggot fly emergence, *Metarhizium* detection, soil characteristics, and corn yield. Seed corn maggot was detected in post-plant emergence traps from all treatments in maize and soybean, with approximately 10 times greater numbers captured from maize compared to soybean. Numbers of flies captured were not related to level of cover crop diversity. *Metarhizium* was detected in all treatments, with similar average detection rates in maize and soybean. Detection of *Metarhizium* was not related to level of cover crop diversity. In multivariate analyses, numbers of emerged flies relates negatively to maize yield and detection of *Metarhizium*. *Metarhizium* detection relates positively to maize yield, soil organic matter, electrical conductivity, and Mg. The negative relationship between emergence of seed corn maggot flies and *Metarhizium* suggests that this fungus is a natural mortality factor for seed corn maggot at this site.

VIRUSES 5Contributed paper. Wednesday, 14:00 **184****Soybean aphid viruses exploit contrasting transmission strategies**

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The soybean aphid, *Aphis glycines* Matsumura, is an invasive pest of primary agricultural importance in North America. Following its introduction in 2000, soybean yields dropped 11%, with the costs of management and yield loss estimated to be \$1.6 billion over a 10 year period. Soybean aphids are managed primarily by application of chemical insecticides. We identified two viruses from the soybean aphid transcriptome and small RNA data that may have potential for use in soybean aphid management: Aphid lethal paralysis virus (ALPV)-Ames (Dicistroviridae) and *Aphis glycines* virus (AGV; unclassified). There is evidence for the presence of ALPV-like viruses in several different insects including the bird cherry-oat aphid, *Rhopalosiphum padi* (Linnaeus), the honeybee *Apis mellifera*, and Western corn rootworm *Diabrotica virgifera virgifera*. AGV has a ~5 kb single stranded RNA (ssRNA) genome and forms a 30 nm particle. The RNA-dependent RNA polymerase (RdRp) of this virus is closely related to that of *Euproctis elaeasa* virus (Tetraviridae), while the AGV coat protein (CP) is similar to those of plant *Sobemoviruses*. Based on RT-PCR of AGV RdRP sequence, AGV-like viruses appear to be present in two other aphid species, the bird cherry-oat aphid, *R. padi* and the green peach aphid, *Myzus persicae* (Sulzer). Notably, ALPV-Ames does not appear to be vertically transmitted in the soybean aphid, while AGV is 100% vertically transmitted. The different transmission strategies of these two viruses and the implications of 100% vertical transmission will be discussed.

Contributed paper. Wednesday, 14:15 **185****Characterization of mechanisms involved in the transmission of a lepidopteran densovirus**

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Densoviruses are small insect parvoviruses infectious per ingestion for several lepidopteran species. Their potential as microbial control agents led us to focus on the understanding of motors driving the transmission of densoviroes in the environment. Natural dynamic of densoviral infections was never investigated although several metagenomic studies revealed the presence of densoviroes in samples from various origins (feces from bats, mosquitoes, marine samples like urchins...). In this study, we qualitatively and quantitatively characterized direct and indirect mechanisms leading to the transmission of a model viral species, *Junonia coenia* densovirus, on the model host *Spodoptera frugiperda*. We showed that cannibalism of infected individuals and bites between infected and uninfected individuals are major events in the transmission of JcDNV while exposition to contaminated feces and/or regurgitations contributes to widely disseminate the

virus throughout a susceptible population. We also found that parasitoid wasps participate to indirect transmission of densoviroes although probably in a non-specific manner. Altogether, these results are a first step toward the construction of a dynamic transmission model for densoviroes.

Contributed paper. Wednesday, 14:30 **186****Discovery of circular single-stranded DNA viruses in top insect predators**

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Viruses with circular single-stranded DNA (ssDNA) genomes that encode a replication initiator protein (Rep) are among the smallest viruses known to infect eukaryotic organisms. Additionally, their rapid evolution rates have led to the emergence of some of these viruses as serious pathogens. Recent research indicates that the host range of eukaryote-infecting circular Rep-encoding ssDNA (CRESS-DNA) viruses, which was previously thought to be restricted to plants and vertebrates, may include insects. To expand our knowledge of circular ssDNA viruses in invertebrates, this study surveyed CRESS-DNA viruses circulating among insect populations by targeting dragonflies (Ephemeroptera). Dragonflies are highly mobile top insect predators that accumulate viruses from their insect prey over space and time and, thus, can be used as 'sampling traps' to explore the diversity of CRESS-DNA viruses found among flying insects. Using degenerate PCR and rolling circle amplification coupled with restriction digestion, 16 CRESS-DNA viral genomes were recovered from eight different dragonfly species collected in tropical and temperate regions. Nine of the genomes are similar to cycloviruses and represent five species within this proposed genus, suggesting that cycloviruses are commonly associated with insects. Three of the CRESS-DNA viruses share conserved genomic features with the recently described fungal virus *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1. The remaining viruses are divergent species representing novel CRESS-DNA viral genera. The novelty of CRESS-DNA viruses identified in dragonflies using simple molecular techniques indicates that there is an unprecedented diversity of ssDNA viruses among insect populations.

Contributed paper. Wednesday, 14:45 **187-STU****Single-stranded DNA viruses in marine crustaceans**

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Metagenomic sequencing has recently revealed the ubiquity of eukaryotic circular single-stranded DNA (ssDNA) viruses in the marine environment; however, a definitive host has not been identified for most of these viruses. Through direct examination of marine shrimp and crab species, this study surveyed the diversity of circular ssDNA viruses in economically and ecologically important crustaceans, linking these newly discovered viruses to their hosts and improving our understanding of the ecological impact of ssDNA viral infection in marine crustaceans. Viral particles were partially purified from specimen homogenates through filtration, DNA was then

extracted and amplified through rolling circle amplification to enrich for small circular ssDNA templates. The concatenated circular genomes were then digested with restriction enzymes and the resulting products (~1– 4 kb) were cloned and sequenced. Thirteen distinct ssDNA viral genomes were recovered from five crab species and three shrimp species. Putative encoded proteins share less than 60% identity with known viral proteins from members of the *Circoviridae*. The detected genomes exhibit four different genomic architectures revealing an incredible diversity of ssDNA viruses in shrimp and crabs. Ongoing work aims to propagate these viruses in insect cell lines and develop a system to assess viral infectivity and modes of transmission.

Contributed paper. Wednesday, 15:00 **188**

Remarkable diversity of endogenous viruses in the genome of an isopod crustacean

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Recent studies in paleovirology have uncovered myriads of endogenous viral elements (EVEs) integrated in the genome of their eukaryotic hosts. These fragments result from endogenization, i.e., integration of the viral genome into the host germline genome followed by vertical inheritance. So far, most studies have used a virus-centred approach, whereby endogenous copies of a particular group of viruses were searched in all available sequenced genomes. Here we follow a host-centred approach whereby the genome of a given species (the crustacean isopod *Armadillidium vulgare*) is comprehensively screened for the presence of EVEs using all viral sequences available as queries. This search and downstream evolutionary analyses revealed that 56 EVEs corresponding to 11 different viral lineages belonging to 5 viral families (*Bunyaviridae*, *Circoviridae*, *Parvoviridae*, *Nimaviridae*, *Totiviridae*) and one viral order (*Mononegavirales*) became endogenized in *A. vulgare*. We show that viral endogenization occurred recurrently during the evolution of isopods, that *A. vulgare* viral lineages were involved in multiple host-switches that took place between widely divergent taxa. Furthermore, 32 *A. vulgare* EVEs have uninterrupted open reading frames, suggesting they result from recent endogenization of viruses likely to be currently infecting isopod populations. Overall, our work shows that isopods have been and are still infected by a large variety of viruses. It also extends the host range of several families of viruses and brings new insights into their evolution. More generally, our results underline the power of paleovirology in characterizing the viral diversity currently infecting eukaryotic taxa.

Contributed paper. Wednesday, 15:15 **189**

Iteraviruses (Densovirinae) from monarch and black swallowtail butterflies and slug caterpillar moths and characterization of their expression strategies

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Iteraviruses belong to a separate genus of the Densovirinae subfamily of the Parvoviridae family and includes three densoviruses, i.e. *Caspalia extranea* Densovirus (CeDNV), *Dendrolimus punctatus* Densovirus (DpDNV) and *Bombyx mori*

Densovirus (BmDNV). In this study, we used a Sequence-Independent Single-Primer Amplification (SISPA) method to detect the pathogens of larvae from three additional insect species (*Papilio polyxenes*, *Sibine fusca* and *Danaus plexippus*), killed by some unknown pathogen. Sequencing of the clones that were obtained and BLAST analysis revealed the existence of three previously unknown densoviruses (provisionally named PpDNV, SfDNV and DppIDV). The genome of the new densoviruses were cloned into pCR2.1-topo or pBluescript(SK-) vectors. These virus sequences (including ITRs) have high identities with CeDNV and BmDNV. The identical genome organizations indicated that these three new densoviruses should be classified in the Iteravirus genus. Together with the infectious clones of CeDNV and BmDNV, we investigated the expression strategies of five different iteraviruses (PpDNV, SfDNV, CeDNV, BmDNV, DppIDV). Total RNA was obtained both from LD cell line transfected by infectious clones of the iteraviruses and virus infected larvae (*Papilio polyxenes*). RACE methods were used to identify the 5' and 3' transcription ends. The nonstructural (NS) and structural (VP) genes were located on the same strand of the genome. The NS cassette consists of two genes with NS1 and overlapping NS2. The NS2 transcripts all start at 7 nts downstream of the NS1 start codon. Transcription starts for NS1 genes are close to the AUG of NSS1. NS and VP transcripts do not overlap. The four VPs were similarly generated by leaky scanning translation of unspliced mRNA. The VP transcripts just start 2nts downstream of the poly (A) motif for NS transcripts. Interestingly, poly (A) signals for VP transcripts all overlap with the stop codons of the VP genes.

Contributed paper. Wednesday, 15:30 **190**

Remarkable genetic diversity of single-stranded DNA viruses in cultured shrimps and crickets

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Single-stranded DNA viruses are among the smallest viruses and include members of the *Parvoviridae* and *Circoviridae* families (linear and circular ssDNA viruses). In the past decades, PstDNV and AdDNV have been well-known viruses that have caused a severe impact on cultured shrimps and crickets. Here, we report the discovery and genome characterization of numerous novel denso- and denso-like viruses and, for the first time, new circoviruses from these hosts. During the last years, we received many cricket samples from North America that were negative for AdDNV. However, denso-like particles have been observed by EM. Complete sequence of different viral genome have been isolated and cloned including one circular ssDNA viruses of 2.5 kb, an ambisense densovirus of 4.9 kb and a segmented brevidenso-like virus (3.3 kb). Meantime, large numbers of new ssDNA viruses were also isolated from cultured shrimp from Vietnam. Characterization of these viruses revealed 3 different, unrelated circoviruses of 1.7, 1.7 (the latter is not using the standard genetic code and may have been ingested) and 1.3 kb. We also discovered a new shrimp parvovirus of about 4.1 kb that is phylogenetically poorly related to any known parvovirus. Near-atomic structures of some cricket and shrimp parvoviruses were obtained by X-ray crystallography as well as their transcription strategy. These results demonstrate a great diversity of ssDNA-viruses infecting these economically important animals. Future work will be focused on molecular features of these viruses for a further insight into the evolution and classification of ssDNA viruses.

Contributed paper. Wednesday, 15:45 **191**

How do vine mealybug, grapevine leafroll-associated virus and grapevine interact on a molecular level?

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Vine mealybug (VMB), *Planococcus ficus*, is one of the most damaging grapevine pests in the world, largely because it is a vector of grapevine leafroll-associated virus (GLRaV). Since interactions among VMB, GLRaV and grapevine are responsible for the extent of VMB damage and GLRaV spread, we investigated the relationships among the three organisms. The effect of GLRaV infection on VMB was determined using cDNA-AFLP analysis and validated with RT-qPCR. It was found that VMB responds to GLRaV by activating only a few genes, and possibly also by endosymbiont mediation. The effect of VMB feeding on grapevine was investigated with microarray analysis and validated with RT-qPCR. Grapevine was found to respond to VMB feeding by mounting a weak response within a narrow window of time. These results are useful for understanding the interaction among the organisms in the VMB system, and limiting the damage caused in vineyards.

CONTRIBUTED PAPERS Wednesday, 14:00-16:00

BACTERIA 4

Contributed paper. Wednesday, 14:00 **192**

Analysis of the bacterial community of the insect pest *Lymantria dispar* during its life cycle

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Gypsy moth (*Lymantria dispar*, Lepidoptera) outbreaks can cause great damage to forestry across Europe. In order to control this pest, there is need for new insecticidal bacterial strains for the development of more effective biopesticides. The insect gut microbiota represents all aspects of microbial relationships, ranging from pathogenic to obligate mutualistic interactions. Latest investigations suggest that there is competition between individual opportunistic pathogens and that they are able to upregulate the production of virulence factors according to their density within hosts, what renders them interesting for use in combination with biocontrol agents.

The objective of this work is to characterize the bacterial midgut community of *L. dispar* and to monitor 1) changes in diversity during its life cycle, 2) changes within larvae from spring to summer and 3) differences between individuals. Microorganisms were first analyzed using a culture dependent approach where midguts were extracted and plated on media. Growing bacteria were analyzed by colony characteristics - color, size, shape, opacity, margin, elevation and viscosity and then by 16S rRNA gene sequencing. In a second step bacterial midgut communities were analyzed by a culture independent method using PacBio technology to sequence full length 16S rRNA genes.

Results showed relatively simple composition of the gypsy moth midgut community. We observed differences between individual larva from the same time point and structural changes of diversity in bacterial communities over the season.

This project is conducted within the frame of the SCIEX program with ETH Zürich and the University of Daugavpils as partners

Contributed paper. Wednesday, 14:15 **193**

Contacting microbe induce grooming behaviour in *Drosophila*

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Insects remove and clean microbes from their surface by grooming behavior, which is considered as a behavioral defense against pathogen/parasite infection in some cases. It is well known that the insects like *Drosophila melanogaster*, which live in an environment littered with bacteria, fungi and other microorganisms developing on decaying material devote a lot of time to self-grooming which seems to contribute cleaning their cuticula from external particles. The mechanisms that trigger this behavior are still ambiguous, although grooming behavior was identified in many insects. In this work, we examined if *D. melanogaster* can sense microbe in their habitat and if they conduct any hygiene behavior like grooming after they have perceived microbe. To follow the behavioral reaction, we focus on a contact chemo-stimulus, which would activate taste neurons. Microbe, microbe-related compounds and standard chemicals were used as stimuli and influence of water and mechanical stimuli were removed by using Gal4-UAS system in control experiments. Grooming seems to be specifically triggered by the activation of taste neurons since flies showed strong cleaning behavior when contacted with taste stimuli.

Contributed paper. Wednesday, 14:30 **194**

Cultivable gut bacteria of scarabs inhibit *B. thuringiensis* multiplication

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The entomopathogen *Bacillus thuringiensis* is used to control various pest species of scarab beetle but is not particularly effective. Gut bacteria have diverse ecological and evolutionary effects on their hosts, but whether gut bacteria can protect scarabs from *B. thuringiensis* infection remains poorly understood. To investigate this we isolated 32 cultivable gut bacteria from *Holotrichia oblita*, *Holotrichia parallela* and *Anomala corpulenta*, and analyzed their effect on *B. thuringiensis* multiplication and Cry toxin stability. 16S rDNA analysis indicated that these gut bacteria belong to the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* phyla. A confrontation culture analyses of the 32 isolates against three scarab specific *B. thuringiensis* strains showed that the majority of the scarab gut bacteria had antibacterial activity against the *B. thuringiensis* strains. The Cry toxin stability analysis results showed that whilst several strains produced proteases capable of processing the scarab-specific toxin Cry8Ea, none were able to completely degrade it. These results suggest that gut bacteria can potentially affect the susceptibility of scarabs to *B. thuringiensis* and that this should be considered when considering future control measures.

Contributed paper. Wednesday, 14:45 **195**

Interactions between the Med fly *Ceratitis capitata* (Wied.) and a new *Bacillus cereus* sensu lato strain

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The Med fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a polyphagous species affecting many species of fruits and vegetables worldwide. Due to its high economical impact on crops, the management of this multivoltine pest is always necessary and mostly based on the application of various synthetic insecticidal formulations as foliage baiting or cover spraying. Besides the use of chemicals, the potential of entomopathogenic microorganisms (i.e. bacteria, fungi) against this pest has been highlighted. The lethal and sub-lethal effects of sporulated cultures of a novel *B. cereus* sensu lato strain lacking detectable cry genes and identified by its morphological and genetic features, have been studied in a larval based bioassay model. Sporulated cultures of this strain significantly reduced immature stages survival and development time, and the size of emerging Med fly adults. The toxicity has been associated to a specific parasporal fraction characterized through a proteomic approach (SDS-PAGE, 2D PAGE, LC MS/MS). The results of these analyses highlighted the possible role of different protein families produced also by other microbial entomopathogens and that have already been specifically associated to an insecticidal action. These proteins include molecular chaperones (GroEL), metalloproteases, aldehyde dehydrogenases, peptidases and other enzymes.

Contributed paper. Wednesday, 15:00 **196**

Long-term effect of *Bacillus thuringiensis* subsp. *israelensis* application on *B. cereus* group populations in Swedish riparian wetland soils

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The *Bacillus cereus* group (Bcg) commonly occurs in soil and includes the pathogens *B. cereus*, *B. thuringiensis* and *B. anthracis*, differing in pathogenicity and disease spectrum. The insect pathogenic *B. thuringiensis* subsp. *israelensis* (Bti) is available in products for augmentation biological control and has been applied worldwide to control larvae of the order Diptera. However, knowledge is limited on how long-term Bti application affects the structure of indigenous Bcg communities as well as the overall abundance of Bti. Based on new primer pairs targeting internal spacers located on the bacterial chromosome, group-specific quantitative PCR assays for Bcg and Bti in environmental samples were developed. On six occasions during the vegetation season, soil samples were collected in forest swamps and wet meadows which have been treated with Bti during the last 11 years as well as in untreated forest swamps, wet meadows and well-drained forests. Preliminary results from two of the time points indicate a decline of Bti abundance over time after the last treatment in wet meadows and forest swamps. These preliminary data also indicate that abundance of Bti in the untreated sites were lower than in the treated, independently of the sampling occasion. This study is coming up with the first

specific PCR-primers for Bcg and Bti that target chromosomal DNA. These new tools will be useful for investigating the abundance and diversity of Bcg members in various environments and thereby for assessing the resident insecticidal potential of this bacterial group.

Contributed paper. Wednesday, 15:15 **197**

Proteomics of *Brevibacillus laterosporus* and its insecticidal action against noxious Diptera

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Brevibacillus laterosporus is a pathogen of invertebrates and an antimicrobial species, morphologically characterized by a typical spore surrounded by a firmly attached canoe-shaped parasporal body (CSPB). The biocontrol potential in agriculture of this bacterial species, is not limited to invertebrate pests (insects in different orders, nematodes and mollusks) but includes also phytopathogenic bacteria and fungi. This broad-spectrum activity is associated to a wide variety of molecules, including proteins and antibiotics, it produces. Whilst there are significant differences among strains in terms of virulence, the results of the recent whole genome sequencing of strains LMG 15441 and GI-9 revealed a conserved potential of this species to produce several polyketides, nonribosomal peptides, and toxins. Among genes encoding for putative toxins some show similarities to *Lysinibacillus sphaericus* mosquitocidal toxins.

Employing a *B. laterosporus*-*Musca domestica* bioassay model, associated to a proteomic and gene expression study, we have analyzed the implication in the microbial action of specific proteins produced during different bacterial life stages. Based on these results, new insights into the pathogenicity against noxious Diptera will be discussed.

Contributed paper. Wednesday, 15:30 **198-STU**

Outer membrane vesicles are vehicles for the delivery of *Vibrio* virulence factors to oyster immune cells

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V. tasmaniensis LGP32, a facultative intracellular pathogen of oyster hemocytes, was shown here to release outer membrane vesicles (OMVs) both in the extracellular milieu and inside hemocytes. Intracellular release of OMVs occurred inside phagosomes of intact hemocytes having phagocytosed few vibrios as well as in damaged hemocytes containing large vacuoles heavily loaded with LGP32. The OMV proteome of LGP32 was shown to be rich in hydrolases (29.8 %) including potential virulence factors such as proteases, lipases, phospholipases, hemolysins and nucleases. One major

caseinase / gelatinase named Vsp for vesicular serine protease, which is homologous to the VesA serine protease of *Vibrio cholerae*, was found to be specifically secreted through OMVs in which it is enclosed. Vsp was shown to participate in the virulence phenotype of LGP32 in oyster experimental infections. Finally, OMVs were highly protective against antimicrobial peptides, increasing the minimal inhibitory concentration of polymyxin B by 16-fold. Protection was conferred by OMV titration of polymyxin B but did not depend on the activity of Vsp or another OMV-associated protease. Altogether, our results show that OMVs contribute to the pathogenesis of LGP32, being able to deliver virulence factors to host immune cells and conferring protection against antimicrobial peptides.

Poster / Bacteria. Wednesday, 16:30. **BA-2**

'Candidatus Rickettsiella isopodorum', a new lineage of intracellular bacteria infecting woodlice

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The taxonomic genus *Rickettsiella* (*Gammaproteobacteria*; *Legionellales*) comprises intracellular bacteria associated with a wide range of arthropods including insects, arachnids and crustaceans. Ultrastructural together with genetic evidence is provided for a *Rickettsiella* bacterium occurring in Germany in the common rough woodlouse, *Porcellio scaber* (Isopoda, Porcellionidae). The new bacterium is found very closely related to a *Rickettsiella* strain from California that infects the pill bug, *Armadillidium vulgare* (Isopoda, Armadillidiidae). Both bacterial isolates display the ultrastructural features described previously for crustacean-associated bacteria of the genus *Rickettsiella*, including the absence of well-defined associated protein crystals; occurrence of the latter is a typical characteristic of infection by this type of bacteria in insects, but has not been reported in crustaceans. As demonstrated by a molecular systematic approach combining multilocus sequence analysis (MLSA) with likelihood-based significance testing, both bacteria - despite their distant geographic origins - form a tight sub-clade within the genus *Rickettsiella*. In the 16S rRNA gene trees, this sub-clade includes other bacterial sequences from woodlice. Moreover, the bacterial specimens from *P. scaber* and *A. vulgare* are found genetically or morphologically different from each of the four currently recognized *Rickettsiella* species. Therefore, the designation '*Candidatus Rickettsiella isopodorum*' has been introduced for this new lineage of isopod-associated *Rickettsiella* bacteria.

Reference: Kleespies R.G., Federici B.A., Leclerque A. Ultrastructural characterization and multilocus sequence analysis (MLSA) of '*Candidatus Rickettsiella isopodorum*', a new lineage of intracellular bacteria infecting woodlice (Crustacea: Isopoda). Systematic and Applied Microbiology, in press.

Wednesday, 16:30-18:30

POSTERS

BACTERIA

Poster / Bacteria. Wednesday, 16:30. **BA-1**

A New Local Bio-Insecticide: Developing, Optimization, Toxicity and Determination of Activity

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The insects belonging to the order Coleoptera are one of the most harmful insect groups in our country and in all over the world. Members of coleopteran cause serious damages in the agricultural fields and the forested areas and the warehouses. So far, efforts to control coleopteran pests have mainly involved the use of chemical insecticides. These agents can have undesirable side-effects on humans, plant and other animal species, particularly predators and parasites of pests. In this study, we proposed to develop a biological preparation (bio-insecticide) against coleopteran pests using an insecticidal isolate of *Bacillus thuringiensis* subsp. *tenebrionis* (Mm2). Our results showed that the isolate has maximum growth at 30°C, at pH 7 in Tryptic Soy Broth containing 1% NaCl. Its sporulation was supported in synthetic medium and the bacterial cell suspension was produced in pilot fermenter. Powder biopesticide was produced using this cell suspension and necessary formulation materials in the spray dryer. The physical and biological properties like wettability, suspensibility, particle size, moisture content, and viable spores of the formulated powder were determined and noted as 24 s, 80%, 10 µm, 5% and 10x10¹² (CFU/gdw), respectively. Insecticidal activity of the product against *Agelastica alni* and *Stophilus granarius* adults in laboratory conditions were investigated. Mortality results were identified as 37% against *S. granarius* and 100% against *Agelastica alni*.

Poster / Bacteria. Wednesday, 16:30. **BA-3-STU**

Analysis and characterization of binary AB toxins in the honey bee pathogen *Paenibacillus larvae*

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The gram-positive spore-forming bacterium *Paenibacillus larvae* is responsible for American foulbrood in honeybees. Four *P. larvae* genotypes could be distinguished via genotyping with ERIC-primers, ERIC I – IV, with genotypes ERIC I and II being frequently isolated from outbreaks worldwide. The most important phenotypic difference between the genotypes are the differences in virulence. Recent studies show that binary AB toxins play an important role in the infection mechanism, presumably in breaching the larval midgut epithelium as crucial step in pathogenesis. AB toxins usually consist of two subunits which are encoded either by the same or different open reading frames (ORF). The A subunit is enzymatically active and modifies a cellular target, e.g. by mono-adenosine diphosphate (ADP)-ribosylation. Contrarily, the B subunit is responsible for cell surface receptor binding and the translocation of the A subunit into the cell. Recently, two binary AB toxins, Plx1 and Plx2, have been identified as virulence factors in *P. larvae* ERIC I. The study on further binary AB toxins in *P. larvae* will be

continued via exposure bioassays with knockout mutants. We aim at analyzing and characterizing the binary AB toxins in *P. larvae* in order to gain further insight into the pathogenic mechanisms of *P. larvae*.

Poster / Bacteria. Wednesday, 16:30. **BA-4**

Interplay of Regulators Controlling Fit Insect Toxin Expression in the

Biocontrol Bacterium *Pseudomonas protegens*

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The root-colonizing biocontrol agent *Pseudomonas protegens* CHA0 protect its plant hosts from fungal diseases by releasing toxic exoproducts into the rhizosphere. Remarkably, this microorganism might also function as a natural insecticide since it is capable of exhibiting potent oral and systemic insecticidal activity against various pest insect species. Recently, our group discovered an insecticidal protein toxin termed Fit, which makes essential contributions to insect killing. The Fit toxin gene *fitD* is located in a virulence cluster coding for a type I secretion system (FitA, FitB, FitC and FitE) essential for toxin transport and also for three regulators (FitF, FitG and FitH) of toxin expression. By using a $\Delta fitF \Delta fitG \Delta fitH$ triple mutant, in which each regulatory gene was individually reintroduced and expressed, we observed that the expression of the *fitABCDE* operon is positively regulated by the LysR-type regulator FitG and repressed by the response regulator FitH. We demonstrate that a phosphorylation of the conserved aspartate residue (D59) in the receiver domain of FitH is necessary to eliminate the repressive activity of the regulator, and that this residue is necessary for Fit toxin expression. Findings of an analysis of the heterologous expression of regulatory genes *fitG* and *fitH* in naturally Fit-locus deficient strains carrying a *gfp* reporter monitoring the *fitA* leader sequence activity strongly suggests that the LysR-type regulator FitG promotes Fit-toxin expression through specific binding to the promoter of the *fitABCDE* operon. These results allowed to improve the model explaining the regulation of Fit toxin expression in *P. protegens* CHA0.

Poster / Bacteria. Wednesday, 16:30. **BA-5-STU**

Identification and Characterization of *Bacillus thuringiensis* Strains with Nematicidal Activity

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Crystal proteins from the soil bacterium *Bacillus thuringiensis* (Bt) are globally used in agriculture as biological control agents against insect pest, but its use as a nematicidal control agent is still under development. In this work, a total of 310 Bt strains were screened for activity against the free-living nematode *Caenorhabditis elegans*. Strains LB1T-596 (serotype darmstadiensis) and LB1T-107 (serotype neoleonensis) showed significant toxicity levels. These strains were characterized by plasmid and RepPCR patterns, and flagellin gene sequencing. Preliminary bioassays of LB1T-596 and LB1T-107 spore-crystal complexes estimated LC₅₀s at 63.36 and 76.33 µg/ml, respectively, and 24.2 and 24.99 µg/ml, respectively, when pure crystals were tested. SDS-PAGE protein content analyses of LB1T-596 crystals showed two proteins (35 and 130 kDa) before activation, which turned into lower molecular-weight proteins (28

and 55 kDa) after activation. LB1T-107 also showed two major proteins of 28 and 70 kDa, before activation. Amplicons from the *cry*-gene conserved blocks and from *cyt1* gene group were cloned and sequenced. Sequence analyses indicated that LB1T-596 contains sequences identified within the *cry5B* and *cyt1A* gene families, while LB1T-107 contains sequences identified within the *cry14* and *cyt1A* gene families. Interestingly one of the amplicons from LB1T-107 showed only 88% identity with the *cry14A* gene. These results indicate a potential use of these toxins against economically important parasitic nematodes.

Poster / Bacteria. Wednesday, 16:30. **BA-6**

Evaluation of Culture media for maximal growth, Cry toxin production and insecticidal toxicity of *Bacillus thuringiensis*

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Bacillus thuringiensis Berliner is a gram positive soil dwelling, aerobic bacterium which produces parasporal crystal (Cry) toxins that are highly specific and effective against insect species. During the course of isolation of native strains, *B. thuringiensis* AUG-05 was found the most effective with a wide range of activity against lepidopterans. Hence, studies were carried out on its fermentation in different media to evaluate the production of maximal Cry toxin as well as spore and colony forming unit (cfu) counts. Increase in concentration of the Luria Bertani [(LB), composed of casein, yeast extract and sodium chloride in 2:1:2 w/w] medium in the fermentation broth from 1 to 2% enhanced cfu, spore and also Cry1Ac and Cry2Ab toxin content. Addition of 1% Wesson salt in 1% LB broth dramatically increased spore, cfu counts, and also that of Cry1Ac but not of Cry2Ab. Spore and cfu counts in media were positively correlated with Cry1Ac and Cry2Ab contents. Bt powders from each fermentation with varying ratios of Cry1Ac and Cry2A toxins were more toxic to the cotton bollworm, *Helicoverpa armigera* than the tobacco caterpillar, *Spodoptera litura* and. Of all media substituting LB with agroproducts, most did well in supporting *B. thuringiensis* culture except for medium VI and VII, suggesting need for balancing qualitative and quantitative nutrients in the medium for optimal growth of the bacterium. Medium consisting of 2% wheat flour, 2% soybean meal and 1% Wesson salt could be considered as an alternative to LB medium to achieve economy of production costs.

Poster / Bacteria. Wednesday, 16:30. **BA-7**

Gene organization of large plasmids of novel mosquitocidal *Bacillus thuringiensis* TK-E6

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A novel Bt strain, TK-E6, isolated from grove soil in Japan, produces a mosquitocidal inclusion body called crystal consisting of several Cry proteins during sporulation phase. We detected twelve genes belong to the *cry* family, by degenerate PCR from Bt. TK-E6. Nucleotide sequences of these genes were determined and deduced ORFs encoding 140 - 145 kDa Cry proteins were cloned into a Bt expression vector carrying *cyt1A* promoter and *cry4A* terminator. Each Cry protein was purified and used for mosquitocidal assay against *Ae. aegypti* larva, any protein, however, did not show the strong activity when used alone. These results suggested that there was a synergistic action with some proteins for mosquitocidal activity. Pulse-field gel electrophoresis analysis showed that Bt. TK-E6 had five

plasmids ranging 66 - 224 mDa. Southern hybridization experiments revealed that twelve genes we detected had been distributed on four of five large plasmids. Interestingly, insertion sequences and transposon structures are also found in the up- and downstream of all genes. It is very possible that some DNA rearrangement of gene amplification occurred in both intra- and inter-plasmids during the evolutional process of *Bt*. TK-E6. Elucidation of structure of *Bt*. TK-E6 large plasmids is very important to know evolution of *Bt*. Therefore, we are analyzing the gene organization of large plasmids by the next generation sequencer.

Poster / Bacteria. Wednesday, 16:30. **BA-8-STU**

Testing of Vip3 proteins for the control of caterpillar pests

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Vip3 insecticidal proteins are produced by *Bacillus thuringiensis* during the vegetative growth phase and most of them have activity against lepidopteran species. Five *B. thuringiensis* Vip3A proteins (Vip3Aa, Vip3Ab, Vip3Ad, Vip3Ae and Vip3Af) and their corresponding trypsin-activated toxins were tested for their toxicity against eight lepidopteran pests: *Agrotis ipsilon*, *Helicoverpa armigera*, *Mamestra brassicae*, *Spodoptera exigua*, *Spodoptera frugiperda*, *Spodoptera littoralis*, *Ostrinia nubilalis* and *Lobesia botrana*. Vip3Aa, Vip3Ae and Vip3Af were the most active proteins. Vip3Af was the protein active against most of the species tested. Contrarily, Vip3Ad was non-toxic to any species. *Agrotis ipsilon* was the species most susceptible to the four active proteins, whereas *O. nubilalis* was tolerant to all Vip3 proteins tested, with just some susceptibility to Vip3Af. The results obtained will help to design new combinations of insecticidal protein genes in transgenic crops or in recombinant bacteria for the control of insect pests.

Poster / Bacteria. Wednesday, 16:30. **BA-9**

Interactions between Cry and Vip proteins from *Bacillus thuringiensis* against different lepidopteran pests

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Second generation *Bt* crops (insect resistant crops carrying *Bacillus thuringiensis* genes) combine more than one gene coding for insecticidal proteins in the same plant to provide a better control of agricultural pests. Some of the new combinations involve co-expression of *cry* and *vip* genes. Since Cry and Vip proteins have different midgut targets and possibly different mechanisms of toxicity, it is important to evaluate possible synergistic or antagonistic interactions between these two classes of toxins. Three members of the Cry1 class and three from the Vip3A class were tested against *Heliothis virescens* for possible interactions. At the level of LC₅₀, Cry1Ac

was the most active protein, whereas the rest of proteins were similarly active. However, at the level of LC₉₀, Cry1Aa and Cry1Ca were the least active proteins, and Cry1Ac and Vip3A proteins were not significantly different. In the experimental conditions used, we found an antagonistic effect of Cry1Ca with the three Vip3A proteins and a slight antagonism of Vip3Af with either Cry1Aa or Cry1Ac. The interaction between Cry1Ca and Vip3Aa was also tested on two other lepidopterans. Whereas antagonism was observed in *Spodoptera frugiperda*, synergism was found in *Diatraea saccharalis*. In all cases, the interaction between Vip3A and Cry1 proteins was more evident at the LC₅₀ than at the LC₅₀ level. The fact that the same combination of proteins may result in a synergistic or an antagonistic interaction may be an indication of different types of interaction with the host depending on the insect species tested.

Poster / Bacteria. Wednesday, 16:30. **BA-10**

Cry1Ac and Cry1F toxicity and binding sites study in two important soybean pests, *Anticarsia gemmatalis* and *Chrysodeixis (=Pseudoplusia) includens*.

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Anticarsia gemmatalis (velvetbean caterpillar) and *Chrysodeixis (=Pseudoplusia) includens* (soybean looper) are two important defoliating insects of soybean that cause economic damage in soybean producing regions in the Americas. Both lepidopteran pests are currently controlled mainly with synthetic insecticides. Alternative control strategies such as biopesticides based on the *Bacillus thuringiensis* (Bt) toxins or transgenic plants expressing Bt toxins can be used and are increasingly being adopted. The studies on the insect susceptibility and mode of action of the different Bt toxins are crucial to determine management strategies to delay insect resistance. Also, these studies are necessary to help design pyramided transgenic plants involving more than one Bt toxin to ensure a crop long term protection. In the present study the susceptibility of both soybean pests to Cry1Ac and Cry1F has been investigated. Bioassays performed in larvae show that both insects are susceptible to these two toxins. Competition-binding studies using brush border membrane vesicles indicate that Cry1F and Cry1Ac share some, but not all, binding sites in midguts of both insects. Incomplete shared binding indicates that there are resistance management benefits from combining the two proteins in Bt soybeans. Additional information on the receptors involved in binding and consequent cross-resistance potential are needed to more fully understand the long-term durability of combinations of Cry1Ac and Cry1F to control these two pests.

Poster / Bacteria. Wednesday, 16:30. **BA-11-STU**

***In vivo* and *in vitro* binding of Vip3Aa to *Spodoptera frugiperda* midgut and characterization of binding sites using ¹²⁵I-radiolabeling**

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Bacillus thuringiensis vegetative insecticidal proteins (Vip3A) have been recently introduced in important crops as a strategy to delay the emerging resistance to the existing Cry toxins. The mode of action of Vip3A proteins has been studied in *Spodoptera frugiperda* with the aim to characterize their binding to the insect midgut. Histological localization of Vip3Aa in the

midgut of intoxicated larvae using immunofluorescence showed that Vip3Aa bound to the brush border membrane along the entire apical surface. The presence of fluorescence in the cytoplasm of epithelial cells seems to suggest internalization of Vip3Aa or a fragment of it. Successful radiolabeling and optimization of the binding protocol for the ^{125}I -Vip3Aa to *S. frugiperda* BBMV allowed the determination of binding parameters of Vip3A proteins for the first time. Heterologous competition was performed using different protein competitors with the aim to determine if they share the same binding sites with Vip3Aa in *S. frugiperda* BBMV and thus select the appropriate candidates to be used in combination with the later in transgenic crops.

Poster / Bacteria. Wednesday, 16:30. **BA-12**

Comparative histopathology of two novel bacterial insecticidal proteins in *Tenebrio molitor* and *Diabrotica virgifera virgifera* larvae

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Larvae of the Western corn rootworm (*Diabrotica virgifera virgifera*) are the most devastating pest of corn in the US. Due to reports of field-evolved resistance, novel insecticidal proteins are needed as alternative candidates for expression in transgenic corn to control this insect pest. A novel insecticidal protein from a Gram negative bacterium (toxA) and a Cry-derived protein (toxB) have been identified and developed, respectively, as candidates for expression in transgenic corn targeting larvae of *D. v. virgifera*. In this work, we used *Tenebrio molitor* larval midgut as a model to characterize toxin binding and histopathology of toxA and toxB proteins in coleopteran larvae, and then compared to histopathology in *D. v. virgifera* larval midguts. While both toxins bind to the midgut brush border membrane, differences observed in H&E stained histological sections and TUNEL assays support differences in the mode of action of these toxins in coleopteran larvae.

Poster / Bacteria. Wednesday, 16:30. **BA-13-STU**

Role of ABC-C2 in the interactions of *Heliothis virescens* with its host plants and Bt toxins

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Bacillus thuringiensis (Bt) Cry toxins are widely used biopesticides for reduction of crop losses caused by larvae from species such as *Heliothis virescens*. Until recently, Cadherin was identified as the major receptor for Bt toxins, albeit Bt resistance was shown to be genetically linked to an inactivating mutation in an ABC transporter. ABC (ATP-binding cassette) transporters are transmembrane proteins that hydrolyze ATP in order to conduct transport and other cellular processes. To date, we have no insights into the physiological role of this specific ABC transporter as well as into its role in the Bt toxin mode of action. We aim to investigate whether ABC-C2, the specific ABC transporter implicated in Bt resistance, acts as a receptor to Cry toxins. Furthermore, we want to find out whether an inactivated (mutated) ABC-C2 could cause a trade-off between Cry toxins and host plant secondary metabolites in Bt resistant insects. To address these two hypotheses, we first heterologously express *H. virescens* Cadherin and ABC-C2 in Sf9 cells. In addition, feeding assays with two *H. virescens* populations, JEN2 (wild

type) and YEE (ABC-C2 mutant), are performed with different host plants as well as host plant secondary metabolites incorporated into artificial diet. The genes of interest were expressed successfully, generating the basis for our ongoing *in vitro* trials. Subsequently, the effect of different Cry toxins on transfected cells will be investigated. Our first feeding assays with homozygous Bt susceptible and homozygous Bt resistant insects revealed a trade-off between Cry toxins and host plant secondary metabolites

Poster / Bacteria. Wednesday, 16:30. **BA-14-STU**

AminopeptidaseN in *Popillia japonica* Newman larvae is putative *Bacillus thuringiensis* Cry8Da toxin receptor

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Cry8Da from *Bacillus thuringiensis galleriae* SDS-502 has insecticidal activity against both the larvae and adult of Japanese beetle (*Popillia japonica* Newman). The receptor determines the specificity of the insecticidal activity of Cry proteins and hence, in order to reveal the mode of action of Cry toxin, receptor identification is a necessary step. However, a receptor for Cry-type toxin has not been identified in the Scarabaeidae family of insects. Therefore, we aimed to identify the receptor of Cry8Da toxin in larvae *P. japonica* BBMV. A ligand blot showed the Cry8Da toxin bound to 110 kDa and 40 kDa protein in the BBMV of larvae *P. japonica*. The 110 kDa protein had higher binding affinity than the 40 kDa protein. In order to identify the Cry8Da toxin binding protein in the BBMV of larvae *P. japonica*, it was purified by column chromatography. The result of mass spectrometry indicated that the Cry8Da toxin binding protein in the BBMV of larvae was aminopeptidaseN which is commonly reported as receptors for Cry toxins in Lepidopteran and Dipteran insects. The 106 kDa APN homologous genes in larvae *P. japonica* could be amplified by PCR using degenerate oligonucleotide primers designed from a conserved sequence of Coleopteran APN. The 106 kDa APN is truncated into two peptides and tested to confirm the ability of binding with Cry8Da toxin. This experiment indicated the APN in larvae *P. japonica* is the receptor for Cry8Da toxin.

Poster / Bacteria. Wednesday, 16:30. **BA-15**

A Whole Genome Approach to Determine Cadherins associated with Bt toxicity in the Diamondback Moth, *Plutella xylostella*

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Diamondback moth, *Plutella xylostella*, is a main pest of Brassicaceae throughout worldwide and was first reported to evolve resistance to Bt toxins in field population. Cadherin has been known to be one of receptors of *Bacillus thuringiensis* Cry proteins and synergizes Cry toxicity against Lepidopteran, Dipteran, and Coleopteran insects by elevating a toxin oligomerization. Full genome analyses of several model insects suggest various number of cadherin genes in an organism and raise a fundamental question on which cadherin(s) is the Bt receptor. In a whole genome sequence of *P. xylostella*, 52 open reading frames were annotated to be cadherins, in which putative Bt receptors were chosen on the basis of three receptor motifs: a signal peptide, cadherin repeat, and transmembrane domains. Compared to other cadherins of *P. xylostella*

(PxCads), *PxCad1* has the highest homology with other lepidopteran insect cadherins previously associated to the Bt mode of action. *PxCad1* was expressed in all developmental stages especially in gut tissue. Expression of *PxCad1* was suppressed by feeding its specific double-stranded RNA (dsPxCad1) in the third instar. The suppression of *PxCad1* expression did not significantly influence on pupal and adult developments of *P. xylostella*. However, the larvae treated with dsPxCad1 (150 ng/larva) significantly reduced susceptibility to *B. thuringiensis* Cry1Ac toxin. In contrast, the dsPxCad1-treated larvae did not show any change in susceptibility to *B. thuringiensis* Cry1Ca toxin. Only one cadherin, *PxCad1*, out of 52 candidate cadherins is the Bt receptor and is responsible for the specificity to Bt toxin, Cry1Ac.

Poster / Bacteria. Wednesday, 16:30. **BA-16**

RNA Interference of Integrin subunit β1 Impairs Development and Immune Responses of the Oriental tobacco budworm, *Helicoverpa assulta* against Bacteria

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Integrin is a cell surface protein that is composed of α and β heterodimer and mediates cell interaction with extracellular matrix or other cells including microbial pathogens. A full length cDNA sequence (2,517 bp) of a integrin subunit β1 (*HalTGβ1*) was cloned from the oriental tobacco budworm, *Helicoverpa assulta*. Phylogenetic analysis showed that *HalTGβ1* was clustered with other insect β integrin subunits with the highest amino acid sequence identity (61%) to β1 of other Noctuidae such as *Spodoptera exigua* and *S. litura*. Structural analysis of the *HalTGβ1* possessed all functional domains known in other insect β1 integrins. RT-PCR analysis showed that *HalTGβ1* was expressed in all developmental stages and all tested tissues of *H. assulta*. Injection of double-stranded *HalTGβ1* RNA (ds*HalTGβ1*) into third instar of *H. assulta* suppressed *HalTGβ1* expression and resulted in significant delay from last larval stage to pupal stage. The ds*HalTGβ1* injection significantly impaired nodule formation of *H. assulta* in response to bacterial challenge and hemocyte adherence. These results suggest that *HalTGβ1* plays crucial roles in cellular immune responses as well as development in *H. assulta*.

Poster / Bacteria. Wednesday, 16:30. **BA-17**

A natural hybrid of a *B. thuringiensis* Cry2A toxin implicates domain I in specificity determination.

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A PCR-RFLP method was used to identify *cry2A* toxin genes in a collection of 300 strains of *Bacillus thuringiensis* were confirmed with *cry2* gene. Of the 81 genes identified the vast majority appeared to be *cry2Aa* (32) and *cry2Ab* (46) on the basis of their RFLP pattern. Three genes showed a different pattern and were subsequently cloned and sequenced. The gene cloned from strain HD395 was named *cry2Ba2*. The proteins encoded by the genes cloned from LS5115-3 and DS415 shared enough similarity with existing toxins that their genes were named *cry2Aa17* and *cry2Ab29* respectively by the

toxin nomenclature committee. Despite this overall similarity these two toxins resembled natural hybrids with *Cry2Ab29* resembling *Cry2Ab* for the majority of the protein but then showing identity to *Cry2Aa* for the last 60 amino acids. For *Cry2Aa17*, domains II and III resembled *Cry2Aa* whilst domain I resembled *Cry2Ab*. The toxicity of the recombinant toxins against three insects was tested, and it was found that the toxicity of *Cry2Aa17* more closely matched the toxicity profile of *Cry2Ab* than that of *Cry2Aa*, thus implicating domain I in specificity determination. Analysis of all publicly available *Cry2Aa* sequences identified other examples of natural hybrids.

Poster / Bacteria. Wednesday, 16:30. **BA-18**

***Bacillus thuringiensis* Cry3Aa toxin increases the susceptibility of *Crioceris quatuordecimpunctata* to *Beauveria bassiana* infection**

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The spotted asparagus beetle, *Crioceris quatuordecimpunctata* (Coleoptera: Chrysomelidae), is one of the most devastating pests of asparagus in China. Sprayed synthetic pesticides have been used to control *C. quatuordecimpunctata* damage, but they pose problems because of residues and harm to natural enemies. Neither the microbial coleopteran-specific toxin from *Bacillus thuringiensis tenebrionis*, Cry3Aa, nor the fungal pathogen *Beauveria bassiana* have sufficient activity to effectively control *C. quatuordecimpunctata* damage to asparagus. However, second instar *C. quatuordecimpunctata* larvae exposed to a sublethal dose of Cry3Aa toxin demonstrated significantly higher larval mortality when exposed to *B. bassiana*. Our results suggest that a combination of Cry3Aa and *B. bassiana* may be effective in reducing damage by *C. quatuordecimpunctata* larvae to asparagus.

Poster / Bacteria. Wednesday, 16:30. **BA-19**

InterVening Sequence (IVS) elements as genetic markers for the differential diagnosis of arthropod-associated *Rickettsiella* bacteria

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Genomic analysis has revealed the presence of insertion sequences within 23S ribosomal RNA encoding genes of arthropod-associated *Rickettsiella* bacteria (*Gammaproteobacteria*). Secondary structure modelling shows that these insertions fulfill the structural criteria for RNase III processed bacterial intervening sequence (IVS) elements.

IVS elements have previously been identified within the rRNA operons of several *Alphaproteobacteria* and occur comparatively frequently within *Enterobacteriaceae*, but not in *Escherichia coli*. In these bacteria, IVS insertion sites have been shown to be conserved with respect to deduced rRNA secondary structures. 23S rRNA gene insertions in *Rickettsiella* occur at one of these conserved loci, more exactly within rRNA helix 25, and at a previously unidentified insertion site within helix 72. Expression of the *Rickettsiella* 23S rRNA genes in the surrogate host *E. coli* by a plasmid replacement approach leads to rRNA fragmentation and thereby confirms that *Rickettsiella* insertion sequences at both sites can function as IVS elements. Given the

lack of sequence similarity with current GenBank database entries, IVS25 and IVS72 give rise to two unprecedented IVS element superfamilies. Whereas the IVS72 element is highly conserved across the full range of investigated *Rickettsiella* species and *Rickettsiella*-like bacteria, the sequence of element IVS25 strongly varies among different *Rickettsiella* strains. Using the sequence information available for both IVS elements, a PCR-based approach for the genus-specific identification and infra-generic characterization of *Rickettsiella* bacteria has been developed.

Poster / Bacteria. Wednesday, 16:30. **BA-20**

Type IV Secretion System (T4SS) substrates as potential virulence factors of arthropod-pathogenic *Rickettsiella* bacteria

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Rickettsiella bacteria (*Gammaproteobacteria: Legionellales*) are intracellular pathogens of arthropods that multiply inside replicative vacuoles within host cells. Delivery of bacterial proteins across the vacuole membrane to the host cell's cytosol is believed to be of key importance for successful infection and pathogenesis.

Comparative genomic analysis of *Rickettsiella* and related bacteria has revealed the presence of a complete set of gene clusters presumably encoding a type IVB secretion system (T4SS) in two *Rickettsiella* strains of the pathotypes '*R. melolonthae*' and '*R. armadillidi*', i.e. infecting, respectively, the European cockchafer and the pill bug. Hypothetical *Rickettsiella* T4SS key components show high similarity to orthologs in the Dot/Icm systems of the related vertebrate pathogens *Legionella pneumophila* and *Coxiella burnetii*, and T4SS gene cluster organization is very similar in these bacteria. In *Legionella* and *Coxiella*, involvement of Dot/Icm systems and several of their substrates into infection and pathogenesis has been demonstrated previously. In *Legionella*, transcriptional regulation of both T4SS structural and substrate genes is most likely mediated by several bacterial two-component systems, but only one of these, PmrAB, seems to be conserved in the genomes of both *Coxiella* and *Rickettsiella*. Expression studies in the surrogate host *Escherichia coli* that lacks an own T4SS, have demonstrated that '*R. melolonthae*' PmrAB drives expression from the promoter regions of the presumed homologous T4SS gene clusters. Comparative *in silico* analysis of PmrAB regulons reveals a very high degree of divergence in hypothetical T4SS substrates sets that is in line with expectations from the specific host-adaptation of these bacterial pathogens

Poster / Bacteria. Wednesday, 16:30. **BA-21**

Unbalanced Polyphosphate Levels Impair Insect Pathogenicity in Plant-Beneficial *Pseudomonas protegens*

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Pseudomonas protegens is a plant-associated bacterium with lifestyles that potentially may be exploited for its use as a biological control agent in agricultural applications. The bacterium is a highly competitive root colonizer and produces antifungal compounds that ward off soil-borne plant pathogenic fungi and oomycetes. *P. protegens* is also capable of killing

larvae of various pest insects following oral or systemic infection. We are exploring global regulatory mechanisms that control insect pathogenicity of the plant-beneficial bacterium. Here, we provide evidence that altering cellular levels of polyphosphate (PolyP) may strongly impair insect pathogenicity in *P. protegens*. The polymer is known for its involvement in regulation of diverse cellular and metabolic processes contributing to bacterial survival and virulence. *P. protegens* mutants with deletions in *ppk1*, encoding a PolyP kinase, or *ppx*, encoding an exopolyphosphatase, had a markedly reduced capacity to kill larvae of the Large White *Pieris brassicae* following oral infection. Oral toxicity could be restored by reintroducing the respective intact alleles into the mutant strains. Deletion of *ppk1* or *ppx* resulted in reduced *in situ* expression of a major virulence factor required for insect pathogenicity in *P. protegens*, i.e. the insecticidal toxin Fit, in insect larvae. We hypothesize that altering PolyP levels affects stress tolerance of *P. protegens* in the insect host thereby impacting virulence of the bacterium.

Poster / Bacteria. Wednesday, 16:30. **BA-22-STU**

***Paenibacillus larvae* and the virulence factor SpIA- an ERIC II specific S-layer Protein**

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Paenibacillus larvae is the causative agent of the notifiable epizootic American Foulbrood of honey bees. Four genotypes, ERIC I - IV of this pathogen do exist, with only ERIC I and II being frequently isolated from outbreaks worldwide. Despite the importance of the disease, molecular and cellular details of pathogen-host interaction during pathogenesis of AFB in honey bee larvae are poorly understood. Recently, the surface layer protein SpIA was identified and functionally characterized as the first virulence factor of the *P. larvae* genotype ERIC II. Through a gene-disruption strategy expression of the *spIA*-gene was successfully interrupted. In infection assays, SpIA-deficient *P. larvae* and the parental wild-type bacteria were compared and it was demonstrated that lack of SpIA expression resulted in a significant decrease in total mortality. To further investigate the role of SpIA in virulence of *P. larvae*, SpIA has been expressed in the natural SpIA-deficient genotype *P. larvae* ERIC I. We will present our most recent data on this SpIA-expressing ERIC I-mutant in respect to growth characteristic in the lab and in larvae and, most importantly, to virulence parameters in exposure bioassays when compared to the parental wild type strain and to naturally SpIA-expressing ERIC II.

Poster / Bacteria. Wednesday, 16:30. **BA-23**

Influence of (varying) population size on host-parasite coevolution: an experimental approach

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Host-parasite interaction is one of the most common and important type of interaction among species, which has a strong impact on species evolution. The signatures of this impact have been identified in genomes, natural communities and on a phylogenetic level. It is not surprising that many aspects of host-parasite relationships have received particular attention from evolutionary biologists. Paradoxically, one indispensable and basic property of host-parasite interaction, population size oscillations, has been overlooked as a factor in host-parasite

coevolution. Parasites, by reducing host fecundity and survival, strongly affect population size of the host, which very often is their only ecological niche. Already in the 1920s Lotka and Volterra showed that antagonistic interactions between species would lead to interdependent oscillations in their population size. However, most of the current models of host-parasite coevolution ignore population size changes or use a deterministic approach which cannot realistically imitate the finite nature of real populations. Similarly, in most experimental studies on host-parasite coevolution the population size is kept constant as a matter of good practice. To enhance a more realistic understanding of the coevolutionary dynamics, we performed laboratory-controlled evolution experiments with the model nematode host *Caenorhabditis elegans* and its microparasite *Bacillus thuringiensis* and specifically varied the factor population size. Here, we will show our results on temporal changes in host fitness and parasite virulence under different population size regimes.

Poster / Bacteria. Wednesday, 16:30. **BA-24**

An *in vivo* experimental evolution system for analyzing bacterial adaptation and evolution of *Bacillus cereus* *sensu lato* in an insect model

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The continuous exposition of a pathogenic bacterium in a host during a serial passage experiment (SPE) may drive the fixation of mutations that favour its growth and multiplication in the host environment¹. These changes that are usually associated with an increase in virulence, can be now traced during an SPE by whole genome sequencing of the evolved variants². Here we describe the set up and initial results of a SPE using a *Bacillus thuringiensis* crystal minus strain (Bt407 Cry-) ³ using *Galleria mellonella* larvae. A new infection protocol has been established which permits bacterial multiplication inside the intestine following force-feeding with spores. The genomes of experimentally evolved bacteria that show significant changes in virulence or persistence will be sequenced and compared with the initial parental strain. Such a global genome based approach of pathogen evolution analysis should allow us to describe the history of the events which arose during the evolution of the *B. cereus* group in one of its natural hosts and explain phenotypic variations based on genotypic differences.

Honey bees (*Apis mellifera*) are essential pollinators of various agricultural crops and fruit but also of many wild plants. Therefore, it is crucial to maintain honey bee health and prevent or cure diseases. The most contagious and fatal bacterial disease of honey bee brood is American Foulbrood (AFB) caused by *Paenibacillus larvae*, a Gram positive, spore-forming bacterium. Infection spreads among the whole hive, eventually leading to the loss of entire colonies resulting in considerable losses in apiculture. Despite the enormous impact of this disease and intensive research, molecular mechanisms involved in the pathogenesis are still not fully understood. Recently we have identified and characterized four genotypes of *P. larvae* (ERIC I-IV) which differ, among other factors, in virulence. Here we present our data on immune inhibitor A (InhA), a metalloprotease which is exclusively secreted by *P. larvae* ERIC II. In homologs of other pathogenic bacteria, InhA has been shown to have multiple functions such as degradation of antimicrobial peptides and cleavage of tight junctions. Here we functionally characterize InhA of *P. larvae* by combining transcriptomic, proteomic and histological studies as well as *in vivo* exposure bioassays with wild type and mutant *P. larvae*, the latter being deficient in InhA expression.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-2**

Awareness and Concept of Insects in a Korean Population

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To investigate the degree of individuals' concept and awareness of insects, a survey study was conducted with students and adults living in Korea. The misconception rate for insects was about 50% for both students and adults, but it was lower for students and people who had experienced insect-related events than for adults and those who had not. The highest misconception rate was obtained in answer to a question about the basic structure of an insect. Most people had a high preference of insects. Significant differences and correlations for the preference of insects were found between students and adults, men and women, people who had experienced insect-related events and those who had not. The experience of an insect-related event most influenced preference of insects. These results suggest that increasing people's interest in insects and utilizing insects in treatment situations may be beneficial for the field of mental healthcare.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-3**

Virus Epizootiology in Managed and Native Bee Populations

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The possible cross host-genus transmission of several honeybee viruses into native bee populations has recently been proposed. Given current pollination practices and the number as well as high levels of different viruses found in honeybees, the cross genus transmission of these viruses could have a dramatic impact on the health of native bees. In order to examine this possibility we initiated a study of the prevalence of the two honeybee viruses; deformed wing virus (DWV) and black queen cell virus (BQCV) in *Apis* and *Bombus* sp. where *Apis* was maintained under different conditions. These included sites

DISEASES OF BENEFICIAL INVERTEBRATES

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-1-STU**

Identification and Characterization of Immune Inhibitor A Metalloprotease of the Honey Bee Pathogen *Paenibacillus larvae*

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where stationary or migratory *Apis* hives were present, and sites where no or few *Apis* were present. Both viruses were found in both bee species in sites where *Apis* hives were present. The level of BQCV was significantly higher than DWV in all sites in both bee species when present. While BQCV reached level of 100% in *Apis* and 80% in *Bombus* in both migratory and stationary sites, DWV levels were only at 60% in *Apis* and 30% in *Bombus* in these sites. In the no or few *Apis* sites, BQCV reached levels of up to 65% in *Bombus* and DWV was never found in more than 10% of these bees. We are currently examining gene sequences of viruses recovered from the different bee species collected at each site to determine if they cluster by bee species or by collection site thereby providing further evidence on the interspecies transmission of these two viral pathogens.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-4**

Honeybee Virus Epizootiology in Bee Populations in Connecticut, USA

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We examined the prevalence of Black Queen Cell Virus (BQCV), and Deformed Wing Virus (DWV) in bee pollinators found foraging on pumpkins (*Cucurbita sp.*) on four farms in Connecticut. The three main groups foraging on pumpkins, *Apis mellifera*, *Bombus sp.* and *Peponapis pruinosa*, were sampled 5 times at each site from early June to late September. Sampling included approximately 20 bees of each group when available. Our initial analysis has focused on BQCV which is the most prevalent of the viruses in bees we have examined to date. Of the ~1,000 bees we have analyzed to date, 46.3% were found to be infected with BQCV. This virus was the most prevalent in *Apis* with 73.2% being infected, while the *Bombus* and *Peponapis* were infected at 37.7% and 3% respectively. The level of virus-positive bees of any species from the different farms ranged between 2.3% and 91.9% and overall our results suggest a correlation between the level of this virus in honey bees and the level of infection of other bee species. On the two farm sites where we found honey bees infected with BQCV at 95% and 75%, *Bombus* bees were at 91% and 40% respectively. At the site where we found only 10% of *Apis* infected with BQCV we were able to detect only 9.5% BQCV infected *Bombus*, suggesting that the infection of *Apis* and *Bombus* is clustered and may be connected in some way.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-5**

High-throughput sequence analysis of the change in expression profile of Ig2-, Ig3- and Ig7- variant domains in *Carcinus maenas* Down Syndrome Cell Adhesion (*CmDscam*) mRNAs in response to pathogenic infection

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Previously, we have identified a DSCAM gene (*CmDscam*) within the shore crab *Carcinus maenas*. This gene codes for a pattern recognition protein and has alternately spliced Ig2-, Ig3- and Ig7- domains and multiple different 3' UTRs. In other decapods evidence that these variable domains are alternately spliced during an immune response has been used to support a

concept of specificity within arthropods. However, these data have been generated using conventional Sanger sequencing of a limited number of clones. This approach has insufficient depth to confirm unequivocally that the transcript profile is changed specifically through infection. Herein we present the first high throughput sequencing comparison of the variant Ig2-, Ig3- and Ig7- domains in response to Gram-negative or Gram-positive bacterial challenge. Haemolymph from individual crabs was sampled before and after a single sub-lethal inoculation with either bacterium to produce a deep haemocytopenia through haemocyte degranulation. Amplicons from each sample were then deep sequenced to test the hypothesis that bacterial infection specifically alters the transcription of *CmDscam* during the immune response. Data are discussed in light of new theories of specificity and memory within the innate immune system of decapods.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-6**,

A novel pathogenic *Paenibacillus* strain of *Biomphalaria glabrata*, an intermediate host for schistosomiasis

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Schistosomiasis is the second most widespread tropical parasitic disease after malaria. To achieve the objective of schistosomiasis eradication in a decade, various research strategies and treatment programs were recommended and supported by WHO. One of these applicable approaches is based on the control of snail vectors in endemic area. Previous field studies have shown that competitor or predator introduction could be effective but no systemic investigation has ever been conducted to identify snail microbial pathogen and evaluate its molluscide effect. In our laboratory, infectious agent was isolated on white nodules from unhealthy *Biomphalaria* snails. Only one bacteria was characterized and identified as *Paenibacillus* sp closely related to *P. alvei* through 16S and rpoB DNA analysis. Histopathological examination has shown massive bacterial infiltration leading to an overall disorganization of snail tissues. Exposure of healthy snails to *Paenibacillus* infected snails led to a massive mortality. Moreover, the number of hatched snails was significant lower in exposed snails than in control whereas the spawning appeared to be unaffected. Embryonic lethality is correlated with the presence of this pathogenic bacteria in eggs. This study reports the first description of a novel *paenibacillus* strain as snail microbial pathogen by affecting both adult and embryonic stages.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-7**

Venom from the ectoparasitic wasp *Habrobracon hebetor* activates calcium-dependent processes of haemocytic degradation in *Galleria mellonella* larvae

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The influents of *Habrobracon hebetor* venom on the cellular and humoral immune reactions of the wax moth larvae (*Galleria*

mellonella) by the naturally envenomation were analyzed. A strong decrease of phenoloxidase (PO) activity in the haemolymph and the number of haemocytes with PO activity of envenomated larvae were recorded. The capsule melanization in the envenomated larvae was twofold less than in control. Production of reactive oxygen species in the haemolymph of envenomated larvae also decreased. The main immune reactions (capsule formation, phagocytosis and coagulation of the lymph) are directly related by emission of calcium ions (Ca^{2+}) into the cytosol and in the pericellular space of haemocytes. The cytosolic calcium concentration in the haemocytes of *G. mellonella* larvae on first and second day after envenomation from *H. hebetor* female was measured (fura - 2 AM used). The increase of Ca^{2+} concentration and phospholipase C activity in haemocytes were registered for two days after envenomation. The addition of the parasitic venom *in vitro* (final concentration of protein 6,2 µg/ml) have induced the decreasing of viability and adhesive capacity of the haemocytes during one hour. The membrane potential was measured with a fluorescent probe. The changes of trans-membrane potential of hemocytes were investigated both *in vitro* and *in vivo* experiments. The degree of trans-membrane potential was in direct dependence of the added venom concentration. The envenomated insects exhibited the decreased potential values.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-8**

Histopathological analyses of different tissues of diseased honey bees (*Apis mellifera*)

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The western honey bee (*Apis mellifera*) is threatened by numerous infectious pathogens (bacteria, viruses and fungi), affecting different life stages of the honey bee and various tissues. This work aims to compare diseased and healthy honey bee tissues in order to detect the pathogens' specific localization and to identify tissue alterations caused by various etiologic agents. For this purpose honey bee larvae were infected with *Paenibacillus larvae*, the causative agent of American Foulbrood (AFB), a notifiable epizootic, by feeding first instar larvae with *P. larvae* spores of different genotypes. Also, white eyed pupae were infected with deformed wing virus (DWV) by injection of virus particles. Adult worker bees were infected with *Nosema apis* and *Nosema ceranae* by the oral uptake of food supplemented with defined spore concentrations. Diseased and control animals were collected at various time points post infection, fixed in formalin and embedded in paraffin. Thin sections of the different body parts were analyzed by fluorescence *in situ* hybridization (FISH) using specific fluorescence dyes labeled oligonucleotide probes for each pathogen and following recently established protocols. *In situ* visualisation of infected cells and tissues will in the end help us to understand the pathogens' life cycles during pathogenesis.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-9**

New findings in genome of *Apis mellifera* filamentous virus

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The honey bee plays an extremely important role as a pollinator of crops and wild plants. Honey bee colony losses noted worldwide since 2006 can heavily impair not only global food production but also ecosystem and biodiversity maintenance. One of the possible causes of this situation is the co-infection of bee colonies with different pathogens including *Nosema apis/ceranae*. This microsporidium is often associated with viruses like Black queen cell virus, Bee virus Y and *Apis mellifera* filamentous virus (*AmFV*). The life span of bees infected with *Nosema* and viruses is shorter than of bees infected with *Nosema* alone.

AmFV is a DNA virus. The size of the enveloped particle is 150-450 nm x 150 nm. On the basis of morphological features, *AmFV* was considered to be related to baculoviruses. In 2012 the first fragment of *AmFV* genome was sequenced (822nt long) and submitted to the GeneBank. Phylogenetic analysis of this fragment supports previous assumptions of similarity to baculoviruses.

In our studies we use the Illumina Next Generation Sequencing approach to sequence much longer fragments of genome of *AmFV*. One of the contig that contains the full sequence of previously described BroN gene comprises another gene, which sequence is highly similar to baculoviral ribonucleotide reductase. Other findings about genome structure and possible theories concerning origin of elusive *AmFV* will be presented during the conference.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-10**

Development of prototypes of rapid molecular diagnostic tests for pathogens of honeybees (*Apis mellifera* L.) on chromatographic NALF platform (Nucleic Acid Lateral Flow)

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Honeybees are of inestimable value as agents of cross-pollination and also as workinsect for beekeeping. Honeybee populations have been decreasing globally in recent years because they are affected by environment, human activities, moreover they are susceptible to many pathogens.

Viroses and nosemosis are widespread in honeybees, but despite the serious economic losses they can cause, these are underestimated by the beekeeping industry. An early diagnosis of the causative agents has great importance for the management of the disease and in the establishment of measures to guide therapy and prophylaxis.

We present the development of diagnostic tests based on the NALF (Nucleic Acid Lateral Flow) technology for the detection of the following pathogens of honeybees: Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV) and the microsporidian *Nosema* (*Nosema ceranae*).

DNA and RNA of the pathogen are amplified by isothermal reactions using LAMP (Loop-mediated Isothermal Amplification) in the presence of at least one primer conjugated to Gold Nano Particles (GNPs) that are used to label the molecules of interest. The result of the isothermal reaction is detected by naked eye, in a few minutes, by means of a NALF device.

The assay has the same sensitivity and specificity of a molecular test but being at the same time quicker, cheaper, waste friendly, adapted to basic laboratory equipment and accessible to ordinary technical personnel.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-11**

What Kind of Insects Do You Like?

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Insect constitute the largest and most diverse group of animals on world and also serve as the hosts or nutrient sources. In addition, several insects have a strong influence on people's emotion. To utilize the preference and interest of insects in the field of mental healthcare, a survey study was conducted with individual living in Korea. As results, the most people had a high preference and interest of insect, but some were disagreeable to the insect itself. The preference and interest of insect were high on male, adult and practician experienced insect-related events than female, student and non-practician, respectively. The most favored insects were familiar or pet insects such as *Papilio xuthus*, *Lucanus maculifemoratus*, *Allomyrina dichotoma* and *Lampyridae*. These results may be useful to develop a healing program for mental healthcare using insects. Further research is needed to determine the effects of these insect in the mental therapy for this purpose.

Poster / Dis. Ben. Invertebr. Wednesday, 16:30. **DB-12**

A muscle-infecting microsporidium infecting pink shrimp (*Pandalus montagui*) from Europe: closing in on the type species of *Thelohania*?

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The type species of the genus *Thelohania*, *T. giardi* was described infecting European brown shrimp (*Crangon crangon*) in the late 1800's. Although never rediscovered, recent work describing *T. butleri*, a similarly octosporous microsporidium infecting Canadian pink shrimp (*Pandalus jordani*), provided evidence that *Thelohania* (containing parasites of marine shrimp, freshwater crayfish, and ants) is polyphyletic and in need of significant revision. This work led to proposals that only marine forms should be considered as true members of the genus and that effort should be applied to rediscover the type species. In this study, we describe a novel microsporidium infecting another pandalid shrimp, *P. montagui* from Europe using histological, ultrastructural and phylogenetic data. Although the parasite does not display the characteristic morphological features of either *T. giardi* or *T. butleri* (8 spores contained within each sporophorous vesicle), phylogenetic analysis places it closest to *T. butleri* (91% similarity, 100% coverage of 937bp fragment of SSU rDNA gene) within the broader microsporidian tree. Previous work from our laboratory has focussed on the potential for morphological plasticity within Microsporidia infecting the musculature of marine crustaceans. To this end, we propose that despite the divergence in form from the type species of *Thelohania*, the close phylogenetic relationship to *T. butleri* suggest that the parasite in *P. montagui* is a species of *Thelohania*. In addition, we provide further evidence that closely related taxa can display wide morphological variance and, that marine thelohandids may display a level of intra-generic plasticity which nullifies the use of morphology in their taxonomy.

FUNGI

Poster / Fungi. Wednesday, 16:30. **FU-1-STU**

Monitoring of entomopathogenic fungi in *Metarhizium* and *Beauveria* treated fields

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Application of entomopathogenic fungal strains for the control of cockchafer grubs was investigated in sour cherry orchards. Safety like possible effect of the inoculum on natural soil microbiota as well as efficacy and fate of these fungi need to be investigated. The applied fungal strains have wide host range, thus we have to determine the risks of their use during repeated long-term applications. Different inoculation methods were compared and the persistence of inoculum was monitored in the soil and on target and non-target organisms. One year after treatments we collected soil samples and grubs from un-treated and treated areas and re-isolated the fungi on selective media. Furthermore we applied PCR analysis for the identification of our *Metarhizium anisopliae* strains. According to Ya Li & Shuang Hu-Cai (2011) we used a species-specific primer for the detection of fungus. We were able to detect the presence of *Metarhizium* strains. Neither another entomopathogens (*Beauveria*, *Lecanicillium*), or other fungi like fusaria gave positive signal with the *Metarhizium*-specific primers. Furthermore, the presence of *M. anisopliae* was detected in about 10 percent of untreated soil samples. It proves that *Metarhizium anisopliae* can be found in the original soil mycobiota, although at a very low frequency. Research was supported by the grant **GOP-1.1.1-11-2012-0059** „Development of environment friendly product with the use of entomopathogenic organisms”..

Poster / Fungi. Wednesday, 16:30. **FU-2**

Distribution of insect-pathogenic soil fungi in agricultural and forest ecosystems in Georgia

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Entomopathogenic fungi naturally occurring in the soil represent a reservoir of antagonists to insect pest. Local strains of such fungi may be adapted to their environment and are of particular interest for usage in biological control. Georgia has a high diversity of altitudes, eco-systems and cropping system and may offer special opportunities for studies of insects pathogens. Soil samples were obtained in 2012-2013 from 8 different geographical sites at different altitudes (600-2200 m a.s.l.), representing different agricultural and forest ecosystems, National parks of Georgia. A total 161 soil samples representing 45 locations were analysed using the insect bait method Waxworm, *Galleria mellonella* L. and Mealworm *Tenebrio molitor*) The following entomopathogenic fungal taxa were found: *Beauveria bassiana* s.l., *Beauveria brongniartii*, *Metarhizium* spp., *Lecanicillium* sp., *Isaria* sp. Also, we isolated *Aspergillus flavus*. The most abundant species was *Beauveria bassiana* (41,4%) and *Metarhizium* sp. (49,4%) from the total number of isolates. Three isolates of both *Metarhizium* and *Lecanicillium*

were found, while only one *Beauveria brongniartii*. Interestingly, no entomopathogenic fungi were isolated from six of the soil samples. In these locations, *B. bassiana* was predominantly recovered more often from soils of natural habitats, while *Metarhizium* spp. were recovered mostly in agricultural habitats. Our study included a limited number of samples, and more extended studies may reveal additional information about the occurrences of these fungi in different habitats and geographical zones of the South Caucasian region.

Poster / Fungi. Wednesday, 16:30. **FU-3**

Diversity of Entomopathogenic fungi in different citrus cropping systems in Brazil

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Biodiversity studies of entomopathogenic fungi (EF) in agroecosystems are very important to understanding the ecology of indigenous populations, their contributions to pest control and the impact of agricultural practices on their populations. The objective of this study was to investigate the natural occurrence of EF in citrus in São Paulo State, Brazil. Samples were collected in four fields with conventional cropping systems (Santa Barbara D'Oeste, Conchal, Nova Europa and Bebedouro), one organic field (Itirapina) and some abandoned fields in Itapetininga, Anhembi, Conchal, Corumbataí, Limeira and Araras during one year (2013-2014). The EF were isolated from soil samples by selective medium and the "Insect Bait" method using *Tenebrio molitor* larvae, and from pest samples by direct transfer onto PDA medium. The Hypocreales fungi isolated from soil by selective medium were *Metarhizium* sp. (18.9% of 174 soil samples) followed by *Beauveria* sp. (14.3%) and *Isaria fumosorosea* (8%). Using the "Insect Bait" method *Metarhizium* sp. was recovered from 75.9% of the soil samples and *Beauveria* sp. from 1.7% of samples. The insect pests found infected by EF were the citrus snow scale, *Unaspis citri* (Hemiptera: Diaspididae) infected with *Beauveria* sp. and *Pochonia* sp., the sharpshooters (Hemiptera: Cicadellidae) with *Beauveria* sp., the whitefly *Dialeurodes citri*, and citrus blackfly *Alurocanthus woglumi* (Hemiptera: Aleyrodidae) with *Aschersonia* sp., green scale *Coccus viridis* (Hemiptera: Coccoidea) with *Lecanicillium* sp., and two unidentified Lepidopteran with *Cordyceps* sp. in organic and abandoned citrus fields. In the abandoned fields the density of EF in the soil was lower than the conventional and organic fields.

Poster / Fungi. Wednesday, 16:30. **FU-4**

The Entomopathogenic Fungus *Isaria* for Pest Insect Control in Vegetables

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The growing area of vegetables in the EU covers more than 3,000,000 ha. BIOCOMES is an EU funded project to provide fundamental information for the development of plant protection products, based on biocontrol agents (BCA). Currently, the common control of various insect pests is mainly by synthetic insecticides. Nevertheless, several pest insects cause considerable damage in agriculture due to resistance to pesticides.

The aim of the BIOCOMES work package is to develop a new

fungal BCA for pest insect control in open field crops and in greenhouses. Presently, we investigate the integration of entomopathogenic fungi into a control strategy. Within different treatments and pre- and post-harvest applications in protected and non-protected cropping systems, we compare the efficacy of at least 10 *Isaria* spp. strains under different laboratory conditions. Moreover, the host range of these strains will be screened, in order to determine the relationship of clade specific differences between virulence and pathogenicity factors. Additionally, the effect on beneficial insects like the predatory mite *Typhlodromus pyri* and the seven-spot ladybird, *Coccinella septempunctata*, will be evaluated to assess the possibility for implementation of entomopathogenic fungi in an integrated pest management strategy. As entomopathogenic fungi are known to produce a wide range of secondary metabolites as, e.g., antibiotics or repellents, selected strains will be screened for secondary metabolites and enzyme activities. Actually, first results will be presented.

Poster / Fungi. Wednesday, 16:30. **FU-5**

Prevalence of *Beauveria pseudobassiana* among tick-associated fungal isolates from the Republic of Moldova

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Human and animal disease transmitting hard ticks (Acari: Ixodidae) are of eminent concern for public health and animal farming. Alternatives to tick control by chemical acaricides are highly solicited, and one intensively evaluated biocontrol strategy is based on the use of tick-pathogenic filamentous fungi. An indispensable prerequisite of the development of tick-derived fungal isolates into registered myco-acaricides is their sound taxonomic characterization.

Using a molecular taxonomic approach based on phylogenetic reconstruction from both internal transcribed spacer (ITS) and protein-encoding gene sequences, a set of fungal strains isolated from ixodid ticks in the Republic of Moldova that had previously been assigned to the species *Beauveria bassiana*, together with further tick-derived fungal isolates from different geographic locations in Europe and the North America was characterized at the genus and species level. All fungi investigated were conclusively assigned to one of the two "hyphomycete" genera, *Beauveria* or *Isaria* (Ascomycota; Hypocreales; Cordycipitaceae). Within the genus *Isaria*, two species, *Isaria farinosa* and *Isaria fumosorosea*, were equally represented. Within the genus *Beauveria*, the species *Beauveria pseudobassiana* was found to strongly prevail among the isolates from Moldova. In particular, the previous classification as *B. bassiana* could not be confirmed for any of the correspondingly characterized tick-pathogens from Europe and North America. The data presented motivate the hypothesis that within the genus *Beauveria* specific adaptation to ticks might have occurred in the species *B. pseudobassiana*. However, to test this hypothesis, a more extensive molecular taxonomic survey carefully reconsidering previous taxonomic assignments of tick-derived fungal isolates is indispensable.

Poster / Fungi. Wednesday, 16:30. **FU-6**

Diversity and abundance of entomopathogenic fungi on strawberry crops in Brazil

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The aim of this study was to characterize the diversity and abundance of entomopathogenic fungi in arthropods on leaves of strawberry and of spontaneous herbaceous plants from the crop borders as well as from soil samples of organic and conventional fields in. The aboveground pests were collected from the crop and from the crop border vegetation at four localities of the Minas Gerais state in Brazil and were incubated in high moisture and inspected for fungi, daily. Two methods were used for isolation of entomopathogenic fungi from soil: selective media (SM) and insect baiting (IB) with *Tenebrio molitor*. No entomopathogenic fungi were observed in the aboveground insect pests, while eight mites were infected with *Neozygites floridana*. Pooling all soils samples revealed that *Metarhizium* spp was the most common fungus (73%-SM / 97.9%-IB), followed by *Beauveria* spp (22%-SM / 1.7%-IB) and *Isaria* spp. (5%-SM / 0.4%-IB). Diversity and abundance of entomopathogenic fungi was not much different between organic and conventional fields. For organic cropping alone the following fungi were isolated: *Metarhizium* spp (58.5%-SM / 97.5%-IB), *Beauveria* spp (34%-SM / 2.5%-IB) and *Isaria* spp. (7.5%-SM / 0%-IB) and for crop border vegetation in organic systems *Metarhizium* spp (82.4%-SM / 96.7%-IB), *Beauveria* spp (17.4%-SM / 2.6%-IB) and *Isaria* spp. (0.2%-SM / 0.7%-IB). For conventional cropping: *Metarhizium* spp (83.2%-SM / 98%-IB), *Beauveria* spp (16.8%-SM / 1%-IB) and *Isaria* spp. (0%-SM / 1%-IB), and for crop border vegetation around conventional crops: *Metarhizium* spp (86.1%-SM / 100%-IB), *Beauveria* spp (4.3%-SM / 0%-IB) and *Isaria* spp. (9.6%-SM / 0%-IB). The on-going studies on the intra-specific diversity will reveal the role of the crop borders as a reservoir of these generalist natural enemies.

Poster / Fungi. Wednesday, 16:30. **FU-7**

Abundance and diversity of *Metarhizium* spp. in an agricultural landscape in Sweden

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Entomopathogenic fungi belonging to the genus *Metarhizium* are important regulators of insect populations, including agricultural pests, and products based on these fungi have been applied in augmentation biological control of different pest insects. As sustainable agriculture and implementation of integrated pest management is gaining attention, the interest in establishing conservation biological control strategies is also growing. In conservation biological control, habitats or agricultural practices are adjusted to enhance the abundance of resident natural enemies, i.e. the biological control agent. Such approaches require a profound understanding of the control agent's life cycle and its ability to survive in different environments. However, abundance and diversity of these entomopathogenic fungi in Sweden have not been evaluated. In this study, we therefore investigate the occurrence of indigenous *Metarhizium* spp. in transects of a cereal field, a permanent grassland and an unmanaged forest site in Uppland, Sweden using cultivation-

dependent techniques as well as quantitative PCR. A collection of new *Metarhizium* isolates from the different habitats will be established, and strains will be characterized by PCR and genotyping. Factors such as soil management and vegetation will be evaluated for their effect on the abundance and diversity of *Metarhizium* spp. This study will generate new information on the potential of using *Metarhizium* for insect pest control in Sweden. Hence, it will facilitate the development of *Metarhizium* based biological control approaches including both augmentation as well as conservation biological control and the use of these approaches in sustainable farming systems in Sweden.

Poster / Fungi. Wednesday, 16:30. **FU-8**

Diversity and distribution of entomopathogenic fungi in Czech Republic soils

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A survey of entomopathogenic fungi was carried out in cultivated and uncultivated soil habitats in Czech Republic. A total of 189 soil samples were collected during October 2013. Two different methods of isolation were employed, selective media containing cicloheximide and SYLLIT 65 WP and *Tenebrio molitor* bait method. Entomopathogenic fungi were detected in all collected soil samples by using selective media, but not with the second isolation method. Eight different taxa belonging to five different genera were encountered by using morphological and molecular identification (ITS and EF 1- α molecular markers). The two more common taxa were unnamed species designated as *Lecanicillium* sp. (14%) and *Metarhizium anisopliae* (44.5%). Additionally, uncultivated soils showed a higher richness in entomopathogenic fungi than cultivated ones.

This is the first time that a monitoring study for the natural occurrence of entomopathogenic fungi was developed covering all Czech Republic. This study constitutes a valuable source for the discovery of indigenous isolates that can be applied in biological control strategies.

Poster / Fungi. Wednesday, 16:30. **FU-9**

Entomopathogenic fungi as plant growth enhancers

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Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium brunneum*, and *Isaria fumosorosea* are primarily used for managing pests. A preliminary study showed that treating the roots of strawberry transplants with *B. bassiana* significantly promoted its growth compared to untreated plants or those treated with a commercial plant growth enhancer. In another study, soil treatment of strawberry plants with *M. brunneum* appeared to help plants withstand twospotted spider mite (*Tetranychus urticae*) infestations compared to untreated plants. These studies suggest that entomopathogenic fungi could be promoting plant health and growth through mycorrhizal interaction. A study was conducting by soil treatment of potted cabbage plants with various commercial products based on entomopathogenic fungi - *B. bassiana*, *M. brunneum*, *I. fumosorosea*, mycorrhizal fungus - *Rhizophagus irregularis*, and a formulation based on bacterial and fungal combination - *Azorhizobium caulinodans*, *Bacillus subtilis*, *Pseudomonas*

phaseoli, *Rhizobium phaseoli*, and *Trichoderma virens*. Impact of these treatments on plant development will be discussed. Preliminary data show superior growth of cabbage plants treated with *B. bassiana*.

Poster / Fungi. Wednesday, 16:30. **FU-10**

The entomopathogenic fungus Beauveria bassiana improves the growth of *Triticum aestivum* and *Triticum durum*

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The main role of Entomopathogenic Fungi (EF) is to kill insect. However, it was recently discovered that many EF, especially hypocrealean ascomycetes, have additional not fully understood ecological roles. This research deals with the effect that EF have on growth, nutritional status, and hormone levels of inoculated plants, *Triticum aestivum* and *Triticum durum*. Three inoculation methods were used using a conidial suspension of *B. bassiana* (Balsamo) Vuill with a concentration of 10^8 conidia mL⁻¹, soil treatment, seed dressing and leaf spraying (2 first leaves of wheat plants 7 days after germination), with 25 plants per treatment either treated / inoculated or control. Plant growth parameters were determined and evolution of the fungal inoculum in the soil and colonisation of plant tissues (leaves and roots) assessed through re-isolation of *B. bassiana* at different phenological states. The fungus was revealed to be rhizosphere-competent, with root re-isolation percentages ranging from 20 to 80% for plants grown on soil treatment and seed dressing. Percentage of fungal re-isolation from leaf tissues was significantly higher in plants inoculated by leaf spraying ranging between 8 and 75%. At the end of the crops, it was detected that the dry weight, the total root length, the quantity of some nutrients and yield of inoculated plants was higher than in control plants. The possible origin of these differences in *B. bassiana* inoculated plants and their implications for pest and disease control and the promotion of plant growth are being investigated.

Poster / Fungi. Wednesday, 16:30. **FU-11-STU**

Interactions between cowpea plants vs. *Metarhizium* spp. entomopathogenic fungi

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In recent years, *Metarhizium* spp. fungi have been reported to associate with plants through rhizosphere competence and endophytic growth. Benefits to both the fungus and the plant, at least in some cases, is beneficial to both members of the pair. In the present study, germination of two important *Metarhizium* species was analyzed after incubation of conidia on young plant leaves. Seeds of *Vigna unguiculata* (cowpea) were planted in sterile soil and incubated with ambient light at room temperature for 10 days. Aqueous suspensions of *Metarhizium robertsii* ARSEF 2575 or *Metarhizium brunneum* ARSEF 1095 were brushed directly onto plant leaves. Control plants, to ensure conidial germination, had their leaves brushed with potato dextrose agar (PDA) plus 0.05% chloramphenicol and 0.002%

benomyl; air dried; then the fungus suspension was brushed on the leaves. After 24h and 48h, 0.5 cm² leaf pieces were examined by scanning electron microscopy (SEM). Conidia of both fungal isolates germinated on cowpea leaves 24h and 48h after inoculation on both PDA treated (control) and PDA not treated (test) leaves. SEM observation showed conidial adherence but with no preferred attachment sites. Each conidium produced one germ tube; and both long and short germ tubes were observed. Their growth over the plant cuticle was random (had no apparent targets). There was no evidence of appressorium formation. However, some *M. brunneum* ARSEF 1095 conidia that germinated on non-PDA-treated leaves had images that suggested direct penetration. Culture studies with surface-sterilized fungus-exposed leaves are underway to verify or deny cuticular penetration.

Poster / Fungi. Wednesday, 16:30. **FU-12**

Biological control in oilseed rape: An attempt to establish the entomopathogenic fungus Beauveria bassiana as an endophyte in oilseed rape plants

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With the rapid spreading of the cultivation of oilseed rape (*Brassica napus* L.), the populations of pest insects of rapeseed also increase, in particular the rapeseed pollen beetle (*Brassicogethes aeneus*) and rape stem weevil (*Ceutorhynchus napi*). Hence, the aim of the investigations within the scope of biological control is to establish the entomopathogenic fungus *Beauveria bassiana* Naturalis ATCC74040 as a systemic endophyte in oilseed rape. Blastospores of *B. bassiana* (10^5 Sp/ml) from Czapek liquid medium were infiltrated into rape leaves. The plants were held with 80% RH and 20°C on long day conditions. Between 3 days and 4 weeks leave samples were taken and examined by fluorescence-microscopy, either with Blankophor or specifically with polyclonal primary antibodies against *B. bassiana*. PCR primers targeting a characteristic partial sequence of a self splicing group-I intron within the 28S rRNA encoding gene of *B. bassiana* Naturalis ATCC74040 were designed and used for strain-specific diagnosis. While the fungus was found to be persistent on the epidermis, only few hyphae could be detected microscopically in intercellular space of the leaves. By means of PCR, *B. bassiana* Naturalis could be proven successfully in rape tissue samples; a clear molecular proof of systemic growth within leaves is still pending. Possible defense mechanisms are discussed.

Poster / Fungi. Wednesday, 16:30. **FU-13**

Azygo- and zygospore formation of *Neozygites floridana* in the two-spotted spider mite (*Tetranychus urticae*) in strains from tropical and temperate regions

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Neozygites floridana is an obligate fungal pathogen of mites in the family Tetranychidae and is an important natural enemy of the two-spotted spider mite (*Tetranychus urticae*). Until now, information about the formation of azygospores remained to be fully confirmed. In this study, we document the formation of

azygospores by a Brazilian *N. floridana* strain and the formation of azygospores and zygospores by a Norwegian *N. floridana* strain both in the host *T. urticae*. Evidence of both zygosporogenesis and azygosporogenesis was also found in the same individual in the Norwegian stains. Further we report the presence of immature azygospores with 1-3 nuclei for the Norwegian strains, immature resting spores (probably azygospores) with 1-8 nuclei for the Brazilian strain, and mature resting spores with 2 nuclei for both the Norwegian and the Brazilian strains (azygo- or zygospores). Our observations suggest that the immature resting spore (prespore) of both strains begins in a multinucleate condition but that the nuclear number is reduced during maturation until mature resting spore is binucleate regardless of its origin as zygospore or azygospore.

Poster / Fungi. Wednesday, 16:30. **FU-14**

Susceptibility of *Biomphalaria glabrata* egg masses to fungal infection

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Aquatic *Biomphalaria glabrata* snails from the neotropics are most common in stagnant or slow-flowing water habitats. Quantities of egg masses are laid near the water surface on submerged substrates but are often eventually exposed to desiccation and natural enemies. Almost nothing is known about fungal pathogens acting against these snails. We report on the ovicidal activity of *Metarhizium anisopliae* (IP 46) and *Beauveria bassiana* (ARSEF 9588). Freshly laid egg masses (5 masses each test of four independent repetitions) were either exposed to water and treated with 2x10⁶ conidia or hyphal bodies/ml of these fungi or treated topically (2x10⁶ conidia or hyphal bodies) and then incubated in a permanent water film in a moist chamber at 25°C. Controls were treated with water only. Egg masses were checked daily for fungal growth and eclosion of juveniles. After application of conidia or hyphal bodies, IP 46 developed distinct mycelium and new conidia on egg masses in water film, and hyphal bodies yielded no later eclosion of juveniles. No mycelium developed when ARSEF 9588 was applied to egg masses exposed in water films and all juveniles eclosed. In water, both fungi developed mycelium after application of conidia or hyphal bodies to egg masses, and juveniles failed to eclose. All juveniles eclosed from uninoculated egg masses exposed in water or film. The results suggest that both *M. anisopliae* and *B. bassiana* may act against *B. glabrata* egg masses, but that the degree of molluscicidal activity depends on the type of fungal inoculum applied.

Poster / Fungi. Wednesday, 16:30. **FU-15**

Antimicrobial, Antioxidant and Anticancer Activity of Culture Filtrates from Entomopathogenic Fungi

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Entomopathogenic fungi are natural pathogens of insects and contribute to the regulation of host insect populations in the environment. Several these fungi produce a wide range of secreted enzymes, secreted protein toxins and secondary metabolites to overcome host defenses and ultimately kill the host, and to defend host resources against competing pathogens and saprophytes. Therefore, this study was performed to select

the antimicrobial activity of entomopathogenic fungi from Korea soils against plant pathogenic bacterium *Ralstonia solanacearum* and plant pathogenic fungus *Botrytis cinerea* using dual culture technique on SDYA. In addition, we also performed to screening of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals scavenging activity compounds from liquid culture filtrates of entomopathogenic fungi and investigate to its anticancer activity. As results, 12 isolates, 6 isolates and 25 isolates showing of these fungal metabolites produced antibacterial, antifungal and radicals scavenging activity compounds, respectively. The preferential antimicrobial, radical scavenging and anticancer activities give evidence that these entomopathogenic fungal metabolites might be useful as a source for plant pathogen control and pharmaceutical interests.

Poster / Fungi. Wednesday, 16:30. **FU-16**

Evolutionary-ecological strategies of *Metarhizium robertsii*

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The species of the entomopathogenic fungus *Metarhizium* include forms characterized by different pathogenic strategies. Two strains of entomopathogenic fungus *M. robertsii* with different strategies were investigated. The strain Mak-1 («growth strategy») is characterized by slow killing speed of different insect species (Orthoptera, Coleoptera, Diptera, Lepidoptera) and abundant sporulation on cadavers. The strain P-72 («toxin strategy») is characterized by significantly rapid killing speed, but sporulation of this strain was detected only on Lepidoptera. Thus the fungi specialization can be associated with necrotrophic (but not biotrophic) phase of life cycle. In addition P-72 is characterized by the higher level of destruxin B, E production, rapid activation of conidia on the artificial media and insect's cuticle. The strain P-72 was more productive in media from plant compounds while Mak-1 - on insects and media of them. Our results show that «non-toxicogenic» strain has higher adaptation to entomoparasitic nutrition, and the «toxicogenic» strain to saprophytic nutrition. We found the change of the defense systems of Colorado potato beetle (*Leptinotarsa decemlineata*) larva (increasing of phenoloxidase in cuticle and detoxificative enzyme in fat body and hemolymph, decreased rate of cells immunity) under infection by the «toxicogenic» strain but not by strain with «growth strategy». Our data support hypothesis that evolution of entomopathogenic fungi *Metarhizium* was directed with a loss association with plants and formation of specialized entomoparasitic forms.

Poster / Fungi. Wednesday, 16:30. **FU-17**

Mycelial and conidial thermotolerance of *Metarhizium anisopliae* s.l. IP 46 and

***Metarhizium robertsii* ARSEF 2575**

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High temperature is a very important environmental stressor that may limit efficacy of fungi in arthropod biocontrol programs; however, formulation of fungal propagules is suggested for increasing performance of fungi. The current study was designed to evaluate the radial growth of colonies of *Metarhizium anisopliae* s.l. IP 46 and *M. robertsii* ARSEF 2575 on PDAY

culture medium incubated at $27\pm1^\circ\text{C}$ (optimum) or $32\pm0.5^\circ\text{C}$ (heat stress) for 15 days. Colonies diameter was measured daily, and at day 15 the conidia produced were quantified, and their viability assessed. In addition, thermotolerance of conidia prepared in different additives was investigated; accordingly, dried conidia were suspended in water solution (Tween 80, 0.01%), commercial emulsifiable or non-emulsifiable oils or carboxymethyl-cellulose gel (CMC), and exposed to $45\pm0.5^\circ\text{C}$ for 4, 6 or 8h. Germination was assessed 48h after inoculation of conidia onto PDAY plates. A significant reduced radial growth and conidial production were shown in colonies incubated at $32\pm0.5^\circ\text{C}$, but conidial viability was high (>98%) for both fungi grown under optimum or heat-stressed conditions. Viability of conidia suspended in water solution, commercial emulsifiable oils or CMC, and exposed to $45\pm0.5^\circ\text{C}$ was drastically low [0% mean relative germination (RG) at 8h exposure]. Conversely, conidia suspended in non-emulsifiable canola or mineral oil had high viability (69.3% and 71.8% RG for ARSEF 2575, and 95.0% and 80.2% RG for IP 46, respectively, both at 8h exposure). In conclusion, oil formulation minimizes the effects of high temperature to conidia of these entomopathogenic fungi, indicating that conidia applied to the field could persist longer in heat-stressed environments and that their development may occur during periods reaching optimum temperatures.

Poster / Fungi. Wednesday, 16:30. **FU-18**

Delayed germination of heat-stressed conidia of *Metarhizium anisopliae* on tick cuticle

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The current study assessed the germination of heat-stressed conidia of *Metarhizium anisopliae* IP 119 on cuticle of *Rhipicephalus sanguineus*. Aqueous conidial suspensions (Tween 80, 0.01%) of *M. anisopliae* IP119 were exposed to 0 h (non-heated control) or 4 h at $45\pm0.5^\circ\text{C}$ (heat-treated test) in a water bath, and then inoculated onto either the dorsal surface of *R. sanguineus* engorged females or onto PDAY culture medium. The samples were incubated at $27\pm1^\circ\text{C}$ and RH>80% for 0, 12, 18, 24, 36, 48 or 72 h. After each incubation time, ticks were dissected, i.e., the dorsal cuticle was removed and immersed in Calcofluor White 2% overnight, then observed by fluorescence microscopy for evaluation of conidial germination. Conidial germination on PDAY plates was assessed using a phase-contrast microscope. A minimum of 300 conidia per cuticle or plate was evaluated, and percent germination calculated. It was found that conidial germination on tick cuticle was delayed in comparison to germination on artificial culture medium, regardless the incubation time. When conidia were exposed to heat, a higher percent germination was detected on PDAY (61.5%) in comparison to the tick cuticle (13%) at 72 h after inoculation. On tick cuticle, appressoria from non-heated (control) conidia were observed 36 h after inoculation, whereas no appressoria were seen from heated conidia (test) at any incubation period after inoculation, including 72 h. In conclusion, heated conidia germinated faster when they were inoculated on PDAY than when they were applied to the tick cuticle. This result suggests that the negative effect of heat on conidial germination was greater when the conidia were applied to arthropod cuticle than would be predicted by *in vitro* (artificial medium) thermotolerance tests. In addition, the technique of fluorescence microscopy proved to be a simple method for visualizing germinated conidia and appressoria on the cuticle of *R. sanguineus*.

Poster / Fungi. Wednesday, 16:30. **FU-19**

Influence of environmental factors on insects resistance to anamorphic fungi

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We studied effect of different factors such as suboptimal temperatures, sublethal bacterial infection (*Bacillus thuringiensis*), synthetically and natural insecticides (pirimiphos-methyl, *Cordyceps militaris*) and venom of parasitoid *Habrobracon hebetor* on defense systems of wax moth *Galleria mellonella* and Colorado potato beetle *Leptinotarsa decemlineata*. Moreover insect susceptibility to fungi *Beauveria bassiana* and *Metarhismium robertsii* under these factors has been examined. We found the decreasing of phenoloxidase activity in hemolymph and cuticle, and detoxicative enzymes activity (nonspecific esterases, glutation-S-transferases) in hemolymph, as well as in encapsulation response. Thus dramatic depression in host's defense systems led to increased susceptibility of insects to fungi from ten to several thousand times. Our data support hypothesis that low specificity of anamorphic entomopathogenic fungi is closely associated with their ability to infect insects with defense system seriously suppressed by various environmental factors.

Poster / Fungi. Wednesday, 16:30. **FU-20**

Intraspecific and interspecific variation in osmotolerance of entomopathogenic fungi

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Entomopathogenic fungi must be capable of cell division under multiple stresses imposed during the various stages of the lifecycle, some of which take place on the insect surface or within the hemolymph. These include the energy-expensive synthesis and retention of compatible solutes to maintain osmotic pressure. The windows for osmotolerance of 24 isolates of entomopathogenic fungi were determined by assessing conidial germination over a range of KCl concentrations. Germination was evaluated on potato dextrose agar (PDA; control) or PDA+KCl using 31 concentrations of KCl from 100 to 3000 mM (after 24 h; 26 °C). *Trichothecium roseum* was the most osmotolerant (≤ 3000 mM KCl), followed by *Lecanicillium aphanocladii*, *Simplicillium lanosorivium*, and *Isaria fumosorosea*. Several fungal species showed moderate osmotolerance (≤ 1700 mM) including *Metarhizium robertsii* (for some isolates), *Metarhizium brunneum*, *Metarhizium anisopliae*, *Tolyphocladium inflatum*, *Tolyphocladium cylindrosporum*, and *Fusarium coccophilum*. Some isolates showed modest levels of osmotolerance (≤ 1400 mM), including one isolate of *M. robertsii*, one of *M. anisopliae*, two of *M. acridum*, and *Beauveria bassiana*. *Aschersonia aleurodis* and one isolate of *M. brunneum* were relatively intolerant to osmotic stress (≤ 1000 mM KCl). These findings indicate high levels of inter- and intraspecific variability in osmotolerance for insect-pathogenic fungi. Eighty percent of *Trichothecium roseum* conidia germinated at 2000 mM KCl (equivalent to 0.928 water activity), with a LC50 at 2300 mM, and some germination at < 0.890 water activity (on 3000 mM KCl). This suggests that *T. roseum* is highly xerotolerant and may therefore be unique amongst the entomopathogenic fungi.

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Poster / Fungi. Wednesday, 16:30. **FU-21**

Different intensities of visible light during mycelial growth induce differently the conidial tolerance to menadione in *Metarhizium robertsii*.

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The threshold of illumination during mycelial growth influenced the conidial tolerance of an entomopathogenic fungus to the oxidative agent menadione. *Metarhizium robertsii* (ARSEF 2575) was grown at 26 °C for 14 days in five treatments: 1) minimal medium (MM) in the dark; 2) potato dextrose agar (PDA) in the dark inside the Panasonic incubator; 3) PDA medium under continuous visible light in the Panasonic incubator; 4) PDA medium in the dark inside the Marconi incubator; 5) PDA medium under continuous visible light inside the Marconi incubator. For the Panasonic incubator, three intensities of light were studied with 1, 3, and 5 lumens. The germination of conidia produced under these treatments was subsequently evaluated on PDA medium supplemented with menadione at the concentrations 0.10 and 0.15 mM. For control, conidia germinated on PDA medium. The germination was evaluated counting at least 300 conidia after 24 h at 26 °C. Each treatment was repeated four times with a new batch of conidia produced for each repetition. Conidia produced on minimal medium were more tolerant to menadione, followed by conidia produced under visible light inside the Marconi incubator. Conidia produced inside the Panasonic incubator at 5 lumens were more tolerant to menadione, but less tolerant than conidia produced under light in the Marconi incubator. Conidia produced in the Panasonic incubator at 1 and 3 lumens showed somewhat increased tolerance as compared with control in the dark. Therefore, growth under visible light produced conidia more tolerant to menadione.

Poster / Fungi. Wednesday, 16:30. **FU-22**

Effect of *Metarhizium* spp. growth media on the accumulation of destruxins in a 10-L stirred tank reactor

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Entomopathogenic fungi secrete a wide range of metabolites, mostly products of secondary metabolism. These metabolites serve different functions depending on the ecological niche of the fungus. Nevertheless, the EU-approach to microbial metabolites is still under discussion and therefore, three *Metarhizium brunneum* production strains were assessed for their secondary metabolite secretion (i.e. destruxin A, B and E) in a 10-L stirred tank reactor. Biomass production on the Sabouraud-4 glucose - complete medium - and on a modified Czabek-Dox media, blended with yeast extract without peptone was tested two-times in batch-

fermentation runs. The aim was to figure out whether secondary metabolite impurities (i.e. destruxin analytes) in the technical BCA products derive from a overdosage of complex nutrient ingredients or if they are routinely formed during the BCA production process. The destruxin A, B and E accumulation considerably decreased for all three production strains by avoiding peptone as nitrogen source. Comparing the three production strains in both culture broth batch-systems it must be concluded that the strains differ in the amount of destruxin accumulation. Crude extract products are now available for the purpose of further risk assessment studies of *Metarhizium* metabolites (a.o. cytotoxicity and genotoxicity studies).

Poster / Fungi. Wednesday, 16:30. **FU-23**

Evaluation of destruxin A production in four strains of *Metarhizium* by capillary electrophoresis

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Destruxin A (dtx A) is one of the main secondary metabolites produced by *Metarhizium* strains that exhibit insecticidal activity. Due to its toxicity, and the fact that it could be a risk to humans and the environment since it is able to enter in the food chain, the interest in learning more about the detection of this metabolite has increased in recent years. In this study the production of dtx A by four different strains (BIPESCO5, EAMa 01/58-Su, ART 2825 and ARSEF 23) was evaluated. These strains were grown in four different culture mediums (CM: semi-synthetic complete medium; MM: minimal medium; OSM: osmotic stress medium; CN2: peptone in water). All analyses were carried out using a powerful separation technique named Capillary Electrophoresis with Ultraviolet detection (CE-UV). The results showed that ARSEF 23 cultivated in MM medium was the only strain which produced dtx A with a maximum concentration of 20.2 mg/L. In CM medium, BIPESCO5, ARSEF 23 and EAMa 01/58-su strains produced dtx A at different concentrations (24.4 mg/L, 9.9 mg/L and 7.8 mg/L, respectively). Under the CE conditions selected, dtxA was not detected in ART 2825 strain. No strains cultivated in either OSM or CN2 medium produced detectable amounts of dtxA. Our results indicate that the production of dtx A by strains depends on the culture medium, probably related to glucose content. Additionally, it can be confirmed that CE coupled with UV detector is a suitable tool to identify and quantify dtx A (at concentrations higher than 0.5 mg/L) in fungal culture medium.

Poster / Fungi. Wednesday, 16:30. **FU-24**

Entomopathogenic fungal genera and the 1F=1N standard: The shape of the future begins to emerge

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Recent changes to the International Code of Nomenclature for algae, fungi and plants compel changes in how pleomorphic fungal genera are named, and they disallow the retention of separate generic names for sexual and asexual morphs of the same taxa. These changes broadly affect many fungi, but

strongly affect the taxonomically complex insect pathogens of Hypocreales. Molecular data and phylogenetic reconstructions are being used to develop community-driven, consensus-based proposals for conservation and rejection of generic names for the affected fungi. These efforts seek to stabilize generic concepts around well-supported monophyletic clades while minimizing disruption to the diverse research and user communities dealing with these fungi. Inevitably, some widely studied genera will be synonymized, and their names will no longer be available except in a descriptive manner (e.g., hirsutelloid morphology rather than *Hirsutella*). Proposals for Ophiocordycipitaceae and some taxa in Clavicipitaceae (notably *Metarhizium* and closely related genera) are now available. The current draft proposal for genera of Cordycipitaceae is presented here. Despite the substantial effort involved in generating the lists presented here, real challenges in resolving some relationships remain; future studies can be expected to justify the recognition of still more segregate genera than are now listed in the proposals. The senior author will continue to update the SIP membership about relevant changes at future SIP meetings and the ARSEF collection's website (<http://www.ars.usda.gov/Main/docs.htm?docid=12125>).

Poster / Fungi. Wednesday, 16:30. **FU-25**

Genotyping of Georgian isolates of entomopathogenic fungi *Beauveria* spp.

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Our research is about genotyping different subspecies isolates of *Beauveria* collected from various regions of Georgia. *Beauveria* spp is one of the most widely spread entomopathogenic fungi in agriculture. It is a producer of toxins and biological active materials, which can cause high mortality in different species of pests. Nowadays, there is high interest towards active strains of *Beauveria*. Use of molecular biology techniques has demonstrated that *Beauveria* spp (7 isolates from different habitats and geographical zones of Georgia) unites unknown species and their determination by traditional conidial morphology is impossible. We have done phylogenetic characterization of *Beauveria Bassiana*: (I) Polymerase Chain reaction (PCR) to differentiate the clades of Georgian strains (It has never been investigated to which clades A, B or C they belong); (II) Sequencing of DNA fragments from ITS region (the rRNA gene cluster) and (the Elongation Factor 1-alpha) EF1 and (the intergenic) Bloc region. At present, we plan to identify proteins that are responsible for the virulence of *Beauveria Bassiana*. This study gives us opportunity to understand population of *Beauveria* and its future applications in effective biocontrol strategy of pathogens. Attention to biocontrol is a breath taking perspectives for sustainable development of the world.

Poster / Fungi. Wednesday, 16:30. **FU-26**

Genetic characterization, fungicide sensitivity, and aphicidal potential of *Lecanicillium* fungi from Argentina

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Mitosporic fungi of the genus *Lecanicillium* (Ascomycota; Hypocreales) are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of single-spore derived *Lecanicillium* strains isolated from a wide range of original hosts. Current species delineation within the taxonomic genus *Lecanicillium* that consists of the three "core species" *Lecanicillium lecanii*, *L. muscarium*, and *L. longisporum* as well as further less closely related species, is not free of ambiguity. For species-level characterization of *Lecanicillium* isolates, a set of five genetic markers comprising one mitochondrial (NMS) and two nuclear (ITS, IGS) ribosomal RNA operon together with one mitochondrial (*nad1*) and one nuclear (*ef1α*) protein-encoding sequences, has been employed. The aggregated information from these markers indicates that fungal isolates from Argentina mainly, but not exclusively belong to the *Lecanicillium* core species. Moreover, the set of *Lecanicillium* strains has been investigated for fungicide sensitivity. Between strain differences in susceptibilities have been found to be important and not necessarily in line with systematics, making careful determination of sensitivity to agriculturally used fungicides an important criterion of biocontrol agent selection. However, the fungicidal polyketide compound soraphen has been found of outstanding activity against a wide variety of isolates from all species investigated. On the basis of these results, a subset of strains has been selected for virulence bioassays against the green peach aphid, *Myzus persicae*, an important agricultural pest in Argentina and other parts of the world.

Poster / Fungi. Wednesday, 16:30. **FU-27**

Species-specific PCR assay to identify and discriminate *M. pingshaense*, *M. anisopliae*, *M. brunneum*, and *M. robertsii*

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Metarhizium comprises important fungal pathogens of insects and several species in the *M. anisopliae* complex are in use for biological control of insect pests. The most recent taxonomic revision of the *M. anisopliae* species complex used a multilocus phylogenetic (EF-1α, RPB1, RPB2 & β-tubulin) approach and nine species are now recognized. Accurate molecular identification of these species is possible using the 5' region of EF-1α or one of seven recently developed nuclear intergenic loci. The goal of this study was to develop a species-specific PCR assay to rapidly identify species of the "PARB" clade, which includes *M. pingshaense*, *M. anisopliae*, *M. robertsii* and *M. brunneum*, without the need to obtain full-length sequence reads. Markers included in the recent multilocus phylogeny (ITS, rIGS, EF1-α, EF1-5', RPB1, RPB2 and β-tubulin) and 5 nuclear intergenic (nuIGS) sequence markers for *Metarhizium* were screened for the presence of species-specific sequence signatures amenable for discriminatory PCR primer design. One primer pair was designed each for *M. anisopliae* (rIGS), *M. robertsii* (rIGS) and *M. pingshaense* (MzIGS2), and two primer pairs were designed for *M. brunneum* (both MzIGS2). Specificity

of the different primer pairs was tested by performing BLAST similarity searches and PCR amplifications on a collection of 65 strains representing 11 different *Metarhizium* species. The approach was further validated by identifying soil isolates collected from a Swiss meadow.

Poster / Fungi. Wednesday, 16:30. **FU-28**

Species identification of entomopathogenic fungi of the genus *Lecanicillium* (=*Verticillium lecanii* s.l.) by mitochondrial gene sequences

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For species identification of entomopathogenic fungi of the genus *Lecanicillium* (former *Verticillium lecanii* Zimm. Viegas) from collection of All-Russian Institute of Plant Protection, sequencing of mitochondrial gene nad1 was exploited. Among 39 isolates, 36 showed attribution to *Lecanicillium muscarium*, 2 – to *Lecanicillium psalliotae* and one – to *Lecanicillium longisporum*. In *Lecanicillium muscarium*, 4 nad1 molecular haplotypes were detected. Only one of them was identical to that already present in Genbank (EF512920). Two novel haplotypes were 99.3-99.7% similar to each other and to the former haplotype. Finally, the fourth haplotype was similar to the other three at the level of 97.9% sequence similarity and was represented by 14% of the isolates under study. The geographic origin and isolation source (partially reflecting the host specificity) were diverse with no consistent pattern among haplotypes. Supported by RFBR # 13-04-01905.

Poster / Fungi. Wednesday, 16:30. **FU-29**

The genomic basis for evolved resistance to *Beauveria bassiana* in *Drosophila melanogaster*

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We use an “evolve and resequence” approach to determine the genomic basis for evolved resistance to the fungal pathogen *Beauveria bassiana* in the genetic model insect, *Drosophila melanogaster*. Entomopathogenic fungi, such as *B. bassiana*, are used in biological control of mosquito vectors of dengue fever and malaria, and of various agricultural insect pests. To better understand mechanisms of insect resistance to *B. bassiana*, we artificially select *D. melanogaster* for increased resistance to this pathogen in very large, replicated experimental populations. The populations that are selected for increased resistance to *B. bassiana* have not evolved cross-resistance to bacterial pathogens, which suggests that selection may be acting on mechanisms outside of core immunity. We genotype the selected and control populations at multiple generations throughout selection to identify relevant genes and to make inferences about the temporal trajectories of adaptive alleles. We are developing novel methods for analysis of pooled sequences from such evolve and resequence datasets that will provide better assessment of technical artifacts and accurately identify regions of the genome that have responded to selection.

Poster / Fungi. Wednesday, 16:30. **FU-30-STU**

**Behavioral control of malarial mosquito by entomopathogenic fungi:
Death as the vector**

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Our previous study clarified infection of entomopathogenic fungi via the proboscis route is important on rapid mosquito death rather than infection route from tarsomere, and death of mosquito highly correlated with fungal invasion to brain. We developed a hypothesis that fungal infection via proboscis route can affect to mosquito behavior, and the aim of this study was to investigate the alteration of host searching behavior of mosquito by entomopathogenic fungi infection until the mosquito dies.

The mosquitoes were inoculated with *B. bassiana* s.l. 60-2, and quantification of the total amount of host searching behavior in a free flight system by using automated-recording device was conducted. Attractiveness of fungus infected mosquitoes and mock mosquitoes to the heat (40°C) and the color (black) were evaluated in this device for 10 days. As a result, attractiveness to the heat was drastically decreased from 3 days post inoculation, whereas attractiveness to the color has a tendency to decrease from 6 days post inoculation. This reduction of response to mosquito attractant might be caused by fungal infection to their head where has various important sense organ to search host. It will inhibit or damage to their heat and visual sensors or sensory neuron, then mosquitoes became less able to recognize host cues (death as the vector). Although conventional vector control has only focused on killing vectors, our results indicate that there need holistic evaluation as disease transmission risk on vector control using entomopathogenic fungi including lethal and sub-lethal effects.

Poster / Fungi. Wednesday, 16:30. **FU-31**

Effect of *Metarhizium brunneum* strain LRC112 and *M. anisopliae* F52 on non-target Carabid Beetles

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Carabid beetles are considered to be among the most important beneficial insects in agricultural ecosystems and are commonly associated with agricultural fields in British Columbia. Sustainable treatment to control click beetles and wireworms should have little impact on non-target carabid beetle populations. In the present study, we examined the effect of the *M. brunneum* strain LRC112 on adult *Agriotes obscurus* and on common local Carabid species. Further, we compared the effects of *M. brunneum* strain LRC112 to the commercial *M. anisopliae* F52 strain. Examined Carabid beetle species were less susceptible to the tested *Metarhizium* strains than *A. obscurus* beetles. Additional assays at multiple spore concentrations of both *Metarhizium* strains were conducted on two common Carabid species: *Pterostichus melanarius* and *Calathus fuscipes*. For both beetle species, significant mortality was observed at the highest *M. anisopliae* F52 concentration, whereas little mortality was observed at the highest *M. brunneum* LRC112 concentration.

Poster / Fungi. Wednesday, 16:30. **FU-32**

Effect of a local strain of the fungus against *Corythucha ciliata* (Say) and *Glyptodes pyloalis* (Walker) in Georgia

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The sycamore lace bug, *C. ciliata* is one of the most destructive pest of plane trees (*Platanus* spp.) all over the world. This pest is also known to be major nuisances in Georgia since plane trees has been very popular in parks and planting of the cities. The lesser mulberry pyralid, *G. pyloalis* which was spread and caused damage to *Morus alba* in recent years in Georgia is very big problem as well.

Isaria fumosorosea isolated from pupae of *Hyphantria cunea* Drury in Georgia was evaluated to determine its potential as a biological control agent of these pests. Second and third instar larvae of *G. pyloalis* were collected in Tbilisi from *Morus alba* trees and *C. ciliata* adults were collected from the bark of *Platanus* trees in Kutaisi, Georgia. A conidial suspension, concentration 10^9 conidia/ml, was used for both experiments. The suspension was applied to bark to expose *C. ciliata* adults and *M. alba* leaves to expose *G. pyloalis* larvae under laboratory conditions. Efficacy, corrected with mortality in the control treatment, was calculated according Schneider-Orelli's formula. *I. fumosorosea* showed 30% corrected efficacy against larvae of *G. pyloalis*, and 50% for *C. ciliata*. The results of this study suggest that larvae of *G. pyloalis* were tolerant to the induced mycoses caused by *I. fumosorosea*, but more effect on the mortality of *C. ciliata* adults. Experiments are needed to determine the IC_{50} of the fungus for the two pests and to develop appropriate application methods if efficacy proves to be sufficient.

Poster / Fungi. Wednesday, 16:30. **FU-33**

The effect of pesticides used in strawberry and soybean on the mite pathogenic fungus *Neozygites floridana*

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Neozygites floridana is an important natural enemy of the two-spotted spider mite, *Tetranychus urticae*. Pesticides used in strawberry and soybean that might affect the conservation and enhancement of this beneficial fungus in Integrated Pest Management (IPM) systems were therefore studied in laboratory. Eighteen pesticides were sprayed on mummified mites killed by the *N. floridana* isolate ESALQ1420 placed on coverslips with alphanumeric coded squares. The effect of these pesticides on the sporulation and capilliconidia production (germination) of *N. floridana* were determined. Recommended concentrations (RC) and half of this concentration (RC/2) were used, and the control was sprayed with distilled water plus 0.05 % Tween 80. The treated cadavers were set to sporulate for 12h in darkness at $25\pm2^\circ\text{C}$ and 100% RH. The acaricide Vertimec (Abamectin) at half dose resulted in a primary conidia production of $1283(\pm169)$ and 38%(±12) of these produced capilliconidia (germinated). RC of Folicur (Tebuconazol) resulted in a primary conidia production of $1558(\pm308)$ and 37%(±11) germination. Further, RC of the insecticide Danimen (Fenpropathrin), resulted in a primary conidia production of $1057 (\pm 201)$ and a resulting 37%(±11) germination. RC of Talcord (Permethrin) resulted in $1292 (\pm 335)$ primary

conidia and 74%(±4) germination and Karate at RC/2 in $2985(\pm 337)$ primary conidia and 83%(\pm) germination. This demonstrates that Vertimec, Folicur, Danimen, Talcord and Karate (Lambda-cyhalothrin) were the five pesticides that had the lowest impact on *N. floridana*. Products containing sulfur even in RC/2 were detrimental to *N. floridana*. Thiovit Jet (sulfur) resulted in a primary conidia production of only $162(\pm84)$ and 0%(±0) germination and no sporulation was observed from mummified mites sprayed with Kumulus (sulfur). These results are important considering that organic farmers extensively use sulfur-based products in order to control phytopathogenic fungi.

Poster / Fungi. Wednesday, 16:30. **FU-34**

Development of a granular formulation of *Metarhizium brunneum* based on mycelial fragments

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The application of the entomopathogenic fungus *Metarhizium brunneum* strain Ma43 (=BIPESCO 5 = F52) against soil dwelling pests like *Otiorrhynchus sulcatus* needs specific requirements on the product. Although the fungus can be grown on solid media the fermentation time in solid state fermenter is long and labor intensive. Additionally, problems with the application of the fermented grain are reported. Therefore, we investigated the possibility of formulating mycelial fragments. Mycelium of Ma 43 was produced in a liquid fermenter and was homogenized to get a flowable suspension. The results demonstrate that humid heat of up to 70°C reduce the viability of the mycelial fragments whereas dry heat of up to 70°C did not influence the viability. Further experiments on fluid bed drying demonstrated that mycelial fragments can be coated on millet at temperatures of 50°C . After coating the fungus was growing and sporulating on the surface of the millet under humid conditions. Protectants like lactose enhanced the viability after fluid bed drying. Further optimization steps and the practicability of mycelial fragments based formulations will be discussed.

Poster / Fungi. Wednesday, 16:30. **FU-35**

Innovative biological products for soil pest control: Outline of an EU project

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Many herbivorous insect pests have soil dwelling larval stages, which are difficult to control. These subterranean insect pests, such as the western corn rootworm, wireworms, black wine weevil, sciariids, white grubs, and tipulids, currently need to be controlled by insecticidal applications. However, in complying with EU directives, several pesticides are or will be phased out in the near future, requesting new and complementary control

strategies. INBIOSOIL explores in detail the recently discovered synergistic effects between entomopathogenic fungi (EPFs), entomopathogenic nematodes (EPNs), and semiochemicals by developing innovative co-formulations, making use of strategies derived from nature. These co-formulations will be based on capsules containing EPFs (*Metarhizium brunneum* or *Beauveria bassiana*) in combination with strains of EPNs (*Heterorhabditis bacteriophora*), or semiochemicals. Additionally, INBIOSOIL will develop integrated pest management (IPM) strategies that exploit synergies between these biocontrol agents and semiochemicals. The overall aim of the project INBIOSOIL is to optimize the use of biocontrol agents in the soil for more efficacious, low input, control of pests in farming systems of major importance in Europe. New crop protection strategies will be developed that will i) reduce pesticide inputs, ii) provide protection in non-sterile soils, eliminating for soil sterilants, iii) reduce production costs, and iv) result in the production of high-quality and safer crops in accordance with theme priority area (Integrated pest management in farming systems of major importance in Europe).

Poster / Fungi. Wednesday, 16:30. **FU-36**

Oxidative stress levels in the entomopathogenic fungus *Beauveria bassiana* growing in very long-chain hydrocarbons

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Broad host range entomopathogenic fungi attack insect hosts via attachment to insect surface, with the subsequent production of degrading enzymes that help penetration through the cuticle. The outermost insect surface is covered by a lipid-rich layer, usually composed of very long-chain hydrocarbons. It is known that *B. bassiana* is able to grow on straight chain hydrocarbons (alkanes) as a sole source of carbon and energy, but it would have to pay a high cost to do so. The aim of this work was to study the oxidative stress levels in alkane-grown *B. bassiana*. For this purpose, we analyzed the gene expression pattern of *sod1*, *sod2*, and *sod3* encoding superoxide dismutases, *catA*, *catB*, *catC*, *catD*, and *catP* encoding catalases, and *gpx* encoding glutathione peroxidase; and the enzymatic activity of SOD, CAT, and GPx in crude homogenates. Fungi grown either in hexadecane (*n*-C16) or octacosane (*n*-C28) showed overlapping but differential gene induction, with a concomitant increment in enzymatic specific activities, compared with controls grown in complete medium. These results confirm that high levels of reactive oxygen species are produced in *B. bassiana* during growth in alkanes, and an antioxidant response is triggered in fungal cells to overcome this drawback.

MICROBIAL CONTROL

Poster / Microbial Control. Wednesday, 16:30. **MC-1-STU**

Fungal strain selection and screenhouse evaluation of the virulent isolate against aphids on crucifer and okra vegetables

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Aphids are major pest problems of crucifer and okra vegetables in sub-Saharan Africa. Biopesticides are now acceptable pest control alternatives to synthetic chemical insecticides. Five isolates of *Metarhizium anisopliae* and three of *Beauveria bassiana* were screened for virulence against the following apterous adult aphids in the laboratory: *Brevicoryne brassicae* and *Lipaphis pseudobrassicae* on kale, and *Aphis gossypii* on okra. *Metarhizium anisopliae* isolates ICIPE 30, ICIPE 62 and ICIPE 69 outperformed the others causing mortality of 85-98%, 83-97%, and 73-77%, in *B. brassicae*, *L. pseudobrassicae* and *A. gossypii*, respectively, at 5 d post inoculation. However, *M. anisopliae* ICIPE 62 had the shortest LT_{50} values of 2.8, 2.1 and 1.9 d; and the lowest LC_{50} values of 5.5×10^5 , 8.1×10^4 and 1.7×10^4 conidia ml⁻¹ against *A. gossypii*, *B. brassicae* and *L. pseudobrassicae*, respectively. It also produced significantly higher conidia on cadavers compared to the other isolates, and was therefore selected for screenhouse experiments. In the screenhouse, aqueous and oil formulations of ICIPE 62 significantly reduced aphid population growth rate (r_i), *B. brassicae* -0.03 and -0.03 and *L. pseudobrassicae* -0.02 and -0.04 on kale, and *A. gossypii* -0.04 and -0.07 on okra, respectively; compared to the control (0.08 and 0.04 for *B. brassicae*, 0.01 and 0.01 for *L. pseudobrassicae*, and 0.03 and 0.01 for *A. gossypii*, respectively). These results are indicative of the potential of isolate ICIPE 62 in the management of aphids

Poster / Microbial Control. Wednesday, 16:30. **MC-2**

Virulence of fungal spores produced in liquid and solid state media on nymphs of *Trialeurodes vaporariorum*

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Quality of spores of five fungal entomopathogens, produced in liquid and on solid media, was assessed on nymphs of whitefly *Trialeurodes vaporariorum*. Isolates of *Lecanicillium attenuatum*, *L. muscarium*, *L. longisporum* and one unidentified *Isaria* sp. were first passed through larvae of *Tenebrio molitor* to enhance virulence. Three-times subcultured pure colonies were used to inoculate liquid or solid media to produce submerged and aerial spores. The liquid medium production system consisted of 250 mL Erlenmeyer flasks containing a mineral solution with a C/N ratio of 10/1 supplemented with yeast extract, placed in an orbital shaker at 180 rpm and 25°C. The solid medium production system consisted of Petri dishes containing PDA, placed in an incubator at 25°C. Spores were collected and suspensions of 1×10^6 germinable spores were prepared. Five tomato leaves, infected with *T. vaporariorum* nymphs at 2nd-3rd instars, were submerged for one minute in the spore suspensions of each isolate, and maintained in 200 mL water-agar glasses in a growth chamber during ten days. The number of dead nymphs was evaluated six and ten days after inoculation. Control treatments consisted of ten leaves infected with the whitefly nymphs and treated with sterile water. Aerial spores of the *Lecanicillium* spp. isolates caused higher mortality than submerged spores. *L. longisporum* was the least affected by the production system. Contrary to *Lecanicillium*, submerged spores of the *Isaria* isolate killed more nymphs than aerial spores six days after inoculation. The production system should be considered during the screening and evaluation of microbial control agents.

Poster / Microbial Control. Wednesday, 16:30. **MC-3-STU**

Development of entomopathogenic fungi in mosquito control: which kind of production for which efficiency?

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Mosquitoes (Diptera: Culicidae) are zoonotic vectors responsible for numerous infectious diseases of medical and veterinary importance such as filariasis, malaria and encephalitis. As part of an integrated vector control, entomopathogenic fungi could be developed as biopesticides in two ways: spores and metabolites recognized as effective virulence factors. Solid-state fermentation enhances spore production and induces the secretion of metabolites quantitatively and qualitatively different from submerged fermentation, which impairs fungal metabolic efficiency. In this context, we showed high spore productivity of solid-state media based on agro-industrial substrates as wheat bran. Spores remained pathogenic, as revealed by classical toxicity tests and electron microscopy. However, the absence of free water makes culture parameter variations difficult to control in large-scale. Recently, we performed a bioreactor design intended for simultaneous spore and metabolite production, combining the technological advantages of submerged and solid-state fermentations. Biofilm fermentation (i.e. growth of fungal biomass on an inert support immersed in a nutrient medium) is a tremendous production system favouring the secretion of insecticidal metabolites in the liquid medium as we showed recently. This is also an interesting tool to provide an overview of the complexity of the metabolic pathways involved in the regulation of extracellular metabolites secretion because corresponding genes are reported to be differentially expressed from classical fermentation systems. Researches in vector control are currently intensified. In this context, the identification of genes and metabolites specifically expressed during biofilm fermentation will help to develop new technologies related both to the design of bioreactor and the production of insecticidal proteins.

Poster / Microbial Control. Wednesday, 16:30. **MC-4**

The basis for rootstock resilient to *Capnodis* species: screening for genes encoding delta-endotoxins from *Bacillus thuringiensis*

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Conventional methods often fail to control the flatheaded borers *Capnodis* spp., major pests of stony fruit trees; the larvae are protected from insecticides and predation because they feed deep in the roots. A potential solution is transgenic trees producing in their roots toxic compounds such as Cry proteins of *Bacillus thuringiensis* (*Bt*). Toxicities against *Capnodis* larvae were demonstrated by exploiting a recently-designed artificial larval diet and an available collection of field isolated *Bt*. An isolate of *Bt tenebrionis* (*Btt*) from commercial bioinsecticide (Novodor) displayed LC₅₀ and LC₉₅ values of 3.2 and 164 mg g⁻¹ respectively against neonates of *Capnodis tenebrionis*, whereas values of the most toxic field isolate K-7

were 1.9 and 25.6 mg g⁻¹ respectively. Weights of surviving larvae after 1 month on diets containing low concentrations of K-7 (0.1 - 1.0 mg g⁻¹) were lower than on *Btt* or untreated larvae. K-7 was also toxic against larvae of *C. cariosa* and *C. miliaris* and found to harbor genes encoding Cry9Ea-like and Cry23Aa/Cry37Aa binary toxins. Larvae of *Capnodis* spp. are susceptible to *Bt* Cry toxins. Expressing cry genes active against these pests thus seems a feasible solution toward production of transgenic rootstock trees resilient to the pest

Poster / Microbial Control. Wednesday, 16:30. **MC-5**

Selection of entomopathogenic fungi for the control of *Aegorhynus nodipennis* (Coleoptera: Curculionidae) under laboratory conditions

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Plum weevil *Aegorhynus nodipennis* is one of the most important native pests of blueberry in Chile. The larvae produce severe damage to the root system, by destroying the plant crown, and causing decay and death of the plant within a few years. Adults are long-lived and feed on twigs during the day and oviposit on the crown of the plants, where they hide during the night. Strains of the insect pathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* were evaluated on adults of the Plum weevil under laboratory conditions. 45 strains of *M. anisopliae* and 50 strains of *B. bassiana*, from the Chilean collection of insect pathogenic fungi where screened. Plum weevil adults were exposed to a dose of 1×10^7 conidia / insect and mortality was assessed every day for up to 10 days. Two strains of *B. bassiana* and *M. anisopliae* were selected as the most effective on adults. The *B. bassiana* strain reached 100% mortality and the *M. anisopliae* was only 80% of control. Attributes such as high performance of the spore, stability and virulence will determine the selection of strains to be evaluated in greenhouse and field trials.

Poster / Microbial Control. Wednesday, 16:30. **MC-6**

Susceptibility of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations to *Bacillus thuringiensis* strain HD1

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The development of insect resistance to *Bacillus thuringiensis* (*Bt*) appears to involve various mechanisms and to be dependent on the type of insect, toxin, and *Bt* strain. The aim of this research was to investigate the factors affecting the susceptibility of insects to *Bt* (protein level, the midgut bacteria, and mutations in the ABCC2 gene), in five Brazilian populations (PC, PA, PX, SBT and BT) and one English population of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae). The BT population of *P. xylostella* showed a high resistance to the HD1 strain, and therefore was used in the molecular assays. Enzymatic and molecular experiments with the guts of larval populations were also conducted to investigate the factors affecting the susceptibility of insects to

Bt. We analyzed total protein, total protease, protease activity, esterase levels, intestinal bacteria, and exon characteristics. Mutations in the *ABBC2* gene may be related to resistance to Bt in various insects, as deletion of this gene occurs in a resistant strain of *P. xylostella*. The exon that has a known mutation in the (NO-QAGE) Bt-resistant population was sequenced. None of the populations showed this or any other mutations in the exon. Gut bacteria may influence the susceptibility of insects to Bt and all sequences had similarities above 99% for the *Enterococcus mundtii* 16S rRNA gene. The tests performed, both enzymatic and molecular, were inconclusive as to the factors that may influence the susceptibility of *P. xylostella* to Bt and further studies should be conducted to elucidate these factors.

Poster / Microbial Control. Wednesday, 16:30. **MC-7**

Sublethal effects of the Cry1Ac toxin of *Bacillus thuringiensis* Berliner in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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The diamondback moth (DBM), *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is a key pest of crucifers. Although can be controlled with insecticides, *P. xylostella* can quickly develop resistance to insecticides, such as those of from *Bacillus thuringiensis*. The objective of this research was to analyze the sublethal effects of *B. thuringiensis* Cry1Ac protein in five Brazilian populations of *P. xylostella* (PC, PA, PX, SBT, and BT). Bioassays examining the sublethal effects of Cry1Ac protein in DBM larvae were conducted using concentrations of 0.1, 0.25, and 0.5 µg/mL for the BT population, and 0.001, 0.005, 0.01, and 0.05 µg/mL for the PA, PX, PC and SBT populations. As a control treatment, autoclaved deionized water and 50 µg/mL Triton-X100® was used. The period of life from the third instar to pupa, pupal period, pupal weight, sex ratio, survival of from the third instar to pupal stage, survival of from the third instar to adulthood, and leaf consumption by the larvae were all evaluated for sublethal effects. Sublethal effects on the Bt population were most significant in prolonging the larval period, for approximately 2 days with a toxin concentration of 0.05 µg/mL, and the emergence of adults was 44% lower than that in the control. For the PA, PC, SBT, and PX populations, the most significant sublethal effects observed were also in prolonging the larval period and adult emergence. No influence on consumption of the larvae was observed, except with the Bt population, where the consumption was significantly lower at all tested concentrations.

Poster / Microbial Control. Wednesday, 16:30. **MC-8**

Effect of *Bacillus thuringiensis* Berliner on biological characteristics of *Orius insidiosus* Say (Hemiptera: Anthocoridae) fed with eggs of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae)

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The Diamondback moth, *Plutella xylostella* (Linnaeus, 1758) (Lepidoptera: Plutellidae), is considered the most important pest of Brassicaceae (Cruciferae) worldwide, occurring throughout the year in Brazil, where chemical control is the most widely used method, justified by the convenience, quick action and efficiency. The indiscriminate use of pesticides can affect non-target organisms, and the growing concern for the environment, the high cost of pesticides and frequent cases of resistance in populations of increased interest in the use of other control tactics as entomopathogenic organisms such as *Bacillus thuringiensis* and predators such as *Orius insidiosus*. The objective of this work is to evaluate the action of *B. thuringiensis* (Agree ®) in biological characteristics of *O. insidiosus*. The predators were fed with eggs of *P. xylostella* treated with distilled water (control) and a suspension Agree® (*B. thuringiensis* aizawai CG91), at a dosage of 0.7 g/0.5L. The nymphal period, consumption and nymphal survival rate were assessed, whereas with adults were measured consumption, the number of eggs per female and egg viability. Parameters were also determined for the construction of fertility life tables for eggs treated and not treated with *B. thuringiensis*. The parameters duration of the second instar, nymph consumption and female longevity of *O. insidiosus* are affected by the presence of Agree®, and females who consume eggs treated have the progeny decreased, resulting in lower population growth rate.

Poster / Microbial Control. Wednesday, 16:30. **MC-9-STU**

Evaluating microbial biocontrol agents: effects of *Metarhizium brunneum* on a non-target arthropod

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The objective of this study was to evaluate the virulence of *Metarhizium brunneum* on non-target arthropods. The strain Met52/BIPESCO 5/F52 is used against various pest insects throughout Europe and North America. However, research related to the ecotoxicology and side effects against non-target organisms are still needed. In this MSc study, a part of the EU supported project INBIOSOIL, we documented that *M. brunneum* had a high virulence against the model insect *Tenebrio molitor* (Coleoptera: Tenebrionidae), whereas it had a much lower virulence against the beneficial arthropod *Atheta coraria* (Coleoptera: Tachynidae), a soil dwelling predator used for macrobiological control. In addition, the virulence of *M. brunneum* was compared to that of another entomopathogenic fungus (*Beauveria bassiana*). Bioassay results showed notable efficacy of the entomopathogenic fungi against *T. molitor*, both at high and low spore concentrations (respectively 1×10^7 and 1×10^5 conidia/ml). Conversely, infection bioassays carried out on *A. coraria* showed significantly lower virulence of the fungal isolates at a high spore concentration. These data suggest that this *M. brunneum* strain does not represent a threat to the non-target arthropod *A. coraria*. Further studies are still needed to evaluate the effects of *M. brunneum* on other non-target arthropods. Nevertheless, based on the results of this study we propose that *M. brunneum* can be considered a 'low risk substance', a novel category of plant protection agents currently considered by the EU Commission.

Poster / Microbial Control. Wednesday, 16:30 **MC-10-STU**

An experimental autoinoculation device to control an invasive Asiatic pest, *Drosophila suzukii*

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Unlike most drosophilids, which typically infest overripe or decaying fruit, it has been observed that *Drosophila suzukii* (Matsumura) also oviposits eggs into the skin of immature and/or ripening fruit through the use of a serrated ovipositor. *Drosophila suzukii* is an important pest of fruit such as strawberry, cherry, blackberry, blueberry, peach, plum, nectarines and grapes. Spotted wing D. suzukii was first found in Spain in 2008. Managing this pest is a challenge, and new methods of control are being developed. In our research, the transmission potential of EAMA 01/58-Su Metarhizium brunneum strain was evaluated against *D. suzukii* adults in experiment cages, using an experimental autoinoculation device which consists in a plastic mineral water bottle with fermented food as lure, and a tissue with the fungal propagules. *D. suzukii* adults entered and exited the autoinoculation device for the 48 h of exposure and became infected with the fungus with 100.0% mortality followed by mycosis. These results show the potential of the lure and infect as a strategic option for the control of *D. suzukii* using EAMA 01/58-Su strain, with the persistence of the inoculum in the device and the time course evolution of the adult fly infection being actually investigated.

Poster / Microbial Control. Wednesday, 16:30 **MC-11**

Use of a commercial *Metarhizium anisopliae* s.l. formulation to control *Rhipicephalus microplus* ticks in pen study

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The present study evaluated the effect of the commercial product Metarril® SP Organic of *Metarhizium anisopliae* s.l. plus 10% mineral oil to control *Rhipicephalus microplus* ticks in a pen study. Three groups were formed with six animals each: the first group was exposed to Metarril® plus 10% mineral oil; the second group was exposed to sterile distilled water plus 10% mineral oil (oil control group) and the third group received no treatment (control group). Fungal formulation contained 1×10^8 conidia mL⁻¹. Each animal was sprinkled with 3L of formulation. Fallen ticks were counted daily and a sample of 20 engorged females per group was incubated for assessment of biological parameters. Throughout the study period, Metarril® oil-based formulation showed an efficiency ranging from 19.20% to 67.39% in comparison with the control group; and from 8.18% to 61.38% in comparison with the oil control group. Average efficiency of Metarril® oil-based formulation was 47.74% and 40.89% in comparison with control and oil control groups, respectively. Changes in the biological parameters of *R. microplus* females were observed in the first three days after treatment. There was statistical significant reduction in females' egg mass weight, larval hatching percent, nutritional index and egg production index. We concluded that Metarril® SP Organic plus 10% mineral oil was efficient against *R. microplus* ticks in

pen studies. Further *in vivo* studies are required in order to increase efficiency of this product aiming establish a protocol for the use of Metarril® in field conditions against the cattle tick.

Poster / Microbial Control. Wednesday, 16:30. **MC-12**

Two Colombian entomopathogenic fungi are highly efficient on *Cerotoma tingomariana*

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The Chrysomelids (Coleoptera: Chrysomelidae) are a limiting soybean pest in Colombia. These insects can affect seeds, nodules, leaves and roots, reducing the yield crop. An amount of 19 species has been registered, but *Cerotoma tingomariana* is the most important, due to its high frequency and distribution. This insect is controlled with insecticides (I - II category) and some of them are forbid in USA or Europe. The aim of this work was to select an efficient entomopathogenic fungus on *C. tingomariana*. Seven isolates of *Beauveria bassiana* (Bv) and six isolates of *Metarhizium anisopliae* (Mt) were biological testing on laboratory. In addition, this isolates were tested on different temperatures (5°C, 15°C, 25°C, 30°C and 35°C), pH values (3, 5, 7, 9) and tolerance to UVB radiation (302 nm) by measuring germination (%), radial growth and Colony Formate Unit (CFU). Mt isolates showed efficiency under 50%. Isolates Bv060 and Bv003 showed an efficiency of 100%. In the UVB radiation test, Bv060 reduced the conidia viability between 75% and 80%, and Bv003 reduced the viability between 65% and 66%. At 5 and 9 pH value, the two isolates (Bv003 and Bv060) showed germination higher than 90% and the faster rate of radial growth. Bv003 showed the best growth at 15°C and 25°C and Bv060 at 25°C and 30°C. These results suggested that Bv060 and Bv003 could be use as an active principle for a biopesticide on *C. tingomariana* control in soybean.

Poster / Microbial Control. Wednesday, 16:30. **MC-13-STU**

Biological control of pollen beetles with the entomopathogenic fungus *Beauveria bassiana*

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Pollen beetles are a main pest in oilseed rape (OSR) throughout Europe, able to cause substantial yield loss. The main damage is caused by adult beetles feeding on pollen in spring during bud stage of inflorescences. There is currently no possibility to control pollen beetles in organic OSR cultivation. In addition, increasing resistance of pollen beetles to commonly used insecticides hampers conventional OSR production and further emphasizes the need for alternative control possibilities.

The application of entomopathogenic fungi (EPF) is a promising tool in biological control of pollen beetles (Hokkanen 2008). Several Swiss isolates of the EPF *Beauveria bassiana* showed promising effects in laboratory experiments, causing up to 80% mortality seven days after application (Kuske 2011). Field treatments showed similar results regarding beetle mortality, but did not result in significantly increased yield so far. To improve their efficacy, synergies of EPF and

other natural compounds, such as stone dusts or vegetable oils, are tested. First laboratory results of combined applications of *Beauveria bassiana* spores and vegetable oil have shown a potential increase in beetle mortality due to improved fungal infection. The exploitation of synergistic effects and innovations in formulation technology should result in a better spore persistence under field conditions and a higher efficacy of the fungal treatments against pollen beetles.

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Poster / Microbial Control. Wednesday, 16:30. **MC-14**

Pathogenicity and virulence of *Beauveria* spp. against mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytidae)

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The mountain pine beetle (MPB), is a forest pest to western Canada and the United States and causes severe disturbance in lodgepole and other pine forests. We evaluate pathogenicity and virulence of number of *Beauveria* spp. including the two commercial strains of *B. bassiana*, GHA and Naturalis against adult MPB. All the 29 isolates tested in the preliminary bioassay proved to be pathogenic to MPB adults. Mean survival times (MST) of MPB adults when treated with 1×10^6 conidia/ml falls between 4.05 to 8.95 days and the commercial isolate GHA is the most virulent (MST 4.05 d), followed by isolates INRS 211 (MST 4.59 d), and INRS 236 (MST 4.82 d) based on the log rank test. Among the 3 different species tested, *B. bassiana* isolates were highly virulent followed by *B. pseudobassiana*. The *B. brongniartii* isolates used in this study were neither virulent nor supported conidia growth on the cadavers. From this initial screening, seven isolates of *B. bassiana* viz., GHA, Naturalis, INRS 211, INRS 236, INRS CFL-A, L49-1AA, and ARSEF 8150, were selected based on their virulence as well as mycosis/condiosis for further dose-wise bioassay. Based on the LC₅₀ values, the commercial isolates, GHA and Naturalis were the most virulent to MPB, however, isolates INRS 236 and INRS CFL-A were the better conidia producer. The result obtained from this study was used in selecting amendable and virulent *Beauveria* isolates to be deployed in managing MPB through classical biological approaches in a trap based auto-contamination-dissemination strategy.

Poster / Microbial Control. Wednesday, 16:30. **MC-15**

The Use of Microbial Plant Protection Agents for Insect Control in Germany

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Micro-organisms play an important role in biological plant

protection in Germany. By the Directive 2009/128/EC on the sustainable use of pesticides, biological control measures are proposed to be enforced in order to reduce the application of chemical pesticides in Europe. To obtain information about the scale of application of biological control agents in Germany, we have performed a survey on their use. Two baculoviruses are registered for tortricid control in Germany. The most important one is the *Cydia pomonella* granulovirus, which is used on about 30% of apple plantations in Germany. Three *Bacillus thuringiensis* (Bt) subspecies (kurstaki, azawai and tenebrionis) are in use and play an important role in organic farming and integrated pest management (IPM). So far, there is no entomofungal product registered as plant protection agent in Germany. However, some strains of *Beauveria bassiana*, *B. brongniartii* or *Metarhizium anisopliae* have been used for research purposes or for restricted use with a specific legal allowance. The data are presented in the Status Report Biological Plant Protection, which is published every five years by the Julius Kühn Institute and represents an indicator of the National Action Plan to monitor the use of plant protection products.

Poster / Microbial Control. Wednesday, 16:30. **MC-16-STU**

Synthesis and secretion of volatile organic compounds by *Triatoma infestans* infected with *Beauveria bassiana*

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Physically disturbed *Triatoma infestans* adults secrete volatile organic compounds (VOC) with alarm and defense function. It is still unclear whether infection with entomopathogenic fungi changes or not the profile of these volatiles. The aim of the present research was to study the effect of *B. bassiana* on secretion of VOC by *T. infestans* and to study the expression of genes potentially involved in the biosynthesis of these volatiles in triatomines infected or not. Volatiles released by *T. infestans* on different periods after treatment (1-4, 6-10, 11-15 days) were quantified and identified employing capillary gas chromatography coupled to mass spectrometry. The expression pattern of *Ti-brnq* and *Ti-bckdc* was analysed by real-time PCR, 4 and 10 days after treatment. Isobutyric acid was the most abundant VOC found (70 to 78% of the total) with no significant effect of the progress of infection on quantitative secretion of this compound. Secretion of propionic acid, however, was highest in the beginning (18.6±5.8%) and decreased distinctly with the progress of infection and at this time did not differ from values found for the control. Highest expression of both genes was found on insects 4 days after treatment. Significant difference was found in *Ti-brnq* expression, with 1.3 ± 0.5 and 3.0 ± 0.4 fold induction over the controls in insects treated with 1×10^6 and 1×10^8 con/ml, respectively. Similar results were observed for *Ti-bckdc* expression, resulting in 1.9 ± 0.3 and 2.5 ± 0.4 fold induction, respectively. The results help to understand better the impact of fungal infection on the chemical ecology of *T. infestans*.

Poster / Microbial Control. Wednesday, 16:30. **MC-17**

Preliminary studies of entomopathogenic microorganisms present in Latvian population of horse-chestnut leaf miner *Cameraria ohridella*

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The first record of horse-chestnut leaf miner *Cameraria ohridella* Deschka & Dimic (Lepidoptera: Gracillariidae) in Latvia was made in summer 2002. In recent years *C. ohridella* has spread across all territory of Latvia. The aim of the study was to acquire preliminary data on mortality factors of horse-chestnut leaf miner *C. ohridella* and identify present entomopathogenic fungi and bacteria. Since 2010 *C. ohridella* population dynamics are monitored in two sampling plots. This work provides information about causes of mortality of *C. ohridella* larvae and pupae and gives first record about bacterial and fungal microflora of collected larvae. Observed larval mortality, caused by pathogens was low (0.2-1.6%). Specimens with symptoms of infection were used for pathogen isolation. Twelve species of entomopathogenic fungi were isolated from collected dead specimens. A pilot experiment to test virulence of fungal isolates *Beauveria bassiana*; *Isaria fumosorosea* and *Metharizium anisopliae* on *C. ohridella* larvae and hibernating pupae was performed. Bacteria were isolated from insects by using standard methodology - dissecting insect and preparing homogenates. Individual bacterial isolates 16S rRNA genes were amplified and sequenced. Results showed that bacterial community is relatively simple and it's similar to composition found in other insect species described by the same methodology. Community was dominated by proteobacteria - *Pseudomonas* sp. and *Pantoea* sp.

Poster / Microbial Control. Wednesday, 16:30. **MC-18**

Toxicity of *Bacillus thuringiensis* BERLINER Cry toxins in different Brazilian *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations

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Plutella xylostella (Linnaeus, 1758) (Lepidoptera: Plutellidae), the diamondback moth (DBM), is a major insect pest of crucifers (Brassicaceae) worldwide. The most common insecticides used to control *P. xylostella* are based on the entomopathogenic bacterium *Bacillus thuringiensis* (Bacillaceae) (Bt). Although many studies have focused on the action of Bt on various agricultural pests, such as DBM, many doubts still persist, particularly regarding the toxicity of Bt proteins. We analyzed the virulence of Cry proteins in Brazilian populations of *P. xylostella*. Bioassays of susceptibility in five Brazilian populations (PC, PA, PX, SBT, and BT) of *P. xylostella* and Cry1Ac, Cry2Aa, and Cry1IE *B. thuringiensis* proteins, estimating the virulence of the toxins, were performed. Seven concentrations, ranging from 0.001 to 1.0 µg/mL, for the PA, PC, PX, and SBT populations, and 0.1 to 2.5 µg/mL for the BT population, were used to calculate the values of LC₅₀. Five replicates were performed, with each replicate being a petri dish containing 20 larvae, totaling 100 insects per concentration for each population. The Cry2Aa and Cry1IE toxins caused no mortality in larvae from any of the populations; therefore, tests were performed only with Cry1Ac. The PC, PA, PX, SBT, and BT *P. xylostella* populations exhibited different levels of susceptibility to the Cry1Ac toxin. The PA, PC, and SBT populations showed LC₅₀ values of 0.02, 0.04, and 0.04 µg/mL. The LC₅₀ estimate for the BT population was 0.78 µg/mL, while that for PX it was

0.01 µg/mL. The LC₅₀ estimated for the BT population was 78 fold greater than that for the PX population.

Poster / Microbial Control. Wednesday, 16:30. **MC-19**

***Bacillus thuringiensis* isolation from Brazilian soil samples: molecular characterization and biological activity against *Plutella xylostella* (Lepidoptera: Plutellidae)**

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Plutella xylostella L. (Lepidoptera: Plutellidae) is one the most important pests of crucifer worldwide, and farmers usually control this pest with pesticides that favors the population resistance development, depletion of natural enemies and environmental pollution. Studies on biological control agents such as the entomopathogenic bacterium *Bacillus thuringiensis* must be carried out aiming to minimize or even replace the pesticides in the field. This research was carried out to isolate *B. thuringiensis* from 40 soil samples, to characterize them by Polymerase Chain Reaction (PCR) and mortality bioassays were performed to verify the *B. thuringiensis* biological activity of each isolate against 100 *P. xylostella* second instar larvae. 50 *B. thuringiensis* isolates were obtained from soil samples. No isolate amplified genes cry1Ab, cry1Ac, cry1Ea, cry1Eb, cry1Fa, cry1Fb, cry2Aa, cry2Ab, cry2Ac, cry9A, vip1, cyt2B and cyt2Ba but isolates named LCMA04, LCMA05 and LCMA29 amplified gene vip2 and the isolates LCMA06, LCMA20, LCMA45 and LCMA46 amplified gene cry1C and vip2. These isolates were pathogenic to *P. xylostella* second instar larvae but the mortality range from 38,0% to 55,5%. This mortality is too low to consider these isolates as promising ones to *P. xylostella* management. This isolation is ongoing to find isolates with high virulence to *P. xylostella*.

Poster / Microbial Control. Wednesday, 16:30. **MC-20-STU**

Effect of endophytic *Beauveria bassiana* on herbivore defence in *Arabidopsis thaliana*

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The entomopathogenic fungus *Beauveria bassiana* can live as an endophyte by colonizing plant tissues without causing disease symptoms. Recent studies in different crop plants indicated that endophyte presence can have a negative effect on herbivorous insects. However, whether this was due to induced plant defence responses has not been reported. We established *Arabidopsis thaliana* as a model plant to find out whether *B. bassiana* colonization increases herbivore resistance by activating/priming the jasmonic acid (JA) or salicylic acid (SA) defence pathways. Three *B. bassiana* strains were applied as conidial suspension to *Arabidopsis* using root dipping. Colonization was assessed through plating on selective medium and through PCR based detection using *B. bassiana* specific SCAR markers. The endophyte was recovered from leaves and inflorescence confirming systemic colonization throughout the plant. Bioassays were carried out to test the effect of endophyte presence on caterpillars of *Plutella xylostella* and the aphid *Myzus persicae*. Endophyte presence did not have any antagonistic effects on the growth of *P. xylostella* and the fecundity of *M. persicae*. The re-

isolated fungus caused 100% mortality when applied topically on caterpillars. This correlated with the finding that JA levels were only induced by caterpillar feeding but were not influenced by the presence of the fungus. No effect by either treatment was found on endogenous SA levels. In conclusion, our results do not confirm that endophytic *B. bassiana* induces plant defences against the selected herbivore species. Further studies are planned to assess the plant's transcriptomic response to the presence of this endophytic entomopathogen.

Poster / Microbial Control. Wednesday, 16:30. **MC-21-STU**

Pathogenicity of Beauveria and Metarhizium to the two stink bug species *Nezara viridula* and *Piezodorus guildinii* (Hemiptera: Pentatomidae) in laboratory and semi-field

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The stink bug species *Nezara viridula* and *Piezodorus guildinii* are troublesome pests of common bean (*Phaseolus vulgaris*). The aim of this study was therefore to determine the pathogenicity of two *Beauveria* isolates (Bb-1 and Bb-18) and two *Metarhizium* isolates (Ma-11 and Ma-30) from Cuba to these two stink bug species. Each fungal strain was tested in the laboratory against adults of the two stink bug species. Further, in a pilot semi-field experiment the two stink bug species inoculated on bean plants with pulses in cages were sprayed with the same four fungal strains. In the laboratory experiment Ma-30 and Ma-11 caused 100% mortality in both stink bug species. The *Beauveria* strains resulted in a lower mortality, however, and Bb-1 caused 85% mortality in both stink bug species, while Bb-18 caused 85 % mortality in *P. guildinii* and 95% mortality in *N. viridula*. In the semi-field experiment the Ma-30 strain caused the highest mortality and 73% of the *N. viridula* was killed by this fungus while only 68% of the *P. guildinii* was killed. The Ma-11 strain caused 65 % mortality in *N. viridula* and *P. guildinii* while Bb-18 and Bb-1 caused 41% and 54% mortality respectively in both stink bug species.

Poster / Microbial Control. Wednesday, 16:30. **MC-22-STU**

Evidence for synergies between Heterorhabditis bacteriophora (Nematoda: Heterorhabditidae) and Metarhizium brunneum (Hypocreales: Clavicipitaceae) in western corn rootworm control

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The western corn rootworm (WCR), *Diabrotica v. virgifera* LeConte (Coleoptera: Chrysomelidae), is one of the most deleterious pests of maize worldwide, and is commonly controlled by chemical insecticides. Currently neonicotinoid-dressed maize seeds are banned in the European Union which highlights the importance of intensified research into suitable alternative control strategies. Field trials using a blend of entomopathogens in conjunction with chemical insecticides were carried out to determine the effect on survival and development of the WCR as well as on grain yield. The entomopathogens included the nematode species

Heterorhabditis bacteriophora Poinar (Heterorhabditidae) and the fungus *Metarhizium brunneum* Petch (Clavicipitaceae). The agents were applied in two naturally heavily WCR-infested maize fields in the province of Styria, Austria, in 2013. Neither the abundance of larvae nor the number of adults showed significant differences between the treatments. However, when both *H. Bacteriophora* and *M. brunneum* were used in combination with untreated seeds, the grain yield was almost equivalently high compared to treatments using neonicotinoid-dressed seeds. The two entomopathogens possibly interact synergistically and could provide a powerful alternative strategy to chemical insecticides for the larval control of *D. v. virgifera*. Nonetheless, a repetition and extension of the trials in 2014 is essential to further evaluate the efficacy of the different agents for WCR control.

Poster / Microbial Control. Wednesday, 16:30. **MC-23**

Evaluation of the effectiveness of the entomopathogens for the management of wireworms (Coleoptera: Elateridae) on spring wheat

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Wireworms, the larval stage of Elaterid beetles are serious soil dwelling pests of small grain, corn, sugar beet and potato crops. *Limonius californicus* (Mannerheim) and *Hypnoidus bicolor* (Eschscholtz) are the predominant wireworm species infesting wheat in Montana, particularly in north-central Montana. Currently available insecticides provide only partial control, and no alternative management tools exist. At two field locations (Ledger and Conrad, MT) in 2013, the fungi, *Metarhizium brunneum* F52, *Beauveria bassiana* GHA, and *Metarhizium robertsii* DWR 346, were evaluated in seed coat, in-furrow granular and soil drench applications, in addition to imidacloprid seed treatment, which is currently being used by growers. Wireworm damage in various treatments was evaluated as standing plant counts, wireworm population survey, and grain yield production. The three fungi applied as formulated granules or as soil drenches, resulted in significantly higher plant stand counts and yields at both locations, than fungus-coated seed treatments and the untreated control. Significant difference was detected among the application methods instead of species of the fungi. All three fungi applied as granules in furrow and in soil drench were paramount to seed-coating treatments in wireworm control, and provided an efficacy comparable or superior to imidacloprid. The fungi used in the current study provided significant plant and yield protection under moderate wireworm pressure, indicating their potential utility in the integrate management of this pest.

Poster / Microbial Control. Wednesday, 16:30. **MC-24-STU**

Using the combination of entomopathogenic fungi and extracts improves control of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Both the virulence and the insecticidal activity of the crude extracts of 26 isolates of the mitosporic ascomycete entomopathogenic fungi *Metarhizium* sp. and *Beauveria* sp. (Ascomycota, Hypocreales) were determined against the second-instar *S. littoralis* larvae (Boisduval) (Lepidoptera, Noctuidae), which is considered a very harmful polyphagous insect pest. All isolates were pathogenic for second instar *S. littoralis* larvae by immersion on fungal suspension, but only four isolates of *Beauveria* (EABb 01/33-Su, EABb 01/88-Su, EABb 01/103-Su, and 3155) and one isolate of *Metarhizium* caused more than 50% mortality of larvae. EABb 01/33-Su and EABb 01/88-Su isolates caused the higher mortalities with 78.33% and 75.00%, respectively, and their average survival time (AST) values were 9.67 and 8.73 days, respectively. The LD₅₀ and LT₅₀ values were 5.69x10⁶ conidia ml⁻¹ and 6.76 days for EABb 01/33-Su and 1.05x10⁷ conidia ml⁻¹ and 7.02 days for EABb 01/88-Su. On the other hand, the crude extracts obtained from the isolates EAMB 09/01-Su and EAMA 01/58-Su caused the highest mortality rates, 80.00 and 66.66%, and the lowest AST values, 5.13 and 4.43 days, respectively. Topical application of the crude extracts did not cause any mortality. Combined treatments of fungal suspensions of isolates EAMB 09/01-Su and EAMA 01/58-Su and their extracts caused higher mortality rates than the single ones, in a dose-dependent manner, with mortality rates reaching 100% for EAMB 09/01-Su isolate and its extract at 1 mg ml⁻¹ and 76% mortality for EAMA 01/58-Su, and its extract at 1 mg ml⁻¹. The combination of the fungus EAMB 09/01-Su at 10⁸ conidia.ml⁻¹ and the crude extracts had a synergistic effect on larvae resulting in 100 % mortality to concentrations 1 mg protein ml⁻¹ and the combination of the fungus EAMB 01/33-Su + extracts EAMB 09/01-Su to concentration of 10⁷ and 10⁸ conidia ml⁻¹ to 1 mg protein ml⁻¹ crude extract also had a synergistic effect on larvae resulting in 93.33 and 100% mortality. The AST ranged between 4.08 and 5.77 days at 10⁸ conidia.ml⁻¹. These results show the potential of using the combination of entomopathogenic fungi with crude extracts for an integrated *S. littoralis* management strategy targeting larvae.

Poster / Microbial Control. Wednesday, 16:30. **MC-25-STU**

Wireworm control with fungus colonized barley kernels in cover-crops

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Insecticide treatments to control wireworms in cover-crops have been a successful strategy to prevent wireworm damage in sensitive crops planted in the following season. One example was the application of Fipronil as a seed treatment of summer oat preceding potatoes. This way, the wireworm population was reduced below the damage threshold already before planting of potatoes.

We tested a similar strategy in a semi-field pot experiment, replacing the insecticide with *Metarhizium brunneum* ART2825, formulated as fungus colonized barley kernels (FCBKs). Pots were treated with four different doses of FCBKs in August 2013 during sowing of summer oat. In addition, pots were artificially infested with *Agriotes obscurus* larvae. In April 2014, potatoes were planted into these pots. Establishment of the fungus in the pots was evaluated by counting colony forming units per g of substrate. Numbers of recaptured wireworms and the percentages of wireworms dying from mycosis were used to estimate efficacy of the treatments. Finally, effect on yield will be as estimated by counting wireworm holes on harvested potatoes.

Preliminary results are promising: The fungus successfully

established in the substrate after a few weeks and up to 70% of wireworms were killed by the treatments, depending on FCBK doses used for application. Results suggest that treating cover-crops with *Metarhizium*-inoculated FCBKs may be a useful tool for biological control of wireworms in potatoes.

Poster / Microbial Control. Wednesday, 16:30. **MC-26**

A resource efficient method to test non target effects of new biocontrol agents in vitro

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As part of the EU supported project INBIOSOIL we developed a protocol to test the non-target effects of microbial biocontrol agents and their formulations. For this purpose we selected four beneficial - predatory arthropods that are widespread and naturally occurring in Europe and are all also commercially available for biological pest control: *Aphidoletes aphidimyza* (Insecta, Diptera, Cecidomyiidae), *Athet a coraria* (Insecta, Coleoptera, Staphylinidae), *Orius majusculus* (Insecta, Hemiptera, Anthocoridae) and *Geolaelaps aculeifer* (Acari, Mesostigmata, Laelapidae). These arthropods have different life cycles, prey, and most important, they inhabit different strata of the plant and soil in the field. The protocol allows a quick assessment of the potential side effects of microbiological biocontrol agents and their formulation components on these representatives of beneficial arthropods – and therefore should be considered standard tests to be done before further resource and time demanding testing in the field.

Poster / Microbial Control. Wednesday, 16:30. **MC-27**

Ultrastructure of midgut of *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) after consumption of prey with the *Bacillus thuringiensis* strain HD1

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The interaction of Cry toxins from *Bacillus thuringiensis* in the midgut of some insect larvae determines their efficacies as insecticides, due to the expression and availability of the sites of action of the toxins in the midgut. Research has highlighted cases of resistance to Cry toxins due to alterations in the binding sites in columnar cell membranes. We analyzed the effects of spraying a *B. thuringiensis* var. *kurstaki* (HD1 Strain) suspension at a concentration 3 x 10⁸ spores/mL, onto leaves that were then offered to the larvae of *Plutella xylostella* (L., 1758) (Lepidoptera: Plutellidae) and subsequently offered as prey to the predator *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae). We examined the ultrastructure of the midgut of predators. *P. nigrispinus* adults, 3 h after consuming prey with the HD1 strain were used for dissection and extraction of the midgut. The villi present in the midgut of the predator were observed in both cross section and as longitudinal sections. At the apex of the intestinal cells, the microvilli were seen. Also visible were remarkable muscle fibers in the lumen of the intestine; these fibers are perceptible only in the anterior and middle intestine, suggesting that they move when moving food into the large intestine during

digestion. The results showed that there were no adverse effects on the predator when the larvae of *P. xylostella* had previously ingested the HD1 strain of *B. thuringiensis*.

Poster / Microbial Control. Wednesday, 16:30. **MC-28**

Control of sugarcane borer, *Diatraea saccharalis*, with formulations of *Beauveria bassiana* and *Metarhizium anisopliae*

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The control of sugarcane borer (*Diatraea saccharalis*), the most important pest of this crop, with entomopathogenic fungi has already been reported in Brazil. However, have been used the pure conidia, which can decrease the efficiency of control due to environmental factors such as temperature and level of ultraviolet radiation. The objective of this study was to evaluate, in laboratory, encapsulated formulations containing *Beauveria bassiana* and *Metarhizium anisopliae*, against this pest. It was used pure conidia of the isolates IBCB 66 (*B. bassiana*) and IBCB 425 (*M. anisopliae*) and the formulation in sodium alginate. The fungi, were applied in two ways, powdered and sprayed, at the concentration 6×10^8 conidia, and the formulation was applied directly in two concentrations 6×10^8 and 1×10^9 . The caterpillars were evaluated at the 7° and 14° day after the application. The jars with insects were kept in air-conditioned room at $25.0^\circ\text{C} \pm 2.0^\circ\text{C}$ and relative humidity around 70%. The bioassay was done with 30 caterpillars per treatment and 5 repetitions. To pure conidia of *B. bassiana*, in the 14° day, the mortality of caterpillars was 96% in sprayed application, while in powdered 87%. In the formulation, the mortality was 57% at the concentration of 6×10^8 and 77% at 1×10^9 . As for the *M. anisopliae*, the mortality of caterpillars in the 14° day, in the sprayed treatment was 47%, and in the powdered 27%, while the mortality in the formulations were 4% at the concentration of 6×10^8 and 24% at a concentration of 1×10^9 .

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Poster / Microbial Control. Wednesday, 16:30. **MC-29-STU**

Identification and functional analysis of two ABCC family genes in *Helicoverpa armigera*

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Bt toxins are widely used for insect control and resistance to *Bt* toxin is a problem that has been presented in recent years. Midgut receptors have been reported as binding proteins for *Bt* toxins and play important roles in toxicity. Recently, mutations in the ABCC2 transporter were reported to take key roles in *Bt* resistance of several species of insects. In this study, we cloned two ABCC genes from *Helicoverpa armigera*, and sequence analysis showed that these genes were quite homologous to ABCC2 and ABCC3 genes from other lepidopteran insects, so were named HaABCC2 and HaABCC3 respectively. Tissue specific expression and instar specific expression analysis showed that the two ABCC genes were mainly expressed in midgut and later instar larvae. RNAi was

done to silence these ABCC genes by feeding dsRNA to *H. armigera*. Bioassays showed that silencing of HaABCC2 in *H. armigera* larvae resulted in increased survival and pupation rates with normal eclosion rate on Cry1Ac toxin-incorporation diet, while silencing of HaABCC3 had no effect. Our research proved that ABCC2 play important role in Cry1Ac toxin pathological mechanism in *H. armigera*.

MICROSPORIDIA

Poster / Microsporidia. Wednesday, 16:30. **MI-1**

Decline of native bumblebees (*Bombus*) and *Nosema* (*Microsporidia: Nosematidae*) infections associated with introduction of the European bumblebee in Northern Japan

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The European bumblebee, *Bombus terrestris* (L.), has been widely established throughout a broad range of Hokkaido, northern Japan since its introduction for pollinating agricultural products in 1991 and has been suggested to cause the decline of native bumblebee species. Recent invasions of *B. terrestris* into the eastern Hokkaido have been reported in 2007. The Notsuke Peninsula is covered with the species-rich maritime grassland that extends along the coast. This region is also one of the restricted distribution ranges of a rare native species, with a highly diverse bumblebee species. Given the features of the geographic region and the species involved, the invasion of *B. terrestris* into the Notsuke Peninsula is assumed to have devastating influence on native bumblebees. Here, we conducted a multi-year survey of bumblebee species to examine the population dynamics of introduced and native bumblebees. We also investigated the prevalence of *Nosema* spp. which may play an important role in the declines of native bumblebee, as well as genetic variation of the *N. bombi* rRNA ITS region for comparison with the European and North American isolates.

Poster / Microsporidia. Wednesday, 16:30. **MI-2**

Development and application of a loop-mediated isothermal amplification method for rapid detection of *Nosema ceranae*

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Loop-mediated isothermal amplification (LAMP), a novel nucleic acid amplification method, was developed for the rapid detection of the major honey bee microsporidia disease, *Nosema ceranae*. The LAMP method amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions using a set of four specially designed primers and a DNA polymerase with strand displacement activity. In this study we designed primers for LAMP assays to detect *N. ceranae* protein coding gene for DNA dependent RNA polymerase II largest subunit (RPB1) and methionine aminopeptidase type 2 (MetAP2), and evaluated the specificity and sensitivity of these assays. The detection limits for both assays was ~200

pg/μl and DNA amplification was completed within 60 min at an optimal temperature of 63°C. The assays detected 6 different geographical isolates of *N. ceranae*, and no cross-reaction was observed with other microsporidia species. The performance of LAMP and PCR was comparable: 100% specific, 100% sensitive, 100% positive predictive value (PPV), and 100% negative predictive value (NPV). In conclusion, the LAMP assay was equally specific but with a shorter detection time when compared to PCR in the identification of *N. ceranae*. The LAMP assay is an easy-to-use method and a promising alternative to conventional PCR for the rapid, cost-effective for specific identification of *N. ceranae* and other microsporidia species. LAMP is considered an appropriate technology that could be used in resource-limited laboratories and the field.

Poster / Microsporidia. Wednesday, 16:30. MI-3

Permanent level of pathogens within ten bark beetles generations

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During the ten generations of spruce bark beetle *Ips typographus* population densities were monitored for 5-10 trap trees at several study sites in the Czech Republic in 2008-2012. On every of the four debarked section number of entry holes of spruce bark beetle were counted and then converted to density per unit area to the size of the studied sections (length about 0.5 m and about half trunk circumference). During the analysis in the field paternal beetles were collected and then stored refrigerated at -5°C. Total of 3,388 *I. typographus* beetles were dissected and checked for the presence of pathogens. In total four pathogenic organisms were detected: intestinal nematodes in 14.8%, microsporidia *Chytridiopsis typographi* in 9.1%, eugregarine *Gregarina typographi* in 0.3% and larvae of endoparasitoids in 4.9% of studied beetles. Relationship between the infection levels of pathogens and population growth of bark beetles from year to year according to the formula for calculating the rate of growth: R = logN_t - logN_{t-1} was studied. Our research has proven that intestinal nematodes, *Ch. typographi* or *G. typographi*, did not influence the population growth of spruce bark beetle at the studied sites and are not as strong and lethal factor during the spruce bark beetle gradation. In contrast, the coefficient of population growth and the rate of beetle infested by endoparasitoids in the population is positively correlated ($y=4.72+10.38*x$; $r=0.68$; $p<0.01$; $r^2=0.47$). Parasitoids are thus able to respond very effectively to increase of the host population.

Poster / Microsporidia. Wednesday, 16:30. MI-4

Microsporidia in beet webworm *Loxostege sticticalis* (Pyraloidea: Crambidae): a survey of 2013

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Local populations of beet webworm in South Western Russia (populations "Slobodka", adults sampled through May to August, "Chertkovskiy" and "Neklinovskiy", larvae sampled in May 2013) and Western Siberia (population "Karasuk", sampled in June and July 2013) were examined for the presence of microsporidia. In South Western Russia, microsporidia were found only in population "Neklinovskiy".

There were three distinct microsporidian species, as proved by SSU rRNA gene sequences: *Tubulinosema* of *loxostegi* (35% prevalence rate), *Nosema* of *granulosis* (4%) and *Nosema ceranae* (3%). The identity of the latter species, a widespread pathogen of honey bees, was established using partial gene sequences of SSU-ITS-LSU and IGS rRNA. Its detection in a lepidopteran host implies a wider host range than though earlier and is logically explained by relatedness of *N. ceranae* to species of *Vairimorpha* which eagerly attack lepidopteran hosts and their hymenopteran parasitoids. In Western Siberia, the same isolate of *Tubulinosema* of *loxostegi* was detected at the prevalence rates of 3% and 30% in June and July, respectively. All three species of microsporidia were able to infect beet webworm larvae in lab assays. For *Tubulinosema* cf. *loxostegi* vertical transmission to infected beet webworm progeny, experimental infection of *Galleria mellonella* and natural infection of tachina fly parasites (Diptera: Tachinidae) emerged from the microsporidia-infected beet webworm population were also confirmed.

Supported by RFBR grants 13-04-00284 and 14-04-91176 and RF President grant MK-1175.2013.4.

Poster / Microsporidia. Wednesday, 16:30. MI-5

Microsporidia from larvae of different lepidopteran species in Bulgaria

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Thirty-five lepidopteran species in 12 families were investigated for the presence of microsporidia in Bulgaria from April 2009 to June 2012. Infections caused by microsporidia in the genera *Nosema* and *Endoreticulatus* were identified in *Tortrix viridana*, *Operophtera brumata*, *Archips xylosteana*, *Orthosia cerasi*, *Orthosia cruda* and *Eilema complana*. The prevalence of *Nosema* spp. was low in host species: 0.3% for *T. viridana*, 2.1% for *O. brumata*, 2.4% for *O. cerasi*, 2.7% for *Archips xylosteana* and 3.3% for *O. cruda*, respectively. Spores of *Endoreticulatus* sp. were observed in 13.5% of collected *E. complana*. The spores of *Nosema* in *O. brumata* were localized in host fat body and phylogenetic studies showed that this microsporidium is relatively distantly related to *Nosema wistmansi*, and the genera *Orthosomella* and *Cystosporogenes*. It is, however, closely related to *Nosema thomsoni*. *Nosema* sp. found in *Orthosia cruda* was detected in the silk glands of host larvae. Phylogenetic analysis confirmed that the microsporidium observed in the gut epithelium of *E. complana* belongs to the genus *Endoreticulatus*; however, it is not identical to other *Endoreticulatus* spp. described from Lepidoptera.

Poster / Microsporidia. Wednesday, 16:30. MI-6

Ultrastructural characterization of a new microsporidium (Opisthokonta: Chytridiopsida) from the pigeon feather mite *Falculifer rostratus* (Astigmata: Pterolichoidea)

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Only about 20 species of microsporidia have been described from mites. All except one species produce typical spores with a long polar filament and a polaroplast. We present the first

study of an atypical microsporidium infection in a feather mite (*Falculifer rostratus*). The infection is restricted to the *colon epithelium* where it leads to hypertrophy of the concerned cells. During sporogony multinucleate plasmodial aggregates are formed within a sporont. The sporonts are in direct contact to the host cell cytoplasm. Merogonial stages were not present. Spores are tiny (3.6 x 2.6 μm), broad ovoid in form and monokaryotic. The spore wall of mature spores has a thickness of about 240 nm and consists of a three-layered endospore and a thin, electron-dense exospore. The polar filament is anisofilar and arranged in 3–4 coils. In cross-sections it has a star-like appearance since the electron-dense core forms rounded compartments for lucent material at its surface. In grazing sections this results in a honeycomb-like pattern. A polaroplast is missing. The life cycle features and atypical spore structures clearly classify the species from the feather mite as a member of the order Chytridiopsida. Its affiliation to one of the known genera is discussed.

Poster / Microsporidia. Wednesday, 16:30. **MI-7**

Infectivity of a *Theholohania* like microsporidian isolated from *Phthonandria atrilineata* to the silkworm,

Bombyx mori

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The pebrine of the silkworm, *Bombyx mori*, is a disease caused by infection with the microsporidium *Nosema bombycis*, also can be caused by cross-contamination of microsporidium from wild insects. We have isolated a *Theholohania* like microsporidian (TMPA) from the *phthomndria atrilineata* in the silkworm rearing region of Zhejiang province, China. The mature spores of TMPA were cylindrical or ovocylindrical in shape with a strong dioptr and glossy surface. The spore size of TMPA was $3.27 \pm 0.14 \times 2.03 \pm 0.16 \mu\text{m}$ with a length/width ratio of 1.61 ± 0.11 μm , similar to those of *N. bombycis*. Therefore, the spores of TMPA were hardly distinguished from the spores of *N. bombycis* under light microscope. In TMPA spores formative stages, sporont produced pansporoblast including 8 nuclears by meiosis, and later 8 spores were formed in pansporoblast. Infection was systemic with mature spores produced in muscular tissue, epithelial cell of trachea, fat body, middle and posterior silkgland, fore and middle intestine, malpighian tubule and germ gland, most extensivest in muscular tissue and epithelial cell of trachea, but not in dermal cells, nerve cells, fore silkgland, posterior intestine and hemocyte cells. The IC_{50} value of TMPA to newly-hatched silkworm larvae was 1.55×10^4 spores/ml, 700-fold higher than that of *N. bombycis*, suggesting a weakly infectiousness. TMPA have transovarian transmissibility in silkworm, the rate of transovarian transmission was 1.74%, which was significant lower than that of *N. bombycis*.

NEMATODES

Poster / Nematodes. Wednesday, 16:30. **NE-1**

First release of the mermitiid *Strelkovimermis spiculatus* in *Culex pipiens* mosquito populations in Argentina

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Mermitids have proved to be effective in parasitizing natural populations of mosquito larvae. However nothing is known about the inoculative introduction of this nematode in natural populations of culicids in our country. We report the results of the first field release of *S. spiculatus* in Argentine. Study area was constituted by house drainage ditches, breeding site of the mosquito *Culex pipiens* where this nematode was not present. The number and stage of mosquitoes were recorded pretreatment. *Strelkovimermis spiculatus* was introduced as second-stage juveniles (J2) obtained from laboratory cultures maintained at CEPAVE laboratory. Release was done in November 2012 (spring). A dose of 10,000 J2 per meter was applied (over a total area of 17 x 0.5 m). The number of J2 was based on previous results. Mosquito larvae were sampled 24 hs post-treatment once a week during a year, to corroborate the presence of nematode by microscopic dissection and emergence from fourth instars larvae. Parasitism by *S. spiculatus* began to be observed at third day post-application (3%). Values ranged between 0.01% and 86.3%. The highest value was recorded at 8 months post-release. This environment remained dry or without larvae during a period of four months. Nevertheless a parasitism of 45.2% was observed after this period during the first larvae collection and reaching levels between 4.8% and 86.3%. Only in three occasions was not observed infected larvae throughout the year of sampling. *Strelkovimermis spiculatus* was able to establish itself in this habitat and cause high levels of infection in *Culex pipiens* larvae.

Poster / Nematodes. Wednesday, 16:30. **NE-2**

Increased infectivity in *Steinerinema websteri* IJ after development in desiccation-stressed hosts

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This study investigates the effect of desiccation during development on entomopathogenic nematode (EPN) infectivity. *Galleria mellonella* hosts infected with *Steinerinema websteri* A10 were allowed to air-desiccate in an environmental chamber set at 23°C for up to 31 days post-infection (DPI) resulting in a host weight loss of approximately 64%. Host carcasses were re-hydrated using reverse-osmosis (RO) water and placed in White traps to collect emergent infective juvenile populations (IJ). IJ were pooled over a three-day time period for time points on days 10, 17, 24, and 31 DPI, respectively. For a randomly chosen sample of 100 IJ for each time point, sine wave movement (number of oscillatory motions completed in one minute) and IJ morphometrics, were measured. To evaluate IJ efficacy, plexiglass "bulls-eye" traps with screens dividing sections into quadrants of specific radii were loaded using sterile soil. Twenty hosts were placed in each quadrant in the outer ring only. A dose of 10,000 IJ from each time point was placed in the center ring. Host mortality was measured over 132 hour time period. Results demonstrated that IJ collected from desiccation-stressed hosts at days 17 and 24 post-infection were significantly smaller while exhibiting greater oscillation compared with controls ($\alpha \leq 0.5$). Furthermore, efficacy experiments using bulls-eye traps demonstrated that the same desiccation-stress IJ populations killed approximately 70% of hosts between 60–72 hours post load as compared 30% mortality between 72–84 hours post load for controls. This study has implications for host delivery systems in field applications.

Poster / Nematodes. Wednesday, 16:30. **NE-4-STU**

Characterization of symbiotic bacteria *Photorhabdus luminescens* subsp. *laumondii* associated with *Heterorhabditis bacteriophora* isolated from Turkey

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The symbiotic bacteria of a novel entomopathogenic nematode *Heterorhabditis bacteriophora* isolate 48-02 was identified as *Photorhabdus luminescens* subsp. *laumondii*. This bacterial isolate did not exhibit typical signs of infection, e.g., red pigment and a gummy consistency in the host was lacking. *P. luminescens laumondii* strain 48-02 was more virulent in percentage mortality and time-to-kill compared with the molecularly similar *P. luminescens laumondii* TT01 strain. In specificity tests, *P. luminescens laumondii* strain 48-02 colonized in *H. bacteriophora* TT01 infective juvenile nematodes but the bacterial symbiont of TT01 did not colonized in *H. bacteriophora* 48-02 infective juveniles.

Poster / Nematodes. Wednesday, 16:30. **NE-5**

Pathogenicity of nematobacterial complexes and its development

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Entomopathogenic nematodes and their associated bacteria comprise together highly pathogenic complex able to invade and kill insect host within two days. Both bacteria and nematodes produce variety of factors interacting with insect immune system that help to overcome host defences. These factors are specific for each of nematobacterial complexes leading to the differences in their pathogenicity. Moreover, we observed difference in pathogenicity also between two isolates of one nematobacterial complex, *Steinerinema carpocapsae* – *Xenorhabdus nematophila*. Ability to invade and kill insect host is low in newly emerged nematodes and develops through the time reaching its maximum after three weeks in complex *Heterorhabditis bacteriophora* – *Photorhabdus luminescens*. Differences in pathogenicity were observed also among particular generations of nematodes released from insect cadaver. Nematodes collected at the beginning of emergence were less pathogenic than subsequent collections. From third week of collection further we did not detect any other significant changes in nematobacterial pathogenicity, which is then influenced only by the survival of nematodes. Data describing development of infectivity and pathogenicity of *Heterorhabditis bacteriophora* – *Photorhabdus luminescens* complex will be used to increase efficiency and reproducibility of experimental infections used to describe immune response of insect to the nematobacterial complexes.

Our research was supported by the project KONTAKT II LH14047 and program CZ.1.07/2.3.00/30.009 co-financed from European Social Fund and the state budget of the Czech Republic.

Poster / Nematodes. Wednesday, 16:30. **NE-6**

Use of entomopathogenic nematodes to control vine weevils on Chilean berry orchards

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Vine weevils (Coleoptera: Curculionidae) are the most challenging pest in Chilean berry crops; they produce severe damage in the root system, decrease fruit yield and the longevity of the orchard. Besides, most of those species are quarantine pests, making obligatory their control to avoid fruit rejections in foreign markets. The control is difficult because larvae are deep into the soil or dwelling the main roots, avoiding pesticides or cultural practices. Entomopathogenic nematodes (EPN) are the most effective alternative to control these insects, because of their ability to search the larvae in the soil and even inside the dwellings. The Chilean collection of EPN (102 isolates) has been screened against the most important vine weevil affecting berries: Fuller's rose weevil *Asynonychus cervinus*, Grapevine weevil *Naupactus xanthographus*, Black vine weevil *Otiorynchus sulcatus*, Plum weevil *Aegorhinus nodipennis* and Raspberry weevil *Aegorhinus superciliosus*. The most effective NEPs have been isolates of *Steinerinema australe*, *S. feltiae* and *S. unicornum*. Average control is about 70% for these pests, measured through adult emergencies. Mass rearing has been accomplished by *in vivo* production in larvae of *Galleria mellonella* and *in vitro* through liquid media, with yields of 30-35,000 dauer/ml. NEP have been formulated in granules, gels and clays and storage up to 6 months, with 78, 80 and 72% of survival and those dauers remain actives against the target insects. Field evaluations shows that NEP are an effective alternative for vine weevil control.

Poster / Nematodes. Wednesday, 16:30. **NE-7**

Nematodes of large larch bark beetle *Ips cembrae* (Coleoptera: Scolytinae)

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Nematodes of pest large larch bark beetle *Ips cembrae* were studied on three localities. Infestation by phoretic nematodes as well as infestation by endoparasitic nematodes in haemocel and intestinum was recorded. Phoretic nematodes were found under elytra, on wings or between body segments, especially between thorax and abdomen. It was the case of genus *Micoletzkya*. In haemocel adult females and juveniles of *Contortylenchus* sp., *Parasylenchus* sp. and members of *Cryptaphelenchus* sp. were found. While in intestine the juveniles of *Parasitophoraditis* sp. and some tylenchid juveniles were found too. The large larch bark beetle gallery content was examined and adults of *Parasitophoraditis*, *Micoletzkya*, *Cryptaphelenchus*, *Bursaphelenchus* and *Laimaphelenchus* genera and some tylenchid juveniles were found.

This study was supported by Internal Grant Agency B0118/004 of Czech University of Life Sciences Prague.

Poster / Nematodes. Wednesday, 16:30. **NE-8**

Natural Occurrence of Entomopathogenic Nematodes (Steinerinemidae and Heterorhabditidae) in the Aydin district of Turkey

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Entomopathogenic nematodes (EPNs) in the families Steinernematidae and Heterorhabditidae are lethal parasites of insects and used for biological control of soil insect pests. Because of favorable climatic conditions, the Aydin district is an important agricultural area which produces several exported valuable crops including strawberries (mostly in grown greenhouses), peaches, citrus, chestnuts, cherries and vegetables. Each product has specific or non-specific pests in the area and farmers have difficulties to overcome some of these pests with insecticides. The objective of this study was to determine the natural occurrence of entomopathogenic nematodes in the Aydin district of Turkey. A total 83 soil samples were collected between 2011-2012 to determine the diversity and distribution of EPNs. Nematodes were isolated using the insect baiting technique. Ten EPN isolates were recovered from 83 soil samples (8.3% positive). According to morphometric and molecular analyses (28S rDNA and ITS) six of the isolates were identified as *Heterorhabditis bacteriophora* Poinar, two isolates were *Steinernema feltiae* Filipjev and one isolate was *S. weiseri* Mracek, Sturhan & Reid.

Poster / Nematodes. Wednesday, 16:30. **NE-9**

Detection of dsRNA virus-like molecules in entomopathogenic nematodes

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Viruses have been largely viewed as pathogens; nonetheless, even if most of the studied viruses are detrimental to their hosts, some of them have been reported to be also beneficial or symptomless. They are ubiquitous, and have been described infecting almost all types of organisms, from other viruses and bacteria, to animals, plants, fungi, or even protzoa. Surprisingly, little is known about viruses which naturally infect nematodes, even if they are among the most abundant animals on Earth. Nevertheless, RNA viruses infecting *Caenorhabditis* species and the soybean cyst nematode have been recently detected thanks to next generation sequencing (NGS) technologies.

Many viruses associated with persistent and symptomless infections are known to have dsRNA genomes. The presence of dsRNA molecules of sizes ranging from 1 to 14 kbp have been used as indicator of virus infection in plants and fungi. This nucleic acid can represent genomes of dsRNA viruses, as well as replicative forms of viruses with ssRNA genomes. According to this, the main objective of this work was the discovery of new viruses among a collection of entomopathogenic nematodes by using dsRNA virus-like molecules detection, which constitutes a cheaper and faster technic if comparing with NGS technologies. At the present time a total of 27 strains belonging to 12 different nematode species were analyzed. Two dsRNA virus-like molecules of approximately 2.4 and 2.3 kbp were detected infecting one of the analyzed species, *Steinernema huense*. These molecules could correspond to the genome of the first identified virus infecting an entomopathogenic nematode.

Poster / Nematodes. Wednesday, 16:30. **NE-10**

Cellular and humoral interactions between the white grub, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes

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The interaction between the white grub larvae, *Polyphylla adspersa* Motschulsky (Col., Melolonthidae) and entomopathogenic nematodes (EPN), *Heterorhabditis bacteriophora* and *Steinernema glaseri* was addressed here. Differential Hemocyte Count (DHC) for the both second and third instar larvae of the white grub, granulocytes (65.25± %2.22) and plasmacytocytes (22.14± %1.14) were most abundant cell types in the circulating hemolymph. Study on hemocyte and humoral reactions of the white grub larvae against the EPNs was performed by injection 20 monoxenic infective juveniles (IJs) of *S. glaseri* and *H. bacteriophora* into the insect hemocoel. The hemocel of the larvae at different hours post injection (hpi) was dissected and showed changes in total hemocyte count (THC), DHC and cell shape. Encapsulation was a typical cellular reaction, which its maximum rate was observed by 8 hpi of *H. bacteriophora* and 12 hpi of *S. glaseri*. The encapsulation reaction in third instar larvae was observed stronger than those of the second instar larvae. Also the encapsulation reaction against the *H. bacteriophora* had significant different with those against *S. glaseri*. In contrast to second instar larvae, third larval stage had higher specific phenoxidase activity when challenged with both EPNs species. It was showed the defense system could create initial melanization at 18 hpi of *S. glaseri* and 12 hpi of *H. bacteriophora*. However, EPNs probably reduced the hemocyte number in circulating hemolymph by their symbiotic bacteria. This occurrence which followed by reduce in THC level decreased the cellular and humoral intensity response of the larvae. Therefore, the immune system of the grub was suppressed by the EPNs while this system was activated in early stage of infection. This study showed weak immunity response of the white grub larvae of *P. adspersa* against EPNs, *S. glaseri* and *H. bacteriophora*. This finding could be helpful for the pest management by select the suitable EPN species in term of virulence and ability to suppress the insect defense system.

Poster / Nematodes. Wednesday, 16:30. **NE-11**

***Oscheius rugaoensis*, new genus and species of insect parasitic nematodes from Iran**

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During 2013, a survey was carried out to determine the pathogens of *Polyphylla adspersa* (Col; Scarabaeidae) in the Mashhad region, North East of Iran. All larval instars of *P. adspersa* with sign of nematode infection were collected and death larvae were transferred to the White trap. By using this method fifteen nematode isolates were isolated. Among the pathogenic agents, entomopathogenic and parasite nematodes had a moderate frequency. The initial identification of the collected nematodes carried out using morphometric data. Subsequently, molecular identification and phylogenetic analysis were performed using DNA sequences of ITS and 18SrDNA genes. The molecular data indicated that wg10 and wg19 isolates belong to *Oscheius* genus with 99% bootstraps support. Also, Nblast analysis introduced two isolates wg10 and wg19 as *O. rugaoensis*. The sequence of 18S gene O.

rugaolensis differed with wg10 and wg19 in 8 and 1 nucleotides, respectively. While on the basis of ITS sequences, 7 nucleotides were differed. The phylogenetic relationship was analysed based on bayesian procedure. In the reconstructed phylogenetic tree, wg10 and wg19 isolates were placed together with *O. rugaoensis* in a clade by 100% bootstraps support. The phylogenetic results from both genes, ITS and 18S, were similar. This is the first report of *Oscheius* genus for Iran. Despite the free living behavior of this species, it had high virulence on some insect species and higher ability to reproduce on the cadavers of *Galleria melonella* rather than healthy larvae. Future studies may provide more data about ability of this species as biocontrol agent.

Poster / Nematodes. Wednesday, 16:30. **NE-12**

Reproduction status of *Tribolium castaneum* affects its response to infection by *Steinerinema feltiae*
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Gender specific reproductive roles are a reason of sexual dimorphism not only in a body size but also in a whole range of physiological traits. We investigated differences between sexes as well as reproduction status (virgin vs. reproducing) of the red flour beetle, *Tribolium castaneum* in defence against infection by the nematode, *Steinerinema feltiae*. Females and males of the beetles either virgin or after copulation were exposed individually to the nematodes. The beetles during infection were kept without food. From each group 20 individuals were sampled after 12, 24, 36 and 48 hours. Ten individuals of each sample were dissected and checked for the presence of the nematodes, ten were frozen for further phenoloxidase activity measurements.

Reproduction strongly affected the response of females – they mortality and parasite load was the highest among all studied group. This group had also the lowest phenoloxidase activity. At the same time, we did not observed differences between virgin beetles as well as between virgin and reproducing males. Surprisingly, eggs production itself did not increase females vulnerability to parasite – we observed eggs also in the body cavity of virgin females. Probably production of unfertilized eggs is less expensive than fertilized ones. The highest parasite load we found just after infection and after 48 hours. Last outcome can be explained by starvation of the beetles so they were weakened and the nematodes more easily infected them. Our results confirm that cost of reproduction may impair defence mechanism and immunological system of *T. castaneum* females..

Poster / Nematodes. Wednesday, 16:30. **NE-13**

Effect of culture type, container type, and temperature on a Korean strain of the entomopathogenic nematode, *Steinerinema carpocapsae*

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A Korean isolate of the entomopathogenic nematode (EPN) *Steinerinema carpocapsae* KCTC 0981BP strain (ScK) is

effective for control of many Korean agricultural and forestry pests. In vitro culture is available for large scale of mass production of commercial EPN, but it is a costly and complicated process, whereas in vivo culture using great wax moth *Galleria mellonella* larvae is simpler for small scale production. However, culture type and storage temperature during in vivo culture may influence harvesting and survival of EPN. We investigated effects of those factors on harvest, survival, and pathogenicity of ScK. Storage period, culture method, and storage container and temperature all influenced ScK survival. ScK survived better in small cultures rather than in mass culture, and better in Zip-lock containers than in tissue culture container. The best storage temperatures for ScK were 13 and 20°C in small scale culture while there were no differences among temperatures in mass culture. The highest yields of ScK were obtained by rearing them in small cultures and keeping them in Zip-lock containers at 20 or 13°C. The pathogenicity of ScK differed among treatment combinations on the 1st day after inoculation, but there were no differences on the 3rd day. The number of established nematodes differed depending on storage temperature and period.

Poster / Nematodes. Wednesday, 16:30. **NE-14**

Steinerinema feltiae* (Nematoda: Steinernematidae) to control fungus gnat, *Bradysia mabiusi* (Diptera: Sciaridae): effect of dosage and application time

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Fungus gnat, *Bradysia* spp., is a pest of worldwide importance for nursery plants. The larvae feed on the roots of emerging plants, becoming potential target to use of entomopathogenic nematodes. This study aimed to determine the best time for the application of *Steinerinema feltiae* after the exposition of the substrate (inside the pot) to the insect adults. Sixteen treatments were considered: the nematode applied at three doses 3, 14 and 70 IJs/cm² (173, 883 and 4417 IJs/pot, respectively), applied soon after the infestation of the substrate with adults, as well as by 7 days, 14 and 21 days after, plus the respective controls. For each treatment, four replications were considered, with each replication composed by a plastic pot (200 ml) containing 50 g of substrate (10% humidity) and 3 grains of black bean (pre-cooked) gathered on the substrate surface, on the center of the pot, for larval feeding. The pots were transferred to inside of a large cage containing the insect rearing and exposed to the adult population for 2 hours to allow insect oviposition. Then, the pots were transferred individually to inside of other chambers (1 liter) containing a double yellow plastic sheets (8.0cm x 8.0cm) covered with insect glue for attracting and capture the emerging adults. The best time for application of the nematode was 3 weeks after the exposition of the substrate to adults, providing 61, 69 and 78% control for the doses of 3, 14 and 70 IJs/cm².

Poster / Nematodes. Wednesday, 16:30. **NE-15**

The non-sterilizing strain of *Deladenus siricidicola* (Tylenchida: Neotylenchidae) and its development on different strains of *Amylostereum* (Basidiomycota: Russulales)

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The nematode *Deladenus siricidicola* Kamona, which sterilizes *Sirex noctilio* females, has been extensively and successfully used as a biological control agent for this woodwasp in the Southern Hemisphere. Curiously, a non-sterilizing (NS) strain of *D. siricidicola* is commonly found in North America and it is thought that the NS strain was introduced with *S. noctilio* when *S. noctilio* was introduced to North America. Finding an appropriate biological control agent in North America has been challenging due to the existence of native species of *Sirex* woodwasps that are not considered pests but are part of the decomposer community in forests. Therefore, evaluation of biological control agents requires studies of host specificity of the nematodes. For this experiment, we evaluated the NS strain of *D. siricidicola*, which is poorly understood and is a potential competitor of *D. siricidicola* Kamona. *D. siricidicola* has two forms: a form that parasitizes *S. noctilio* and a mycophagous form that feeds on the fungal symbiont of *S. noctilio*, *Amylostereum*. The goal of this study was to investigate associations between the NS nematodes and different isolates of the symbiotic fungus, mainly to evaluate the ability of the nematodes to develop and reproduce on different isolates of *Amylostereum* associated with *Sirex* in North America.

Poster / Nematodes. Wednesday, 16:30. **NE-16**

Use of entomopathogenic nematodes in the biological control of gypsy moth *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae)

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The gypsy moth is one of the major insect pests, commonly distributed in west Georgia. Insect damages mainly foliage trees and spread easily from forest vegetation to fruit orchards. The aim of the research was to study efficacy of two species *S. carpocapsae* and local *S. thesami* against larvae of the gypsy moth in field conduction. Nematodes were reared produced in vivo, *Galleria mellonella* larvae. (Temperature=23°C and hygrometry=88-92%). Experiments against larvae of the gypsy moth were carried out in June, in the area adjacent to the deciduous forests of the Tbilisi National Park. Small, young crab-apple and wild pear trees were chosen for experiment. The average number of pest specimens on 1 m² branch of the each experimental plant was 74.3±4; 58.6±5; 85.2±6 and 78.3±5 on the control plant. About 30 liters of nematode suspension was used to treatment of experimental trees. One part of plants was treated with *S. carpocapsae* suspension 1500 IJs/ml of water, and the second part with the same dose of *S. thesami*. Experiments on the same pests were performed with increased concentration - 3000 IJs/ml of water.

The calculation of the insect mortality in field conduction was carried out on the 7th day after treatment. The larval mortality rate was 77.5% - 63.3% where low concentration of nematodes was used. In the case of double concentration mortality was 88.6 and 76.3% respectively.

On the basis of the results obtained it can be noted that *S. carpocapsae* proved to be more efficient (10-12%) compared with the local species *S. thesami*.

Poster / Nematodes. Wednesday, 16:30. **NE-17**

The susceptibility of Colorado potato beetle *Leptinotarsa decemlineata*, and mulberry moth *Glypodes pyloalis* to entomopathogenic nematodes, *Steinerinema carpocapsae* and *Steinerinema feltiae* in Georgia

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Colorado potato beetle, *Leptinotarsa decemlineata* and mulberry moth, *Glypodes pyloalis* are the major pest insects of vegetable and urban horticulture crops in Georgia. The aim of this study was to determine the efficacy of entomopathogenic nematodes *Steinerinema carpocapsae* and *Steinerinema feltiae* against *L. decemlineata* and *G. pyloalis* larvae under laboratory and field conditions. In the laboratory, *S. carpocapsae* and *S. feltiae* caused 92% and 62% larval mortality on *L. decemlineata*, respectively. *S. carpocapsae* also caused high mortality (74%) than *S. feltiae* (52%) in the field study. For *G. pyloalis*, *S. carpocapsae* induced greater larval mortality (82 and 72%) than *S. feltiae* (65 and 61%) under the laboratory and field conditions, respectively. In conclusion, *S. carpocapsae* exhibited significantly greater efficacy than *S. feltiae* against both insect species. The results suggest that *S. carpocapsae* has a great biological control potential against *L. decemlineata* and *G. pyloalis* larvae in Georgia. However, the efficacy of *S. carpocapsae* should be tested in large-scale field studies.

Poster / Nematodes. Wednesday, 16:30. **NE-18**

Co-infection interactions between entomopathogenic fungi and *Steinerinema feltiae* using *Tenebrio molitor* as a model system

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Prior studies have been conducted investigating additive, synergistic, or antagonistic interactions between multiple types of biocontrol agents when co-infecting an insect host. Fewer studies have focused on combining entomopathogenic nematodes (EPNs) and entomopathogenic fungus (EF) to control weevils and scarab grubs. None of these studies have investigated interactions between *Steinerinema feltiae* and EF. The present study investigates co-infection interactions between commercially produced *S. feltiae* and two isolates of EF, using *Tenebrio molitor* (Coleoptera) as a model host system. *T. molitor* larvae were infected with either *Beauveria* or *Metarhizium* isolated from naturally infected insects collected in strawberry fields in Denmark. At different intervals following EF infection, larvae were exposed to *S. feltiae*. The impact of fungal infection on the nematode was measured by counting the number of infective juveniles that penetrated the host in comparison to the number of infective juveniles that penetrated control larvae with no prior EF exposure. Daily mortality was recorded, and cadavers from nematode treatments were monitored for mycosis and placed on white traps in order to compare the total number of *S. feltiae* offspring produced in the presence of fungal infection. We discuss the use of *T. molitor* as a model system and the extrapolation of these results for the control of strawberry blossom weevil, *Anthonomus rubi*.

Poster / Nematodes. Wednesday, 16:30. **NE-19**

Some observation on morphology and ecology of mollusc-parasitic nematode *Alloionema appendiculatum*

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Alloionema appendiculatum is a common larval parasite of many terrestrial molluscs. Its 3rd stage larvae (dauer juveniles) invade foot muscle of snails and slugs. Dauer juveniles

develop in to the 4th stage larvae, that leaves slugs. Later they mature and reproduce in the soil. Despite the fact this nematode is a parasite of snails in heliciculture and also an invasive slug *Arion vulgaris* (syn. *A. lusitanicus*), that is one of the most serious pest in agriculture and horticulture, the knowledge about morphology and ecology of this nematode are very poor. We performed some studies of this nematode with a goal to provide new information about morphology, phylogeny and ecology of this species. This work brings, above all, the complete redescription of *A. appendiculatum*, include molecular biological characterisation suggesting high intraspecific variably in ITS region. Results of ecological studies provided new information about the saprobic life cycle and natural prevalence, but also show that, in standard conditions, *A. appendiculatum* has very weak influence on mortality and feeding activity of slugs *A. vulgaris*, while in other stressful conditions it might be an important agent controlling population density. But we concede that this can be also strongly influenced by bacterial associates, even though the role of bacteria in nematode development is questionable.

Poster / Nematodes. Wednesday, 16:30. **NE-20**

Osmotic stress tolerance and infective juvenile production of entomopathogenic nematodes subject to fast host-desiccation treatments

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Entomopathogenic nematodes (EPN) are being used commercially in several countries for the control of soil dwelling pests. However, their effectiveness is affected by environmental stresses such as low soil moisture. An alternate method for ensuring nematode's survival and infectivity is to apply them in the cadavers of *Galleria mellonella* used to reproduce them. It has been reported that the IJ's emerging from cadavers have increased infectivity and higher tolerance to low soil moisture and high temperatures. To determine the optimum time post infection and intensity of desiccation for higher IJ's production and their effects on osmotic stress tolerance in these EPN a laboratory experiment was carried out. Our results showed that timing to start desiccation (2, 4 and 6 days post-infection) and intensity (1, 2 and 4 days in a desiccator) affected weight reduction, especially in *S. glaseri*, which resulted in higher death rates of the IJ's. The total number of nematodes, however, was not related to the opportunity or intensity of the stress treatments, but to nematode species and initial weight of the hosts. In an evaluation of survivorship in a 30 % PEG-8000 solution, pre-conditioned *Heterorhabditis bacteriophora* showed a significantly higher tolerance to osmotic stress than *Steinernema glaseri* and showed an increase in tolerance 100 % larger than the observed with the last nematode species. The higher percent of survivorship was obtained with IJ's from hosts where desiccation treatments initiated 2 days post-infection in both EPN.

Poster / Nematodes. Wednesday, 16:30. **NE-21**

Assessing entomopathogenic nematode population genetics: a research and teaching approach

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While entomopathogenic nematodes (EPN) are important components of ecosystems, relatively little is known about the genetics of individual EPN populations in natural settings. We are combining an attempt to answer the question "How related are EPN found in natural settings?" with an integration of EPN into an undergraduate Genetics course module on population genetics. We used Random Amplified Polymorphic DNA (RAPD) approaches, and are working with lab maintained geographic isolates of EPN to identify appropriate primers and develop methodology. We have tested our technique by first assessing the genetic variability of a single geographic isolate of a single EPN species, and then exposed waxworms to a combination of geographic isolates of that species. We then assessed the genetic variability of the IJs that emerged from "mixed-isolate" waxworms. RAPD has been effective at identifying markers for individual geographic isolates, and for assessing the population genetics from "mixed-isolate" populations. RAPD is also a standard technique taught in Genetics labs, meaning that a high throughput of samples is possible and that undergraduates are exposed to real-world questions in the classroom. Once this technique has been fully developed for laboratory isolates, we plan to move this research effort into the local ('natural') environment, where we will answer the original question regarding the population genetics of local EPN isolates pre- and post-infection. This may improve our understanding of how natural populations are structured, and hopefully will provide insight that is relevant to the use of these organisms for biological control.

VIRUSES

Poster / Viruses. Wednesday, 16:30. **VI-1**

High-level Expression of Foreign Protein Using the Partial Polyhedrin-fused Baculovirus Expression System

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Polyhedrin is the major component of the nuclear viral occlusions produced during replication of the baculovirus *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV). To enhance the production efficiency of foreign protein in baculovirus expression system, the effects of various polyhedrin fragments were investigated by fusion expressing them with the enhanced green fluorescent protein (EGFP). Recombinant viruses were generated to express EGFP fused with polyhedrin fragments based on the previously reported minimal region for self-assembly and the KRKK nuclear localization signal (NLS). The marked increase of EGFP production by these fusion expressions was confirmed through protein and fluorescence intensity analyses. Among the fusion-expressed protein in nucleus and cytoplasm, the most hyper-expression was observed in the fusion of amino acids 19 to 110 and 32 to 59 of polyhedrin. The marked increase of production of several other foreign proteins was proved by the fusion expression with these polyhedrin fragments. This study suggests a new option for higher expression of useful foreign recombinant protein by fusion expression with the partial polyhedrin in baculovirus.

Poster / Viruses. Wednesday, 16:30. VI-2

A natural recombinant between *S. frugiperda* MNPV and *S. litura* NPV

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A Colombian SfMNPV isolate (SfCOL) and its genotypic variant (SfCOL-A) have shown great potential as active ingredients for a biopesticide product to control the fall armyworm *S. frugiperda* in Colombia. The complete genomic sequence of SfCOL-A was determined and analyzed in the present study, consisting of 134,239 bp, encoding 144 putative open reading frames. Gene synteny maps showed great colinearity with genomes of other sequenced SFMNPVs. SfCOL-A genome displayed a ~1470 bp deletion localized within the main variable region among SFMNPV geographical isolates and their genotypic variants previously described. Interestingly, a ~2970 bp sequence block insertion, carrying two ORFs which lacked any similarity with previously described SfMNPV genes, was also found in this region. The highest identity values and codon usage similarity within the inserted sequence suggested the idea of a recombination event between SplitNPV-II (or a similar virus) and a wild type Colombian SfMNPV. Two bioinformatics approaches (relative similarity and bootscanning analysis) were used to explore the recombination hypothesis. Both analyses supported the hypothesis, showing a recombination event involving the C_{term} region of the chitinase ORF and the N_{term} region of the gp37 ORF. This event resulted in the deletion of a genomic region including the Homologous Region 3 (HR3) and the Sf23 ORF; and an insertion of ~2970 bp carrying the split020 and split021 ORFs from SplitNPV-II. Breakpoints seemed to be localized within the frames of chitinase (near position 21,471) and gp37 (near position 24,443) genes in SfCOL-A, restoring the integrity of both frames. These results suggested a natural recombination between heterologous baculoviruses involving genes that encode non-essential proteins and affect the viral phenotype.

Poster / Viruses. Wednesday, 16:30. VI-3

Host specificity and PIFs based phylogeny of Betabaculovirus isolates from Gelechiidae family

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Tecia solanivora, *Phthorimaea operculella* and *Tuta absoluta* are insect species belonging to the potato tuber moth complex (Lepidoptera: Gelechiidae) and are considered the main pests of potato and tomato crops. In this sense, baculovirus constitutes a useful tool for the biological control of these insects. In the viral cycle, a protein complex known as PIF (Per Os Infectivity Factors) are responsible for the virus entry into the mid gut cells determining the host range. In the present work the heterologous host infection of three viral isolates (recovered from moths of each insect species *T. solanivora*, *P. operculella* and *T. absoluta*) was determined by oral inoculation of each specie larvae with occlusion bodies (OBs). Infection by each of the three

Granuloviruses in the three different host species produced a fatal disease. Additionally, comparative sequence analysis of pif genes was assessed. Seven pairs of degenerated primers were designed to amplify sequences of *p74*, *pif1*, *pif2*, *pif3*, *odv-e28*, *odv-e56* and *pif6*. The PCR products were cloned and sequenced. Comparative analysis of *pif* sequences of three isolates revealed high similarity with *Phthorimaea operculella* granulovirus (PhopGV) previously reported in the Genbank. The topology of phylogenetic tree using concatenated deduced aminoacid sequence of seven PIFs was consistent with previously published trees for Baculoviruses using three conserved genes or complete genomes. The three isolates evaluated were grouped with PhopGV. These results suggest a potential use of Granuloviruses isolated from different species of Gelechiidae family for biological control in heterologous species and showed the utility of *pif* (core genes) for phylogenetic studies in Baculoviruses.

Poster / Viruses. Wednesday, 16:30. VI-4

Diagnosing the unknown – advancing the taxonomy of aquatic invertebrate viruses

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Few viruses from marine invertebrates, in particular crustaceans, have been assigned to a virus family with certainty because biochemical, biophysical and immunological data are incomplete or lacking, essentially due to the lack of crustacean cell cultures. Particularly, we have very limited information on viral infections in non-commercial crustacean species or in other invertebrates that may be living in the same environment. Crustacean viruses have so far been tentatively assigned to families based upon morphological and developmental characteristics and the location within the cell. The need to complete full characterisations and harmonise the naming of new viruses using International Committee on Taxonomy of Viruses (ICTV) guidelines is evident throughout the literature with many different names or abbreviations being used to describe the same virus. We present the identification of a virus infection from wild caught *Crangon crangon* (brown shrimp), the optimisation of viral purification techniques from these samples, and the application of next generation sequencing to characterise the viral genome. Similarities between crustacean viruses and those described in other invertebrates including insects may assist in classification of this novel virus. The data obtained will be also be used to develop a diagnostic tool.

Poster / Viruses. Wednesday, 16:30. VI-5

Proteomic analysis of the occluded *Tipula oleracea* nudivirus (ToNV)

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The *Nudiviridae* family has been recently established by the International Committee on Taxonomy of Viruses (ICTV). Although six fully sequenced genomes are now available in databases and protein profile from nudivirus particles were mainly characterized by polyacrylamide gel electrophoresis, only few direct matches have been published between genomic and proteomic data to the exception of the major occlusion body protein (mOBp) from *Penaeus monodon* nudivirus (PmNV) and four nucleocapsid proteins from *Helicoverpa zea* nudivirus 2 (HzNV-2). Function of nudiviral predicted proteins is still inferred from what is known from their baculovirus sister-group and the occluded nature of virions remains incidental to the *Nudiviridae* family. *Tipula oleracea* nudivirus (ToNV) is one of the causative agents of crane fly nucleopolyhedrosis. The dsDNA virus genome was recently sequenced. Phylogenetic analysis revealed ToNV is related to Betanudivirus clade representatives and distantly related to another Diptera-infecting nudivirus representative, the *Drosophila innubia* nudivirus (DiNV). Electronic microscopes showed occlusion bodies are irregularly shaped and measure from 2 to 5 µm in length and 2 µm in mid-diameter. They are filled with rode-shape virions containing a single nucleocapsid within a tri-layered envelope. Quantitative proteomic analysis using on-line nanoflow liquid chromatography tandem mass spectrometry (nanoLC-MS/MS) revealed ToNV occlusion bodies are composed of 47 viral proteins, of which the most abundant are the functional homolog of baculovirus major occlusion bodies proteins and the homologs to two HzNV-2 predicted ORFs corresponding to virion structural proteins.

Poster / Viruses. Wednesday, 16:30. VI-7

Regulation and activation of two effector caspases that affect Sindbis virus replication in *Aedes aegypti* mosquitoes

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The yellow fever mosquito (*Aedes aegypti*) proteins CASPS18 and CASPS19 are closely related effector caspases that are hypothesized to be involved in midgut escape of Sindbis virus (SINV). Silencing CASPS18 and 19 expression results in decreased SINV titer in *A. aegypti* following oral infection, while overexpression of CASPS19 by recombinant SINV in *A. aegypti* causes increased virus replication. Furthermore, levels of the midgut basal lamina proteins collagen IV and laminin are increased in infected mosquitoes when CAPS18/19 are silenced, and decreased by overexpression of CASPS19, consistent with a role in midgut escape. CASPS18 lacks a typical caspase active site motif (QACRG) and has no enzymatic activity, but is able to directly enhance the activity of CASPS19. To investigate the mechanism of enhancement, we examined whether the two proteins interact and found that CASPS18 co-immunoprecipitated with CASPS19 when expressed in Sf9 cells. Under these conditions, both CASPS18 and 19 underwent a proteolytic processing event that released the small subunit. The intact CASPS19 catalytic site was required for processing of both proteins. Recombinant purified CASPS18 enhanced the activity of purified active CASPS19 in vitro, but was not able to induce the activation of unprocessed CASPS19, indicating that the enhancement of CASPS19 activity by CASPS18 occurs after CASPS19 activation. Recombinant CASPS19, alone or with CASPS18, could not directly cleave collagen IV or laminin, suggesting that the effect of these caspases on the levels of midgut basal lamina proteins is not through direct cleavage, but may instead be an indirect effect.

Poster / Viruses. Wednesday, 16:30. VI-6

Nucleopolyhedrovirus and Microsporidia in Winter Moth (*Operophtera brumata*, L.) and Bruce Spanworm (*O. bruceata*, Hurst) populations in the Northeast US

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The winter moth (WM, *Operophtera brumata*, L.), a polyphagous geometrid affecting mainly deciduous tree species was accidentally introduced to the Northeast United States from Europe in the 1990s. Although WM has been in a continuously outbreaking population since its introduction, the native congener, Bruce spanworm (BSW, *O. bruceata*, Hurst) rarely exhibits outbreaks. We propose that this difference in population dynamics exists because BSW is experiencing a different set of pathogens, which exist at a higher prevalence. Field collected WM and BSW larvae were reared in the lab and percent mortality was noted. Cadavers were examined microscopically for evidence of *Microsporidia* and nucleopolyhedrovirus (NPV) infections. DNA was extracted from BSW samples that were positive for NPV, and amplified by PCR to detect and characterize polyhedrin gene sequences. Of 433 BSW larvae, 51.5% did not survive to the pupal stage while only 1.1% of the 15,677 WM larvae died prior to pupation. BSW had a higher prevalence of *Microsporidian* infection than WM (63.0% compared to 3.3%) while WM experienced a high prevalence of NPV (93.3% compared to 14.1%). Polyhedrin sequence from BSW was only 88% identical to that of OpbrNPV, indicating that the NPV infecting these insect species are different. In conclusion, WM and BSW are experiencing different pathogens and at a different prevalence. Understanding the controls of epizootics on BSW may provide valuable insight into possible biological controls for WM.

Poster / Viruses. Wednesday, 16:30. VI-8

Proteomic analysis and *in vivo* differential gene expression of *Trichoplusia ni* granulovirus (TnGV)

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Nucleopolyhedrovirus (NPV) and *Granulovirus* (GV) infections on *Trichoplusia ni* larvae is regulated by the expression of the virus genes. There are very few reports about the differential expression of the baculovirus genes in the host. This report deals with the proteomic analysis to detect the differential expression of viral genes. Also, macroarrays were prepared to analyze total proteins from infected *T. ni* larvae with the granulovirus TnGV, which were compared with those from non-infected larvae, obtained at different post-infection (p.i.) periods. When the expressed proteins from infected and non-infected larvae were compared, no significant change in the protein pattern was observed at 24 hs p.i.; however, when compared at 48, 72, 90, and 120 hs p.i., differential protein bands were detected in the infected larvae, not present in the non-infected larvae. Additionally, subtractive libraries were constructed in order to identify those genes expressed differentially at different p.i. periods. Libraries were obtained with 36, 21, 16, 13, and 23 clones at 24, 48, 72, 96, and 120 hs p.i., respectively. In these macroarrays a decrease of the hybridization intensity was observed as the p.i. periods were increasing. This observation may suggest that, due to the TnGV infection in the *T. ni* larvae tissues, the expression of normal proteins of the host decreased. That is, there might be an expression repression of the larval

genes. This report it sets the bases to understand the induction and repression mechanisms of the insect genes, when a GV infection occurs..

Poster / Viruses. Wednesday, 16:30. **VI-9**

Recombinant Iridovirus IIV-6 expresing the Cn-10 neurotoxin from *Centruroides noxius* scorpion
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Was established the methodology to obtain a recombinant Iridovirus *in vivo*, using the microparticle bombardment. Genes encoding for the green fluorescent protein GFP, and the protein Cn10, was cloned into the 295L gene Iridovirus, strain IIV -6. Were standardized optimal conditions for micro-projectile bombardment cotransfection of the vector DNA TOPO-295L-GFP- Cn10 and wild DNA from Iridovirus IIV-6, being the ratio of 3:1 (vector: wild DNA) the more useful for obtained recombinant Iridovirus. Recombinant Iridovirus IIV-6, was obtained by cotransfection of vector DNA TOPO-295L-GFP-Cn10 and wild DNA Iridovirus IIV-6, using the technique of biolistic to co-infecting *Galleria mellonella* larvae. This recombinant Iridovirus expressed both proteins, the GFP and Cn10. Furthermore, using fluorescent microscopy, were detected a green fluorescent staining in few portions of fat tissue of *G. mellonella* larvae. The potential expression of GFP and Cn10 proteins, was corroborated by SDS-PAGE, restriction analysis and PCR. This is the first report of the production of a recombinant Invertebrate Iridovirus, expressing a reporter gene (GFP) and a virulence gene (Cn10) and represents a model system for the genetic improvement of Invertebrate Iridovirus. More studies are needed at the molecular level, such as the sequencing of the genome of recombinant Iridovirus IIV-6 and performing Western Blot tests to detect, for one hand, the insertion of both genes (GFP and Cn10) into the genome of IIV-6 Iridovirus, and on the other hand, to verified the correct expression of both proteins in the tissues of the infected insect larvae..

Poster / Viruses. Wednesday, 16:30. **VI-10**

Genomic sequencing and analysis of *Sucra jujuba* nucleopolyhedrovirus

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The complete nucleotide sequence of *Sucra jujube* nucleopolyhedrovirus (SujuNPV) was determined by 454 pyrosequencing. The SujuNPV genome was 135,952 bp in length with an A+T content of 61.34%. It contains 131 putative open reading frames (ORFs) covering 87.9% of the genome. Among these ORFs, 37 were conserved in all completely sequenced baculovirus genomes, 25 conserved in lepidopteran baculoviruses, 64 were found in other baculoviruses, and 5 were unique to SujuNPV genome. Seven homologous regions (*hrs*) were identified in the SujuNPV genome which can be classified into two groups. SujuNPV was identified to contain several duplicated or multiple copy genes, as it contains two copies of helicase, DNA binding protein gene (*dbp*) and *cg30*, 3 copies of inhibitor of apoptosis gene (*iap*), and 4 copies of baculovirus repeated ORF (*bro*). Phylogenetic analysis suggest that SujuNPV

belongs to a subclade of group II alphabaculovirus, interestingly different from other baculoviruses, all the nine members of this subclade contain a second copy of *dbp*.

Poster / Viruses. Wednesday, 16:30. **VI-11**

Functional analysis of exonuclease gene (012L) of *Chilo iridescent virus*
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Chilo iridescent virus (CIV) encodes an open reading frame (ORF 012L) homologous to exonuclease II of *Schizo-saccharomyces pombe*. In the current study, we focused on the characterization of exonuclease gene of CIV. The target gene was cloned into the pET28a vector, expressed in *E. coli* strain BL21 (DE3) Lys with an N-terminal His tag and purified to homogeneity by using Ni-NTA affinity chromatography. Biochemical characterization of the purified CIV-exonuclease protein (CIV-Exo) confirmed that this viral protein is a functional 5'-3' exonuclease that digests 3'-biotin-labelled oligonucleotides and linear double-stranded DNA molecules from their 5'-termini in a highly processive manner. CIV-Exo has also a potent endonuclease activity *in vitro*. The CIV-Exo converted supercoiled plasmid DNA (replicative form I, RFI) into the open circular form (RFII) and then open circular form into linear form (RFIII). Both exonuclease and endonuclease activities of CIV-Exo are optimal at pH 8.0 in the presence of 10 mM MgCl₂, 2 mM dithiothreitol and 100 µg BSA ml⁻¹.

Poster / Viruses. Wednesday, 16:30. **VI-12**

Identification of a new multiple nucleopolyhedrovirus isolated from the Jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt

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A new multiple nucleopolyhedrovirus was isolated from diseased larvae of the jasmine moth, *Palpita unionalis* (Hübner) (Lepidoptera: Pyralidae) in Egypt. The virus caused typical symptoms of a baculovirus infection, and it was possible to propagate the causative agent in larvae of the homologous host. Light microscopy studies showed polyhedral occlusion bodies (OBs). Electron microscopy of ultrathin sections of polyhedral OBs showed multicapsid virions identifying the virus as a multiple embedded nucleopolyhedrovirus. Therefore, this virus was termed *Palpita unionalis* multiple nucleopolyhedrovirus (PaunNPV). The identity of the isolated virus was confirmed by sequencing of a 452 bp fragment of the *polyhedrin* (*polh*) gene that was amplified using degenerate primers. Blast search showed that it was closely related to *polh* genes in *Dirphia peruviana* NPV, *Pterolocera amplicornis* NPV, and *Nepytia phantasmaria* NPV. A neighbour-joining phylogenetic tree was constructed based on the predicted amino acid sequences of the *polh* genes of the selected closely related NPVs. Phylogenetic distances suggested that PaunNPV should be considered to belong to a novel species within the genus *Alphabaculovirus*. Preliminary bioassay data showed that the virus was active against either 2nd or 4th instars of jasmine moth. The calculated

LC₅₀ was 1.3x10³ and 3.1x10³ OBs/ml for the tested 2nd and 4th instars, respectively. The study reports a new baculovirus that might be used as a promising agent for biological control of the jasmine moth.

Poster / Viruses. Wednesday, 16:30. VI-13

A single baculovirus for the production of recombinant Adeno-Associated Virus 8 vectors

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We have developed a single baculovirus, named "Monobac", for the production of recombinant Adeno-Associated Virus vectors of serotype 8 (rAAV8) using the Sf9 cell/baculovirus system. In an AcMNPV bacmid devoid of the *chitinase* and *cathepsin A* genes, the AAV *rep2* and *cap8* genes have been inserted at the *egf* locus, while the recombinant AAV was cloned in the Tn7 site. This system was used for the production rAAV8 encoding the human γ -sarcoglycan gene, of clinical interest for the treatment of LGMD2C (γ -sarcoglycanopathy) myopathy disease. Enhanced rAAV8 productivity was observed in the cell culture and was maintained after purification, compared to production system based on the use of 2 baculoviruses. The produced rAAV8 capsids displayed a reduced degradation profile of the capsid proteins VP1/VP2 due to the elimination of the baculovirus *cathepsin* protease gene. This optimized system allows the production of an improved quantity of rAAV vectors with improved vector quality, resulting in enhanced infectivity of the rAAV. .

Poster / Viruses. Wednesday, 16:30. VI-14

Determining the role of P10 during baculovirus infection through the development of novel mutants in *Autographa californica* multicapsid nucleopolyhedrovirus

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P10 is a fibrous protein that forms complex networks of filaments and a distinct perinuclear tubular structure around the nucleus during the later stages of infection of cells with baculovirus. Previous research has suggested possible roles of P10 in nuclear stability, polyhedron formation and cell lysis, but distinct functional roles for the protein have yet to be determined. In order to investigate the role of P10 during infection, a variety of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) mutants have been constructed, which include a *p10* deletion and associated rescue virus, phosphorylation mutants and a virus in which the AcMNPV *p10* coding region has been replaced with that from *Spodoptera frugiperda* (Sf)NPV. Mass spectrometry was used to confirm the phosphorylation of P10 serine 93. Mutation of serine 93 to alanine affected the structure of P10 tubules as evidenced by confocal microscopy. The distinctive tubular structure surrounding the nucleus that is observed in wild-type virus infected cells failed to form correctly. Circular dichroism analysis confirmed a distinct change in the protein secondary structure. These data suggest that phosphorylation plays a key role in P10 function. Replacement of the AcMNPV *p10* coding region with that from SfNPV resulted in a virus with low budded virus titre and aberrant rearrangement of microtubules in comparison to AcMNPV-infected cells, suggesting that the SfNPV P10 may be affecting microtubules and translocation of nucleocapsids to the plasma membrane for budding.

Poster / Viruses. Wednesday, 16:30. VI-15

Evaluation of the transcriptional transactivation of betabaculovirus regulatory elements in transformed cell lines by alphabaculovirus transcription factors

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The narrow host range of baculoviruses is one of their advantages for sustainable pest control of a single insect species with minimal or no effect on non-target organisms. However, the limited host range may be less desirable from the economical point of view since more than one pest can be present at the same time on most crops. Engineering baculovirus genomes with an expanded host range by design would be an answer to this type of scenarios. Nevertheless, genetic determinants of host range have not been widely characterized and the mechanisms of host recognition are still not well understood. In this context, the generation of hybrid or chimaeric baculoviruses may be an empirical approach to generate viruses with expanded host range. The expression of viral genes in this context will require the transcriptional transactivation of their promoters by heterologous transcription factors (TFs). However, the recognition of baculovirus promoters in different species has not been systematically studied so far. The aim of our work is to evaluate the transcriptional transactivation of betabaculovirus promoters by alphabaculovirus TFs. It has been noted before that late promoters require the replication of the DNA to be activated. Therefore, we generated stably transformed cell lines expressing the red fluorescent protein (DsRed) as a reporter gene under the control of immediate-early, early and late gene promoters of the *Anticarsia gemmatalis* nucleopolyhedrovirus (AgMNPV) and *Epinotia aporema* granulovirus (EpapGV), respectively. These cell lines were infected with AgMNPV to evaluate the transcriptional transactivation of these promoters. Our results showed that the AgMNPV transcription factors activate early and late EpapGV promoters.

Poster / Viruses. Wednesday, 16:30. VI-16

Enhancin Genes of *Lymantria dispar* NPV Do Not Increase Potency Via Metalloprotease Activity

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The baculovirus encoded enhancins characterized so far are metalloproteases that digest proteins in the peritrophic matrix (PM) of their host midgut, increasing viral potency in many systems. *Lymantria dispar* NPV has two *enhancin* genes (E1 and E2) that are distributed in the ODV envelopes, placing them in a position to interact with the PM and the midgut cells. Deletion of either *enhancin* or both reduces viral potency 2-fold and 12-fold, respectively, compared to wildtype. Removal of the PM with optical brightener treatment did not alter these differences in potencies, suggesting that the enhancins do not affect the PM. The results of an *in vitro* PM digestion assay found that although the PM was degraded, it was not affected by inhibitors of metalloproteases, whereas treatment with serine protease inhibitors showed little or no PM degradation. Mutant LdNPV viruses were generated by altering the region that encodes the zinc binding site of the metalloprotease; this region of E1 and E2 was either deleted or altered by homologous amino

acid substitution to attempt to retain a functional enzyme. Bioassays showed that the deletion or alteration of just the zinc binding site of the metalloprotease, but not the entire enhancin gene, did not change viral potency. For example, a construct with E1 deleted/E2zinc modified had the same potency as E1deleted/E2 intact. These results suggest that the enhancins of LdNPV do not improve viral potency through the activity of metalloproteases, but appear to have a different mechanism, which has yet to be identified.

Poster / Viruses. Wednesday, 16:30. **VI-17**

A Cypovirus VP5 Displays the RNA Chaperone-like Activity that Destabilizes RNA Helices and Accelerates Strand Annealing

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For double-stranded RNA (dsRNA) viruses in the family Reoviridae, their inner capsids function as the machinery for viral RNA (vRNA) replication. Unlike other multishelled reoviruses, cypovirus has a single-layered capsid, thereby representing a simplified model for studying vRNA replication of reoviruses. VP5 is one of the three major cypovirus capsid proteins and functions as a clamp protein to stabilize cypovirus capsid. Here, we expressed VP5 from *Helicoverpa armigera* cypovirus-5 (HaCPV-5) in a eukaryotic system and determined that this VP5 possesses RNA chaperone-like activity, which destabilizes RNA helices and accelerates strand annealing independent of ATP. Our further characterization of VP5 revealed that its helix-destabilizing activity is RNA specific, lacks directionality, and could be inhibited by divalent ions, such as Mg²⁺, Mn²⁺, Ca²⁺ or Zn²⁺, to varying degrees. Furthermore, we found that HaCPV-5 VP5 facilitates the replication initiation of an alternative polymerase (i.e. reverse transcriptase) through a panhandle-structured RNA template, which mimics the 5'-3' cyclization of cypoviral positive-stranded RNA. Given that the replication of negative-stranded vRNA on the positive-stranded vRNA template necessitates the dissociation of the 5'-3' panhandle, the RNA chaperone activity of VP5 may play a direct role in the initiation of reoviral dsRNA synthesis..

Poster / Viruses. Wednesday, 16:30. **VI-18**

A recombinant *Autographa californica* nucleopolyhedrosis virus expressing a Cyt1A/GFP chimera in *Trichoplusia ni* larvae

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A novel approach was followed in order to achieve the expression of the Cyt1A toxin of *Bacillus thuringiensis* in a recombinant strain of AcNPV, trying to increase its virulence. First, the reporter GFP protein was used as a means to identify recombinant viruses; and second, biolistic was used to achieve co-transfection. The recombinant construction pAccyta-GFP, containing the *B. thuringiensis* gene *cyt1A* fused with the GFP gene under the control of the p10 promoter from the pacuW31 vector was generated. Successful co-transfection by biolistics was achieved with the AcNPV genome, when neonate *Trichoplusia ni* larvae were bombarded with DNA-coated gold micro-projectiles. Treated larvae showed the typical NPV infection symptoms, although only a thorough inspection detected fluorescent points in the fat body. Microscopic corroboration indicated that a

recombinant AcNPV (AcNPV-cyt1a-GFP) was generated, showing glowing polyhedra in the infected cells, under fluorescence microscopy. This observation may indicate that the putative chimeric protein is incorporated into the polyhedron structure during its integration. Interestingly, the nuclei holding the recombinant polyhedra appeared less compact than those holding the wild-type polyhedra. A series of purification cycles of AcNPV-cyt1a-GFP rendered larvae showing fluorescence throughout the whole body. Preliminary observations indicate that AcNPV-cyt1a-GFP kills larvae faster than the wild-type strain; however, accurate LT₅₀s are still to be estimated.

Poster / Viruses. Wednesday, 16:30. **VI-19**

iLOV baculovirus: Using a novel small fluorescent protein for imaging virus proteins during infection

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Imaging of living cells is now a common place approach in cell biology and virus research, however addition of conventional fluorescent proteins such as green fluorescent protein (GFP) and its derivatives, can lead to alterations in the location and behaviour of target proteins. Such problems in mis-targeting have previously been observed on fusion of GFP to *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) P10, thus hindering imaging of P10 dynamics during virus infection of insect cells. Here we report use of iLOV, a small (~11kDa) genetically encoded fluorescent protein based on the LOV domain of plant phototropin 2, fused to P10 in AcMNPV. Expression of the P10-iLOV fusion during infection showed presence of filaments and nuclear structures, comparable to those seen in previous immunofluorescence images. We have also looked at the fluorescence lifetime of iLOV in P10 structures, where we established that the P10-iLOV fusion shows a very long fluorescence decay of ~4ns, compared to ~2.5ns for GFP. This work shows the successful use of iLOV in the baculovirus system, and provides an opportunity to tag proteins where GFP has previously failed. In addition the long fluorescence lifetime makes iLOV a promising candidate for use in protein interaction studies using Förster resonance energy transfer-fluorescence lifetime imaging (FRET-FLIM).

Poster / Viruses. Wednesday, 16:30. **VI-20**

Expression analysis of the *nsd-2* gene encoding the putative densovirus receptor in the midgut

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Bombyx mori densovirus type 2 (BmDNV-2) is a pathogen that replicates only in the midgut columnar cells and causes fatal disease in the silkworm. The resistance to BmDNV-2 is determined by a single gene, *nsd-2*, which is characterized as non-susceptibility irrespective of the viral dose. Previously we have identified *nsd-2* by positional cloning and found that this gene encodes a putative amino acid transporter which might work as a receptor for BmDNV-2. In this study, we investigated the relationship between the part of the midgut expressing *nsd-2* and the BmDNV-2 infection. To investigate the expression pattern of *nsd-2* in the midgut, we divided the midgut into three parts, anterior, middle, and posterior part, and performed the RT-PCR analysis with total RNA isolated from each part. *nsd-2* transcript

was strongly expressed in the posterior part of the midgut. However the expression levels of *nsd-2* were very low or no-detection in the anterior and middle parts. This regional expression pattern of *nsd-2* was common to all the investigated silkworm strain. On the other hand, the BmDNV-2-derived transcript was clearly detected in the posterior part of the midgut, but significantly lower in the anterior and middle parts. These results suggested that BmDNV-2 infection depended on the expression levels of *nsd-2* in the midgut. In insects, there is little information regarding the host's own factors in virus infection, therefore, we expect that our result will contribute to understanding the infection mechanism of insect virus. .

Poster / Viruses. Wednesday, 16:30. **VI-21**

Simultaneous covert infections with three different RNA viruses in the Lepidoptera *Spodoptera exigua*

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Viral covert infections in invertebrates have been traditionally attributed to sublethal infections that did not reach enough viral titer to establish an acute infection. Recent studies are revealing that, although true for some viruses, other viruses may follow the strategy of establishing covert or persistent infections without producing the death of the host. In the last years, a large number of viruses causing covert infections in all type of hosts have been identified, mostly due to the revolution in the sequencing technologies. The beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae) is a worldwide pest that causes significant loses to agricultural and ornamental plant industries. A comprehensive transcriptome analysis of the larval stage of *S. exigua* revealed the presence of an important number of unigenes belonging to novel RNA viruses, most of them from the order *Picornavirales*. In order to characterize *S. exigua* viral complex, we have completed the genomic sequences of three picorna-like viruses, two of them representing new members of the family *Iflaviridae* and a third one defining a new family. Additional studies have been performed to determine their morphology, infectivity, tissue distribution and abundance in the larval hosts. Influence of these viruses on the insect fitness as well as their effect on other viral and bacterial entomopathogens used for the control of this pest is also discussed.

Poster / Viruses. Wednesday, 16:30. **VI-22-STU**

A novel baculovirus-derived promoter with high activity in the Baculovirus Expression System

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In this work, we describe a novel baculovirus promoter for heterologous protein expression in insect cells using the

baculovirus expression system. The promoter sequence is derived from the *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) genome and it was identified as potential promoter after transcriptional studies of the SeMNPV interaction with its host. First, an open reading frame (ORF) of SeMNPV was identified between the most highly abundant sequences in the transcriptome of *S. exigua* larvae infected with SeMNPV. Moreover, microarray-derived data showed high transcriptional activity of that ORF at different time points during the infective process. Different regions upstream of that ORF were tested for their promoter activity in the AcMNPV baculovirus expression system. Their ability to drive the expression of the GFP protein was compared against the polyhedrin (polh) conventional promoter in different cell lines, Sf21, Hi5, and Se301 and larvae from *S. exigua* and *Trichoplusia ni*. Although we found high levels of GFP expression with several regions, the strongest promoter activity was defined by 120 nt upstream the translation start site. GFP expression was up three times higher than the expression obtained with the polh promoter. Additionally, we also tested the activity for the combination of this sequence of 120 nt with the polh promoter revealing an additive effect over the polh promoter activity. This new promoter improves the conventional baculovirus expression system, allowing a considerable increase in the ability of producing large quantity of recombinant protein.

Poster / Viruses. Wednesday, 16:30. **VI-23**

Construction and Characterization of a Recombinant Invertebrate Iridovirus

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This study describes the construction and characterisation of a recombinant Chilo iridescent virus (family *Iridoviridae*) encoding the green fluorescent protein (GFP). We showed that homologous recombination is a valid method to make CIV gene knockouts and to insert foreign genes. The CIV 157L gene, putatively encoding a non-functional inhibitor of apoptosis (IAP), was chosen as target for foreign gene insertion. The *gfp* open reading frame preceded by the viral *mcp* promoter was inserted into the 157L locus by homologous recombination in *Anthonomus grandis* BRL-AG-3A cells. Recombinant virus (rCIV-Δ157L-gfp) was purified by successive rounds of plaque purification it was confirmed by PCR, sequencing and restriction analysis. One-step growth curves for recombinant and wild-type CIV were similar. Also slot blot analysis showed that DNA's of both recombinant and wild-type CIV started replication at the same time. Hence, CIV157L can be inactivated without altering the replication kinetics of the virus. Consequently, the CIV 157L locus can be used as a site for insertion of foreign DNA, e.g. to modify viral properties for insect biocontrol.

Poster / Viruses. Wednesday, 16:30. **VI-24**

RNA interference and insect-virus interactions

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Gene silencing via dsRNA has become a powerful tool to explore functional genomics in a wide variety of eukaryotic organisms.

However, RNA interference (RNAi) especially in Lepidoptera is not straight-forward and as efficient as in other insects and it is difficult to establish robust methods. So far, several potentially limiting factors for RNAi in Lepidoptera have only been proposed for *Bombyx mori*. An important role in the somewhat random success of RNAi in Lepidoptera could be the tissue-specific gene silencing effects, and also how the dsRNA is delivered to that tissue. To address this highly variable RNAi efficiency, we focused on the RNAi pathway (miRNA-pathway and siRNA-pathway) genes, and genes related to dsRNA transport or spreading in the Lepidopteran *Helicoverpa armigera* and *Heliothis virescens*. When analyzing RNAi-related gene expression levels in different larval tissues, we found that R2D2 is transcribed at very low levels in all tissues except testes, whereas Loquacious is transcribed at very high levels in all tissues. These results suggest that, despite appropriate design, dsRNAs could fail to enter the siRNA pathway, and to knock-down genes of interest due to the observed very low levels of R2D2. As the siRNA pathway is also known as the "antiviral pathway" and defends the organism against RNA and DNA viruses, we also aim at analyzing RNAi related genes (in vivo and in vitro) to an infection with *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Helicoverpa armigera* single nucleopolyhedrovirus (HaSNPV) - both as wild type and modified forms.

Poster / Viruses. Wednesday, 16:30. **VI-25**

Studies on existing and new isolates of *Cryptophlebia leucotreta* granulovirus (CrleGV) on FCM populations from a range of geographic regions in South Africa

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Considering the possibility of some geographic populations of the false codling moth, *Thaumatotibia leucotreta* (Meyrick) developing a reduced susceptibility to the baculovirus biopesticides, Cryptoran and Cryptex, as was the case with codling moth (CM), *Cydia pomonella* (L.) to the codling moth virus (CpGV) in Germany, the search for new isolates of the *T. leucotreta* baculovirus (CrleGV) become eminent. Here we report on the successful induction of a latent baculovirus infection in five geographic populations of *T. leucotreta* and the subsequent recovery of five new CrleGV isolates. These include the Ado, Cit, Mbl, Nels and MixC isolates. These isolates were shown to be genetically different from each other and from the commercial isolates, Cryptex and Cryptoran, using restriction enzyme analysis. The new isolates have been named CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl, CrleGV-SA Nels and CrleGV-SA Mix isolates. Sequence analysis of the *granulin* and *egt* genes of all isolates revealed single nucleotide polymorphisms (SNPs) in both genes. Significantly, SNPs in the *egt* genes of these isolates resulted in a change in amino acid sequence. DNA profiles from RFLPs, as well as phylogenetic analysis based on *granulin* and *egt* sequencing showed the presence of two CrleGV-SA genome types. Cryptex and CrleGV-SA Ado, CrleGV-SA Cit, CrleGV-SA Mbl and CrleGV-SA Mix have been placed as members of Group one CrleGV-SA, and Cryptoran and CrleGV-SA Nels isolate placed into Group two CrleGV-SA. Studies on the comparative biological activity of the isolates also revealed significant differences between the relative potencies of the viral isolates against *T. leucotreta* from the Ado and MixC colonies..

Poster / Viruses. Wednesday, 16:30. **VI-26**

Effects of the baculovirus fibroblast growth factor on Sindbis virus replication

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Fibroblast growth factors (FGFs) are conserved among vertebrate and invertebrate organisms and function in cell proliferation, cell differentiation, tissue repair, and development. Many baculoviruses encode functional viral fibroblast growth factor (vFGF) homologs that stimulate cell motility of insect cells and activate host FGF receptors. During baculovirus infection of midgut lepidopteran cells, expression of vFGF leads to caspase activation and remodeling of tracheal epithelial cell basal lamina. Tracheal cell basal lamina remodeling results in structural discontinuities that allow baculovirus midgut escape. We hypothesized that vFGF would assist in midgut escape of the arbovirus Sindbis virus (SINV) during infection of mosquitoes. We first verified that vFGF stimulated cell motility in two mosquito cell lines, C6/36 and Aag2. Utilizing an alphavirus transducing system for SINV, we then constructed recombinant SINVs expressing *vfgf* (MRE/vFGF, TE/vFGF), and control viruses with the same insert in antisense orientation (MRE/vFGFas, TE/vFGFas). TE-based viruses replicate in cell cultures but poorly infect mosquito midguts, while MRE-based viruses infect midguts efficiently. Replication of each vFGF-expressing virus and its control virus was similar in both cell lines. Female *Aedes aegypti* mosquitoes orally infected with each of the recombinant viruses had no significant replication differences, measured by determining infectious viruses in individual mosquitoes, mosquito midguts, or carcasses. Thus, it does not appear that expressing vFGF affects SINV replication and dissemination.

Poster / Viruses. Wednesday, 16:30. **VI-27**

Sensitivity and vertical transmission of nuclearpolyhedrovirus in various populations of gypsy moth *Lymantria dispar*

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The gypsy moth is known as the most biologically and economically important pest species. Nucleopolyhedrovirus (LdNPV) is one of the key factors that influence on the gypsy moth population density. The different sensitivity of larvae gypsy moth LdNPV from different nature population was registered. This sensitivity of insects may depend on percentage of occult virus in insects populations. High susceptibility of larvae to virus was registered in population with high level ($91 \pm 7\%$) occult virus as compared to population with lower level ($48 \pm 5\%$) of occult virus. In addition for detection of virus transmission during several generations the larvae of parents generation were infected with high (modeling of epizootic) or low doses (modeling of sporadic death) of the LdNPV. Enhanced insects mortality caused by spontaneous virus infection in three progeny generations has been shown for parents infected by both doses of virus compared to non infected control. The level of occult virus was in 2-fold decreased to third generation for all cases. However occult virus has been detected up to sixth generations just in case of parents'

infected with high dose of virus. Possibly exogenous insect virus may be activator of viral infection and lead to epizootic. However sometimes exogenous virus produces transgenerational occult form.

Poster / Viruses. Wednesday, 16:30. **VI-28**

Establishment of SeMNPV Persistent Infection and Screening of Persistent Infection Associated Genes in Baculovirus

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Persistent baculovirus infection is observed in insect populations. Persistent infection can be transformed to a replicative and infective state and plays an important role in epizootiology of baculoviruses. However, the molecular mechanism of baculoviral persistence is unknown. *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) was serially undiluted passaged in Se301 cells to reduce virulence. Upon infection of Se301 cells with the SeMNPV up to passage 8, a few cells survived even if most of cells died due to virus infection. The surviving cells were passaged and designated as P8-Se301 cells. The cells continually released infectious progeny virus and show a typical character trait of baculovirus persistent infection. Using limited dilution method, a cell clone was isolated and designated as P8-Se301-C1. The cells were morphology similarly to the Se301 cells, and no polyhedra or viral particles were observed. However, incomplete SeMNPV genomes and low level SeMNPV transcripts presented in P8-Se301-C1 cells. It was suggested that a latent-like viral infection is present in the P8-Se301-C1 cells. To screen and identify the persistent infection associated genes in baculovirus, The total protein was extracted and isolated through 2-D gel electrophoresis, the differential expression were analyzed between the P8-Se301-C1 cells and the healthy Se301 cells. It would provide a basis for further exploring the molecular mechanisms of baculoviral persistence..

Poster / Viruses. Wednesday, 16:30. **VI-29-STU**

Larvicidal activity of an ascovirus from *Spodoptera litura* against parasitoid wasps

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When the endoparasitoid *Cotesia kariyai* (Hymenoptera: Braconidae) parasitizes *Mythimna separata* larvae infected with *M. separata* entomopoxvirus (MySEV), the parasitoid larvae die in the infected host. Death is caused by a 28-kDa polypeptide, named parasitoid killer toxin (PKT), which is encoded by the MySEV genome and secreted from MySEV-infected fat body cells into the hemolymph of an infected larva. *pkt* gene homologues are found not only in entomopoxviruses but also in other insect viruses including granuloviruses, nucleopolyhedroviruses and ascoviruses (AVs). AVs are double-stranded DNA viruses and mainly infect noctuid larvae, producing symptoms that include stunted growth and opaque white hemolymph. A unique characteristic of AVs is their poor *per os* infectivity; in nature, AVs are transmitted by the ovipositors of female parasitoid wasps. Since AV transmission thus coincides with wasp oviposition, parasitoid wasp larval mortality in an AV-infected host has sometimes been attributed to the AV, although no known

mechanism explains such larvicidal activity. To elucidate whether the *pkt* homologue in AVs is involved with this larvicidal phenomenon, we sequenced *pkt* homologue in an AV isolated from *Spodoptera litura* in Japan, and found that its predicted amino acid sequence displayed identity with MySEV PKT in a 750-bp partial sequence. Hemolymph from AV-infected larvae showed larvicidal activity against *C. kariyai* and *Microplitis* sp. (Braconidae) larvae. These results suggest that PKT expressed from the AV genome can cause death of braconid parasitoid larvae in hosts infected with the AV isolate.

Poster / Viruses. Wednesday, 16:30. **VI-30**

"11K" genes family *sf68*, *sf95* and *sf138* modulate transmissibility and insecticidal properties of *Spodoptera frugiperda* multiple nucleopolyhedrovirus

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The "11K" protein family is notable for having homologs in both baculoviruses and entomopoxviruses. These genes are classified as either type 145 or type 150, according to their similarity with *ac145* or *ac150* of *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV). One homolog to *ac145* (*sf138*) and two homologs to *ac150* (*sf68* and *sf95*) are present in *Spodoptera frugiperda* multiple nucleopolyhedro-virus (SfMNPV). Recombinant viruses lacking *sf68*, *sf95* or *sf138* (Sf68null, Sf95null and Sf138null, respectively), and the respective repair viruses, were generated from a bacmid comprising the complete virus genome. Occlusion bodies (OBs) of the Sf138null virus were ~15-fold less pathogenic to insects, which was attributed to a 100-fold reduction in ODV infectious titer/OB. Inoculation of insects with Sf138null OBs in mixtures with an optical brightener failed to restore the pathogenicity of Sf138null OBs to that of the parental virus, indicating that the effects of *sf138* deletion on OB pathogenicity were unlikely to involve an interaction with the gut peritrophic matrix. In contrast, deletion of *sf68* and *sf95* resulted in a slower speed-of-kill by ~7%, and a concurrent increase in the total production of OBs/larva. Phylogenetic analysis indicated that *sf68* and *sf95* were not generated after a duplication event of the *ac150* gene. We conclude that type 145 genes modulate primary infection process of the virus, whereas type 150 genes appear to have a role in spreading systemic infection within the insect.

Poster / Viruses. Wednesday, 16:30. **VI-31**

Characterization of two ORFs undergoing positive selection in a genotype of *Chrysodeixis chalcites* single nucleopolyhedrovirus from the Canary Islands

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The availability of genome sequences of different genotypes from a single isolate could help explain phenotype differences related to changes at genome level. Here we report the complete genome sequence of five genotypes of a *Chrysodeixis chalcites* single NPV isolate from the Canary Islands (named ChchSNPV-TF1-A, -B, -C, -G and -H). The whole genome sequences of the

ChchSNPV-TF1 genotypes are 99% identical to the previously reported ChchSNPV strain from The Netherlands (ChchSNPV-NL). ChchSNPV-TF1-A, -B, -C, -H genomes did not present ORF 53 of unknown function that is unique to ChchSNPV genomes. Major regions of variability among ChchSNPV genomes was identified in the *hoar* and *bro-d* genes. In an effort to identify genes potentially involved in virulence or in determining population level adaptations, selection pressure analysis was performed. Five ORFs were identified as undergoing positive selection; *chch55* (*bro-a*), *chch65* (*chitinase*), *chch69* (*bro-b*), *chch143* and *chch144*, the last two of which are of unknown function. Strong selection for *bro* and *chitinase* genes indicates that viral replication and liquefaction processes are critical points at which adaptation acts during transmission of these viruses. Among the unknown ORFs, *chch143* exhibits a high degree of similarity with the metalloprotease superfamily and with the previously characterized *sf29* of *Spodoptera frugiperda* multiple nucleopolyhedrovirus involved in ODV packaging. Experiments are in progress to determine the function of *chch143* and *chch144* in the transmission of ChchSNPV.

Poster / Viruses. Wednesday, 16:30. **VI-33-STU**

Analysis of genetic interactions among four non-essential genes of BmNPV

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Nucleopolyhedroviruses (NPV) produce copious amounts of polyhedrin (Polh) by the end of a replication cycle and form a lot of polyhedra in the nuclei of infected-host insect cells. This characteristic feature of NPV is a beneficial trait as the gene expression vector. To further develop baculoviral applications, deep insight into the functions and interactions of viral gene products concerning the explosive expression of Polh is necessary. We constructed a library of single gene knockout BmNPVs and showed that 86 out of 141 viral genes were dispensable for expression of the polyhedrin gene and production of infectious viral progenies (Ono et al., 2012). However, it has not been examined how these non-essential genes in combinations contribute to the viral infection. We then started a study to understand the genetic interactions among the non-essential genes. In this present study, we constructed BmNPVs lacking multiple non-essential genes and analyzed the expression of EGFP under the control of the polyhedrin gene promoter. Synergistic, compensatory, and additive relationships were observed in the genetic interaction analysis between pairs of adjacent genes in the *orf11-12-13-14* gene cluster. The results in this study revealed complex genetic interactions among the non-essential genes of BmNPV.

Poster / Viruses. Wednesday, 16:30. **VI-32**

Genome sequence and organization of a *Betabaculovirus* pathogenicto cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae)

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The cassava hornworm, *Erinnyis ello ello* (Lepidoptera: Sphingidae), is a very severe pest in cassava (*Manihot esculenta*) due to its worldly geographical distribution and high capacity of leaf consumption. It is also the most serious pest of the rubber tree (*Hevea brasiliensis*) in Latin America. The *Baculovirus* *Erinnyis* has been shown to be an economically viable and safe biopesticide for controlling this pest in South America. In the present work the complete sequence of the *Erinnyis ello* *granulovirus* (ErelGV) genome was determined. The viral DNA was extracted from a viral isolate collected in South Brazil, in 1986. Analysis by transmission electron microscopy showed granular occlusion bodies with single virions inside the protein matrix, confirming that this pathogen is a *Betabaculovirus*. The genome is 102,759 bp with G+C content of 38.7%, being larger than the previous estimation of 90,000 ± 5,000 bp based on restriction mapping for a Colombian isolate. A total of 130 putative ORFs were found encoding at least 50 amino acids. Eight of these were shown to be unique (*ErelOrf-11*, *ErelOrf-15*, *ErelOrf-27*, *ErelOrf-53*, *ErelOrf-59*, *ErelOrf-70*, *ErelOrf-90*, *ErelOrf-102*), and all the predicted protein had no significant similarity to any other sequences in GenBank. ErelGV is closely related to *Choristoneura occidentalis* *granulovirus* (ChocGV) and *Pieris rapae* *granulovirus* (PiraGV). No typical homologous regions (hrs), *cathepsin* or *chitinase* genes were detected. *Alphabaculovirus* horizontal gene transfer, such as *he65* and *p43* homologous genes, was found. Moreover, a nucleotide metabolism-related gene and two genes acquired probably from *Densovirus* were also detected.

Poster / Viruses. Wednesday, 16:30. **VI-34 STU**

Comparative fitness of a granulovirus mutant possessing larger occlusion bodies

than wild type *Adoxophyes orana* granulovirus
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During host infection, the virus particles of baculoviruses become embedded within a large proteinaceous occlusion body (OB). Outside the host, the OB protects virus particles from environmental factors including ultraviolet light (UV). However, the mechanism(s) that determine the morphology (size and shape) of OBs are not understood. We isolated a novel mutant of *Adoxophyes orana* granulovirus (AdorGV) from an *A. honmai* larva in a tea field in Japan. This mutant AdorGV produced cube-shaped OBs with edges of approximately 1.0 µm, whereas a wild type (WT) AdorGV isolated in the UK produces typical ellipsoidal OBs of approximately 0.5 µm in length. According to its full genome sequence, the mutant AdorGV was closely related to WT AdorGV. Since such giant OBs should be more costly for the virus to produce than the smaller WT AdorGV OBs, the mutant AdorGV may exhibit a trade-off in production fitness to acquire other adaptive traits. In this study, the UV tolerance of mutant AdorGV was compared to that of WT AdorGV. The persistence of the mutant AdorGV was four times longer than that of the WT AdorGV. The UV tolerance of OB-derived virus particles of mutant and WT AdorGV showed no significant difference. Thus, we elucidate that mutant AdorGV have high UV tolerance to produce giant OBs. This trait may be trade-off of some cost of mutant AdorGV such as production cost of giant OBs.

Poster / Viruses. Wednesday, 16:30. VI-35

Granulovirus detection in larvae of sugarcane borers

Diatraea spp. (Lepidoptera: Pyralidae) in Colombia

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Panela is a solid piece of unrefined sucrose obtained from evaporation of sugarcane juice, a very important industry and source of employment in Colombia. Panela yield depends of sucrose content in sugarcane, characteristic seriously affected by the presence of the stem borers complex, difficultly controlled by chemical insecticides, being an alternative the use of biological agents as granuloviruses. In this sense, the objective of this work was to isolate granulovirus naturally infecting *Diatraea* spp. larvae in sugarcane crops for panela production in Colombia. Larvae were collected from three different production areas and maintained in quarantine until dead. A total of 445 larvae were collected, 227 in Boyacá, 130 in Santander and 88 in Nariño. From collected larvae, 39 individuals died showing disease symptoms. Five dead larvae showed fungal mycelium growth and 34 presented sings of viral infection, which were analyzed by granulin gen QPCR, complete granulin gen PCR with degenerated primers and Dot Blot by using polyclonal antibodies for granulin produced in hen eggs. Two samples from Boyacá and two samples from Santander were positive by molecular and immunological methods, being three detected by QPCR and Dot Blot simultaneously and one from Boyacá positive by the three evaluated techniques. Only the 0.89% of collected larvae evidenced viral infection by granulovirus, which were detected by using very low volumes of crude samples. All methods showed to be promising for detecting granulovirus in field samples and four detected virus will be amplified in the insect for a further characterization and biopesticide development.

Poster / Viruses. Wednesday, 16:30. VI-36

Earthworm-mediated dispersal of baculovirus occlusion bodies in soil: a laboratory study

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Soil is an important environmental reservoir of baculovirus occlusion bodies (OBs). The multiple nucleopolyhedrovirus (SfMNPV, genus *Alphabaculovirus*) of *Spodoptera frugiperda* has attracted attention as a potential biological insecticide for control of this pest in maize and sorghum crops in the Americas. This study examined the potential role of the earthworm *Eisenia fetida* as a possible disperser of SfMNPV OBs in a model laboratory system. A soil incorporation bioassay technique was calibrated using *S. frugiperda* second instars that fed on an OECD artificial soil (70% sand, 20% kaolin, 10% peat) contaminated with SfMNPV OBs (5×10^4 - 5×10^9 OBs/ml). The LC₅₀ value was estimated at 2.3×10^8 OBs/ml. The gut pH of *E. fetida* was estimated to be pH 5.0-6.0 using pH indicators. Earthworms burrowed 22.5 cm into experimental soil in a 72 h period. Earthworms redistributed SfMNPV OBs vertically by up to 22 cm in artificial soil over periods of 1, 7 and 15 days. Incubation of earthworms in OB treated soil for 7 days did not significantly affect the insecticidal activity of the OBs compared to OBs in soil in the absence of earthworms ($P > 0.05$). This represents a previously unrecognized mechanism of baculovirus dispersal in

the environment that is likely to have important implications in the persistence of OB populations in soil reservoirs.

Poster / Viruses. Wednesday, 16:30. VI-37-STU

Effects of rearing temperature on the susceptibility of larvae of the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae) to *A. honmai* nucleopolyhedrovirus

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Many environmental factors, such as ultraviolet, humidity and temperature, can affect the susceptibility of insect hosts to entomopathogens. Among these factors, temperature is one of the most important factors for both insect susceptibility and multiplication of entomopathogens in the host. The smaller tea tortrix, *Adoxophyes honmai*, is one of the most important pests of tea plants in Japan and occurs four or five times in a year. In addition, larvae of *A. honmai* live in a wide range of temperature from 0°C to 35°C. Here, we examined the effects of high temperature on the susceptibility of *A. honmai* larvae to *A. honmai* nucleopolyhedrovirus (AdhoNPV). Fifth instar larvae of *A. honmai* were exposed to AdhoNPV by the modified droplet feeding method and reared on artificial diet at 25°C, 28°C, 31°C or 34°C. The susceptibility of *A. honmai* larvae was reduced with an increase in rearing temperature. No AdhoNPV-infected larvae were observed when larvae were reared at 34°C. The infection rates of *A. honmai* fifth instar larvae that were reared at 34°C were significantly lower than those of larvae that were reared at 25°C, 28°C and 31°C when budded viruses of AdhoNPV were injected.

Poster / Viruses. Wednesday, 16:30. VI-38

Characterization of Nodaviral Protein A Revealed RNA Synthesis and Terminal Nucleotidyl Transferase Activity

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Nodaviruses are a family of positive-stranded RNA viruses with a bipartite genome of RNAs, RNA1 and RNA2. Protein A, which is recognized as an RNA-dependent RNA polymerase (RdRP), is encoded by genomic RNA1 and functions as the sole viral replicase protein responsible for its RNA replication. Although nodaviral RNA replication has been studied in considerable detail, the mechanism(s) governing the initiation of nodaviral RNA synthesis have not been determined. In this study, we characterized the RdRP activity of Wuhan nodavirus (WhNV) protein A and Flock House virus(FHV) in detail and determined that these nodaviral protein A initiates RNA synthesis via a de novo mechanism. Moreover, we uncovered that both of WhNV protein A and FHV protein A possess terminal nucleotidyl transferase (TNTase) activity. We subsequently found that the TNTase activity of WhNV protein A and FHV protein A could function in vitro to repair the 3' initiation site, and may function as a rescue and protection mechanism to protect the 3' initiation site, and ensure the efficiency and accuracy of viral RNA synthesis. Furthermore, we determined the cis-acting elements for RdRP or TNTase activity at the 3' end of positive- or negative-strand RNA1. Altogether, our study establishes the de novo initiation mechanism of RdRP and the terminal rescue mechanism of TNTase for WhNV and FHV protein A, and represents an important advance toward understanding nodaviral RNA replication.

BACTERIA WORKSHOP Wednesday, 20:00-21:30

Non-Target Effects on Biological Pesticides Transgenic Crops

Workshop paper. Wednesday, 20:00 **199**

The impact of herbicide tolerant crops on non-target organisms

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Tolerance to broad spectrum herbicides is the most worldwide cultivated transgenic trait and millions of hectares have been sown with herbicide tolerant (HT) soybeans, maize, and canola. Among potential effects of this kind of genetically modified (GM) crops on the environment are those on non-target organisms (NTOs). A NTO is any species that is not the direct target of the GM crop and may include non-target plants (particularly in the margins and nearby habitats), plant pathogens, arthropods birds and wildlife, and a diversity of soil organisms. The impact of HT crops on non-target organisms may be exerted through three main mechanisms: (i) the direct effect of the trait introduced into the plant on the NTO, (ii) the effect of the herbicide on the NTO, and (iii) through the food web. While there are no records in the literature of any effect through the first mechanism to our knowledge, and relatively very few through the second one, more effects have been described through trophic relationships mainly originated by the alteration of the abundance, composition and phenology of weed flora. This presentation is mainly focused on this third mechanism and particularly on weed- arthropods relationships as the first trophic interaction that leads to build complex food webs in agroecosystems. According to the experience of Spanish field trials with HT maize, few changes in NTO populations may be expected if modifications of weed flora are not dramatic. Potential benefits derived from the flexibility of timing broad spectrum herbicide sprayings are discussed.

Workshop paper. Wednesday, 20:15 **200**

Your Right to Know What You Eat: On the Occurrence of Viable *Bacillus thuringiensis* in Commercial Food Products

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It is widely recognized in the scientific community that genetically engineered crops are safe for human consumption. Yet serious concerns continue about the safety of these foods, despite consumption of approximately two trillion meals by people over the past decade with no known ill effects. During 2013, for example, new laws were proposed in California and Washington State to label foods containing genetically modified organisms (GMOs), the rationale being that people have a right to know what they eat. Although both laws failed, there is little doubt the public remains concerned about GMO food safety. Unknown to the public and many scientists is that *Bacillus thuringiensis* (Bt), the source of the insecticidal proteins used in insect-tolerant crops such as Bt corn and Bt soybeans, occurs naturally and commonly on many vegetables, grains, and nuts, including products based on these such as flour and flour products (bread, pasta), cereals, soup, salami, candy and puddings. Moreover, and ironically,

the only insecticides permitted for use on organic crops are Bts that contain viable spores and the same Cry proteins used in GMO crops. Whether due to natural occurrence or the use of Bt insecticides, these foods can contain hundreds to thousands of viable spore/crystal mixtures per gram or cm². In this presentation, I will review the data showing that Bt occurs naturally and commonly in our food supply, and that the diversity of strains and insecticidal proteins which people consume is much greater than those used in commercial Bt insecticides or GMO crops.

Workshop paper. Wednesday, 20:30 **201**

Environmental risk assessment of genetically engineered crops for spiders

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Before genetically engineered (GE) crops can be grown commercially, potential risks to the environment need to be assessed. Environmental risk assessment (ERA) ensures that desired ecological functions (protection goals), such as biological control, pollination, and decomposition are not harmed. We will present the process of non-target ERA for GE plants producing insecticidal proteins derived from *Bacillus thuringiensis* (Bt). Spiders are among the most abundant biological control agents in arable systems and we will use examples from our research to illustrate the different ERA steps.

The populations of species associated to the ecosystem services to be protected represent assessment endpoints for the ERA. Knowledge on the community inhabiting the GE crop grown in a certain region (receiving environment) is combined with knowledge on potential exposure and sensitivity to the insecticidal compound to focus the assessment and to formulate relevant risk hypothesis to be tested. The different risk hypotheses are then addressed in the analysis phase of the ERA following a tiered approach. Early-tier testing is conducted under worst-case exposure conditions in the laboratory. Surrogate test species are selected that are most likely to reveal an adverse effect. More complex and realistic semi-field or field studies supplement the ERA when uncertainty about the level of risk to non-target species remains high after early tier laboratory studies are conducted. We will discuss important criteria to consider when designing non-target studies, which can only inform the ERA if they are reproducible, reliable, and test clearly defined risk hypotheses.

Workshop paper. Wednesday, 20:45 **202**

Conclusions from 10 years of accumulated evidence from publicly funded field trials research with Bt-maize in Germany

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Publicly funded research into the environmental risks of genetically modified plants has been performed in Germany for more than a decade. The Bt-maize events MON810, MON88017 and MON89034 x MON88017 were assessed in field trials. Each of the trials lasted for 3 years and lead to the further refinement of trial designs and assessment methods. The combined results on non-target organism effects show that a) the assessed Bt-maize events do not harm the communities of NTOs typical for maize; b) conventional treatments with

insecticides can have profound negative impacts; c) conventionally bred maize varieties can differ substantially in their impact; d) different management practices have profound impacts on populations on-crop and off-crop. A number of conclusion can be drawn from the assessments: 1. The NTO ERA for Bt-maize should more strongly rely on early tier experiments; 2. Field trials are only sensible if results from earlier tiers show the possibility for negative NTO impacts; 3. A comparative approach to ERA is without alternative, also looking at conventionally bred varieties and alternative management approaches; 4. The methods and trial designs used are able to detect differences in impact of different maize varieties; 5. To fully assess the potential impacts of the cultivation of Bt- and other genetically modified plants a systems approach is needed, that also takes into account the benefits of using these plants; 6. A decision is needed on what we really want to protect and thus need to assess.

THURSDAY - 7 August

SYMPORIUM 7 (Dis. of Benef.I Inverteb.) Thursday, 8:00-10:00 Emerging Tools for Aquatic Pathogen Discovery and Description

Symposium. Thursday, 8:00. **203**

Early mortality syndrome is an infectious disease with a bacterial etiology

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Beginning in about 2009, a new, emerging disease called "Early Mortality Syndrome or EMS" (more descriptively called Acute Hepatopancreas Necrosis Syndrome or AHPNS) began to cause significant production losses in shrimp farms southern China. By 2010 the range of affected farms in China had expanded, and by 2011 EMS/AHPNS was confirmed in Vietnam and Malaysia, and in Thailand in 2012. EMS/AHPNS disease has caused serious losses in the areas affected by the disease, and it has also caused secondary impacts on employment, social welfare, and international market presence. EMS/AHPNS was first classified as an idiopathic disease because no causative agent had been identified. Preliminary studies conducted in Vietnam in 2012 by the Laboratory of Aquaculture Pathology at the University of Arizona (UAZ-APL) have indicated that EMS/AHPNS is infectious. Since early in 2013, the UAZ-APL was able to isolate and identify the causative agent of EMS/AHPNS in pure culture. In several separate challenge experiments, the same EMS/AHPNS pathology was reproduced consistently in experimental shrimp. In addition, the same identical agent was recovered from the challenged animals and several subsequent challenge tests using the recovered agent could also reproduce EMS/AHPNS pathology with very consistent results. The agent was identified as a unique strain of *Vibrio parahaemolyticus*

that is commonly found in marine environment. Hence, EMS/AHPNS has a bacterial causative agent that satisfies Koch's Postulates to be a typical infectious disease. Further studies focusing on the agent of AHPNS revealed that the agent could produce toxin(s) causing the primary pathology in affected shrimp.

Symposium. Thursday, 8:30. **204**

Policy, phylogeny, and the parasite

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Animal diseases gain political attention by their inclusion on lists of global bodies such as those of the World Organisation for Animal Health. Currently, the OIE lists 116 diseases caused by viral, bacterial, fungal, protistan, and metazoan pathogens. Each is afforded a specific chapter in the regularly updated OIE 'Manual of Diagnostic Tests' series. Of these, 30 diseases are caused by eukaryotic (fungal, oomycete, protistan, and metazoan) parasites. Inclusion necessitates national governments to report outbreaks promptly but may lead to trading restrictions between nations in an attempt to limit spread. Detection therefore has consequences that may directly impact from farm to state levels. Here, we consider current approaches to discrimination of listed parasites from related, but unlisted, counterparts. We outline problems with defining 'species', propose the necessary drivers that should be required for discrimination of important taxa, and highlight how this process may be influenced by national policies. Further, we propose a set of 'best practice' measures, broadly based upon current taxonomic philosophies for protists and metazoans that should be applied when defining taxa for listing as notifiable. We will illustrate these principles with topical issues associated with the taxonomy and listing of aquatic invertebrate pathogens.

Symposium. Thursday, 9:00. **205**

The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics

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Commercial crustacean fisheries on the Atlantic coast of Canada represent over \$(CAD) 1 billion annually. The American lobster (*Homarus americanus*) fishery alone represents over \$(CAD) 600 million with harvests in recent years breaking records for amount of lobster that has been landed. The Canadian and Maine USA lobster populations remain healthy but the once vibrant lobster fisheries in Southern New England USA have been devastated by a mixture of disease, ocean acidification, global warming and anthropogenic stressors. Conventional gross anatomic, microscopic and histological analysis remain the backbone of

crustacean health and disease assessment but new molecular genetic techniques are beginning to be integrated into this assessment. Modern genomics and transcriptomics have revolutionized the discovery of diagnostic and prognostic markers in human and terrestrial medicine and promise to drive crustacean health and diagnostic molecule discovery. We have recently begun to apply high-throughput transcriptomic techniques, such as microarray and RNA-Seq, to investigate American lobster health, disease and response to physiological and anthropogenic stressors. Our studies highlight the incredible potential that modern molecular biological approaches have for advancing our understanding of crustacean immunology and disease biomarker discovery.

Symposium. Thursday, 9:30. **206**

Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns

Hanna Hartikainen^{1,5}, Grant D. Stentiford^{2,3}, Kelly Bateman^{2,3}, Stephen W. Feist³, David M. Stone³, Matt Longshaw^{3,4}, Georgia Ward¹; Charlotte Wood¹; Beth Okamura¹ and David Bass¹

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The increasing application of massively parallel sequencing technology to environmental DNA samples (e.g. from water, sediment, soils, whole animals) is providing unprecedented resolution of microbial community structure, diversity and functioning. Application of general and specific primer approaches, amplicon sequencing and metagenomics have enormous potential for the detection of known, novel and otherwise cryptic pathogen lineages. We use such techniques to detect invertebrate pathogens of potential significance to fisheries and aquaculture. Using specific-primer approaches, we have revealed unknown diversity of haplosporidian parasites from eDNA and show shifts in parasite communities along an offshore gradient. At the other end of the spectrum, we have used a metagenomic approach to identify a mikrocytid pathogen of juvenile edible crabs that had eluded molecular characterization using specific- and general primer approaches. We highlight the current methods for discovery and detection of potential pathogens in eDNA samples and show how such studies can inform on ecology, life-cycle and transmission dynamics of aquatic pathogens. Finally, we predict a re-emergence in the importance of classical approaches to disease investigation (e.g. histopathology, electron microscopy) to enable meaningful links to be drawn between presence within the matrix and outcomes in hosts. eDNA analyses should therefore be considered as a 'tool in the box', rather than the toolbox per se, for investigating pathogens of concern to aquatic hosts.

CONTRIBUTED PAPERS Thursday 8:00-10:00

Nematodes 3

Contributed paper. Thursday, 8:00. **207**

The Role of biocontrol agents within IPM of *Tuta absoluta* on tomato in Egypt

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Since its arrival in Spain, the tomato leafminer *Tuta absoluta* has rapidly spread around Europe and has become an extremely important pest of tomato crops in Mediterranean Basin countries. This pest arrived to Egypt early in 2010 and there soon followed an outbreak in many tomato-planted areas where it caused extensive damage by mining in tomato leaves, stems and fruit. Egyptian entomopathogenic nematode species (EPN) induced 89.3-96.4% mortality to *T. absoluta* larvae. Also, the other biocontrol agents *Trichogramma achaeae* and *Macrolophus pygmaeus* are suggested as effective components within a new control strategy against the insect on tomato in the present study. *M. pygmaeus* may prey on *T. absoluta* eggs and larval stages, but due to more suitable climate of Egypt to *T. achaeae*, earlier release of the latter bug is preferable in order to start the control on the first generations of the pest eggs. EPN have both foliar and soil applications in the strategy. On the foliage, EPN can control efficiently feeding larvae of *T. absoluta* in and outside the leaf galleries while the soil nematodes kill both last instar larvae, when they slide down from the leaves to pupate, and emerging adults from the buried pupae. In addition to such natural enemies, the strategy is supported by prophylactic measures, light and pheromone traps, and IPM compatible insecticides.

Contributed paper. Thursday, 8:15. **208**

Insecticidal activity of *Heterorhabditis bacteriophora* Shandong toward *Brontispa longissima* and *Cryptothelea variegata*

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Heterorhabditis bacteriophora nematodes kill many insect species, but its potencies toward *Brontispa longissima* and *Cryptothelea variegata* pests are unknown. Initially, four isolates of *H. bacteriophora*, UV resistant *H. bacteriophora* Shandong (HbSD), Hb I, Hb II, and Hb III were bioassayed against standard insect *Galleria mellonella*. The UV resistant HbSD isolate was chosen for next bioassay against the last-instar of *B. longissima* and *C. variegata* compared with *G. mellonella* in the laboratory. After exposure of insects to infective juveniles of nematodes (IJs) for six days, mortality was correlated with dosage, and the LC₅₀ was ≈ 9.35 IJs for *B. longissima* and ≈ 11.76 IJs for *C. variegata*, as compared with that ≈ 8.56 IJs for *G. mellonella*. There are no statistically different in potency among these three hosts. Thus, the insecticidal potencies of the nematodes to these three pests

were: *C. variegate* = *B. longissima* = *G. mellonella*. However, there is a significant dose-response in each treatment of the insect species. Two field trials were conducted in local residence yards in the Wanning City suburb of Hainan province, P. R. China. The results showed that after spraying *H. bacteriophora* SD IJs in the period of March and April, *Cinnamomum camphora* trees is significant difference in the survival rate between the treatment and untreated control ($p < 0.05$). The technology presented may be of substantial interest to biological pesticide producers.

Contributed paper. Thursday, 8:30. **209**

Prospects for using Entomopathogenic Nematodes to Control the Vine Mealybug, *Planococcus ficus*, in South African Vineyards

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Planococcus ficus (Signoret), the vine mealybug, is regarded a major pest insect of the South African grape industry. Mealybugs are difficult to control with chemicals due to their cryptic lifestyle of hiding in crevices, under bark and below ground on roots where chemicals battle to reach. Another problem in the use of chemical pesticides is the water repellent waxy secretions and the ability of mealybugs to rapidly build up resistance. Entomopathogenic nematodes of the families Heterorhabditidae and Steinernematidae can potentially be used within an integrated pest management system to control the vine mealybug, which not only occur mostly on the aerial part of plants, but also on the roots. Both local *Heterorhabditid zealandica* and *Steinernema yirgalemense* were able to move 15 cm downward in sand columns to infect *P. ficus*, with respective mortalities of 82% and 95%. Laboratory persistence of *S. yirgalemense* in sterile, moist sand in the laboratory remained high (> 85%) after 6 months, while that of *H. zealandica* dropped to 5%. When *S. yirgalemense* was applied to the soil of two vineyards with adult female *P. ficus*, contained in pierced Eppendorf tubes, buried at a depth of 15 cm in the soil, mortalities of up to 50% were obtained after 48 h. Persistence of *S. yirgalemense*, measured using codling moth larval mortality, was found to be zero in one vineyard, while in the other 70%, 12 weeks after application. These studies showed that entomopathogenic nematodes, specifically *S. yirgalemense*, have promising potential as biological control agents for *P. ficus* soil populations.

Contributed paper. Thursday, 8:45. **210**

New data on *Steinernema ichnusae* distribution in the Mediterranean Area

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The species *Steinernema ichnusae* (Tarasco, Mráček, Nguyen & Triggiani, 2008) have been isolated till now only from Sardinia (Tarasco et al., 2014; doi: 10.1017/S0022149X

14000194). Recent molecular studies carried out on some strains isolated in other Mediterranean areas revealed this species is also present out of Sardinia island. Five strains of *S. ichnusae* were identified coming from different coastal sites in Algeria (ALG2, ALG3, ALG15, ALG 16 and ALG18), one from continental Italy (Campania Region, MU1) and two from Sicily (EMA 2 and CT026). All these strains had previously been only partially identified as belonging to a species of the *S. feltiae* group. The molecular studies showed that all the strains examined shared with *S. ichnusae* some nucleotide changes in the ITS1 region, including a very conserved 10 bp composite deletion. This makes it easy to setup a molecular assay to discriminate *S. ichnusae* from the close species *S. feltiae*. These new results show that this species is not endemic of Sardinia, as previously believed, and it might be widespread in other Mediterranean Countries as well.

Contributed paper. Thursday, 9:00. **211-STU**

Evaluation of entomopathogenic nematodes for control of the diapausing overwintering codling moth population

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In South Africa codling moth (CM) (*Cydia pomonella*) is the most important insect pest of apples and pears. During the winter months, from April to August, no fruit is on the trees, and the total CM population overwinters as diapausing larvae. During this period, entomopathogenic nematodes (EPNs) can be applied to reduce the number of emerging moths in the following season. The impact of aerial EPN application, and environmental conditions, on CM larvae mortality was investigated in an apple orchard. CM larvae were used to culture infective juveniles, used in the different field trials. As containment method, wire-mesh cages filled with apple tree bark and 20 last-instar CM larvae were used, while different nematode species and concentrations were used as treatments. The cages were kept moist, while temperature and moisture levels were recorded during 24 h in the field, after which they were retrieved, and the CM larvae removed and washed. After four days, infection was confirmed by dissection. Five *S. yirgalemense* concentrations and three nematode species (*Steinernema yirgalemense*, *S. feltiae* and *Heterorhabditis bacteriophora*) were investigated. *Steinernema yirgalemense* caused the highest level of mortality of CM larvae, with no significant difference being found between *S. yirgalemense* concentrations investigated.

Contributed paper. Thursday, 9:15. **212-STU**

A new entomopathogenic *Oscheius* (Nematoda: Rhabditidae) from Italian cave

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Specimens of nematode belonging to *Oscheius* genus was isolated through the *Galleria* bait method from soil collected

in a karst cave of Tuscany (Central Italy). Molecular and morphological analyses were performed. Total DNA was extracted from individual nematodes and the mitochondrial COI, the ITS containing region and the 18S rRNA gene were amplified and sequenced. BLAST search at NCBI discriminate this new taxon, similar to other *Oscheius*. This species belongs to Dolichura group. Cuticle is finely annulated, stoma is short and cheilorhabdion is simple, not well cuticularized. Female body is almost straight upon fixation, the reproductive system is didelphic and tail is short, conoid with pointed tip. Males are rare and similar to female in general morphology except for smaller size. Male body is straight when heat-killed, testis is single, ventral reflexed. They show peloderan bursa, tail short rounded and spicules slender and small. Infective Juveniles are slender with elongate tail and have stoma morphology similar to adult. The nematodes were cultured in Petri dishes on several substrates: Nutrient Agar, *Escherichia coli*, *Botritis cinerea*, meat baby food, without satisfactory results. Only Petri dishes method with *G. mellonella* larvae produced IJs, suggesting the entomopathogenicity of this new taxon.

Contributed paper. Thursday, 9:30. **213**

Genetic improvement of the entomopathogenic nematode *Heterorhabditis bacteriophora*

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Abstract-The entomopathogenic nematode *Heterorhabditis bacteriophora* has been genetically improved in beneficial traits, like heat and desiccation tolerance, by cross breeding and genetic selection. For instance, a final overall increase in mean heat tolerance of 5.5°C was achieved with *Heterorhabditis bacteriophora* by cross breeding the most tolerant five strains and then selecting for heat resistance. Success of breeding programmes largely depends on the heritability of the investigated traits. Advances in enhancement of desiccation and heat tolerance often have been lost again during mass production. For heterorhabditid nematodes methods have now been developed to stabilize the traits by selection of tolerant inbred lines. This technique provides a pathway to genetic improvement of commercial strains which will maintain the improved characters also during in vitro mass production. The methodology to produce stable inbred lines for steiner nematids needs further investigation, as these nematodes are amphimictic and production of inbred lines is much more laborious. The reproduction potential in liquid culture was also successfully increased. Future targets for genetic improvement are prolongation of shelf life and field persistence and enhancement of virulence.

Contributed paper. Thursday, 9:45. **214-STU**

Perspectives of new nematode formulation technology for biological control to pest insects in Georgia

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In the result of route investigations the soil samples for searching of entomopathogenic nematodes (EPNs) have been collected in several agroecosystems of different regions of Georgia. Samplings of testing material were done by using of

recent methods in insect nematology (Stock & Goodrich-Blair, 2012). According to preliminary data some active strains of *Steinernema* sp. have been obtained. EPNs extract efficiency was established on laboratory culture of *Galleria mellonella*. Further research directions for the identification of local strains (under the Project CRDF/DTRA/GRDF #GMG-01/13) have been conducted at the University of Arizona, laboratory of Entomology by two different ways: morphological and molecular diagnostic methods. It was established that four local EPN isolates belong to the genus *Steinernema*. Furthermore partial sequencing of the ITS rDNA gene revealed they are closely related to the species *Steinernema feltiae*. This conventionally called - "Georgian strain", considered as a raw material will be base for local production of bioformulation - "Geo-nema". Provided technological product - environmentally safe nematode insecticide will be used for biological control to the pest insects of agricultural crops and ornamental plants. The researches will be continued under the projects CRDF/STEP and SRNSF/STCU financial support. The usage of nematode insecticide will take an important place in IPM (integrated pest management) system for agricultural crop protection in Georgia.

CONTRIBUTED PAPERS Thursday, 8:00-10:00

Viruses 6

Contributed paper. Thursday, 8:00. **215**

Interactions between salivary gland hypertrophy virus and tsetse microbiota

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Many species of tsetse flies are infected by a hytrosavirus that causes Salivary Gland Hypertrophy (SGH) syndrome. Flies with SGH have a reduced fecundity and fertility. Due to the deleterious impact of the salivary gland hypertrophy virus (SGHV) on *Glossina pallidipes* colonies, several approaches have been investigated to develop a virus management strategy including the exploitation of endogenous microbiota. Tsetse flies harbor three symbiotic bacteria (*Wigglesworthia glossinidiae*, *Sodalis glossinidius* and *Wolbachia*) in addition to trypanosome, the causative agent of sleeping sickness disease in human and nagana in livestock. The interaction of the tsetse microbiota (gut bacteria and symbionts) with the SGHV and / or trypanosome is largely unexplored. In the present study, we show that ampicillin treatment of *G. pallidipes* impedes the transgeneration transmission of the SGHV suggesting the involvement of tsetse microbiota in the virus transmission. Quantitative-PCR analysis of the levels of SGHV and *Wolbachia* in wild tsetse flies (mainly *G. morsitans morsitans* and *G. austeni*) clearly indicated a negative interaction between SGHV and *Wolbachia*: flies heavily infected with *Wolbachia* presented significantly low viral titers. In addition, injection of GpSGHV into different *Wolbachia*-infected *Glossina* species did not result to the transgeneration transmission of SGHV as normally occurs in *G. pallidipes* colony, which is free of *Wolbachia*. Taken together, these data

suggest that *Wolbachia* may interfere with the establishment and transmission of this important DNA virus (SGHV), which represents a major hurdle for the application of SIT strategies for the control of tsetse flies and trypanosomosis in sub-Saharan Africa.

Contributed paper. Thursday, 8:15. **216-STU**

Mechanisms of tree-top disease induced by the specialist baculovirus SeMNPV

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Many parasites alter host behavior to enhance their transmission or survival. An intriguing example is the altered behavior of insect larvae infected by a baculovirus, e.g. their movement to elevated positions. This phenomenon (tree top disease or Wipfelkrankheit) is already known for over a century. However, the underlying mechanisms leading to this behavioral adaptation are still largely enigmatic. Here we studied tree-top disease induced by the baculovirus *Spodoptera exigua* multiple nucleopolyhedrovirus (SeMNPV) in *S. exigua* larvae. We show that infected *S. exigua* caterpillars all climb to elevated positions prior to death. Furthermore, we investigate the role of the ecdysteroid UDP-glucosyl transferase (*egt*) gene from SeMNPV in tree-top disease. This gene is known to be important in tree-top disease in another baculovirus-host system, although the mechanism by which it exerts this effect is unknown. We hypothesize that the SeMNPV *egt* gene may directly trigger tree-top disease or induce this phenomenon indirectly by prolonging the larval time to death.

Contributed paper. Thursday, 8:30. **217**

Temporal proteomics to study virus infection and function in the host cell

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Invertebrate iridescent virus 6 (IIV-6) is a nucleocytoplastic virus with a 212 kb-long linear double-stranded DNA genome that encodes 215 putative open reading frames. The IIV-6 virion proteome consists of at least 54 virally-encoded proteins. One of our previous findings showed that most of these proteins are encoded by genes from the early transcriptional class. This indicates that these structural proteins may not only function in the formation of the virion, but also in the initial stage of viral infection. In the current study, we followed the protein expression profile of IIV-6 over time in *Drosophila* S2 cells by label-free quantitation using nanoLC-FTMS. A total of 95 viral encoded proteins were detected in infected cells, of which 37 are virion proteins. The expressed IIV-6 virion proteins could be categorized into three main clusters based on their expression profiles. These clusters were: 1) proteins with stably low or 2) exponentially increased expression levels during infection, and 3) proteins that were initially highly abundant, and then showed slightly reduced levels after 48 hours (h) post infection (p.i.). The study supported that temporal expression patterns did not share direct correlation with protein expression classes

phenomena, suggesting that both proteomic and transcriptomic approaches will be required to obtain a detailed understanding of the viral expressomics (infectome). Here, we provide novel information on the kinetics of virion and infected cell-specific protein levels that assists in understanding gene regulation in this lesser known DNA virus model.

Contributed paper. Thursday, 8:45. **218**

Characterization of an atypical fast-killing ascovirus: *Spodoptera frugiperda* ascovirus 1d (SfAV-1d)

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Ascoviruses (AVs) are large double-stranded DNA viruses that attack lepidopterans, mainly noctuid larvae. One of the unusual features of AVs is their mode of transmission via parasitoid wasps. AVs are poorly infectious *per os* compared to other insect viruses such as baculoviruses and cypoviruses. Additionally, AV infection results in production of a characteristic milky-white hemolymph due to accumulation of virion-containing vesicles produced by a modified apoptotic response in infected cells. Virtually all ascoviruses cause a chronic disease wherein larvae survive for as long as 28 days after infection, which enables an extended period of transmission among larvae by wasps. Here, we report characterization of *Spodoptera frugiperda* ascovirus 1d (SfAV-1d) isolated from a *S. frugiperda* larva. SfAV-1d killed *S. litura* 4th instar larvae within 3 days when compared to another AV (SfAV-N), which took as long as 23 days to kill larvae. Larvae infected with SfAV-1d contained the characteristic white hemolymph. Electron microscopy revealed that both SfAV-1d and SfAV-N infected the fat body but not the tracheal matrix or other tissues. Interestingly, despite the difference in the rate at which SfAV-1d and SfAV-N killed larvae, there was no apparent difference in the kinetics of viral DNA replication. The primary difference between these two isolates was that SfAV-1d formed and accumulated virion-containing vesicles in the hemolymph much more rapidly than SfAV-N. Our future studies will focus on characterizing the genetic differences between these viruses to identify determinants that influence their pathobiology, particularly as it relates to rate of kill.

Contributed paper. Thursday, 9:00. **219-STU**

Two nucleopolyhedroviruses isolated from the genus *Adoxophyes* inhibit juvenile hormone (JH) esterase activity but not JH epoxide hydrolase activity

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Insect metamorphosis is predominantly regulated by two hormones, juvenile hormone (JH) and ecdysone. During the final instar, a dramatic decrease in JH titer is required for the induction of pupation. JH is metabolized by two enzymes, JH

esterase (JHE) and JH epoxide hydrolase (JHEH). *Adoxophyes honmai* (Lepidoptera: Tortricidae) is susceptible to two nucleopolyhedroviruses (NPVs), *A. honmai* NPV (AdhoNPV) and *A. orana* NPV (AdorNPV), which are genetically closely related but differ in killing speed. AdhoNPV kills the host only in the final instar, whereas AdorNPV kills more quickly (5 to 8 days). When 4th instars of *A. honmai* are orally inoculated at >LC₅₀ (1.0 x 10⁹ OBs/ml), AdhoNPV and AdorNPV prevent pupation and kill the host in 10 and 8 days, respectively. In contrast, mock-inoculated larvae pupate in 7 days. Baculoviruses are known to prevent pupation through endocrinological regulation. Here, we monitored both JHE and JHEH activities in AdhoNPV-, AdorNPV-, and mock-infected larva of *A. honmai*. Mock-infected larvae showed increased JHE activity in the hemolymph and fat body during the final instar, with the highest activity found on the 3rd day of the 5th instar. Both AdhoNPV- and AdorNPV-infected larvae did not show JHE activation. On the other hand, JHEH activity in fat body was constant and no differences were found between treatments. Our data suggest that AdhoNPV and AdorNPV prevent pupation by specifically down-regulating JHE but have no effect on JHEH activity. Our data also suggest that JH titers remain relatively high during the final instar of baculovirus infection.

Contributed paper. Thursday, 9:15. **220**

Mechanism underlying virus-induced hyperactive behavior: Substrate identification of the baculovirus protein tyrosine phosphatase

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Many parasites alter the behavior of their host to maximize their transmission and survival. However, the underlying mechanisms are largely unknown. Baculoviruses manipulate the behavior of their caterpillar hosts by inducing hyperactivity and climbing behavior. Previous work demonstrated that a protein tyrosine phosphatase (PTP) encoded by the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was involved in the induction of hyperactive behavior in *Spodoptera exigua* larvae. This finding prompted us to investigate which viral and/or host proteins interact with the baculovirus PTP enzyme and might be involved in altered host behavior. Using affinity-tag purification of a substrate-trapping mutant of AcMNPV PTP incubated with extracts of infected cells followed by proteomic analysis of the trapped protein, we identified six viral and six host proteins that co-purified with PTP. Several of these proteins are known to be important in cellular signaling and in behavior in other insects/organisms, and are therefore potentially involved in PTP-mediated hyperactivity of infected larvae. For one of these identified host proteins, the 14-3-3 ζ protein, RNA expression levels were significantly higher for AcMNPV wild type-infected larvae as compared to AcMNPV Δ ptp-infected larvae, indicating that 14-3-3 ζ expression levels are dependent on the presence of the baculovirus ptp gene. The 14-3-3 ζ protein is known to be important for the synthesis of serotonin and dopamine, which are neurotransmitters that play important roles in many behavioral pathways. It is hypothesized that baculovirus ptp targets 14-3-3 ζ at both the RNA and protein level, which consequently leads to baculovirus-induced hyperactivity.

Contributed paper. Thursday, 9:30. **221-STU**

The genome of a baculovirus isolated from *Lonomia obliqua* (Lepidoptera: Saturniidae) reveals a new transcription terminator factor possibly acquired from the host

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Lonomia obliqua (Lepidoptera: Saturniidae) is a poisonous larva of medical importance due to the severity of accidents occurred in Brazil caused by the contact of these larvae with the human skin. The possibility of controlling these populations is being evaluated by using pathogens such as a nucleopolyhedrovirus isolated from *L. obliqua*. In this work, we have sequenced the genome of the baculovirus *LoobMNPV* and analyzed its genomic composition and evolutionary history. The genome is 120,022 bp long, comprising 135 putative ORFs. Furthermore, in an evolutionary context, based on analysis that include the core gene from 93 sequenced baculovirus, *LoobMNPV* fell into a basal position related to the *Alphabaculovirus* group I (lepidopteran-infective NPV). Interestingly, one ORF showed significant identity (*e*-value equals to 3e10⁻¹¹) to a eukaryotic transcription terminator factor (TTF2) from the lepidoptera *Danaus plexippus* (GenBank: EHJ68439.1). On the other hand, when restricting this search only to baculoviruses, this ORF also demonstrated identity (*e*-value of 1e10⁻⁶) to the Global Transactivator (GTA) gene from *Antheraea pernyi* nucleopolyhedrovirus (Genbank: YP_611073.1). Phylogenetic analysis were performed with the TTF2 gene from various organisms, as well as with the GTA from baculovirus. These results indicated two hypothesis: (i) this gene may have been independently acquired from the host through horizontal transfer, acting as an inhibitor of the host's transcriptional machinery in order to benefit viral translation; (ii) or it is a divergent variation of the GTA gene that has undergone positive selection.

Contributed paper. Thursday, 9:45. **222**

The essential baculovirus protein VP1054 is a hijacked cellular PUR α , a nucleic-acid-binding protein specific for GGN repeats

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The baculovirus VP1054 protein is a structural component of both budded virus (BV) and occlusion-derived virus (ODV), but its exact role in virion morphogenesis is poorly defined. We reveal sequence and functional similarity between the baculovirus protein VP1054 and the cellular purine-rich element binding protein PUR-alpha (PUR α). The data strongly suggest that gene transfer has occurred from a host to an ancestral baculovirus. Deletion of the AcMNPV *vp1054* gene completely prevented viral cell-to-cell spread. Electron microscopy data showed that assembly of progeny nucleocapsids was dramatically reduced in the absence of VP1054. More precisely, VP1054 is required for proper viral DNA encapsidation, as deduced from the formation of numerous electron-lucent capsid-like tubules. Complementary searching identified the presence of genetic elements composed of repeated GGN trinucleotide motifs in baculovirus

genomes, the target sequence for PUR α proteins. Interestingly, these GGN-rich sequences are disproportionately distributed in baculoviral genomes and mostly occurred in proximity to the polyhedrin gene. At the same time they encode crucial proline-rich domains in p78/83, an essential gene adjacent to the *polyhedrin* gene in the AcMNPV genome. We further demonstrate that the VP1054 protein specifically recognizes GGN-repeats and are currently analyzing the significance of these GGN motifs for DNA packaging. Together, whilst some viruses like human immunodeficiency virus 1 (HIV-1) and human JC virus (JCV) utilize host PUR α protein, baculoviruses encode the PUR α -like protein VP1054, which is crucial for viral progeny production.

SYMPOSIUM (Special) Thursday, 8:00-10:00
DFG Priority Program
Host Parasite Coevolution

Symposium. Thursday, 8:00 **223**

Escaping parasite manipulation: Apoptosis and host-parasite co-evolution in *Apis mellifera*

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Programmed cell death (apoptosis) does not only play an important role in the development of multicellular organisms, but also in the protection against pathogens. Nevertheless, numerous intracellular pathogens have evolved diverse strategies to interfere with and overcome the apoptotic machinery of their hosts. Yet, little is known about the actual mechanisms and how hosts might counter act. We here study the interaction of the intestinal microsporidian parasite *Nosema ceranae* in a susceptible and tolerant honeybee host under laboratory controlled conditions, to understand the importance of apoptosis in this case of host-parasite co-evolution. We visualize apoptotic processes in the gut epithelium using TUNEL assays; relate this to the expression levels of key genes in the apoptotic cascade over the course of the infection, and consequences for metabolic energetics affecting honeybee performance.

Symposium. Thursday, 8:15 **224**

Overcoming external immunity: An increase in virulence as a result of host-parasite coevolution in *Beauveria bassiana*

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An increase in virulence is a trait often observed as a result of host-parasite coevolution. Specific immune responses overcome in order to achieve increased virulence can, however, be difficult to elucidate. We carried out a coevolution experiment with the red flour beetle, *Tribolium castaneum*, and the general entomopathogenic fungus, *Beauveria bassiana*. After just seven host generations of evolution we saw a substantial increase in virulence in all evolved isolates of *B. bassiana*. Furthermore, we were able to show that this increase in virulence was a result of the *B. bassiana* isolates evolving resistance to the external immune defences of the *T. castaneum* beetles, who are able to secrete antimicrobial compounds into their environment. This is a rare example of a virulence increase seen as a result of a coevolution experiment where the exact barrier of host immune defence that the parasite has gained resistance to in order to achieve the increase in virulence has been described.

Symposium. Thursday, 8:30 **225**

Rapid adaptation of *Bacillus thuringiensis* to its nematode host *Caenorhabditis elegans*

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Antagonistic interactions between host and pathogen can produce very high selection intensities. They are often one of the main driving forces during evolution, especially if the interactions persist across time. We specifically assessed the evolutionary impact of these interactions for the pathogen, using evolution experiments with the Gram positive biocontrol agent *Bacillus thuringiensis* and one of its animal hosts *Caenorhabditis elegans*. Our results demonstrate that differences in the experienced selection conditions during the evolution experiment favour distinct characteristics across the pathogen life cycle: (i) pathogen adaptation to a co-evolving host associates with high virulence; (ii) pathogen adaptation to a non-changing host increases infection load; whereas (iii) adaptation without host favours environmental persistence. Concomitant genomic changes in the pathogen were observed at two levels: (i) the different evolution conditions caused clonal selection of distinct, broad-scale genotypes, while (ii) one of these with high virulence showed additional nucleotide changes, including copy number variations of nematocidal toxin genes. Based on one of the most comprehensive data sets collected for an experimentally evolved pathogen, we conclude that sustained coevolution is distinct from other types of selective constraints in shaping pathogen genome and life-history characteristics. Surprisingly, our findings also suggest that sustained virulence, as desired for pest control, may be contingent on the unwanted co-adaptation of the target host.

Symposium. Thursday, 8:45 **226**

Intra-host parasite interactions between co-infecting *Bacillus thuringiensis* strains

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Hosts and parasites are expected to influence each others evolution due to antagonistic interactions, potentially leading to host-parasite coevolution. However, many studies focus on the interactions between hosts and parasites, ignoring that within one host different parasite genotypes may interact and may thus feed-back on the coevolution between host and parasite. The interactions between parasite genotypes may range from competition between genotypes for limited host resources to cooperation for more efficient host exploitation. Using *Caenorhabditis elegans* as host and the bacterial microparasite *Bacillus thuringiensis* we found indications for diverse interaction strategies between the bacteria, ranging from public good to spiteful bacteriocin production. However, it remains unclear how stable these strategies are over the course of time, i.e. when hosts have to be repeatedly infected and when hosts may also adapt to these parasite strategies. For this reason, we performed a laboratory-based selection experiment in which either single *B. thuringiensis* genotypes or a mixture of strains coevolved with hosts. After 10 host generations, we found differences between the evolution treatments. Most interestingly, mixed infections strongly lost virulence. Whether this is caused by a trade-off between host-exploitation and bacterial competition or by division of labour between bacterial clones remains to be shown. Importantly, these results have strong implications for epidemiology, since the evolution of bacteria and its consequences for the host depend on the multitude of infection.

Symposium. Thursday, 9:00 **227**

Experimental evolution *in silico*: host-parasite coevolution versus parasite adaptation

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Bacillus thuringiensis is a widely distributed natural pathogen of invertebrates and plays an important role in (agricultural) pest management. The bacteria kill the host by CRY toxins and other virulence factors. Recent experimental studies on the evolution of virulence revealed that one-sided adaptation of *B.t.* with non-evolving hosts (*Caenorhabditis elegans*, *Tribolium castaneum*) selects for intermediate or no virulence, sometimes coupled with parasite extinction. In contrast, host-parasite co-evolution selects for high virulence and for hosts with strong resistance against *B.t.* However, a sound theoretical explanation is missing. Here, we propose a new mathematical model that mimics the experimental set-up. We consider two bacterial strains, a virulent "toxin producer" and an avirulent "non-toxin producer". Bacterial evolution is modeled as an iterated process of intra-host dynamics and bacterial transmission between hosts. The intra-host dynamics are described as a two-phase process, where the

first phase covers the period from beginning of infection until host death and the second phase the period from host death until depletion of host resources. Increase in host resistance is simulated by extending the first phase. Our model analysis revealed, in general, the same basic trends as the above-mentioned experimental studies. Especially, we could show that resistant hosts select for highly virulent bacterial strains. Moreover, we found (1) that the evolved level of virulence is independent of the initial level of virulence, and (2) that the bacterial dosage significantly affects the evolution of virulence with low dosage selecting for highly virulent strains. These predictions can be tested in future experiments.

Symposium. Thursday, 9:15 **228**

Immune priming with *Bacillus thuringiensis* in *Tribolium castaneum*

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There is accumulating evidence for a memory-like phenomenon in the immune defence of invertebrates. Such 'immune priming' can be rather specific, and might be transmitted from parents to offspring. Invertebrates do not possess the machinery of the vertebrate adaptive immune system, and the mechanistic underpinnings of immune priming are still largely unknown. In the red flour beetle *Tribolium castaneum*, immune priming for resistance against the entomopathogen *Bacillus thuringiensis* has been demonstrated, both within and across generations. Immune priming arose after septic 'pricking' as well as oral pathogen exposure. Moreover, not only mothers, but also fathers were able to transmit such resistance to their offspring. In this talk I will present our recent approaches to deepen our understanding of the evolutionary relevance and mechanistic underpinnings of immune priming in this host-pathogen system.

Symposium. Thursday, 9:30 **229**

Rapid reciprocal adaptation between the red flour beetle and *Bacillus thuringiensis* bacteria during experimental coevolution

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The antagonistic interaction between hosts and parasites is a powerful evolutionary force that may drive rapid evolutionary adaptation. It can lead to coevolution by reciprocal adaptation and counter-adaptation of hosts and parasites. However, in natural populations, it is very difficult to exclude other selective forces that may influence the interaction and to identify true coevolution. We thus performed experimental coevolution in the laboratory between the red flour beetle and *Bacillus thuringiensis* bacteria. We made use of an experimental design that included control treatments in which either of the antagonists was allowed to adapt to a non-evolving host or parasite, respectively, and we also controlled for a possible adaptation to laboratory conditions. We here report on evolved differences in the phenotypes of host and parasite,

and in particular an observed increase in parasite virulence and host resistance. Moreover, we found a potential for parasite local adaptation under coevolution.

Symposium. Thursday, 9:45 **230**

Means of fast virulence adaption: the plasmid and prophage equipment of selected *Bacillus thuringiensis* strains

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Strains of *Bacillus thuringiensis* (Bt) are used since decades as pest control in crop protection. A descriptive feature of the species is the existence of paracrystal bodies, which consist of δ-endotoxins, acting against specific classes of invertebrates. Over the years a solid amount of research has been achieved on the activity of δ-endotoxins on invertebrates as well as on the diversity of cry-toxin genes. In contrast surprisingly little is known on the genomic loci which encode this diversity of δ-endotoxins. Furthermore the knowledge on other invertebrate virulence factors encoded by Bt as well as on host adaptation factors is rather fragmentary. The observation of phenotypes that differ between strains indicates that they are encoded within the pan-genome of *Bacillus thuringiensis*. Since a pan-genome consists of the genes that are not shared by all members of species many of them are encoded on strain specific extra chromosomal elements. Here we present a comparative analysis of more than 40 extra chromosomal replicons such as plasmids and prophages of three nematocidal and two insecticidal Bt strains.

signalling peptides PapR, NprX and Phr, respectively. Altogether our results indicate that these three cell-cell communication systems, acting sequentially, coordinate virulence and adaptive properties with the general physiology of the bacterial cells. The PlcR-PapR complex induces the production of virulence factors allowing the bacteria to kill the insect. NprR-NprX activates transcription of genes allowing the bacteria to switch from a virulence state to that of survival in the host cadaver. Ultimately, the inhibition of the Rap proteins by the Phr signalling peptides triggers sporulation, thus allowing the bacteria to disseminate and to persist in the environment.

Symposium. Thursday, 14:30. **232**

The interplay of *Paenibacillus larvae* with honey larvae during infection

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Honey bees are attacked by numerous pathogens, some of them just causing covert infections others causing overt disease symptoms and even death of individuals and entire colonies. Among the latter group is the bacterium *Paenibacillus larvae*, the etiological agent of the epizootic American Foulbrood of honey bees (AFB). As the name suggests, AFB is a bacterial disease affecting only the larval stages of honey bees. *P. larvae* is an obligate killer because death of larvae and conversion of larval biomass into bacterial biomass are prerequisites for disease transmission within and between colonies. Hence, *P. larvae* must have evolved effective means to attack larvae, to circumvent the larval immune response and to finally kill and decompose larvae. We recently identified and characterized some of these virulence factors of *P. larvae*. We will present a model for molecular pathogenesis of *P. larvae* infections built upon these novel findings in order to further the understanding of the molecular basis of pathogen-host-interactions in American Foulbrood disease.

Symposium. Thursday, 15:00. **233**

Antimicrobial defense and persistent infection in insects revisited

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Antimicrobial peptides are mainly produced and used by multicellular organisms such as insects to interact with pathogenic and mutualistic micro-organisms. Antibiotics are mostly produced by single cell eukaryotes and bacteria. Here we provide a possible explanation for this dichotomy. Our hypothesis is based on the observation that antibiotics elevate bacterial mutation rates and we show that AMPs do not elevate bacterial mutation rates. Nevertheless we also found that bacterial resistance evolves readily against single AMPs *in vitro*, but the situation is already more complicated by the simultaneous action of two AMPs. I will contextualize these findings in the light of the immune responses of the beetle *Tenebrio molitor* and will use these findings to discuss some of the multiple roles AMPs have in host-microbe interactions: policing and killing.

SYMPORIUM 8 (Cross-Divisional) Thursday, 14:00-16:00

Host – Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing

Symposium. Thursday, 14:00. **231**

The *Bacillus thuringiensis* way of life: communicate to kill and survive in the insect host

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At the end of exponential growth, bacteria of the *Bacillus cereus* group (*i.e.* *B. thuringiensis* and *B. cereus*) produce virulence factors allowing the bacteria to invade their host. In the insect gut, genes controlled by the PlcR quorum sensor allow the bacteria to damage the intestinal barrier and to gain access to the haemocoel. After the death of the insect, PlcR activates transcription of a gene encoding a second quorum sensor, NprR. NprR induces production of degradative enzymes and of a biosurfactant allowing the bacteria to survive in the insect cadaver and eventually to sporulate. The development of the sporulation process is controlled by the master regulator Spo0A, whose activity is regulated by Rap proteins. PlcR, NprR and Rap are quorum sensing regulators belonging to the RNPP family. Their activity depends on the

Symposium. Thursday, 15:30. **234**

Vibrio and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes

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Vibrio tasmaniensis LGP32 is a *V. splendidus*-related strain pathogenic for *Crassostrea gigas* oysters. We recently showed that LGP32 invades the oyster immune cells, the hemocytes, through phagocytosis. Oyster hemocytes are professional phagocytes harboring microbial activities including a potent oxidative response. Interestingly, the phagocytosed LGP32 survives inside the oyster hemocytes, evading the host defense by preventing acidic vacuole formation and limiting reactive oxygen species production. When hemocytes were invaded by numerous LGP32, we observed cytotoxic effects such as membrane disruptions and cytoplasmic disorders. Cytotoxicity was shown to entirely depend on LGP32 entry into hemocytes, as cytochalasin D was sufficient to inhibit hemocyte death. By developing a transcriptomic approach based on RNA sequencing, we identified a series of *Vibrio* antioxidant genes whose expression is strongly induced within oyster hemocytes. We also observed an overexpression of genes involved in cation efflux. Overexpression of these molecular functions in the intraphagosomal stage was confirmed by RT-PCR. To determine how far those LGP32 genes are involved in resistance to intracellular killing and subsequent virulence, we constructed isogenic deletion mutants for two overexpressed antioxidants and two overexpressed cation transporters. Those mutants were phenotyped for intracellular multiplication, cytotoxicity and virulence in oyster experimental infections. Our data show that resistance to reactive oxygen species and efflux of cations are two important functions required for LGP32 intracellular survival, cytotoxic effects and virulence.

Vuillemin (Ascomycota: Hypocreales) is known to survive as an endophyte in a wide range of plants and offer protection against an increasing number of insect pests. Although recent discoveries suggest that the fungus can also protect plants against plant pathogens, no studies are currently available on the efficacy of endophytic *B. bassiana* against plant viruses. We conducted experiments to determine whether endophytic *B. bassiana* could provide protection against Zucchini Yellow Mosaic Virus (ZYMV), one of the most economically important diseases of cucurbits worldwide. Four selected *B. bassiana* strains were able to successfully colonize squash plants following the foliar inoculation of plants with the conidial suspension of each respective strain. Disease incidence and severity, sampled weekly following the challenge inoculation of plants with ZYMV, were significantly lower in *B. bassiana*-inoculated plants as compared to control plants; irrespective of the *B. bassiana* strain being inoculated. Our study demonstrates, for the first time, that endophytic *B. bassiana* has the biocontrol potential for managing plant viruses. Further studies should be conducted to determine whether such endophytic *B. bassiana*-mediated protection against ZYMV in squash extends to other cucurbits.

Contributed paper. Thursday, 14:15. **236**

Bean plant *Phaseolus vulgaris* endophytically colonized by *Beauveria bassiana* and *Hypocrea lixii* acquires protection against *Liriomyza huidobrensis* (Diptera: Agromyzidae) in the field

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Field trials were carried out for two cropping seasons in two sites (Sagana and Narumoro, Central province of Kenya) to evaluate the prospects of endophyte isolates of *Beauveria bassiana* and *Hypocrea lixii* for the control of leafminer *Liriomyza huidobrensis* in *Phaseolus vulgaris*. Autodissemination device treated with conidia of *Metarhizium anisopliae* was also added as a treatment. The effects of endophytes on leafminer infestation (punctures and mines), number of pupae and parasitoids, and yield were evaluated. Both isolates successfully colonized different parts of *P. vulgaris* plants; however, colonization was greater with *H. lixii* than *B. bassiana* in both sites. Leafminer infestation was not significantly different during the first season while it was higher in the controls than in endophyte treatments at both sites during the second season. The number of pupae varied between 150-250 and 320-400 in endophyte and control treatments, respectively, during the first season; and from 100-200 and 350-500, respectively, in endophyte and control treatments during the second season. The number of parasitoids that emerged from pupae did not differ significantly among the treatments. Higher yield was obtained in endophyte than in control treatments. With exception to yield during season two, the inclusion of autodissemination device treatment did not have significant effect on all the parameters evaluated. There were no significant differences between the fungal isolates. Results of the present study suggest that both endophyte fungal isolates hold potential and could be considered for the control of leafminer. There is the need however to confirm these results on large-scale trials.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

MICROBIAL CONTROL 4

Contributed paper. Thursday, 14:00. **235**

Establishing the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cucurbits for managing Zucchini Yellow Mosaic Virus (ZYMV)

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The fungal entomopathogen *Beauveria bassiana* (Balsamo)

Contributed paper. Thursday, 14:30. **237**

Colonized plants with entomopathogenic fungi produce mortality in *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae

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This study aimed to evaluate the ability of entomopathogenic fungi to colonize endophytically plants for *Spodoptera littoralis* (Boisduval) (Lepidoptera, Noctuidae) larvae control, which is a polyphagous insect of economic importance with a wide range of host plants. The five isolates of *Beauveria* (3) and *Metarhizium* (2) (Ascomycota, Hypocreales) were able to colonize endophytically tomato (*Lycopersicon esculentum* Mill), melon (*Cucumis melo* L., hybrid F1 Galia) and alfalfa (*Medicago sativa*) plants. The tissues colonization of the evaluated plants was determined by the fungus re-isolation of leaves, stem and roots. Two fungal strains, EAMb 09/01-Su and Bb04, showed an increasingly colonization presenting from 4.0 to 24.3 % of colonization of the root tissues by 24 to 96h, and 43.3 to 98.0 % of stem and leaves by 24 to 72h. The potential of this fungus as a mycoinsecticide for the control of *S. littoralis* was also evaluated in present study. In the first step, the larval mortality was determined after topical application of conidial suspension of higher virulent isolates, which showed mortality percentage of 41.6% for EAMb 09/01-Su and 76.6% for EABb 01/33-Su. The ingestion by larvae of alfalfa leaves colonized endophytically showed a significant larval mortality by 25.0% and 31.6% respectively. No differences in leaf consumption between treatments and controls were found, so the possibility of a repellent or a feeding deterrence effect is not appreciated. In conclusion, this study provides evidence for the ability of fungi to colonize internal tissues of tomato, melon and alfalfa, as well as to control *S. littoralis* larvae.

Contributed paper. Thursday, 14:45. **238**

***Beauveria bassiana* and California strawberries: endophytic, mycorrhizal, and entomopathogenic interactions**

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A greenhouse study in 2010 showed that *Beauveria bassiana* colonized strawberry plants and persisted in various tissues for up to 9 weeks. Another greenhouse study in 2013 evaluated the impact of soil and foliar applications of *B. bassiana* on green peach aphid, *Myzus persicae* feeding on potted strawberry plants. Small plot and field studies in 2013 and 2014 indicate that root or soil treatment with *B. bassiana* promotes the strawberry plant growth and development. Treating the roots of the strawberry transplants with *B. bassiana* before planting significantly improved the plant health compared to untreated control and a microbial plant growth enhancer in a 2013 study. Preliminary data from a field study that is currently under investigation also indicate a positive impact of soil treatment of *B. bassiana* on plant growth. Plant canopy is larger in treated plants compared to the grower standard practices alone. A large strawberry field study in 2013 demonstrated the role of *B. bassiana* in strawberry IPM. Results of various studies will be discussed in exploring the role of entomopathogens in pest management and promoting plant development.

Contributed paper. Thursday, 15:00. **239**

Perceptions, trust, terminology and influence: What do consumers think about biological control?

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Those of us who work in the field of biological control (in the broadest sense, including GM crops and micro-organisms) inherently believe these practices to be environmentally benign and significantly safer than conventional alternatives. We tend to engage in terminology describing the science, even when communicating with the general public, although many are unfamiliar with the field and may have only a rudimentary understanding of the concepts and science behind these technologies. Yet if we are to promote use beyond 'niche' markets and advance broader acceptance, technological developments aside, we need to fully engage the public as key partners driving change. Greater understanding of the consumer mind-set allows us to communicate concepts more effectively, and to potentially use biocontrol to positively influence purchase decisions. Here, data from several consumer studies will provide insights into general perceptions of biological control and how these are influenced by trust in science and technology; considerations when phrasing pest management practices to communicate information to consumers; and effects of pest management practices on the likelihood of consumers' purchasing floral or edible crops grown using different pest management practices. Within the parameters of the study, price and pest management practice were consistently the most important factors influencing consumer purchase intention. Findings highlight the importance of using every-day terms when engaging the general public, but also clearly show that there are opportunities to positively influence peoples' choices for products grown using 'natural' methods – as long as we can talk with them in a language they understand.

Contributed paper. Thursday, 15:15. **240**

A phylogenetic survey of protistan parasites

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The advent of molecular biology techniques has shown that parasitism has evolved many times in protists and that all of the eukaryotic supergroups contain several major radiations of parasites. It is hypothesised that much of the vast diversity revealed by environmental sequencing studies also derived from so far uncharacterised parasites; evidence in support of this hypothesis is growing. Some parasitic lineages are relatively well known and the subject of research foci, both at the level of individual taxa and of emerging groups that are being studied for their evolutionary interest. However, other lineages, although known to harbour a significant diversity of parasites, are rarely studied or factored into ecological and parasitological studies of potential hosts. This talk will review the diversity of parasites across the eukaryotic tree of life as a whole, and point to groups that are perhaps worthy of

increased attention and vigilance, as well as underlining the range of parasites expected in many systems.

Contributed paper. Thursday, 15:15. **241**

Bacillus thuringiensis toxins vs baculovirus: differential induction of immune system related genes in

Spodoptera exigua

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Spodoptera exigua Hübner is a polyphagous pest native from Asia that has been spread worldwide. It is a major threat not only for field or flower crops, but also for greenhouse vegetable cultivations. To reduce losses due to *S. exigua* damage, growers often opt for biological control, such as using insecticidal products based on *Bacillus thuringiensis* Berliner (Bt) or baculovirus. Both pathogens act by ingestion and lead to insect death within few days. To counteract the infection, *S. exigua* relies on its immune system response, and production of antimicrobial peptides (AMPs) and proteins is an important part of the innate immune defense cascade triggered by pathogens. In this study, *S. exigua* transcriptome was mined for the presence of unigenes encoding for AMPs and lysozymes, resulting in the identification of a wide and diverse spectrum of these types of defense molecules. Then we compared their transcript abundance in larval midguts after ingestion of different Bt toxins (such as Cry1C and Vip3Aa) or *S. exigua nucleopolyhedrovirus* (SeMNPV) occlusion bodies. Results showed that both Bt proteins triggered a similar pattern of response, which included the specific overexpression of around 80% transcripts tested. In contrast, after SeMNPV ingestion, expression of AMPs decreased or did not change. The possible meaning of *S. exigua* physiological response to different pathogens employed in biological control is discussed.

expression of various target genes classified various categories. Indeed, the viral H4 can join to a nucleosome in *in vitro* reconstruction assay. A chromatin immunoprecipitation (ChIP) assay indicates that the viral histone H4s are located at AT-rich regions near to the inducible genes, such as immune, detoxification, and metabolism. The truncated viral histone H4 loses almost inhibitory activity on host immunity. A series of truncated mutants or point mutations at Lys indicate that a specific Lys at 6th from N terminal is crucial to exhibit its epigenetic control of host immunity.

Contributed paper. Thursday, 14:15. **243**

Heat-shock protein 90 is a broadly active regulator for baculovirus infection

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Cellular chaperon Hsp90 plays important roles in diverse biological processes, including signal transduction, protein folding and trafficking, etc. Many viruses, including HCV, HSV and Influenza virus, are dependent on host Hsp90 either for efficient replication or proper intracellular transfer. A recent proteomics study revealed that Hsp90 is incorporated into the budded virions (BVs) of baculovirus, we therefore investigated the role of Hsp90 in the life cycle of baculovirus. By using Hsp90 inhibitor geldanamycin (GA) and RNA interfering, the levels of viral DNA replication, infectious BV production, as well as ODV and polyhedra morphogenesis of baculoviruses were significantly reduced in AcMNPV infected cells. Further studies demonstrated that GA inhibited the expressions of certain viral proteins at transcriptional levels. The nuclear imports of several nucleocapsid- and ODV envelope proteins were also hindered by GA. Interestingly, when the function of Hsp90 was disturbed by GA, virus-triggered nuclear F-actin network essential for assembly of progeny AcMNPV was absent. Taken together, our data suggest that Hsp90 regulates baculovirus replication and morphogenesis from at least three different aspects: 1) promoting the expression of viral proteins; 2) facilitating the intracellular trafficking of viral structural proteins; 3) participating in the nuclear polymerization of host actin which is required for progeny baculovirus production.

CONTRIBUTED PAPERS Thursday, 14:00-16:00

VIRUSES 7

Contributed paper. Thursday, 14:00. **242**

Lysine residues in N-terminal tail of a viral histone H4 are crucial in controlling host gene expression

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An endoparasitoid wasp, *Cotesia plutellae*, parasitizes young larvae of the diamondback moth, *Plutella xylostella*. Parasitized larvae undergo significant immunosuppression and developmental alteration. Various parasitic factors have been identified from a polydnavirus, *C. plutellae* bracovirus (CpBV), and teratocytes. A viral histone H4 is identified from CpBV episomal genome. It encodes 141 amino acid residues and shares high sequence homology (82.5%) with host histone H4. Its extended N-terminal region (38 residues) contains 9 Lys residues. Pull-down assay showed that CpBV-H4 interacted chromatin remodeling apparatus, such as SWI/SNF complex. Subtractive suppressive hybridization showed that its expression in nonparasitized host alters the

Contributed paper. Thursday, 14:30. **244**

Development and immunity-related microRNAs of the lepidopteran model host *Galleria mellonella*

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MicroRNAs (miRNAs) are small non-coding RNAs which have been recognized as key elements in the regulation of protein synthesis at the post-transcriptional level. Our knowledge about their function in regulating complex physiological processes is limited, but rapidly expanding. The larvae of the greater wax moth *Galleria mellonella* have emerged as a powerful and surrogate model hosts for pathogens capable of infecting insects or humans. Complementary to our previously published comprehensive *G. mellonella* transcriptome, here we screened development and immunity-related miRNAs in order to further advance the suitability of this model host. To screen for miRNAs that are differentially expressed in *G. mellonella* either during metamorphosis or upon natural infection with entomopathogenic bacteria or fungi we designed

a microarray spotted with probes of more than two thousand miRNA sequences known from insects. Relative to untreated last instar larvae which were used as a reference, we determined numerous miRNAs to be expressed in prepupae (1037), pupae (981) or pathogen infected last instar larvae (965). Taking advantage of our transcriptomic data base, we were able to identify potential 3' UTRs for determining miRNA-mRNA duplexes by considering both base pair complementarity and minimum free energy (MFE) hybridization. We confirmed the co-expression of selected miRNAs such as miR-71, miR-263a, miR-236b, and their predicted target mRNAs in *G. mellonella* by RTPCR. This is the first study addressing the identification of miRNAs which are predicted to regulate genes that are expressed during metamorphosis or in response to infection of the lepidopteran model host *G. mellonella*.

Contributed paper. Thursday, 14:45. **245**

The sf122 gene of *Spodoptera frugiperda* nucleopolyhedrovirus modulates key aspects of insect-to-insect transmission and post mortem host liquefaction

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The *sf122* gene present in the longest genotype (SfMNPV-B) of the Nicaraguan isolate of *Spodoptera frugiperda* multiple nucleopolyhedrovirus (SfMNPV) was previously identified as undergoing positive selection. A recombinant virus (Sf122null), lacking *sf122*, was generated by homologous recombination from a bacmid comprising SfMNPV-B. Transcriptional analysis revealed that *sf122* is a late gene. Sf122null DNA was two-fold less infective when injected into *S. frugiperda* larvae and occlusion bodies (OBs) of the deletion recombinant were 15-fold less pathogenic (in terms of 50% lethal concentration), speed-of-kill was slower by 20 hours and OB production was reduced 3-fold, compared to the parental virus. The infectious titre of occlusion derived virions (ODVs) of Sf122null was reduced by >100-fold compared to that the parental or *sf122*-repaired viruses. OBs from each virus did not differ significantly in DNA content or gross morphology. Larvae that died from Sf122null infection did not show liquefaction. Similarly, SfMNPV isolates from the United States and Colombia, containing the shorter variant of the protein, only produced partial larvae liquefaction post mortem. Finally, expression of the *chitinase* and *cathepsin* genes was significantly reduced in larvae infected with the Sf122null virus. We conclude that positive selection on the *sf122* gene is most likely related to its marked role in modulating larval liquefaction and virus transmission.

Contributed paper. Thursday, 15:00. **246**

Effect of a Viral Encoded Protein Kinase on Gene Expression in *Amsacta moorei* Entomopoxvirus Infected Cells

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Insect-born entomopoxviruses (EPVs, Family: *Poxviridae*) are potentially significant biotechnological tools. In comparison to some other insect viruses, the function of relatively few EPV gene has been characterized. In this study, a serine/threonine (Ser/Thr; ORF AMV197) protein kinase gene of the *Amsacta moorei* entomopoxvirus (AMEV, type species of *Betaentomopoxvirus*) was characterized in terms of regulation of expression relative to some other AMEV genes. A recombinant virus (AmΔPK/gfp) was constructed by deleting ORF197 from AMEV genome via homologous recombination. Transcription of wild type virus and recombinant virus genes was compared by whole-genome gene expression microarray. The results showed that the expression levels of 126 genes representing 55.7% of all the viral genes were impacted significantly in the deletion mutant virus. Of these, 88 (69.84 %) transcripts were up-regulated and 38 (30.15 %) were down-regulated. Specifically, transcripts responsible for DNA repair, replication, nucleotide metabolism, and transcription and RNA modification were up-regulated in AmΔPK/gfp-infected cells. The results of this study indicate that the product of AMV197 may have significant effects on the assembly and/or infectivity processes of progeny viruses. However, more detail experiments are necessary to identify the exact role of this gene in AMEV replication.

Contributed paper. Thursday, 15:15. **247**

FP25K acts as a negative regulator in the infectivity improvement of AcMNPV Budded viruses

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Baculoviruses can produce two phenotype virions in the replication cycle, the budded virus (BV) and the occlusion-derived virus (ODV). The regulation of forming these two phenotypes virions is an important process in infection, but the mechanism is still unclear. The *fp25k* gene was reported to be responsible for the regulation of BV/ODV formation. The gene mutation results in a decreased number of normal ODV and an increased production of BV. In this study, we unraveled the mechanism of improved infectivity of *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) BVs by *fp25k* deletion. The investigation of BV titer, copy number of BV genome and electro-microscopy observation indicated that the increase of BVs titer of the *fp25k* knock out recombinant is a result of higher infectivity of virions but not the amount of BVs. The identification of protein associated to the virions showed that more BV envelope protein was incorporated into the gene knock out recombinant BVs. However, the infectivity of BVs was confirmed be not increased when GP64 was over expressed in our study. From the transfection and transformation of BV genome DNA into insect cells and *Escherichia coli*, the results suggested that better integrity genome DNA was packaged in the *fp25k* knock out recombinant BVs. Our study proposed that FP25K is a multifunctional protein in baculovirus life cycle. The virus genome with better integrity might be the major reason of infectivity enhancement and FP25K acts as a negative regulator in this process.

Contributed paper. Thursday, **248**

The leucines in the transmembrane domain of *Autographa californica nucleopolyhedrovirus* Ac76 are important for intranuclear microvesicle formation

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Our previous study has shown that the *Autographa californica nucleopolyhedrovirus* (AcMNPV) *ac76* gene is essential for both budded virion (BV) and occlusion-derived virion (ODV) development. However, the exact role by which *ac76* affects virion morphogenesis remains unknown. In this report, the oligomerization status of Ac76 was investigated and its critical amino acids for intranuclear microvesicle formation were identified to further understand the functional role of Ac76 in virion morphogenesis. Ac76 contains an α -helical transmembrane domain (TM), and phase separation showed that it is an integral membrane protein. In AcMNPV-infected cells, Ac76 was detected as a stable dimer that was resistant to SDS and thermal denaturation, and only a trace amount of monomer was detected. A co-immunoprecipitation assay demonstrated the dimerization of Ac76 by high-affinity self-association. Covalent cross-linking results showed that higher-order oligomers of trimer, tetramer, hexamer and octamer as well as the stable dimer were detected in virus-infected cells. Bioinformatic analysis suggested that the leucine- and isoleucine-rich sequence in the TM helix of Ac76 likely forms a leucine/isoleucine zipper to mediate the helix-helix interaction of Ac76 with itself. A recombinant virus in which L²⁶, L²⁹ and L³³ in the TM of Ac76 were all substituted with alanines was constructed. Analysis of the mutant revealed that the leucines in the TM of Ac76 are important for infectious BV production and normal-appearing intranuclear microvesicle formation.

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Contributed paper. Thursday, 15:30. **249**

High-throughput purification of dsRNA against sacbrood virus disease in honey bees *Apis cerana* (Hymenoptera: Apidae)

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The importance of honey bees to the world economy does not hang on bee products, but pollination of 80% food crops. However, like other animals, honeybee is inevitably subject to infection by a wide variety of pathogens that are responsible for significant colony losses. Sacbrood virus (SBV) is a serious hazard disease to honey bees (*Apis cerana*). Relying heavily on chemical agent for the control of this disease, problems of resistance and pollution are perplexing beekeeping. Therefore, beekeeping calls for environmentally friendly technology of disease management, especially the antiviral bee breeding. Using RNA interference technology is a cost-effective approach for disease bio-control. To address this issue, large-scale and pure dsRNA is in great need. A length of 699 bps *Vp1* gene of SBV was selected to be expressed with L4440 plasmid in *Escherichia coli* HT115 (DE3). After ultrasonic disruption and ethanol precipitation, *Vp1*-dsRNA molecules were purified with anion exchange chromatography utilizing convective interaction media (CIM) monolithic columns. RNAi was performed to prevent bees from SBV under laboratory conditions. Comparing with bees without dsRNA, *Vp1*-dsRNA prevented 49% to 75% larval mortality of *A. cerana* from SBV infection. The result may provide a model in large-scale use of RNAi for SBV control.

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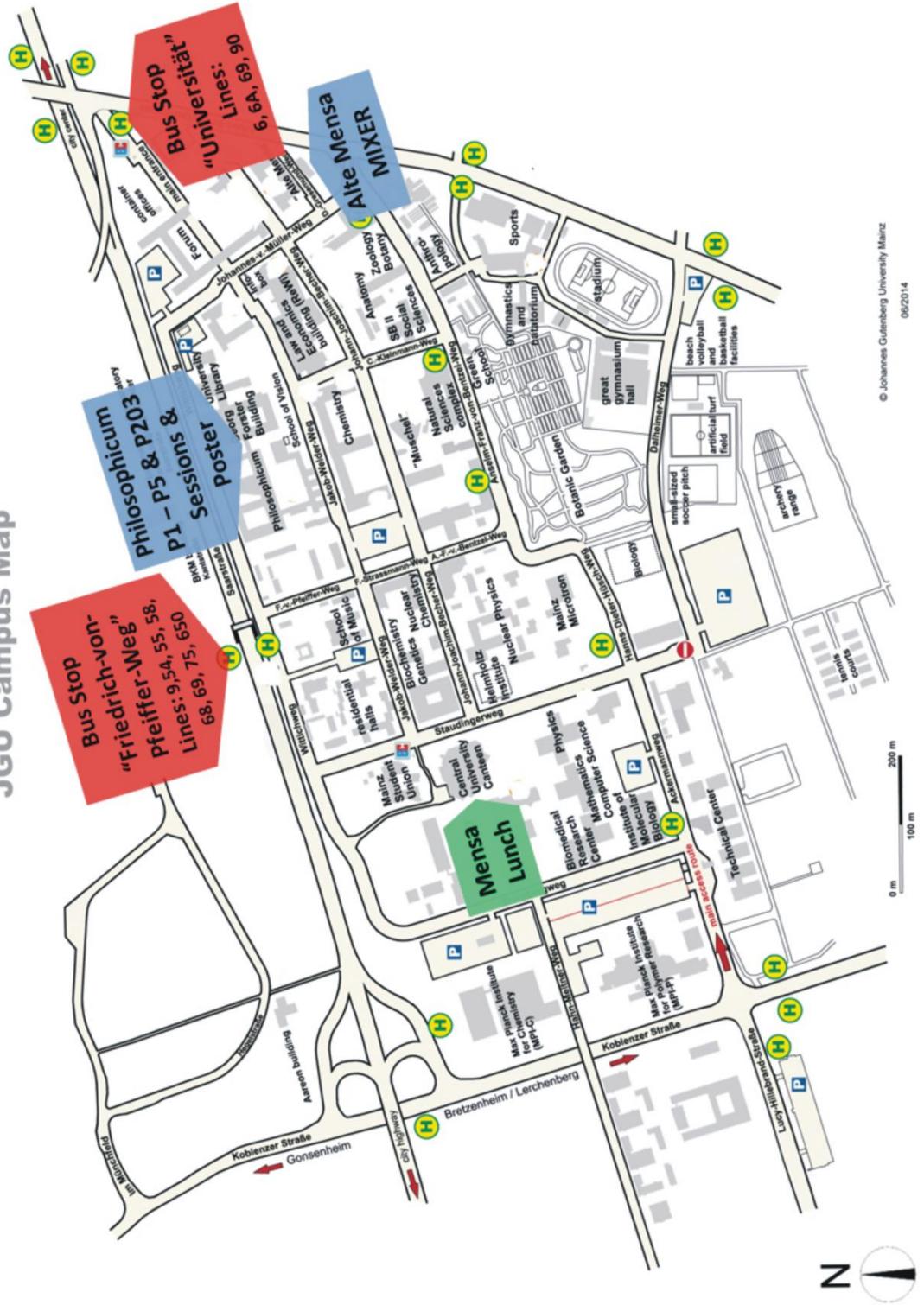
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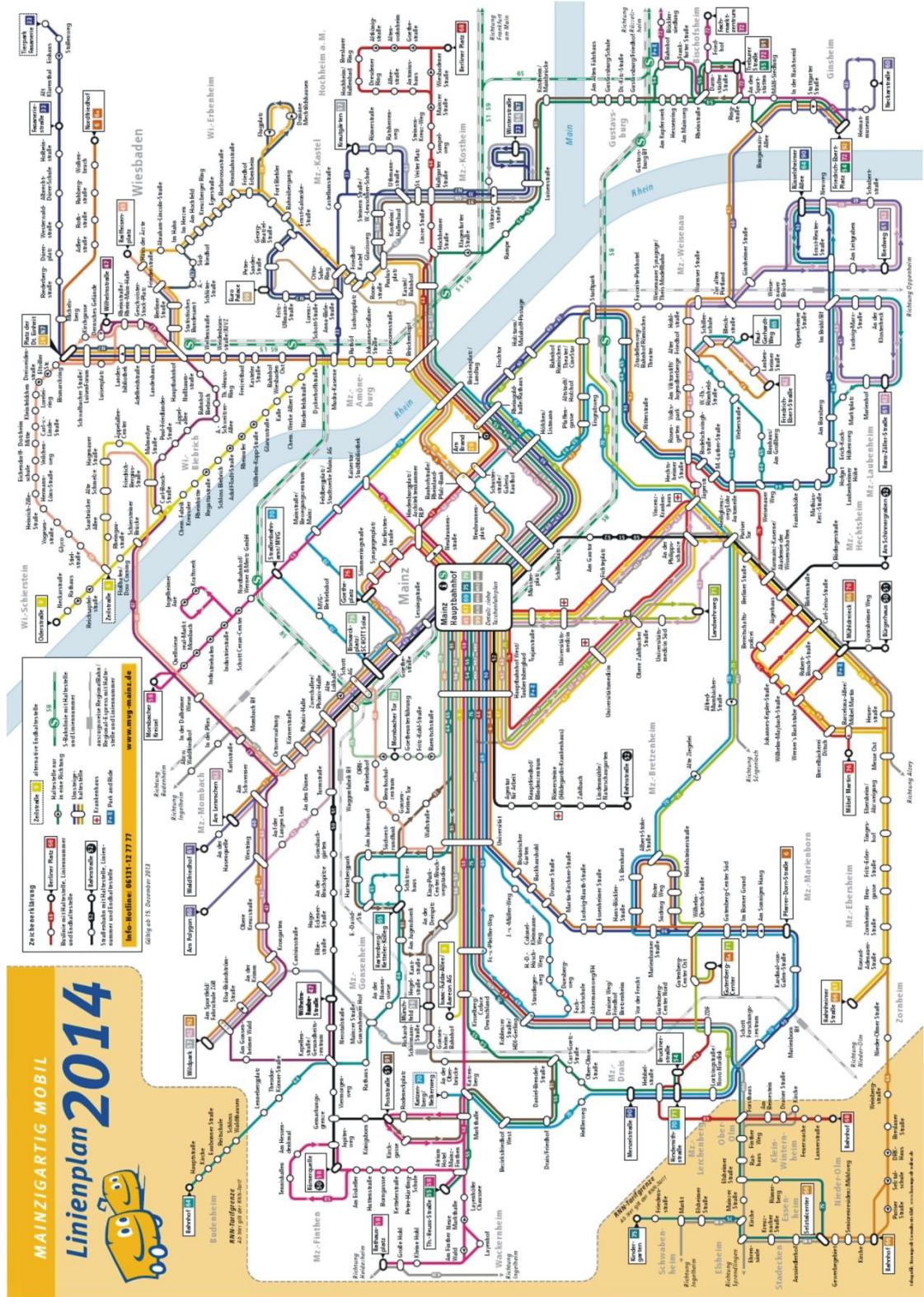
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Thursday - 7 August		
7:30-16:30	Registration	P1
8:00-10:00	Symposium 7 (Diseases of Beneficial Invertebrates) Emerging Tools for Aquatic Pathogen Discovery and Description Early mortality syndrome is an infectious disease with a bacterial etiology <i>Loc Tran</i> Policy, phylogeny, and the parasite <i>Grant D. Stentiford</i> The Next Generation of Crustacean Health: Disease Diagnostics Using Modern Transcriptomics <i>K. Fraser Clark</i>	P2
	Environmental DNA as a tool for detection and identification of aquatic parasites: known unknowns and just plain unknowns <i>Hanna Hartikainen</i>	
8:00-10:00	Symposium (DFG Priority Program) Organizer: Joachim Kurtz Host Parasite Coevolution	P5
8:00-10:00	Contributed Papers Nematodes 3 Viruses 6	P4
10:00-10:30	Break	P1
10:30-12:30	SIP Annual Business Meeting Presiding: Jørgen Eilenberg	P1
12:30-14:00	Lunch	Mensa
14:00-16:00	Symposium 8 (Cross-Divisional) Host-Pathogen Ecology at the Molecular Level: Gene Regulation and Environment Sensing The <i>Bacillus thuringiensis</i> way of life: communicate to kill and survive in the insect host <i>Didier Lereclus</i> The interplay of Paenibacillus larvae with honey bee larvae during infection <i>Elke Genersch</i> Antimicrobial defense and persistent infection in insects revisited <i>Jens Rolff</i> Vibriobacter and the intraphagosomal environment: how an oyster pathogen evades intracellular killing in oyster hemocytes <i>Delphine Destoumieux-Garzon</i>	P2
14:00-16:00	Contributed Papers Microbial Control 4 Viruses 7	P3
16:00-16:30	Student Business Meeting	P4
18:30	Departure from Hotels to Banquet	
19:00-1:00	Reception and Banquet	Alte Lokhalle

Seeing you in Vancouver for SIP 2015!

5.5.7 Improved traps for the coconut rhinoceros beetle, *Oryctes rhinoceros*

Please see next page.

Improved traps for the coconut rhinoceros beetle, *Oryctes rhinoceros*

Improved traps for
the coconut
rhinoceros beetle

Moore, Quitugua,
Siderhurst and
Jang

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Pheromone Traps

Vaned bucket traps
Ultraviolet light
emitting diodes
(UVLEDs)
Pan traps

Fish Net Traps

Mark-Release-
Recapture

Conclusions

Aubrey Moore

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Roland Quitugua

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Virginia

Eric Jang

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Entomological Society of America Annual Meeting,
Portland OR, November 19, 2014

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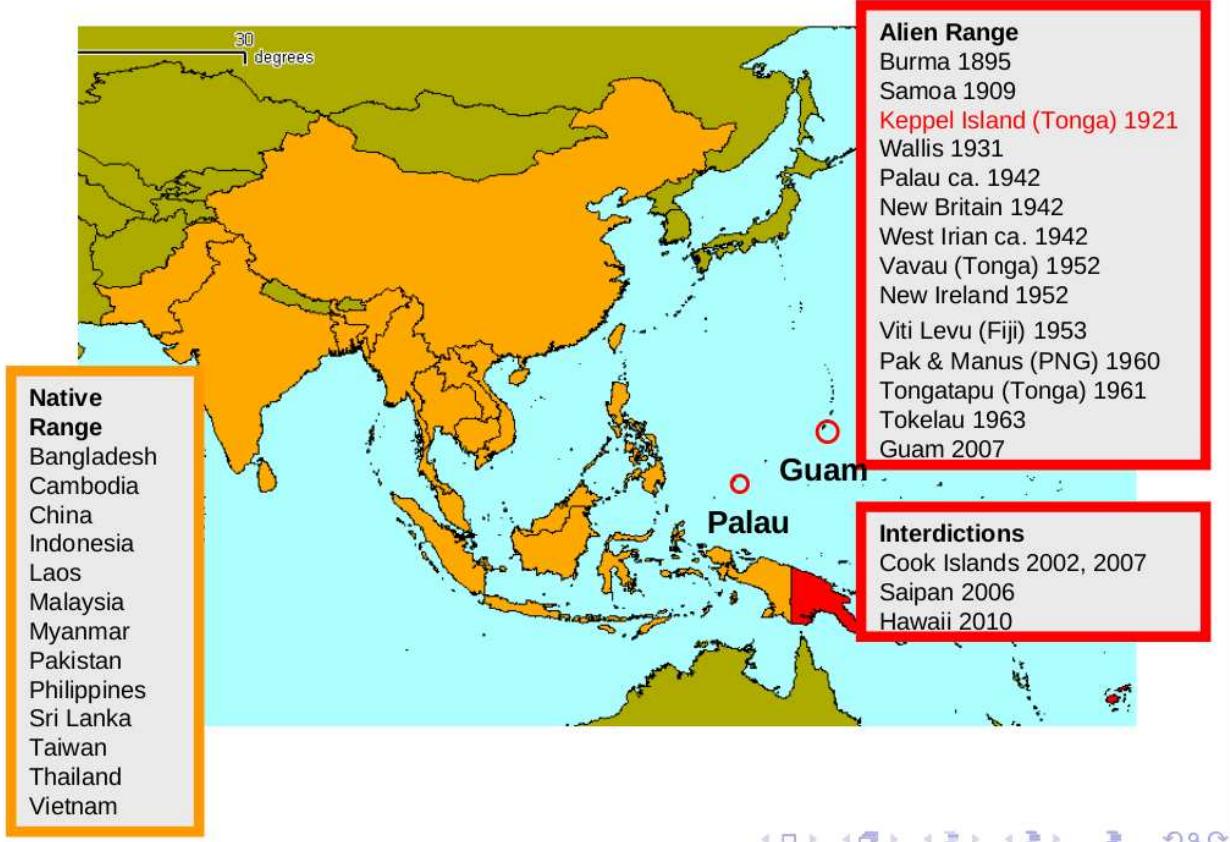
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Oryctes rhinoceros Distribution



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Coconut rhinoceros beetle damage



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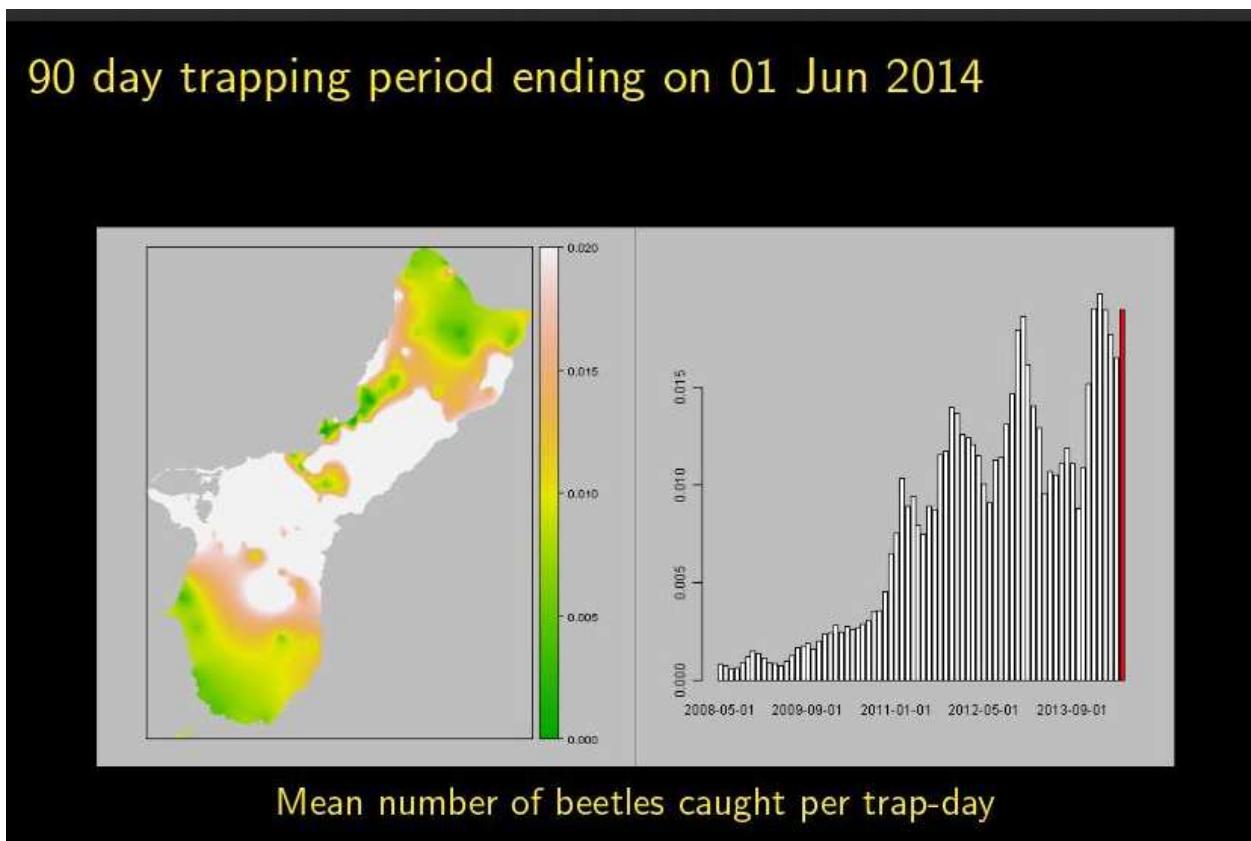
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