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Foreword

This compendium contains the abstracts of all the contributions to be presented at the 8th International Symposium on Aphids being held in Catania, Italy.

The symposium is organized by the Department of Phytosanitary Science and Technology (Entomology Section), Faculty of Agriculture, University of Catania (Italy) on behalf of the Italian National Academy of Entomology, the Italian Entomological Society, the University of Catania, the Gioenia Academy of Natural Science and the Italian Association for Plant Protection.

Its main aims are to allow contacts and interactions among aphid scientists from different countries around the world and to improve knowledge about this group of insects, of which several species are pests to forestry and cultivated plants.

The Symposium is organized into four sessions:

Session 1: Aphid genomics, molecular genetics and evolution

Session 2: Aphid biodiversity and systematics

Session 3: Aphid biology and ecology

Session 4: Aphids in agriculture, horticulture and forestry

Each of the above-named sessions will hold one or two plenary lectures by invited speakers, followed by a number of offered oral presentations and posters, both about research on session topics. In addition to the relevant session contributions and to those concerning the Opening and Closing Sessions, the Symposium will host one Working Group Meeting.

Contributions, *in extenso*, will be published on "Redia", Journal of Zoology issued in Florence (Italy).

The Organizing Committee wishes to thank all sponsoring institutions and organisations, the Session coordinators and plenary lecturers as well as all scientific contributors for their much-appreciated cooperation. The local Committees wish all the participants a profitable meeting and a pleasant stay in Sicily.

Sponsors

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Organized by

DEPARTMENT OF PHYTOSANITARY SCIENCE AND TECHNOLOGY

University of Catania

On behalf of

ITALIAN NATIONAL ACADEMY OF ENTOMOLOGY

ITALIAN ENTOMOLOGICAL SOCIETY

UNIVERSITY OF CATANIA

GIOENIA ACADEMY OF NATURAL SCIENCE

ITALIAN ASSOCIATION FOR PLANT PROTECTION

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PROGRAMME

MONDAY JUNE 8TH

08.30 – 10.00: Arrival, registration

10.00 – 10.30: Welcoming addresses

- Local Organizer – Sebastiano Barbagallo
- Rector of the University of Catania – Antonino Recca
- Dean of the Faculty of Agriculture – Agatino Russo
- Sicilian Agriculture Assessorship - Giovanni La Via

10.30-12.00: Opening Session

Chair: A.F.G. Dixon, *Co-Chair*: A. Binazzi

- A.F.G. DIXON - History of Aphid Symposia
- S. BARBAGALLO, A. BINAZZI & L. LIMONTA – A short historical account on aphids and aphidologists in Italy

SESSION 1. APHID GENOMICS, MOLECULAR GENETICS AND EVOLUTION

Chair: A.C.C. Wilson, *Co-Chair*: C.D. von Dohlen

Plenary Lecture

12.00: D. TAGU, S. JAUBERT-POSSAMAI, F. LEGEAI, J.-P. GAUTHIER, C. RISPE, O. EDWARDS & THE INTERNATIONAL APHID GENOMICS CONSORTIUM - The pea aphid genome to study phenotypic plasticity

12.45 – 14.00: Lunch

Oral presentations

- **14.00:** J.C. CAROLAN, A.E. DOUGLAS, K. REARDON, C.I.J. FITZROY & T. WILKINSON - Proteomic profiling of pea aphid saliva and salivary glands
- **14.15:** T. CORTÉS, B. ORTIZ-RIVAS & D. MARTÍNEZ-TORRES - The circadian clock system in the pea aphid *Acyrthosiphon pisum*
- **14.30:** J-J. ZHOU, X.L. HE, R. LIU & L.M. FIELD - Odorant-binding proteins in aphids
- **14.45:** D. SRINIVASAN, L. AÑO, B. FENTON, S. JAUBERT-POSSAMAI & D. STERN - Molecular mechanism of facultative parthenogenesis in the Pea Aphid, *Acyrthosiphon pisum*
- **15.00:** T.K. WALSH, J.A. BRISSON, K. GORDON, H.M. ROBERTSON, S. JAUBERT-POSSAMAI, D. TAGU & O. EDWARDS - DNA methylation in the pea aphid
- **15.15:** A.C.C. WILSON, P. ASHTON, F. CALEVRO, H. CHARLES, S. COLELLA, G. FEBVAY, G. JANDER, P. KUSHLAN, S. MACDONALD, J. SCHWARTZ, G. THOMAS & A. DOUGLAS - Genomic insight into the amino acid relations of the pea aphid with its symbiotic bacterium *Buchnera aphidicola*
- **15.30:** N.M. GERARDO, B. ALTINCICEK, C. ANSELME, H. ATAMIAN, S. BARRIBEAU, M. DE VOS, J.D. EVANS, T. GABALDÓN, M. GHANIM, A. HEDDI, I. KALOSHIAN, A. LATORRE, C. MONEGAT, A. MOYA, A. NAKABACHI, B.J. PARKER, V. PÉREZ-BROCAL, M. PIGNATELLI, Y. RAHBÉ, J. RAMSEY, C. SPRAGG, J. TAMAMES, D. TAMARIT, C. TAMBORINDEGUY & A. VILCINSKAS - Uncovering the limitation of the aphid immune response

- **15.45:** Y. RAHBÉ, D. COSTECHAREYRE, S. BALMAND & G. CONDEMINE - A model aphid bacterial pathogen: the phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi*) and its insect-specific virulence factors
- **16.00:** F. FRANCIS, E. DE PAUW & E. HAUBRUGE - Application of proteomic tools to investigate the respective role of aphid and symbiotic bacteria in relation to host plant
- **16.15:** D. CARAGEA, O. EDWARDS & G. REECK - Identification and annotation of predicted secreted salivary proteins in the pea aphid, *Acyrthosiphon pisum*

16.30 – 17.00: Coffee break

Chair: C. Favret, *Co-Chair*: D. Tagu

Plenary Lecture
17.00: C.D. VON DOHLEN - Aphid molecular systematics: History, progress and prospects

Oral Presentations
- **17.45:** M. CABRERA-BRANDT, A.X. SILVA, E. FUENTES-CONTRERAS, G. LE TRIONNAIRE, D. TAGÚ & C.C. FIGUEROA - Response in the aphid *Myzus persicae* to insecticide pressures: searching for genetic targets of selection
- **18.00:** M. KUTSUKAKE, H. SHIBAO, K. UEMATSU & T. FUKATSU - Molecular basis of self-sacrificing gall repair by soldier aphids in the social aphid, *Nipponaphis monzeni*
- **18.15:** A. FORNECK, R. MAMMERLER & M. GRIESSER - No "most successful" clones for Grape Phylloxera in European leaf feeding habitats - Why?
- **18.30:** H.D. LOXDALE & W. WEISSER - Why are there so few aphid clones?
- **18.45:** S. THOMAS, P. MISTRAL, V. CHAREYRON, B. BARRAL, N. BOISSOT & F. VANLERBERGHE-MASUTTI - Genetic diversity of the melon aphid *Aphis gossypii* Glover in different melon growing areas of France
- **19.00:** H. KIM & S. LEE - Phylogenetic relationships of the known species-groups of the genus *Aphis*, based on molecular and morphological characters with evidence of cryptic speciation in the *gossypii* group
- **19.15:** G. COCUZZA, V. CAVALIERI, L. ZAPPALÀ & S. BARBAGALLO - Genetic relationship among species of the *Aphis frangulae/gossypii* group based on mitochondrial DNA sequences

19.30: Shuttle bus to hotel

TUESDAY JUNE 9TH

SESSION 2. APHID BIODIVERSITY AND SYSTEMATICS

Chair: J.M. Nieto Nafria, *Co-Chair*: F.W. Quednau

Plenary Lecture
09.00: O.E. HEIE & P. WEGIEREK - A classification of the Aphidomorpha (Hemiptera: Sternorrhyncha) under consideration of the fossil taxa

Oral Presentations
- **09.45:** F.W. QUEDNAU - Phylogenetic aspects of the evolution of the Saltusaphidinae
- **10.00:** Z. ZHANG & Y. HONG - Fossil aphids found in China (Hemiptera, Aphidomorpha) with special introduction to the oldest aphid from the Triassic
- **10.15:** A.V. STEKOSHCHIKOV & S.V. BUGA - Aphid fauna of arctic and subarctic regions
- **10.30:** L. JIANG, G. QIAO, G. ZHANG & T. ZHONG - Species diversity and fauna of Aphids in Northeast China

- **10.45:** X.-L. HUANG & G.-X. QIAO - Relationship between patterns of species richness and sampling effort: a case study of aphids in China

11.00 – 11.30: Coffee break

- **11.30:** S. BARBAGALLO, A. BINAZZI, V. CAVALIERI, A. LA PERGOLA & L. LIMONTA - Biodiversity and chorological outlines for Italian aphid fauna
- **11.45:** D. MIFSUD, A. TABONE & S. BARBAGALLO - Aphids (Hemiptera: Aphidoidea) associated with trees in the Maltese Islands (Central Mediterranean): A preliminary check-list
- **12.00:** R. RAKAUSKAS - Describing cryptic species – is the game worth candles?
- **12.15:** R.G. FOOTTIT - DNA Barcodes to Explore Diversity in Aphids
- **12.30:** A. COEUR D'ACIER, F. DORKELD, S. HUDAVERDIAN, J-C.SIMON & J-Y. RASPLUS - Toward a molecular identification tool for European Aphididae
- **12.45:** D.M. LAGOS, R. GIORDANO, F. SOTO-ADAMES & D.J. VOEGTLIN - Preliminary results of the molecular phylogeny and morphological evaluation of the genus *Aphis* (Hemiptera: Aphididae) in North America

13.00 – 14.30: Lunch

14.30-15.30: Poster Session 1 & 2

Chair: R. Foottit, *Co-Chair*: O.E. Heie

Plenary Lecture

15.30: S. CHAKRABARTI - Diversity, distribution and endemism of Aphids (Hemiptera) in Indian subregion of Oriental realm

Oral Presentations

- **16.15:** G. GORUR, B. AKYUREK, U. ZEYBEKOGLU, H. AKYILDIRIM & İ. TEPECIK - New additions to the Turkey Aphid (Hemiptera:Aphidoidea) Fauna
- **16.30:** J. TURCINAVICIENE & R. RAKAUSKAS - *Macrosiphum* on *Knautia* in Central Europe – molecular data support the synonymy of *M. silvaticum* and *M. knautiae* (Hemiptera: Aphididae)
- **16.45:** B. ORTIZ-RIVAS, N. PÉREZ HIDALGO & D. MARTÍNEZ-TORRES - Molecular phylogenetics and systematics of Iberian Fordini

17.00 – 17.30: Coffee break

- **17.30:** M. SANO & S. AKIMOTO - Phylogenetic position and biogeography of gall-forming aphids on *Zelkova*
- **17.45:** C. FAVRET & D.C. EADES - Introduction to Aphid Species File
- **18.00:** J.M. NIETO NAFRÍA, C. FAVRET, S. AKIMOTO, S. BARBAGALLO, S. CHAKRABARTI, M.P. MIER DURANTE, G. MILLER, N. PÉREZ HIDALGO, G.-X. QIAO, M. SANO, A.V. STEKOLSHCHIKOV & P. WEGIEREK - Several nomenclatural clarifications on genus-group names in the Aphididae (Hemiptera Sternorrhyncha)
- **18.15:** J.M. NIETO NAFRÍA (coord.) Presentation and Discussion on "Part of available names of Aphidoidea taxa of genus group"

18.30: First shuttle bus to hotel (two buses available)

18.30 – 20.00: "EXAMINE" Working Group Meeting
Chair: R. Harrington, *Co-Chair*: P. Verrier

20.00: Second shuttle bus to hotel (one bus available)

WEDNESDAY JUNE 10TH

8.00 – 19.00: Social Excursion (Mount Etna Parkland)

THURSDAY JUNE 11TH

SESSION 3. APHID BIOLOGY AND ECOLOGY

Chair: P. Kindlmann, *Co-Chair*: N. Mills

Plenary Lecture

9.00: S. AKIMOTO **-** Effects of inbreeding and outbreeding on aphid biology

Oral Presentations

- **09.45:** A. RABATEL, N. BENDRIDI, G. DUPORT, S. COLELLA, J. BERMINGHAM, Y. RAHBÉ, T. WILKINSON, H. CHARLES, G. FEBVAY & F. CALEVRO - Metabolic requirements in essential amino acids in parthenogenetic pea aphid embryos
- **10.00:** F. TJALLINGII - Plant penetration variables; how to use them?
- **10.15:** A.C.C. WILSON, K.B. HURLEY, S.U. CHAN, D.H. JONES & L. DA S. L. STERNBERG - Complete trophic signature reversal by aphid parasitism
- **10.30:** J.M. ALVAREZ, B. SRINIVASAN & F. CERVANTES - Potato viral infections affect the biology and behavior of aphid vectors

10.45 – 11.15: Coffee break

- **11.15:** S. POINTEAU, S. BANKHEAD-DRONNET, X. PINEAU, A. SALLÉ & F. LIEUTIER - Role of temperature on the development and fecundity of the emergent species *Phloeomyzus passerinii* (Aphididae: Phloeomyzinae)
- **11.30:** A.F.G. DIXON - Thermal tolerance and resource partitioning in aphids
- **11.45:** C. VORBURGER, C. SANDROCK, A. GOUSKOV, L. GEHRER & P. RODRIGUEZ - Symbiont-mediated coevolution in aphid host-parasitoid systems
- **12.00:** H.F. VAN EMDEN - Artificial diet for aphids – thirty years' experience
- **12.15:** M. LA SPINA & J.A. SÁNCHEZ - Defensive behavior of four *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) clones during a parasitoid attack
- **12.30:** M. GISH, A. DAFNI & M. INBAR **-** Pea aphids drop off the plant to evade incidental predation by mammalian herbivores

12.45 – 14.15: Lunch

14.15 - 15.15: Poster Session 3 & 4

Chair: A.F.G. Dixon, *Co-Chair*: S. Akimoto

Plenary Lecture

15.15: N. MILLS & D. LATHAM - Quantifying the role of predation in the seasonal dynamics of mealy plum aphid populations in California

Oral Presentations

- **16.00:** W.W. WEISSER - Aphid alarm pheromone: costs and benefits
- **16.15:** T. THIEME & A.F.G. DIXON - Do plants affect the population dynamic of aphids?

16.30 – 17.00: Coffee break

- **17.00:** O. AMEIXA & P. KINDLMANN - Testing the assumptions of cage exclusion experiments in field conditions

- **17.15:** P. KINDLMANN & O. AMEIXA - The true role of predators in man-made ecosystems
- **17.30:** E.L. CLARK, A.J. KARLEY, T.J. DANIELL, J. WISHART & S.F. HUBBARD - The community composition and influence on aphid performance of the bacteria associated with the cabbage aphid (*Brevicoryne brassicae*)
- **17.45:** S. BOQUEL, A. AMELINE & P. GIORDANENGO - Potato plant acceptance of apterous and alate morphs of the aphids, *Myzus persicae* and *Macrosiphum euphorbiae*
- **18.00:** E. JOUSSELIN & A. COEUR D'ACIER - Life-cycle evolution and host plant use in the genus *Brachycaudus*: insight from a molecular phylogeny
- **18.15:** C.-A. DEDRYVER, V. FIÉVET, J.S. PIERRE, M. PLANTEGENEST & A. VIALATTE - A synthetic overview of *Sitobion avenae* population functioning in France
- **18.30:** J.R. BELL, R. HARRINGTON, S. WELHAM, S. CLARK & J. PICKUP - The GAMes aphids play

18.45: Shuttle bus to hotel

20.30: Social dinner

FRIDAY JUNE 12TH

SESSION 4. APHIDS IN AGRICULTURE, HORTICULTURE AND FORESTRY

Chair: H. van Emden, *Co-Chair*: N. Mills

Plenary Lecture

09.00: S.L. CLEMENT - Fungal endophytes in temperate grasses: important or insignificant mediators of host plant resistance to aphids?

Oral Presentations

- **09.30:** J.C. REESE, G.R. REECK & H. TRICK - Host plant resistance to aphids: a new strategy
- **09.45:** G. POWELL, S. STEWART, S. KANVIL, N. ISMAIL, J. MANSFIELD, B. FEYS, J.-M. PROSPERI, T. HUGUET, C. BEN & L. GENTZBITTEL - The pea aphid and *Medicago truncatula*: a wealth of interactions
- **10.00:** S.A. STEWART, S. HODGE, J.M. MANSFIELD & G. POWELL - Clone-specific resistance in *Medicago truncatula* against the pea aphid
- **10.15:** T. ABDULRAZAK & S. JAYARAJ - Effects of genotypes of inter/border crops on aphid incidence and their natural enemies in a rain fed cotton ecosystem
- **10.30:** G. ARADOTTIR, A. KARP, S. HANLEY, I. SHIELD, C. WOODCOCK, S. DEWHIRST, C.M. COLLINS, S. LEATHER & R. HARRINGTON - Host selection of the giant willow aphid (*Tuberolachnus salignus*)
- **10.45:** L.S. MCMENEMY, S.A. MACFARLANE, S.E. HARTLEY & S.N. JOHNSON - Co-operation between plant enemies – do raspberry viruses attract more aphid vectors?

11.00 – 11.30: Coffee break

- **11.30:** A. CHERQUI, H. SAMAHA, F. BAILLIEUL, P. GIORDANENGO & C. RUSTERUCCI - Aphid saliva effects on plant defense
- **11.45:** R. HARRINGTON, M. STEVENS, D. COX, S. FOSTER, P. HALLSWORTH, S. PARKER & M. TAYLOR - Complementary methods for monitoring sugar beet aphids to improve risk management of virus yellows
- **12.00:** S. BOQUEL, P. GIORDANENGO, P. LASUE & A. AMELINE - Do non-colonising potato aphids exhibit behaviour which facilitates non-persistent virus transmission?

- **12.15:** M. UZEST, D.GARGANI & S. BLANC - Towards the characterization of the functional role of the common duct in aphid stylets
- **12.30:** S. LIU & B.C. BONNING - A plant virus transmission-blocking peptide
- **12.45:** R. VAN TOOR, G. MALLOCH & B. FENTON - A concept for management of virus vectors and insecticide resistance in *Myzus persicae* on potatoes

13.00 – 14.30: Lunch

Chair: R. Harrington, *Co-Chair*: S. Clement

14.30: T. THIEME – Clip by Urs Wyss "Aphids and other phloem feeding insects - when it rains sugar"

Oral Presentations

- **15.15:** M. DANIELS, J.S. BALE, H.J. NEWBURY, R.J. LIND & J. PRITCHARD - Impairment of xylem-feeding behaviour in response to a sublethal dose of thiamethoxam is associated with dehydration and reduced performance in the bird cherry-oat aphid (*Rhopalosiphum padi*)
- **15.30:** A.M. PUINEAN, S.P. FOSTER, L. OLIPHANT, N.S. MILLAR, M.S. WILLIAMSON & I. DENHOLM - Characterisation of neonicotinoid resistance in the peach–potato aphid, *Myzus persicae* (Hemiptera: Aphididae)
- **15.45:** D. VUKAŠINOVIĆ, O. PETROVIĆ–OBRADOVIĆ, J. JOVIĆ & A. VUČETIĆ - Morphological and molecular identification of apple pests *Aphis spiraecola* and *Aphis pomi* in Serbia
- **16.00:** M. BEN HALIMA KAMEL - Efficacy of *Lysiphlebus testaceipes* in control of *Aphis gossypii* on pepper
- **16.15:** Z. BASKY - Aphids on ragweed

16.30 – 17.00: Coffee break

17.00: Closing session

Chair: H. van Emden, *Co-Chair*: S. Barbagallo

- **Election of a Standing Committee**
- **Proposal for the next Symposium**
- **Agreements on "Part of available names of Aphidoidea"**
- **Closing addresses**

18.30: Shuttle bus to hotel….

….and to the 9th ISA!!!

List of Posters

SESSION 1. APHID GENOMICS, MOLECULAR GENETICS AND EVOLUTION

S1.1. O. CHRISTIAENS & G. SMAGGHE - Cloning and characterization of the ecdysone cascade with ecdysone receptor (EcR) and Ultraspiracle (Usp) in the pea aphid.

S1.2. O. CHRISTIAENS & G. SMAGGHE - The *Acyrthosiphon* genome contains at least 19 nuclear receptors with the ecdysone cascade revealing an increase in evolutionary rate.

S1.3. C. FITZROY, J. CAROLAN & T. WILKINSON - Evidence for the conservation of salivary proteins among aphid species.

S1.4. A. ISHIKAWA, Y. OKUMURA, Y. NAKAGAWA & T. MIURA - Regulations of polyphenic wing development in the vetch aphid *Megoura crassicauda*: morphogenesis, tradeoffs, and gene expressions.

S1.5. S. COLELLA, A. VELLOZO, L. COTTRET, G. FEBVAY, F. CALEVRO, Y. RAHBÉ, M.F. SAGOT & H. CHARLES - AcypiCyc (*Acyrthosiphon pisum* Cyc database) and CycADS (Cyc Annotation Database System): moving from genome sequence annotation to metabolic network analyses.

S1.6. R.N. RAO, E.J.M. VAN DAMME, B. GESQUIÈRE, K. GEVAERT & G. SMAGGHE - Proteomic analysis of GNA binding proteins in the pea aphid.

S1.7. S.A.K. RAO, T. WILKINSON & J. CAROLAN - Characterization of salivary proteins from cereal aphids.

S1.8. K. REARDON, J. CAROLAN & T. WILKINSON - Characterization of the salivary gland proteome of the Pea Aphid (*Acyrthosiphon pisum*) and other aphid species.

S1.9. M. ALEOSFOOR, K.A. IZADPANAH, M. SADEGH SADEGHI, M. MARDI, M. MASOOMI, M. SAEED MOSSADEGH & M.A. OMIDBAKHSH FARD - Genetic diversity of *Rhopalosiphum padi* L. (Hom.: Aphididae) using microsatellite markers.

S1.10. B. BEJI, M. MEZGHANI-KHEMAKHEM, S. BOUHACHEM, H. HARBAOUI, M. MAKNI & H. MAKNI - Polymorphism of *Aphis fabae* in Tunisia assessed by RAPD markers.

S1.11. M. MEZGHANI-KHEMAKHEM, I. KHARRAT, D. BOUKTILA, H. MAKNI & M. MAKNI - Tunisian *Schizaphis graminum* biotype inferred by COI sequences.

S1.12. T. KANBE & S. AKIMOTO - Allelic and genotypic diversity in asexual populations of the pea aphid *Acyrthosiphon pisum* in Japan.

S1.13. J. WANG & G. QIAO - Identifying species in the subtribe Aphidina (Hemiptera Aphididae: Aphidinae) using DNA sequences and resolving some species complex problems.

SESSION 2. APHID BIODIVERSITY AND SYSTEMATICS

S2.1. N. BAKHTADZE, SH. BARJADZE, N. KINTSURASHVILI, G. BAKHTADZE, N. ZHUKOVSKAYA & N. CHAKVETADZE - Biodiversity of the aphid fauna (Hemiptera: Aphidoidea) of Georgia.

S2.2. S. BARBAGALLO, G.E. COCUZZA & P. SUMA - An Eriosomatine aphid relict: *Zelkovaphis trinacriae*, living in Sicily on *Zelkova sicula*.

S2.3. SH. BARJADZE & N. GRATIASHVILI - Zelkova-feeding Eriosomatinae from Georgia (Hemiptera: Aphidoidea).

S2.4. S. BELLA, D. MIFSUD, N. PÉREZ HIDALGO & S. BARBAGALLO - *Greenidea ficicola*: is it an example of rapid colonization due to climatic changes?

S2.5. S. CHAKRABARTI & D. DAS - Aphid fauna of Bhutan and their host association.

S2.6. A. COEUR D'ACIER, N. PÉREZ HIDALGO, L. SOLDATI & O. PETROVIC-OBRADOVIC - The European inventory of Alien aphids.

S2.7. O.E. HEIE & P. WEGIEREK - An attempt to make a phylogenetic classification all aphids, both extinct and extant taxa (Hemiptera).

S2.8. L. JIANG, G. QIAO, G. ZHANG & T. ZHONG - Distribution pattern of Aphids in Northeast China.
S2.9. S.M. MADJDZADEH, M. MEHRPARVAR & F. ABOLHASANZADEH - Morphometric discrimination of host-adapted populations of *Brachycaudus helichrysi* (Kaltenbach) (Hem.: Aphididae).
S2.10. M.P. MIER DURANTE, J. ORTEGO & J.M. NIETO NAFRÍA - Aphids from Argentine Northwest (*NOA*).
S2.11. B. ORTIZ-RIVAS & D. MARTÍNEZ-TORRES - Combined nuclear and mitochondrial molecular data support the existence of three main lineages in the phylogeny of aphids.
S2.12. B. OSIADACZ - To the problem of the genus *Uroleucon* Mordv. in Europe.
S2.13. N. PÉREZ HIDALGO, W. VILLALOBOS MULLER, M.P. MIER DURANTE, X. ESPADALER & J.M. NIETO NAFRÍA - Biodiversity of aphids in Costa Rica.
S2.14. S. POINTEAU, F. LIEUTIER & S. BANKHEAD-DRONNET - Morphology and morphometry of the development instars in *Phloeomyzus passerinii*, the poplar woolly aphid (Aphididae: Phloeomyzinae).
S2.15. K. WIECZOREK - Siphini Mordvilko, 1928 (Aphidoidea, Chaitophorinae) – taxonomy, bionomy and distribution.

SESSION 3. APHID BIOLOGY AND ECOLOGY

S3.1. S. CHAKRABARTI & M. DEBNATH - Diversity of aphids *vis-a-vis* aphidophagous predators in Northwest and Western Himalayas, India.
S3.2. K. DANCEWICZ & B. GABRYŚ - Stylet penetration of *Adelges laricis* (Vallot) on its secondary host *Larix decidua* Mill.
S3.3. P.I. KERCHEV, C.H. FOYER, B.FENTON & R.D. HANCOCK - Reactive oxygen and antioxidants modulate the interaction between *Myzus persicae* (Sulzer) and plant hosts.
S3.4. B. KORDAN, W. SŁOMKA, B. GABRYŚ & K. DANCEWICZ - Feeding site dependant probing behavior of the pea aphid *Acyrthosiphon pisum* on two species of lupines *Lupinus* sp.
S3.5. M. KUTSUKAKE, H. SHIBAO, K. UEMATSU & T. FUKATSU - Wound repair and regeneration of gall tissue by soldier aphids in a social aphid, *Nipponaphis monzeni*.
S3.6. M. LA SPINA & J.A. SÁNCHEZ - Intraspecific variation between *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) clones in development, longevity, fecundity and other biological parameters.
S3.7. B. LESZCZYŃSKI, R. KRZYŻANOWSKI & A. GADALIŃSKA-KRZYŻANOWSKA - Side effect of bird cherry tree on development of *R. padi* local colonies.
S3.8. M.B. PONSEN & W.F. TJALLINGII - Morphology of Aphid salivary glands.
S3.9. C. SEMPRUCH & B. LESZCZYNSKI - Accumulation of putrescine within triticale attacked by grain aphid, *Sitobion avenae* (F.).
S3.10. F.-H. SOMAYEH & A. HOSSEIN - On the biological differences of *Schizaphis graminum* Rond. (Homoptera: Aphididae) in wheat varieties: A comparative survey of 2 equations.
S3.11. F.-H. SOMAYEH & A. HOSSEIN - The pea aphid; *Acyrthosiphon pisum* (Harris) (Aphididae: Homoptera) from developmental to reproductive aspects on broad bean.
S3.12. SPRAWKA, S. GOŁAWSKA & B. LESZCZYŃSKI - Effect of lectin PHA on feeding behavior of grain aphid.
S3.13. D.K. SUZUKI, Y. FUKUSHI & S. AKIMOTO - Do aphid galls provide good nutrients for the aphids?: comparisons of amino acid concentrations in galls among *Tetraneura* species (Aphididae: Eriosomatinae).
S3.14. K. UEMATSU, M. KUTSUKAKE, T. FUKATSU, M. SHIMADA & H. SHIBAO - Colony defense by post-reproductive adults in a social aphid.
S3.15. H.F. VAN EMDEN, J. S. KILBANE & J. PETTERSSON - Changing the host selection responses of aphids through a short experience of a novel secondary plant compound.

S3.16. F.J. VERHEGGEN, E. HAUBRUGE, F. FRANCIS, C.M. DE MORAES & M.C. MESCHER - Variation of alarm aphid pheromone production: impact of the social environment.

S3.17. D.G.M. VITALE, M.V. BRUNDO, L. SOTTILE, R. VISCUSO & S. BARBAGALLO - Morphological and ultrastructural investigations of the male reproductive system in aphids: observations of *Tuberculatus* (*Tuberculoides*) *eggleri* Börner (Hemiptera: Aphidoidea).

SESSION 4. APHIDS IN AGRICULTURE, HORTICULTURE AND FORESTRY

S4.1. T. ABDULRAZAK & S. JAYARAJ - Effect of certain botanicals and entomopathogenic fungi against cotton aphids.

S4.2. J.S. AMEY, A.O. O'REILLY, M.S. WILLIAMSON, L.M. FIELD, B.A. WALLACE & T.G.E. DAVIES - Molecular identification of the *Myzus persicae* voltage gated sodium channel.

S4.3. S.V. BUGA & A.V. STEKOSHCHIKOV - Aphids as pests of fruit- and berry-producing plants in Byelorussia.

S4.4. J.D. BURD & G.J. PUTERKA - Plant resistance management strategies for greenbug (*Schizaphis graminum*) in wheat-sorghum cropping systems.

S4.5. P. CRAVEDI, G.C. MANICARDI, S. CASSANELLI, V. TALESA, C. DELBUONO, D. BIZZARO, E. MAZZONI - Insecticide resistance in Italian populations of the peach potato aphid *Myzus persicae* (Hemiptera: Aphididae).

S4.6. S.KR. GHOSH, G.S.S. MAHAPATRA & G. CHAKRABORTY - Field efficacy of plant extracts and microbial insecticides against aphid (*Aphis gossypii*) infesting okra (*Abelmoschus esculentus*).

S4.7. C. GILSENAN, J. CAROLAN, G. PURVIS, T.L. WILKINSON & M.F. RYAN - Does plant resistance to cereal aphids increase under a bi-cropping regime?

S4.8. E. HUUSELA-VEISTOLA - Variation in the abundance of *Rhopalosiphum padi* in Finland.

S4.9. R. JAFARI & S. MODARES - Study of the population fluctuations of the cabbage aphid *Brevicoryne brassicae* in Sistan (Iran).

S4.10. S.M. KIRCHNER, L. HILTUNEN, E. VIRTANEN, T.F. DÖRING & J.P.T. VALKONEN - *Potato virus Y* transmitting aphids in a Finnish seed potato area.

S4.11. P. LASUE & V. PINCHON - Comparison of two types of yellow water traps for sampling alate aphids.

S4.12. N.C. LAWO, M. GRIESSER & A. FORNECK - Rootstock-phylloxera interaction.

S4.13. S. LIU, Z. WANG, S. SIVAKUMAR, L. GEORGIEVSKA, G.F. KING, W.A. MILLER & B.C. BONNING - Toward aphid-resistant transgenic plants.

S4.14. I. MARKKULA, M. LESKINEN, P. PYLKKÖ, J. KOISTINEN, S. OOPERI, K. TIILIKKALA, H. OJANEN & S. RAISKIO - Detection of aphid migrations in Finland.

S4.15. L. MDELLEL & M. BEN HALIMA KAMEL - Aphids on almonds and peach: biology and life cycle in different area of Tunisia.

S4.16. B. MONSION & V. BRAULT - Annotation of *Acyrthosiphon pisum* genes potentially involved in Luteoviridae transcytosis: yeast two-hybrid system to confirm interaction between aphid and virus proteins.

S4.17. J. OLBRECHTOVÁ & A. KÖHLER - Autumn migration of *Rhopalosiphum padi* in the Czech Republic 1994 – 2008 and the risk of spread *Barley yellow dwarf virus* (BYDV).

S4.18. Y. PELLETIER & X. NIE - The importance of the behaviour of the vector in PVY transmission.

S4.19. L. POLJAKOVIC-PAJNIK & S. ORLOVIĆ - Physiological response of different poplar clones to aphid colonization.

S4.20. L. POLJAKOVIC-PAJNIK & O. PETROVIC-OBRADOVIC - Poplar aphids in Serbia.

S4.21. J. POMPON, D. QUIRING, P. GIORDANENGO & Y. PELLETIER - Physiological impact of xylem consumption on *Macrosiphum euphorbiae*.

S4.22. X. PONS & B. LUMBIERRES - Monitoring aphids in urban green areas: a simple method for evaluating aphid damage.
S4.23. M. RIVI, E. MAZZONI, A. CRINITI, S. CASSANELLI, D. BIZZARO & G.C. MANICARDI - Karyotype variation and insecticide resistance in Italian populations of the peach–potato aphid *Myzus persicae* (Hemiptera: Aphididae).
S4.24. M. RUSZKOWSKA - Aphids on cereals and wild grasses in different environments.
S4.25. E. SCHLIEPHAKE - Evaluation of plant genetic resources of wheat and barley for aphid resistance.
S4.26. S. SHAHIDI-NOGHABI, E.J.M. VAN DAMME & G. SMAGGHE - The carbohydrate-binding activity of the elderberry protein SNA-I is a determining factor for its insecticidal activity.
S4.27. R. THIEME, M. HEINZE, T. THIEME, J. SCHUBERT & U. HEIMBACH - Analysis of the behaviour of virus transmitting Peach–potato Aphid, *Myzus persicae,* feeding on wild potato, *Solanum tarnii*, interspecific somatic hybrids and their progeny.
S4.28. J. VITOU & O. EDWARDS - *Diuraphis noxia* overwintering strategy can affect its performance on resistant and susceptible wheat.
S4.29. B. XU - The occurrence and control of cucumber aphid (*Aphis gossypii*) in Liaoning province, China.
S4.30. G. XU, P. LIU & L. XU - Control of cucumber aphid in greenhouse with biological methods in early spring.
S4.31. S. ZHANG - Control of *Myzus malisuctus* depending on natural enemies.
S4.32. Y. ZHAO & G. XIWU - Status of cotton bollworm and cotton-melon aphid resistance to insecticides and a pesticide management strategy in China.

Opening Session

A short historical account of aphids and aphidologists in Italy

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Entomologists' interest in aphids has quite a long tradition in Italy beginning more or less simultaneously with that in other European countries.

As far as we know, the first Italian who correctly named aphids, using the newly proposed Linnean nomenclature, was G.A. Scopoli. This entomologist, in his well known book "Entomologia carnioloca" published in 1763, described and annotated ten aphid species all belonging to the genus *Aphis*; six of which are well known modern taxa and include *A. fabae* and *A. genistae*.

For a new Italian taxonomic approach to aphids we have to wait until the middle of the following century. Around that time and lasting for forty to fifty years small groups of aphidologists started compiling a comprehensive database on the Italian aphid fauna. Among them was C. Rondani, who in three papers written between 1847 and 1852 described a few well-known aphid taxa, such as *Protaphis terricola*, the wheat greenbug, *Schizaphis graminum* and the Fordine genus *Baizongia*.

Slightly later, G. Passerini, esteemed professor of Botany at University of Parma, published ten papers on aphids over a period of more than twenty years (1856-1879), In 1863, he produced the first Italian catalogue of aphids ("Aphididae italicae hucusque observatae"), established several new genera (such as *Macrosiphum*, *Myzus*, *Myzocallis* and others) and described about fifty aphid species, more than half of which are still valid taxa. Close contemporaries of Passerini were P.M. Ferrari from Genoa and L. Macchiati from Camerino (Macerata). The former was a physician, and passionate naturalist, and published two papers in 1872 in which he described about thirty species of aphid, half of which are still considered valid taxa. Macchiati produced about fifteen papers, mostly on Sardinian aphids, and described more than twenty species few of which are still regarded as valid.

At the end of the 19th century, the eclectic and applied entomologist A. Targioni Tozzetti, director of the Institute of Agricultural Zoology in Florence, published the results of a study on Phylloxerinae, which includes an account of the grape phylloxera and descriptions of three new taxa of oak-feeding species.

One of Targioni Tozzetti's pupils was G. Del Guercio, who worked for many years as a scientist at the same research station in Florence. He published several taxonomic and/or biological papers on aphids, including the applied aspects of controlling pest species. Del Guercio worked for nearly forty years, during which he established quite a large number of aphid genera, only about a dozen of which are currently considered to be valid (e.g., *Anuraphis*, *Drepanaphis*, *Eulachnus*, *Essigella*, *Cavariella*, *Macrosiphoniella*). He also described approximately 150 species, but few of them (about thirty taxa) are still valid.

The great biologist G. Grassi and collaborators, devoted several years to studying the biology and taxonomy of Phylloxerine in Rome, the results of which were published in a large illustrated book in 1912.

Later in the twentieth century, there were more studies on aphids in Italy carried out by an increasing number of scientists at Universities and other national Institutions, some which still exist. Among them, D. Roberti, over more than fifty years, published several papers, including an annotated catalogue of Italian aphids in 1993. A contemporary of his was M. Martelli, who published a monograph on corn aphids and made several other valuable contributions to our knowledge of this group of insects.

Furthermore, it is worth highlighting the effective contributions of several foreign aphidologists, which we are proud to mention, in particular, F.V. Theobald and D. Hille Ris Lambers; the later made valuable additions to the fauna and taxonomic knowledge of aphids in Italy.

Key words: aphids, historical survey, aphidologist, Italy

Session 1

Aphid genomics, molecular genetics and evolution

Plenary Lecture

The pea aphid genome to study phenotypic plasticity

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During 2009, the genome of the pea aphid genome *Acyrthosiphon pisum* will be published. The pea aphid provides the first genome of an hemipteran and an hemimetabolous insect, of a host plant specialist, of an insect able to reproduce both sexually and asexually, and that has coevolved with an obligate bacterial symbiont. This project is the outcome of a long and tremendous effort by a large community, through the International Aphid Genomics Consortium. In 2005, the American National Human Genome Research Institute funded the sequence of the 530 Mb pea aphid genome. In December 2007, the Baylor College of Medicine (Houston, TX, USA) released the first assembly of the *A. pisum* genome (http://www.hgsc.bcm.tmc.edu/projects/aphid/). This raw material was then transferred into the hands of computer scientists and biologists to extract information such as genes coding proteins, genes coding RNAs, repeats, etc. The annotation effort was initiated by running automatic algorithms aimed at predicting putative genes, and culminated by launching the manual annotation by expert biologists in a workshop organized at the University of Princeton, July 2008. After several months of hard work by a large community (around 100 people all around the word), several thousands of genes have been manually curated and are discussed in the main genome paper and in more specific companion papers.

The sequenced pea aphid genome corresponds to 464 Mb, covering a bit more than 85% of the expected 530 Mb. The sequenced fragments have been assembled in approximately 22,800 scaffolds. In the absence of genetic and physical maps, we are still unable to assign scaffolds to the 4 haploid chromosomes. Analysis of the annotated genome revealed i) a large wave of gene duplication and expansion and ii) specific loss of gene families. Some of the major gene expansions include genes involved in chromatin modification, the miRNA synthesis pathway and sucrose-related uniporters. Gene losses include genes central to the IMD immune pathway, selenoprotein utilization, the whole urea cycle, and the purine salvage pathway. Compared to other insect genomes, these features reflect an unusually high level of gene content flexibility.

Analysing a genome requires a database to store, manage and display genomic features. In our lab, we have developed AphidBase as the international aphid genomics database (http://www.aphidbase.com).

Having in hand an annotated genome is a first step, equivalent to an anatomical map of an organism. Next we need to understand how these parts work together. Post-genomics approaches need to be developed and applied to address biological issues. Such post-genomic tools include microarrays of transcripts, proteomic maps, and gene disruption technologies through RNAi. All these strategies are under development by members of the International Aphid Genomics Consortium.

Herein, we will give some examples of how this genome knowledge and the associated post-genomics tools have progressed our understanding of phenotypic plasticity in aphids.

The success of aphids as pests is related to their peculiar life history traits, notably the variation in their reproductive mode between asexual and sexual reproduction which allows extremely high rates of population increase and fast adaptation to environmental change. Aphids reproduce by a viviparous apomictic (clonal) process. Embryos develop within the ovaries of aphid females from diploid oocytes that escape meiosis, in the absence of males. Viviparous parthenogenesis allows adaptation of aphids to local environmental changes by phenotypic plasticity. Phenotypic plasticity requires concomitance between an input signal (the environmental cue) and a critical period of development where the developing organism (usually an embryo) is able to respond to this input. When parthenogenetic, a female adult aphid contains embryos at all stages of differentiation and development, ensuring that at least some embryos (among the youngest) will respond to an environmental trigger. Why do aphids seasonally change their reproductive mode? The answer is to survive cold winters. As cool-blooded animals, aphids do not survive freezing temperatures. By producing over-wintering eggs in autumn, aphids overcome this limitation. Aphid eggs are produced and laid by sexual oviparous females after male fertilization. But how can parthenogenetic individuals produce sexual individuals? In autumn, the decrease of day-length is a sufficient and necessary signal (input) sensed by aphid brains to trigger a developmental switch. The reproductive tract within embryos of aphids submitted to short day conditions switches from the production of diploid oocytes to the production by true meiosis of functional gametes. Thus, phenotypic plasticity of reproductive mode in aphids is dependent on re-programming of developmental pathways, including sensing of the input, transduction of the signal, and effect (or output) on differentiation (gametogenesis and oviparity).

In our lab, we have developed a transcriptomic approach to identify mRNAs that are regulated during the switch of reproductive mode in the pea aphid. We show that in aphid heads, cuticular proteins as well as proteins involved in neuronal physiology are regulated by the shortening of photoperiod. Based on theses observations, we proposed the hypothesis that the insulin pathway might be regulated: by annotating the genome, we identified an expansion of insulin coding genes in the pea aphid.

In parallel, we identified microRNAs in the pea aphid and showed some of them to be regulated by shortening of the photoperiod. Unexpectedly, we showed by annotating the genome that 3 genes (*pasha*, *dicer* and *argonaute*) specifically involved in microRNA synthesis and maturation were duplicated, and all these copies were expressed in the different pea aphid morphs. One of them (*argonaute-1*) is under positive selection, and all these expansions occurred within a brief evolutionary period.

In conclusion, access to an aphid genome has opened new avenues of research not only for understanding the molecular bases of a specific mechanism, but also for understanding population adaptation and ecology. In the very near future, new aphid genomes will be available and one can bet that evolutionary biology as well as population genetics of aphids will benefit from this advance.

Key words: development, insect, miRNA, photoperiod, polyphenism

Plenary Lecture

Aphid molecular systematics: History, progress and prospects

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The aphid lineage (Aphidoidea in the broad sense) contains over 4500 described species and three families, Adelgidae, Phylloxeridae, and Aphididae, the latter accounting for > 95% of species diversity. Historically, most systematists recognized and agreed on a majority of subgroups within Aphididae, but have not always agreed on how subgroups are classified into higher taxa. Most disagreement concerns minor taxa such as *Thelaxes* and *Mindarus*, and major groups represented by *Drepanaphis*, *Phyllaphis*, *Calaphis*, *Panaphis*, *Myzocallis*, *Saltusaphis*, and *Chaitophorus*. In contrast, most systematists have agreed on the tribal composition of Eriosomatinae, Hormaphidinae, and Aphidinae. A recent classification split several former groups into separate subfamilies (Remaudière & Remaudière, 1997).

Classifications are most enduring if taxa are based on monophyletic groups. Traditionally, systematists relied on morphology to construct classifications; however, aphid morphology is problematic for higher-level classification due to morphological reductions and widespread homoplasy. Thus, hypotheses of aphid phylogeny based on morphology have been rare. In the 1990s, DNA sequences emerged as a new class of characters and promised almost unlimited data for phylogeny reconstruction.

Since the mid-1990s, molecular characters have been applied to questions of aphid phylogeny and systematics at several hierarchical levels. Many studies were designed to test an evolutionary question, not classification, with taxon sampling designed accordingly. I review how these studies nevertheless have contributed to a better understanding of aphid relationships and classification. I also discuss where molecular data have so far failed to yield confident relationships, as well as the limitations of molecular data, and the need for effective data analysis. I highlight problems needing further study, and recommendations for future informative studies.

Approximately a dozen published studies have used mainly mitochondrial DNA sequences to examine relationships within aphid genera. About half concern Aphidinae, which contains over 50% of aphid species. Examples include studies of the morphologically homogeneous *Aphis* (Aphidini), and *Brachycaudus* (Macrosiphini), which found support for some subgenera and other subgroups, but ambiguous relationships between subclades (e.g., Coeur d'Acier *et al.*, 2008; 2007). Within *Uroleucon* (Macrosiphini), major subgenera *Uroleucon* and *Uromelan* were paraphyletic (Moran *et al.*, 1999). In addition to elucidating relationships among known species and validating or invalidating subgenera, molecular data also have been used to synonymize taxa and/or aid in species discovery (e.g., Favret & Voegtlin, 2004). Other studies examined character evolution in a subset of *Pemphigus* (Eriosomatinae), *Chaitophorus* (Chaitophorinae) and *Tamalia* (Tamaliinae). While not phylogenetic in intent, aphid barcoding studies contribute important information for alpha taxonomy (Foottit *et al.*, 2008).

At higher taxonomic levels, both mitochondrial and nuclear gene sequences have helped inform aphid relationships. Tribal-level studies have examined relationships within Fordini and Pemphigini (Eriosomatinae), Cerataphidini and Hormaphidini (Hormaphidinae), and Aphidini. In Pemphigini, molecular data suggested that the tribe is paraphyletic (H. Zhang & Qiao, 2007a). Studies on Fordini support the validity of three subtribes, Fordina, Baizongiina, and Melaphidina (e.g., H. C. Zhang & Qiao, 2007b). Phylogenies of Aphidini show high support for Aphidina and Rhopalosiphina, while so far failing to resolve relationships among most subgroups of Aphidina (e.g., Kim & Lee, 2008). Relationships within subfamilies have been studied for Aphidinae, Eriosomatinae, Hormaphidinae, and Lachninae. In some cases, traditionally recognized tribes were paraphyletic and need redefinition (Normark, 2000; von Dohlen *et al.*, 2006).

Within Aphididae as a whole, relationships of major lineages remain largely elusive. An early study found support for most tribes and some subfamilies, but no resolution of relationships among them (von Dohlen & Moran, 2000). A later study found tentative support for three major lineages: Lachninae, Aphidinae + Drepanosiphinae + Chaitophorinae + Calaphidinae, Eriosomatinae+Thelaxinae+Anoeciinae; Eriosomatinae was paraphyletic (Ortiz-Rivas *et al.*, 2004). These studies supported a sister relationship of Drepanosiphinae + Chaitophorinae, Calaphidinae + Saltusaphidinae, and Aphidinae + Pterocommatinae (subfamilies *sensu* Remaudière & Remaudière, 1997).

Collectively, aphid molecular systematic studies reveal several patterns. Lack of resolution among well-defined lineages at various hierarchical levels poses a continuing challenge. Many such instances parallel areas of aphid classification where morphology is ambiguous. Where problematic long branches are evident in the phylogeny, difficulties may be resolved by more strategic taxon sampling. In other cases, data from additional genes evolving at appropriate rates may help. Genes from *Buchnera* endosymbionts have been used successfully, but continued monitoring for horizontal gene transfer or departures from co-speciation is important. Sequence data emerging from the Pea Aphid Genome Project should aid greatly in exploration for informative nuclear genes. Some cases of low

resolution may be a result of rapid diversification; these are problematic because increased sampling (taxa or genes) might not resolve the associated short internodes. However, rapid bursts of speciation can be interesting phenomena in and of themselves.

While subject to some of the same limitations as morphology, molecular characters have demonstrated utility for aphid systematics. Many lineages remain to be investigated and several problems need more attention. Relationships within several major subfamilies are as yet little studied, or unstudied (e.g., Chaitophorinae, Drepanosiphinae, Calaphidinae, Saltusaphidinae, Greenideinae). The monophyly of some subfamilies is in question (Eriosomatinae, Hormaphidinae), or has never been tested (e.g., Anoeciinae). Many minor subfamilies have not been sampled or included in family-level studies. Lack of resolution among subfamilies and tribes may indicate an ancient, rapid radiation, which, if resolvable, will require extensive sampling of all extant lineages and multiple nuclear genes.

Key words: Aphids, classification, mitochondrial DNA, nuclear DNA, phylogeny, taxonomy

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Oral Presentation

Proteomic profiling of pea aphid saliva and salivary glands

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Genomic resources resulting from the pea aphid genome sequencing project are resulting in the application of proteomic and mass spectrometry methodologies to aphid biology. For example, insight into the plant-aphid interaction has been afforded by the direct identification of salivary proteins in aphid saliva. Proteomic analysis of pea aphid saliva indicated that at least 9 proteins are present (Carolan et al., 2009). Four of these proteins were identified by their sequences and included 2 metalloproteases a glucose-methanol-choline oxidoreductase and regucalcin (senescence marker protein 30). The other five proteins were not homologous to any previously described sequence and included an extremely abundant salivary protein, termed Sheath Protein (SHP) due to the likelihood that it is a component of the salivary sheath. The high proportion of "novel" proteins (no homologues in current databases) is most likely reflective of the highly specialised feeding habit demonstrated by aphids.

The salivary gland proteome was also investigated using shotgun proteomic and multi-dimensional protein identification technologies resulting in the production of a non-redundant catalogue of the pea aphid secretome. In total over 500 proteins (supported by multiple peptides), were identified, 57 of which were novel. Signal P analysis identified over 50 proteins with secretion signals and expands the current list of proteins that could potentially act as effectors within the plant. Gene Ontology was assigned to the salivary gland proteins using the Blast2Go suite (http://www.blast2go.org/). Mass spectrometry data has been deposited in the PRIDE public repository (www.ebi.ac.uk/pride) and the 2D gel images have been deposited in the WORLD 2D PAGE database (world-2dpage.expasy.org/repository). On-line access to the data will offer fundamental insights into the biology of aphids and will facilitate applied research targeting sap-feeding insect pests. It is anticipated that these datasets and reference gels will stimulate the submission of additional proteomic analyses to form the basis of a reference proteome and proteomic repository for the pea aphid.

CAROLAN J.C., FITZROY C.I.J., ASHTON P.D., DOUGLAS A.E., WILKINSON T.L., 2009. The secreted salivary proteome of the pea aphid *Acyrthosiphon pisum* characterised by mass spectrometry. Proteomics, in press.

Oral Presentation

The circadian clock system in the pea aphid *Acyrthosiphon pisum*

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Circadian clocks are instrinsic timekeeping systems that help most organisms adapt to the daily cycling in environmental conditions. Basic elements constituent of animal circadian clocks are highly conserved evolutionary and have been best characterized in *Drosophila* and the mouse. The core of the clock mechanism in a diversity of organisms can be described by a transcription-translation feedback-loop model with positive and negative elements. In *Drosophila*, the central circadian clock is composed of six transcription factors, organized into two feedback loops. The *Per/Tim* feedback loop is based on rhythmic expression of genes *Period* and *Timeless*, encoding transcription factors PER and TIM which repress their own transcription. Expression of these genes is promoted by CLOCK-CYCLE heterodimers that bind to promoter regulatory elements. In the second feedback loop, the transcription of genes *Vrille* and *Pdp1* is also activated by the CLK-CYC heterodimer, and the transcription factors produced, VRI and PDP1, regulate the expression of gene *Clock*. Other genes are involved in regulating the stability, subcellular localization and degradation of these proteins. In *Drosophila*, photoperiod entrainment is accomplished with the participation of the photoreceptor *Cryptochrome*, which along with the ubiquitin ligase JETLAG mediates TIM degradation.
In the present study, we searched the recently sequenced pea aphid (*Acyrthosiphon pisum*) genome for homologs of circadian genes in insects. The pea aphid genome encodes a single homolog of each of the core clock genes: *Clk, Cyc, Per, Tim, Pdp1* and *Vri*. Both *Drosophila* and mammalian type cryptochromes are also found, the latter (*Cry2*) being duplicated. Complete characterization of these genes was performed at the cDNA level. Sequence analysis revealed a high divergence for some aphid clock genes when comparing to other available insect sequences. Homogeneity tests revealed significant differences in amino acid composition, especially for *Per* and *Tim* sequences. Moreover, relative rate tests performed on amino acid sequences showed that these genes were evolving at significantly accelerated rates with respect to their respective insect orthologs. Additionally, taking into account that the NLS motifs necessary for nuclear import are rather divergent in *A. pisum* PER, we hypothesize that in *A. pisum* clockwork gene *Cry2* instead of *Per* is the main repressor of CLK-CYC transcriptional activation. Real time PCR showed that the expression levels of some of these circadian genes oscillated weakly along the day-night cycle. Most importantly, in some cases the amplitude of oscillations were higher under short-day conditions. These results suggest that transcriptional regulation of circadian clock genes may have a role in aphid photoperiodism.

Oral Presentation

Odorant-binding proteins in aphids

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Odorant-binding proteins (OBPs) are a family of small water-soluble proteins present at high concentration in the aqueous fluid in the antennae of insects. They are involved in the first step of olfaction, carrying semiochemicals, such as pheromones and host odours to the olfactory receptors (ORs). The OBPs have been classified into groups: classic OBPs (6 conserved cysteines), plus-C OBPs (8 conserved cysteines, one conserved proline), atypical OBPs (9 to 10 conserved cysteines) and chemosensory proteins (CSPs; 4 conserved cysteines). Sequences encoding putative OBPs can be identified by the predicted proteins having: 1) a six α-helix pattern, 2) the conserved cysteine residues with the expected spacing between them, 3) a globular water-soluble nature and 4) the presence of a signal peptide. We have identified genes encoding putative OBPs in the genome in the pea aphid *Acyrthosiphon pisum* from ESTs, the whole genome sequence database and its predicted gene set. This has identified 13 Classic and 2 Plus-C OBPs and 13 CSPs. The *A. pisum* OBP and CSP genes tend to be clustered in the aphid genome and have more and longer introns than their counterparts in *Drosophila*. We have also identified homologous sequences in 9 other aphid species and compared the aphid OBPs with those of other insect species. This showed that although insect OBPs are very divergent within a species and between different Orders, there is a high similarity between the OBPs of species within a genus. For some of the CSPs we have shown that the proteins are expressed in antennae and could therefore be involved in olfaction.

Oral Presentation

Molecular mechanism of facultative parthenogenesis in the Pea Aphid, *Acyrthosiphon pisum*

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Phenotypic plasticity allows organisms to quickly adapt in response to changing environments. However, little is known of the genetic and epigenetic contribution to the expression of alternative adaptive developmental outcomes. To this end, we studied the reproductive polyphenism in the pea aphid, *Acyrthosiphon pisum* (Harris), wherein genetically identical individuals reproduce either sexually (meiosis) or asexually (parthenogenesis) depending on the photoperiod during maternal development. To understand the evolution of and cellular and molecular basis of facultative asexuality in aphids, we determined meiosis and cell division gene activity in sexuals and asexuals.

We found that the pea aphid genome encodes single copies of homologs for the majority of the core meiotic machinery, suggesting that meiotic plasticity is not due simply to gene loss or expansion. Next, we determined if these core meiosis genes are expressed by *in situ* analysis and PCR from cDNA isolated from asexual and sexual ovaries. Surprisingly, genes thought to act solely in meiosis in other organisms (e.g., *Spo11*, *Hop2*, *Mnd1*, *Msh4* and *Msh5*) are expressed not only in asexual ovaries but also in somatic tissue and an obligately asexual aphid strain. Interestingly, the *Spo11* PCR product from asexual ovaries contained intronic sequence, thus representing unspliced mRNA. Using quantitative PCR, we determined that germline expression of *Spo11*, *Mnd1* and *Hop2* is similar in sexuals and asexuals. Preliminary results identified candidate DNA methylation sites in the *Spo11* locus, indicating a possible epigenetic basis for this expression difference.

Furthermore, the pea aphid genome contains lineage-specific duplications of several regulators of mitosis, such as *Cdk1*, *Polo*, *Wee1*, *Cdc25* and *Aurora*. Several of these mitotic kinase paralogs are differentially expressed between the different reproductive morphs. Together, the duplications and differential expression of genes involved in meiosis and cell division may allow aphids to express alternate reproductive phenotypes. Further characterization will help us better understand the evolution of and molecular and epigenetic mechanisms underlying this adaptive facultative plasticity.

Key words: polyphenism, meiosis, evolution

Oral Presentation

DNA methylation in the pea aphid

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DNA methylation of cytosine residues is common in many eukaryotic genomes and is usually a suppressive mechanism, either of specific genes, selfish genetic elements or entire chromosomes as in X inactivation. Evidence of DNA methylation in insects has until recently been limited to a few examples. Recent evidence from the honeybee (*Apis mellifera* Linnaeus) and several other insect species has suggested a greater than hitherto suspected role for DNA methylation in insects. Using a bioinformatic approach the machinery for methylation has been found in the pea aphid: two *Dnmt1* genes, the maintenance methyltransferases, *Dnmt2*, a suspected tRNA methyltransferase and *Dnmt3*, the *de novo* methyltransferase are all present. We have identified twelve different genes that are methylated in the pea aphid, the most identified in any insect. Calculations of the CpG ratio of the coding sequences from the genome suggest that many more genes could be methylated and that the pattern and functional significance of DNA methylation in insects is different to that found in vertebrates and plants.

Key words: DNA methylation, epigenetics, DNA methyltransferase

Oral Presentation

Genomic insight into the amino acid relations of the pea aphid with its symbiotic bacterium *Buchnera aphidicola*

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The symbiosis of aphids and their obligate bacterial symbiont *Buchnera aphidicola* has a nutritional basis. There is persuasive experimental evidence that these bacteria provide aphids with essential amino acids (*i.e.* amino acids that contribute to protein but cannot be synthesized *de novo* by animals), supplementing the limited supply of these nutrients in the aphid's phloem sap diet. Consistent with these functional data, the genome of *Buchnera* includes most genes required for essential amino acid biosynthesis, but lacks the genes for amino acid degradation and the synthesis of most nonessential amino acids (Shigenobu *et al.*, 2000). These nutritional, metabolic and genomic observations suggest that aphid metabolism supports a substantial flux of nonessential amino acids from the insect to the symbiont and of essential amino acids in the reverse direction. It has widely been assumed that amino acid biosynthetic reactions that are not encoded in the *Buchnera* genome were lost from the ancestral *Buchnera* symbiont through genetic drift and relaxed selection because the host aphid catalyzes those reactions. In other words it has been assumed that the two partners are functionally complementary. Availability of the *Acyrthosiphon pisum* genome provides a unique opportunity to test the hypothesis of genomic complementarity.

We annotated genes that contribute to amino acid biosynthesis and degradation in the genome of *A. pisum*. Genome annotation revealed orthologs of most genes in the amino acid biosynthetic and degradative pathways of *Drosophila melanogaster*. Comparison between the gene complements of the *A. pisum* and *B. aphidicola* genomes revealed strong complementarity. In particular we identified five instances where either 1) the genetic capacity of the pea aphid differs from that of other insects with completely sequenced genomes or 2) pea aphid genes contribute to pathways that are also represented by *Buchnera* genes, leading to the potential for shared metabolic pathways between the two partners. These five instances relate to pea aphid aminotransferases, the synthesis of branched chain amino acids, the synthesis of the sulfur amino acids and the amino acids tyrosine and arginine.

Key words: pea aphid genome, symbiosis, amino acid biosynthesis

SHIGENOBU S., WATANABE H., HATTORI M., SAKAKI Y., ISHIKAWA H., 2000. Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. Nature, 407: 81-86.

Oral Presentation

Uncovering the limitation of the aphid immune response

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Due to the ubiquity of microbes in the environment, it is assumed that most organisms must be able to protect themselves from microbial pathogens. Recent genomic analyses of defense mechanisms in arthropods suggest conservation of key elements underlying responses to pathogens and parasites. At the center of pathogen-induced immune response are signaling pathways triggered by the recognition of fungal, bacterial and viral signatures. These pathways signal the production of response molecules, such as antimicrobial peptides and lysozymes, which degrade or destroy invaders. We have used a combination of gene annotation of the recently available pea aphid (*Acrythosiphon pisum*) genome, functional genomics and classical immunological approaches to compare the immune response of pea aphids to that of other insects. Strikingly, pea aphids appear to be missing genes thought critical for recognition, signaling and microbial degradation in insects. In line with results of gene annotation, experimental analyses designed to characterize response of immune-challenged aphids uncovered evidence for few immune-related products. Several proposed hypotheses to explain this lack of response, including a microbe-free habitat and rapid reproductive rate despite infection, can be largely discounted, and the limitation of the immune response may instead be closely tied to the dependence of these aphids on bacterial symbionts.

Key words: immunity, symbiosis, signaling pathways, genome annotation, host-parasite interactions

Oral Presentation

A model aphid bacterial pathogen: the phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi*) and its insect-specific virulence factors

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As most Aphidoidea, the pea aphid (*Acyrthosiphon pisum* Harris) is feeding on an almost germ-free diet, the plant phloem sap. In spite of its abundant symbiotic bacterial inhabitants, aphids were never described as prone to bacterial pathogens, until recently. We described an experimental pathosystem where an aphid is efficiently killed by *Dickeya dadantii* (syn. *Erwinia chrysanthemi*), a typical enterobacterial phytopathogen which possesses canonical insect-killing toxins from the *B.thuringiensis cyt* family (Grenier *et al.* 2006). We further analysed this pathosystem and characterized both the infection routes of the pathogen, and many regulators of its virulence against the pea aphid. These studies identified the digestive tract as the main target of the *cyt* toxins, but not the main host tissue of the bacteria, and showed both similarities and striking differences in the genetic regulation balance by key regulators involved in insect *vs* plant pathogenesis. We will discuss the impact of harbouring insect-targeted virulence factors for a phytopathogen, and the potential ecological importance of bacteria as aphid pathogens.

Key words: *Acyrthosiphon pisum*, bacterial pathogenesis, *Bacillus thuringiensis*, pectinolytic bacteria, cytolytic toxins

GRENIER AM, DUPORT G, PAGES S, CONDEMINE G, RAHBÉ Y., 2006. The Phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) Is a Pathogen of the Pea Aphid. Appl. Environ. Microbiol. 72: 1956-65.

Oral Presentation

Application of proteomic tools to investigate the respective role of aphid and symbiotic bacteria in relation to host plant

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Recent progress has been made in the field of proteomics due to significant improvements in the methodology and accessibility protein separation and identification methods. Further advances can be made by coupling proteomic and genomic approaches. Molecular entomologists now have a panel of tools to address the proteome patterns related to differential adaptation of insects to cope with plant defence mechanisms. The aphid-plant relations will be used as a case study. Indeed, aphids represent a wonderful model in the study of plant adaptation.

We investigated the adaptation and metabolic changes of aphids in relation to host plants focusing on the role of the bacterial endosymbionts. Use of artificial diet including diverse antibiotics but also the comparison of proteomes related to whole aphid and respective purified bacterial symbionts were studied to identify the respective origin and function of proteins constituting the studied proteomes. Diverse methods including traditional two dimension electrophoresis, 2D-Differencial In Gel Expression (2D-DIGE), liquid chromatography (LC) coupled with mass spectrometry (ElectroSpray Ionisation, ESI, and Matrix-Assisted Laser Desorption/Ionisation with time-of-flight mass spectrometer, MALDI-TOF) and data bank investigations were developed.

From the proteome investigation and identification from aphid fed with particular antibiotics but also from proteomes of whole aphid and related extracted bacterial endosymbionts, particular proteins of interest were selected and accurately characterised with both fundamental but also applied views. This broad proteomic approach will be discussed as an interesting and reliable tool to study the biologically involved proteins from aphids in response to several environmental changes, and particularly the insect - host plant interactions.

Oral Presentation

Identification and annotation of predicted secreted salivary proteins in the pea aphid, *Acyrthosiphon pisum*

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The results of a transcriptomics approach to identification of pea aphid (*Acyrthosiphon pisum*) salivary gland-secreted proteins are presented. A dataset was analyzed consisting of 9509 ESTs obtained from two cDNA libraries constructed from RNA of dissected pea-aphid salivary glands. From these we assembled 826 contigs, leaving 2662 singletons. Here we analyze the EST-contigs that have a statistically significant expression bias in salivary glands *and* can be clearly tied to genes in scaffolds of the recently assembled pea aphid genome. These include contigs/genes that encode proteins that are "annotatable", predicted proteins that can be identified as homologs of previously predicted proteins in organisms other than aphids, and those that are "non-annotatable". The annotatable contigs include proteins/enzymes that are members of well-documented protein families, including hydrolases, oxidoreductases, and binding proteins. One non-annotable protein is Protein C002, which we have previously demonstrated to be needed for survival of pea aphids on a host plant. We suggest that others of the non-annotatable pea aphid salivary-gland contigs may encode proteins of important (though unknown) functions in aphid/plant interactions. Interestingly, several of the non-annotatable EST-contigs do show sequence similarity to (unannotated) ESTs from plant EST projects. Using the TargetP program, we have identified N-terminal signal-secretion peptides as indicators of likely secretion into saliva. We compared salivary-gland-enriched EST contigs with NCBI-deposited transcripts from salivary glands of other organisms and found relatively few commonalities outside of aphids. This could indicate a requirement for unique proteins in the aphid salivary gland secretome, forming as it does, a key evolutionary interface between aphid and plant.

Key words: EST, saliva, effector, aphid-plant interactions

Oral Presentation

Response in the aphid *Myzus persicae* to insecticide pressures: searching for genetic targets of selection

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The artificial selective pressures by insecticides on aphid populations produce different responses in terms of detoxification mechanisms. With this goal, *Myzus persicae* aphids were exposed during 12 hours to a sub-lethal dose (LC50) of the insecticide pirimicarb using water as control. Immediately after, the mRNA from each condition was isolated, the cDNA synthesized, labeled reciprocally with Cy3 and Cy5, and hybridized against a heterologous cDNA microchip containing 5,760 aphid genes (5,126 from *Acyrtosiphum pisum*, 378 from *Myzus persicae* and 256 control genes). The results of hybridizations were normalized and then subject to statistical analysis. Thirty eight transcripts appeared to be regulated ($p<0.05$; genANOVA) by insecticide (13 up and 25 down regulated genes). In order to corroborate the regulation, transcript levels were quantified by real-time PCR using two up-regulated genes (Glutathione-S-transferase, which participate in detoxification, and Acyl-CoA binding protein, which degrade neurotransmissors), and two down-regulated genes (heat shock protein, which participated in a stress response and Ubiquinone oxidoreductase, which participate in mitochondrial respiratory chain). These results give new insights about the mechanism of insecticide resistance mediated by gene regulation in *Myzus persicae*, and suggests some putative genetic targets of selection.

Key words: *Myzus persicae,* insecticide resistance, selection, gene regulation

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Oral Presentation

Molecular basis of self-sacrificing gall repair by soldier aphids in the social aphid, *Nipponaphis monzeni*

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In social aphids, it has been reported that soldier castes perform three kinds of altruistic behavior, colony defense, gall cleaning and gall repair. Gall repair was reported from a gall-forming aphid, *Nipponaphis monzeni*, in which monomorphic first-instar nymphs (i.e. no remarkable morphological differences between soldiers and normal individuals) repair their gall in a self-sacrificing manner (Kurosu *et al*., 2003). *N. monzeni* forms completely closed galls on the tree *Distylium racemosum*. Since the wall of growing galls is still soft in early spring, gall-feeders such as lepidopteran larvae often invade the gall by tunnelling through the wall. When a hole was bored in the gall wall, soldier nymphs of *N. monzeni* immediately gathered around the hole, discharged a large amount of body fluid from their cornicles on the damaged area, and mixed the fluid with their legs. The discharged fluid soon became viscous and solidified, whereby the hole was filled up completely. In an attempt to understand the molecular basis of gall repair, especially of the solidification, we analyzed proteinacous components of the body fluid. We found that the body fluid consisted of six major proteinacous components, one of which was phenol oxidase, a key enzyme involved in melanization and hemolymph clotting in insects. Molecular and enzymatic analyses revealed that the expression level of the phenol oxidase in the gall-repairing soldiers was much higher than those in the non-repairing individuals. Other components in the fluid were novel proteins, that contained highly repetitive sequence motifs and showed no sequence similarity to protein sequences deposited in the databases. It seems likely that these proteins are cross-linked due to the action of highly reactive intermediate substances in the melanin pathway, represented by quinones, that results in solidification of the body fluid. From these results, we suggest that the aphid's innate immune and wound-healing mechanisms have been recruited to the social task, gall repair, in the lineage leading to *N. monzeni*.

Key words: social aphid, soldier nymph, gall repair, melanization, hemolymph clotting

KUROSU U., AOKI S., FUKATSU T., 2003. Self-sacrificing gall repair by aphid nymphs. Proc. R. Soc. B 270, S12-S14.

Oral Presentation

No "most successful" clones for Grape Phylloxera in European leaf feeding habitats - Why?

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The genetic structure of ten Austrian grape phylloxera populations, *Daktulosphaira vitifoliae* (Fitch) (Homoptera: Phylloxeridae) was analyzed using six SSR markers (*Dvit1-Dvit6*). The populations were sampled from four distinct viticultural areas in the West and South of Austria in spring of 2006 and 2007: Burgenland, Weinviertel, Vienna and Steirmark. Only leaf-feeding populations were chosen from similar ecological habitats, where susceptible rootstock hosts have overtaken scions in abandoned vineyards and produce long-lived grape phylloxera populations. These populations have been known for years and have not undergone pesticide treatments.

To study population structures and test for potential "super clones", population genetic measures were performed. The genetic diversity detected within the entire set of 305 genotypes was very high, with 152 multilocus individual genotypes. An excess of heterozygotes and significant deviations from HW equilibrium clearly indicated that the major reproduction mode in these populations is asexual, confirming previous studies from populations in Germany and France. Very few overlapping genotypes (three genotypes each connecting two populations) and strongly negative *Fst* values confirm the sessile nature of the insect and indicate that the main mode of insect dispersal is by infested plant material. Multi copy genotypes were extremely rare with the exception of G52 (7), G120 (11) and G145 (19), which were each limited to one population, indicating that no "most successful" genotype was found within the leaf-feeding habitats studied. Possible reasons for such unique population structures are discussed and may relate to the ecology and biology of these habitats, excessive mutation rates within the populations/clones studied, or sampling errors.

Key words: Grape Phylloxera, SSR, population structure, super clone

Oral Presentation

Why are there so few aphid clones?

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In Europe, aphids are generally thought to contribute significantly to the so-called 'aerial plankton' during the spring through to autumn months (growing season), although the actual flight behaviour of individuals has been found, especially from molecular ecological studies, to be species-specific in terms of migratory ambit (range). Thus some species are mostly short distance fliers (average flight distance < 30 km), other medium distance (< 50km), and other species still long distance fliers (sometimes > 100 km). Many of these species individuals may be assumed to belong to clonal asexual lineages, that is, derived from a single asexual foundress. We are presently studying two specialist tansy (*Tanacetum vulgare*)-feeding aphids from samples collected in Jena, Germany – *Macrosiphoniella tanacetaria* (Kaltenbach) (MA) and *Metopeurum fuscoviride* Stroyan (ME) using microsatellite markers. On plotting the number of multilocus genotypes (MLGs) against the number of multiple clonal repeats (1, 2, 3 copies, etc.), it was found that the populations mostly consisted of single rather than multiple copy MLGs. To test this further, microsatellite data collected from a previous study on MA in Germany and France in the year 2000 and on samples of the grain aphid, *Sitobion avenae* (Fabricius) collected in the UK in 1997/8, the latter both in the field and by 12.2. m high suction trapping, were examined in the same way. Again, most MLGs appeared as individual copies. The data are briefly discussed in the light of our evidence, as well as that of other similar studies on other aphid species, relating molecular genetic data to aphid life history, behaviour and ecology.

Oral Presentation

Genetic diversity of the melon aphid *Aphis gossypii* Glover in different melon growing areas of France

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Aphis gossypii (Glover) is a cosmopolitan pest of crops with population genetic diversity structured in relation to its host plants (Carletto *et al.*, in press). Aphids colonizing cucurbits are distributed into two genetic clusters on the basis of eight microsatellite markers. The first cluster comprises a single genotype called NM1 that has been only observed in Southeast France. The second cluster comprises all the other genotypes observed on Cucurbits, including the C9 genotype found all over the distribution area of *A. gossypii*. In melon, the *Vat* gene confers resistance to the *A. gossypii* colonization (Pitrat & Lecoq, 1982). The resistance appears to be complete for clones having a genotype NM1 and partial for clones having a genotype C9 (Boissot *& al.*, 2008).

In this study, our aim is (1) to describe the genetic structure of *A. gossypii* populations infesting Cucurbits in geographically distant melon -producing areas and (2) to evaluate the selection pressure that the *Vat* gene is exerting on *A. gossypii* populations.

We sampled apterous *A. gossypii* (isolated or in colony) from melon crops of susceptible and resistant cultivars over the course of the crop cycle. Samples were collected in four fields of three regions located in Southeast France, Southwest France, and in the French West Indies. Every one of the 1409 apterous aphids sampled was genotyped using eight microsatellite markers to assess its multilocus genotype (MLG).

We discriminated 33 frequent and 71 other MLGs present in only one copy. In France, 5 MLGs were frequently observed over all the areas: NM1, C11, C9, CUC2 and MTB. The dominant MLGs were C9 (24.6%) and CUC1 (27.9%) in the Southeast while it was MTB (55.9%) in the Southwest. In the French West Indies, Guadeloupe, the most frequent MLGs, were C6 (15.8) and GWD (83.8%) that had never been observed in France. Both MLGs observed in Guadeloupe and 13 of those observed in France were found to belong to the second genetic cluster described by Carletto *& al.* (in press). The 3 other MLGs detected in France were close to NM1 and therefore belong the first cluster. The diversity of *A. gossypii* appeared to be reduced in Guadeloupe, a small, tropical, geographically isolated island in the Caribbean Basin, as compared to temperate France where sexual reproduction generates genetic variability as suggest the HW equilibrium in the great majority of the loci when we considered site-associated populations. However, the FST analysis did not reveal any structuring effect of French localities.

In addition, we compared the global MLG frequencies in aphids collected on melon plants with the *Vat* gene and on melon plant without the *Vat* gene. NM1, C9 and MTB were significantly less frequent on melon with the *Vat* gene than on melon without the *Vat* gene (χ^2=29.70, 10.05 and 28.45 respectively, $p<0.01$). This decrease is consistent with the known effects of the *Vat* gene on NM1 and C9 (Boissot & *al.*, 2008). On the other hand, the frequencies of CUC1 and C6 increased significantly on melon plants with the *Vat* gene (χ^2=19.69 and 27.36 respectively, $p<0.0001$). We have therefore identified some MLGs able to develop on resistant cultivars. These results should be confirmed with several years' trials to evaluate the selection pressure that the *Vat* gene is exerting on *A. gossypii* populations.

Key words: *Aphis gossypii,* genetic diversity, microsatellites, *Vat,* resistance gene, *Cucumis melo*

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Oral Presentation

Phylogenetic relationships of the known species-groups of the genus *Aphis*, based on molecular and morphological characters with evidence of cryptic speciation in the *gossypii* group

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The genus *Aphis*, with more than 500 species worldwide (Remaudière & Remaudière, 1997), includes species complexes converged on particular morphological types. Many species of *Aphis* have been considered to be possibly clustered into four species-groups (*gossypii*, *craccivora*, *fabae*, and *spiraecola* groups) in many taxonomic studies. Recently, we ascertained that the morphological differences and similarities within the four species-groups are considerably congruent with their phylogenetic relationships, based on three mitochondrial and nuclear DNA fragments: 12S/16S, tRNA/COII, EF1α (Kim and Lee, 2008). As a result, they were separated into two main lineages; the *gossypii* group and *craccivora* + *fabae* + *spiraecola* groups. In addition, the genetic distances between the members of *gossypii* group were quite small. Nevertheless, the morphology-based analysis has not been explored to clarify the objective characteristics determining the groups.

We performed phylogenetic and morphometric analyses using morphological characters in order to reconstruct their relationships as well as to evaluate the relative contributions of the characters to determine the groups. Based on the two different algorithm-based analyses, we confirmed that the *gossypii* group could be morphologically separated from the other three groups. However, the *spiraecola* group was not clustered separately, and the *fabae* and *craccivora* groups were mixed due to some similarities such as dorsal pigmentation. These three groups may likely be treated as one because they occurred in the same clade, on the basis of several characters in the phylogenetic analysis, and all separated together from the *gossypii* group in the morphometric analysis. The *gossypii* group was supported by reduced or regressive morphological characters, while the other groups exhibited conspicuous differences with high variation on those characters. Most characters of the *gossypii* group, especially meristic ones, were less variable than those of the other groups. These morphological affinities within the *gossypii* group are consistent with their genetic similarities. This suggests that the species of the *gossypii* group recently diverged from a common ancestor.

Key words: *Aphis*, DNA, morphometric analysis, phylogeny, species-group

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REMAUDIÈRE G., REMAUDIÈRE M., 1997. Catalogue of the world's Aphididae – Homoptera Aphidoidea. INRA, Paris, 473 pp.

Oral Presentation

Genetic relationship among species of the *Aphis frangulae/gossypii* group based on mitochondrial DNA sequences

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In the genus *Aphis* L. (Rhynchota Aphididae) there are currently more than 600 valid species (Remaudière & Remaudière, 1997). Most of these are associated with a wide range of wild hosts, mainly belonging to the Dycotiledones, and some are injurious crop pests.

The taxonomy of the genus is not simple, since it lacks the clear morphological characteristics necessary for distinguishing one species from another. They are often clustered into 'complex' groups which include a number of specific and subspecific taxa. One of these is the so-called *frangulae/gossypii* complex, morphologically characterized by reduced or absent (at least in apterae) dorsal body sclerifications, short antennal and crural hairs, sclerified medium-sized siphunculi, and usually a pale cauda with a few hairs.

The ancestor taxon of the *A. frangulae/gossypii* complex has Ramnaceae as primary host and several secondary host plants. This latter diversification is at the origin of the split into different taxa of the group, and the process is probably still ongoing. Normally, the speciation process happens with genetic and biological differentiation, followed by some distinguishable morphological characteristics. Studies by several authors have highlighted difficulties in discriminating species and sometimes advanced the possibility of considering them as subspecies or biotypes of *A. frangulae*.

A molecular investigation was carried out on that aphid complex, considering a group of twenty-five putative species, by sequencing mitochondrial cytochrome oxidase subunit II (COII) and including COI sequences previously acquired (Cocuzza *et al*., 2008). The analyzed species were morphologically identified in advance. Genomic DNA was extracted by using Chelex-100 resin. Amplification of COI was obtained using primers C1-J-2195 and TL2-N-3014, whereas the primers used for COII were mt2993+ and A3772. Genetic distances were estimated using the 2-parameter Kimura model as implemented in Mega4, whereas phylogenetic reconstruction was achieved by maximum parsimony criterion as implemented in PAUP* 4.0b10.

A total of 1217 bp were obtained from COI (537) and COII (680). The results confirm that *A. frangulae* and *A. gossypii,* with their mostly allied taxa, are genetically separate at the specific level. Interestingly, the status of some postulated species are questionable. There is minimal genetic distance among postulated species in the *gossypii* subgroup. This could mean that the group is subdivided into several biotypes which have adapted to various host plants. This biotype hypothesis is supported by the strict relationship of *A. gossypii* sensu stricto (collected from *Citrus x sinensis* and *Cucurbita pepo*) with the other taxa examined. The elevated adaptability of the *gossypii*-group taxa to a wide range of host plants is undoubtedly an ecological advantage that permits their survival in different environments. Following molecular analysis, it is unjustified to consider *A. myopori*, *A. catalpae*, *A. sedi*, *A. capsellae* and *A. brunellae* as separate species from *A. gossypii.*

Among the postulated species of the *frangulae* subgroup, genetic distance is greater. Only *A. symphyti* (collected from *Anchusa italica*) shows a strict link with the two samples of *A. frangulae* s.s. (from *Rhamnus alaternus* and *Lamium purpureum*, respectively). The other taxa we analysed (*A. chloris* from *Hypericum hircinum*, A. *helianthemi* from *Helianthemum nummularium*, *A eupatorii* from *Eupatorium cannabium*, *A. punicae* from *Punica granatum* and *A. origami* from *Origanum vulgare*) appear genetically separate.

Molecular investigation is still in progress and other postulated species are being considered for a deeper understanding of the complex.

Key words: Aphids, cryptic groups, taxonomy, molecular genetics

COCUZZA G., CAVALIERI V., BARBAGALLO S., 2008. Preliminary results in the taxonomy of the cryptic group *Aphis frangulae/gossypii* obtained from mitochondrial DNA sequence. Bulletin of Insectology, 61 (1): 125-126.
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Poster S1-1

Cloning and characterization of the ecdysone cascade with ecdysone receptor (EcR) and Ultraspiracle (Usp) in the pea aphid

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Ecdysteroids are the key insect hormone in insect growth and development and they manifest their activity via interaction with the nuclear receptor complex of ecdysone receptor (EcR) and Ultraspiracle (Usp). These two nuclear receptors form the start of the ecdysteroid cascade. We report here the full length coding sequence of *EcR* and *Usp* in the pea aphid *Acyrthosiphon pisum*. Then we also investigated the presence of transcripts from both genes during the development of a nymphal stadium. The converted amino acid sequences were compared with those of other insects and we examined the phylogenetic relationships of EcR and Usp genes in the hemi-, holo- and ametabolous insect orders Hemiptera, Orthoptera, Lepidoptera, Diptera, Hymenoptera, Coleoptera, and Collembola, and also Crustaceans. In addition we used the amino acid sequence to make an initial *in silico* 3D model of the ligand-binding pocket of EcR docked with ecdysteroid hormone. Finally, we investigated the phenotypes that can result from silencing of EcR and Usp in RNAi experiments. Our results support the advent of *Acyrthosiphon* as a powerful model allowing a better understanding of insect growth and development.

Key words: *Acyrthosiphon*, nuclear receptor, ecdysone cascade, RNAi

Poster S1-2

The *Acyrthosiphon* genome contains at least 19 nuclear receptors with the ecdysone cascade revealing an increase in evolutionary rate

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Screening the *Acyrthosiphon pisum* genome indicates the presence of at least 19 nuclear receptors, representing all of the seven known nuclear receptor subfamilies (NR0-NR6), in this hemipteran species. This first complete nuclear receptor set for a hemimetabolous insect reveals differences towards the nuclear receptors found in holometabolous insects. One nuclear receptor, HR96, that is found in most insects and that also has a vertebrate homolog, was not found in the *A. pisum* genome. Afterwards, phylogenetic trees were made based on these nuclear receptors and those of other species from the major insect orders such as Orthoptera, Lepidoptera, Diptera, Hymenoptera, Coleoptera, Collembola, and also Crustaceans. Most *A. pisum* nuclear receptors showed close relationship to those of the body louse, *Pediculus humanus humanus*, another member of the Paraneoptera, and remarkably those of the coleopteran *Tribolium castaneum*, a member of the Endopterygota. In a second part we extended our study to examine the classic ecdysone transcriptional regulators that control normal molting and reproduction. The data obtained suggest that some of them (*e.g.* the ecdysone receptor complex EcR and Usp) underwent an increase in evolutionary rate at the base of the Mecopterida lineage.

Poster S1-3

Evidence for the conservation of salivary proteins among aphid species

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The role of salivary proteins in aphid-plant interactions is recognized as crucial in allowing aphids to feed for prolonged periods from their host plants, but the mechanistic basis is little understood. We have adopted a comparative approach to explore the role of salivary proteins in both specialist and generalist aphid species from two tribes: *Acyrthosiphon pisum* (Harris), *Megoura viciae* Buckton and *Myzus persicae* Sulzer from the Macrosiphini; and *Aphis fabae* Scopoli from the Aphidini. The salivary protein profiles for each were observed on 1DE gels and samples for all four species were then subjected to LC MS-MS. Nine strongly supported proteins were identified from the saliva of *A. pisum*, and the most strongly supported proteins, including a putative sheath protein, were investigated further. Using primers designed for *A. pisum*, RT-PCR was carried out using cDNA from all four species to investigate if genes encoding salivary proteins are conserved between the species. Preliminary results indicate that some genes are indeed conserved: an ance-like gene is conserved across all four species, whereas an M1 metalloprotease and the putative sheath protein have been found in *A. pisum*, *M. viciae* and *M. persicae*. Another protein, regucalcin, appears to be uniquely found in the pea aphid. Further validation work is currently being carried out for the putative sheath protein using specifically designed antibodies. The identification and characterization of aphid salivary proteins, together with a better understanding of how they interact with plants, may result in specific and novel methods of controlling these global pests.

Key words: aphids-plant interaction, salivary proteins, conserved genes

Poster S1-4

Regulations of polyphenic wing development in the vetch aphid *Megoura crassicauda*: morphogenesis, tradeoffs, and gene expressions

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Depending on environmental conditions, many aphid species show various phenotypes in their annual life cycles. However, the developmental mechanisms underlying polyphenism remain to be unraveled. In this study, to understand the developmental regulation of wing polyphenism, we examined the postembryonic development in winged and wingless individuals of the vetch aphid, *Megoura crassicauda* Mordvilko. At the first-instar stage, the wing primordia were observed both in the future winged and wingless aphids. Developmental differences are seen from the second-instar stage, when wing primordia degenerate in the wingless nymphs, while they become more thickened in the winged nymphs. We also examined differences in gene expression by real-time qPCR for the genes *wingless* (*wg*), *decapentaplegic* (*dpp*) and *hedgehog* (*hh*), that are responsible for wing patterning. The levels of gene expressions of *hh* were significantly suppressed in the wingless aphids. Further, we also found that there is a trade-off relationship between ovarian and flight-apparatus development in the nymphal instars. Our molecular experiments suggest that the insulin-signaling cascade might modulate the tradeoff by energy allocation during their development.

Key words: wing polyphenism

Poster S1-5

AcypiCyc (*Acyrthosiphon pisum* Cyc database) and CycADS (Cyc Annotation Database System): moving from genome sequence annotation to metabolic network analyses

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The pea aphid (*Acyrthosiphon pisum*) genome was recently sequenced and the annotation of identified genes is being performed by the International Aphid Genomics Consortium. Moving from sequence to knowledge is a long process and the genome annotation effort will continue in the future. The availability of the genome sequence for the pea aphid allows the development of several genomics approaches to study different aspects of this model organism biology. We are particularly interested in the intimate symbiosis association with *Buchnera* to better understand the molecular mechanisms underlying the metabolic integration of the two symbiotic partners. *Buchnera* is known to provide essential amino acids to the aphid, but the full metabolic network between the symbiotic partners has not been completely elucidated. To study this intimate metabolic relationship using a global systems biology approach, it is paramount to collect all genomics data from the host and the symbiont in a format adapted to different and complementary research approaches. To this end, we developed a BioCyc database dedicated to the pea aphid (AcypiCyc) that in its present version integrates also the metabolic databases for *Buchnera* and, in an updated version, the one for *Drosophila melanogaster*.

As genome annotation will be evolving over time, we developed CycADS (Cyc Annotation Database System): an automated annotation management system to allow the integration of the latest sequence annotation information into the metabolic network reconstruction and analysis. CycADS is centred on an *ad hoc* SQL database, complemented by a set of Java scripts to import and export relevant information. Data from GenBank and from different metabolic gene annotation tools (such as KAAS, BLAST2GO, Phylome, etc.) are collected into the database and later extracted to generate a complete input file to build and/or update AcypiCyc using the 'Pathway tools' software (BioCyc). The CycADS pipeline will allow an easy update of the AcipyCyc database over time.

The AcypiCyc database offers a framework for the analysis of the integrated metabolic network shared between the aphid and its symbiotic bacterium *Buchnera*. All genes annotated are present in the database and different query tools allow the users to visualize different metabolic reactions and pathways. All pages are complemented with hyperlinks to different information resources including genomics (AphidBase and GenBank), phylogeny annotations (PhylomeDB) and metabolism (KEGG orthology, BRENDA, ENZYME) databases. Metabolism comparisons tools are available and further developments are planned following the model implemented in the "SymbioCyc" database developed for a collection of symbionts. Furthermore, the AcypiCyc database represents a key resource for computational systems biology research and its open platform formats (BioPAX, SBML) will also allow the integration of the data into other tools to perform complex genomics data analysis.

Key words: *Acyrthosiphon pisum*, *Buchnera*, genome, symbiosis, metabolism, systems biology

URLs
AcypiCyc: http://pbil.univ-lyon1.fr/software/cycads/acypicyc/home
SymbioCyc: http://pbil.univ-lyon1.fr/software/symbiocyc/

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Poster S1-6

Proteomic analysis of GNA binding proteins in the pea aphid

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A proteomics approach was used to investigate the proteome of the pea aphid *Acyrthosiphon pisum* and improve our understanding of the important proteins that bind to an affinity column with immobilized *Galanthus nivalis* agglutinin (GNA), a lectin that specifically interacts with high mannose N-glycans and shown to be very effective against several insect species. Purified proteins were directly in-solution digested using trypsin and the resulting peptides identified by Electrospray ionization-ion trap mass spectrometer. Using the Mascot search algorithm and the Swissprot database (version 14.9) we found around 100 proteins in the *A. pisum* samples. These proteins were then categorized into different groups based on their function in digestion, metabolism, transport, protein biosynthesis, stress response, proteolysis, etc. Using the Scan Prosite tool available at http://www.expasy.org, motifs for N-glycosylation consensus sites were analyzed. The current investigation contributes to our understanding of these *A. pisum* proteins that specifically interact with GNA and may contribute to the insecticidal activity of GNA.

Poster S1-7

Characterization of salivary proteins from cereal aphids

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The objective of this study is to identify the key salivary proteins that allow aphids to feed on cereal crops. These proteins are thought to disrupt the plant wound response by preventing blockage of the feeding site, and therefore allow aphids to feed for long periods. Our focus is on salivary proteins from three cereal aphid species (*S. avenae, M. dirhodum* and *R. padi*). Proteins will be collected using artificial membrane systems and separated by gel electrophoresis. Protein bands or spots will be excised and sequenced using mass spectrometry to obtain identity and/or unique peptide mass fingerprints. We will present preliminary data that compares our protein sequences with other salivary proteins from different aphid species, and will address questions such as "do monocot feeders and dicot feeders differ in their salivary proteins?" and "are there common proteins in saliva that are essential for aphid feeding?"

Poster S1-8

Characterization of the salivary gland proteome of the Pea Aphid (*Acyrthosiphon pisum*) and other aphid species

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The aphid's ability to feed for prolonged periods of time, seemingly un-perturbed by their plant host, has long been associated with proteins and bioactive agents present in oral secretions. Recent mass spectrometry analysis of pea aphid saliva (Carolan *et al*., 2009) has indicated the presence of 9 proteins, many of which are novel to the aphid. It is unlikely however that this list is comprehensive and in an attempt to identify other proteins that may be associated with aphid saliva we have undertaken a proteomic/MS analysis of the aphid salivary glands. 1DE and 2D fractionated salivary gland proteins from *A. pisum* were identified using LC-MS/MS and Maldi-Tof/MS. Of the c.800 proteins identified in the 1DE fractionated samples about 400 were supported by two or more unique peptides and were further characterised (based on biological process, molecular function and cellular component) using BLAST2GO. In addition to identifying many of the proteins demonstrated in secreted saliva, a new list of candidate secreted proteins was obtained based on the presence of a secretion signal. Identified proteins have been deposited to World 2DPAGE.

A number of the identified salivary proteins have been characterised functionally using immunochemistry. An antibody was designed for a unique protein in aphids thought to comprise the salivary sheath (SHP). Western blot analysis of salivary sheath homogenates with anti-SHP demonstrated that SHP was a component of the salivary sheath. Additionally SHP was demonstrated by probing anti-SHP against *Vicia faba* protein lysates, prepared from plants that had been fed upon by aphids. To our knowledge this is only the second demonstration of an aphid salivary protein *in planta* (see Mutti *et al*., 2008). The distribution of the salivary proteins identified in *A. pisum* has also been investigated in other species (*Myzus persicae* Sulzer, *Megoura viciae* Buckton and *Aphis fabae* Scopoli) using immunochemistry. SHP was detectable in the salivary glands of all four aphid species investigated to date.

Key words: Plant-aphid interactions, salivary glands, salivary sheaths

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Poster S1-9

Genetic diversity of *Rhopalosiphum padi* L. (Hom.: Aphididae) using microsatellite markers

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The aphid *Rhopalosiphum padi* is an important pest of cereal crops worldwide. Because of having both sexual and asexual populations, it has been the target of extensive ecological and genetic studies. In this paper we report on genetic variation of *R. padi* in Iran based on microsatellite markers. Genetic diversity of thirty five *R. padi* clones, collected from various cultivated and wild host plants in different regions in Iran, was studied using eight microsatellite markers. Out of eight markers, five showed 16 polymorphic bands, varying from 2 to 5, with an average of 3.2. Phylogenetic analyses using microsatellite markers revealed that Iranian aphid clones collected from northern, north-eastern, north-western, southern, south-western and central region of Iran were clustered into four distinct groups. A significant correlation was observed between aphid clones and geographical regions in most cases. According to our results, geographic populations differed significantly and their genetic distance could be mainly explained by geographic separation. Apparently geographic barriers hinder gene flow and genetic variation among Iranian *R. padi* clones.

Key words: cereal, aphid clones, Phylogenetic analyses, geographic populations

Poster S1-10

Polymorphism of *Aphis fabae* in Tunisia assessed by RAPD markers

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Aphis fabae is a common polyphagous aphid, recorded on a wide range of plant species. It represents a complex of at least four heteroecious holocyclic subspecies that use the same primary host *Evonymus europaeus* and a wide secondary host plant range, comprising *Vicia faba, Tropaeolum majus, Cirsium sp. and Solanum nigrum*. These plants are the exclusively specific hosts of *A. f. fabae, A. f. mordwilkoi, A.f. cirsiiacnthoidis* and *A. f. solanella* respectively.

In this study, we used RAPD-PCR to assess the genetic diversity in *A. f. fabae* and *A. f. solanella.* Five primers, OPH-02, OPH-03, OPH-04, OPH-06 and OPD-01 were used and a total of 106 different bands ranging in size from 120bp to 1580bp were scored for analysis. Among the primers, OPH-02 and OPH-03 revealed, the highest polymorphism in *A. f. fabae.* However for individuals of *A. f. solanella* OPH-02 and OPH-06 were the most polymorphic. The level of polymorphic fragments detected by the five primers ranged from 85.7 % to 100% for the population of *A. f. fabae* and from 93.3 to 100% for *A. f. solanella*. Genetic distance estimated between individuals of *A. f. fabae* and *A. f. solanella* ranged from 0.209 to 0.712. These results suggest a holocyclic reproduction mode of *A. fabae* in Tunisia.

Key words: Aphids, RAPD, *Aphis fabae fabae*, *A. fabae solanella*, *Vicia faba*, *Solanum nigrum*

Poster S1-11

Tunisian *Schizaphis graminum* biotype inferred by COI sequences

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The greenbug, *Shizaphis graminum*, is an important vector of viruses that cause barley yellow dwarf disease and it is considered one of the major pests of wheat worldwide. Greenbugs are classified in biotypes based on host plant response. To date, several biotypes of *Schizaphis graminum* have been reported and their phylogenetic relationships inferred using the mitochondrial COI gene. In Tunisia, no data are available on biotype identification and distribution. Because the effectiveness of greenbug resistant cultivars is biotype dependent, correct biotype identification is essential to develop and implement pest management programs. To establish the biotypic identity of the Tunisian greenbug a fragment of 648 bp of COI gene was sequenced. Comparison of the Tunisian *S. graminum* sequences revealed only one variable position. The sequences of the Tunisian greenbug biotype were also compared with twelve sequences available in Genbank. The phylogenetic analyses showed that individuals from Tunisia clustered together with "agricultural biotypes" (I, J, E, K and C).

Key words: Aphids, *Shizaphis graminum*, Biotypes, Phylogeny, Cytochrome Oxidase I

Poster S1-12

Allelic and genotypic diversity in asexual populations of the pea aphid *Acyrthosiphon pisum* in Japan

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The pea aphid, *Acyrthosiphon pisum* (Harris), has been intensively studied in Europe and North America. In Asia, this species is indigenously distributed in association with several leguminous host species. In warm-temperate Asia, the populations of this species have been know to reproduce parthenogenetically all year round on *Vicia angustifolia*. Furthermore, in northern regions, a host race with distinct morphology is found on *Vicia cracca*. In the present study, we examined the population genetic characteristics of *A. pisum* populations in central and northern Japan, using microsatellite makers.

Many aphid species exhibit geographic variation in the mode of reproduction that ranges from cyclical parthenogenesis with sexual phase to obligate parthenogenesis. Theoretical studies predict that organisms reproducing asexually should maintain higher allelic diversity per locus but lower genotypic diversity than organisms reproducing sexually. To corroborate this hypothesis, we evaluated genotypic and allelic diversity in the sexual and asexual populations of the pea aphid, *A. pisum*. Microsatellite analysis revealed that populations in central Japan are asexual, whereas populations in Hokkaido, northern Japan, are obligatorily sexual. Laboratory rearing experiments indicated that clones from Hokkaido produced sexuales under the conditions of low temperature and short day length, but clones from central Japan did not. No mixed populations were detected in our study sites. Phylogenetic analysis using microsatellite data and mitochondrial COI gene sequences revealed a long history of asexuality in central Japan and negated the possibility of a recent origin of the asexual populations from the sexual populations. Asexual populations exhibited much lower genotypic diversity but higher allelic richness per locus than did sexual populations. Asexual populations consisted of a few predominant clones that were considerably differentiated from one another. Sexual populations on alfalfa, an exotic plant in Japan, were most closely related to asexual populations associated with *Vicia angustifolia*. The alfalfa-associated sexual populations harbored one COI haplotype that grouped within the clade of asexual populations. Available evidence suggests that the sexuality of the alfalfa-associated populations has recently been restored through the northward migration and colonization of alfalfa by *V. angustifolia*-associated lineages.

Key words: *Acyrthosiphon pisum*, population genetics, asexual, sexual, microsatellite

Poster S1-13

Identifying species in the subtribe Aphidina (Hemiptera Aphididae: Aphidinae) using DNA sequences and resolving some species complex problems

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Aphid species in the subtribe Aphidina are concentrated in north-temperate latitudes. The relative lack of useful morphological characters is a large obstacle for Aphidina classification. DNA-based identification systems represent a promising approach to resolve this taxonomic impediment. We demonstrate that the sequence diversity in a partial segment of the mitochondrial gene COI is highly effective in Aphidina species discrimination. Aphid samples were collected mainly from China. Forty-five species of Aphidina were correctly identified in a neighbor-joining phylogenetic tree. The results indicated the average intraspecific sequence divergence in subtribe Aphidina is 0.58%, from 0.00%–1.72%, whereas the average interspecific divergence is 6.87%, mostly from 3.50%–8.00%. In addition, we analyzed the reasons for some overlap between mean nucleotide divergence at the intra- and inter-specific levels.

Key words: Aphididae, Aphidina, DNA barcoding, COI

Session 2

Aphid biodiversity and systematics

Plenary Lecture

A classification of the Aphidomorpha (Hemiptera: Sternorrhyncha) under consideration of the fossil taxa

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A classification of all aphids is presented. It is similar to the classification used by Heie (1980), Foottit & Richards (1998) and several others, but different from that used later by Quednau & Remaudière (1994), Remaudière & Remaudière (1997) and others. Apart from all the recent aphids with viviparous parthenogenetic females it also covers the extinct taxa and the recent aphids with oviparous parthenogenetic females.

We call the group containing all aphids Aphidomorpha Becker-Migdisova & Aizenberg, 1962 as the name of an infraorder below the suborder Sternorrhyncha and call most of the subfamilies of Quednau & Remaudiére (1994) families. The infraorder is divided into eight superfamilies, five extinct ones and three still living. Aphidoidea contains all aphids with siphunculi, and reasons for not incorporating Adelgoidea inside Aphidoidea are given. The number of known fossil aphids has grown considerable during the last twenty years, and gradually we are arriving towards a better understanding of the evolutionary history and phylogenetic classification of the aphids. In the Cretaceous more families were represented than in any other period of the world history. Most or probably all recent families and subfamilies evolved at that time or perhaps a little earlier.

We have among the extinct superfamilies included Naibioidea Shcherbakov, among the aphids, though Shcherbakov (1990) placed his Naibiidae closer to Coccomorpha than to Aphidomorpha and later indicated a relationship between Naibiidae and Sinojuraphididae, which we therefore have placed in the same superfamily together with a still unnamed fossil from Triassic deposits in China, which is the oldest aphid known until now. Members of these three families are known from whole bodies of fossils and differ only a little from the typical aphid body.

Especially the aphids called drepanosiphines by Quednau have given us difficulties. In the above mentioned catalogue by Remaudière & Remaudière (1997) they are spread over several subfamilies given the same rank as the eriosomatids and lachnids, but we prefer to put them together into one family Drepanosiphidae, though most of its characters apparently are plesiomorphies, except at least one, viz. the presence of a wishbone-shaped stiffening at the base of second segment of rostrum. This character is only known among the drepanosiphids, however not in all of them.

The apparently most primitive subfamilies, Mindarinae and Neophylladinae, have never acquired this character. It has obviously been lost in other subfamilies without this character, among these Chaitophorinae, which previously has been excluded from the drepanosiphids though it clearly is the sistergroup of Drepanosiphinae. Among the other subfamilies of Drepanosiphidae there are some members that obviously also have lost it, while their close relatives still have the structure.

It is difficult to draw a phylogenetic tree. The situation seems to be that the picture looks more like a puzzle or a mosaic than a tree, because several characters show up in several groups apparently not depending on relationship, to disappear again in some cases. The reason may be that some genes always are present, but dormant during short or long periods. We have anyway tried to tell how the ancestor of all aphids looked like.

Among the changes we have made, the following can be mentioned: Baltichaitophorinae Heie, 1980, has been split up into a subfamiliy Parachaitophorinae inside Drepanosiphidae and a subfamily Baltichatophorinae inside the Aphidoidea. Anoeciidae is regarded as very different from Aiceonidae and related to Eriosomatidae.

We have tried to explain the evolutionary history, and some peculiarities are discussed, e.g. the speed of evolution compared with other animals. The similarities between aphids millions of years old and aphids from our time are explained as a result of few possibilities for change of morphology, but instead possibilities for change of senses and choice of host plant and possibilities for escape from enemies.

Key words: Aphidinea, Aphidomorpha, aphid classification, phylogeny, evolution, fossil aphids, Drepanosiphidae

Plenary Lecture

Diversity, distribution and endemism of Aphids (Hemiptera) in Indian subregion of Oriental realm

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The Himalayas are traditionally considered as the boundary between the Oriental and the Palaearctic realms. The Oriental realm has four subregions viz., Indian, Ceylonese, Indo-Chinese and Indo-Malayan. Biogeographically Indian subregion has five areas viz., Northeast Himalaya, Northwest Himalaya, Penninsular India, Gangetic plain and Indus plain. It has at least 13 phyto-geographic regions. Each such areas and regions is unique with regards to climate, physiography and vegetation which has direct influence on the floral and faunal characteristics in general and aphid fauna in particular. These subregions have 3 hotspots viz., Himalayas, Indo-Burma (within Greater Himalaya) and the Western ghat (within Penninsular India). The Himalayan areas exhibit richer floral and faunal composition than the Penninsular area, while the Gangetic plain and Indus plain are relatively poor in this respect.

This account restricted only on the aphid species under the family Aphididae of the superfamily Aphidoidea. A total of 829 species under 223 genera and 18 subfamilies are known from these areas. The Himalayan areas extending from Northeast to Northwest through Central (Nepal) part show species richness. Here 818 species under 221 genera representing 17 subfalimies are found. This shows that only 11 species and 2 genera are not represented in the Himalayan areas. The Penninsular area supports 115 species under 58 genera, Gangetic plain with 67 species under 32 genera and Indus plain having 27 species under 14 genera of aphids. The species of the subfamily Aphidinae has the largest representative with 474 species (57.18 % of all the species together) under 114 genera. The Gangetic plain and Indus plain have not supported many groups of aphids such as Lachninae, Greenideinae, Eriosomatinae and many other small groups. The overall generic diversity in these areas is 1:3.7 where as in different areas such diversities are: Penninsular (1.5), Gangetic plain (2.0), Indus valley (1.9). However, in different subfamilies these diversities are different and least diversities are noticed in Anoecinae (10.0), Greenideinae (9.33), Pterocommatinae (7.0) and Chaitophorinae (5.0).

Endemism is very high among aphids of Indian subregion. About 49.91% species are endemic. Of which 49.22% are in the Himalayas. At least 32 endemic genera are present here. Except *Aspidophorodon* Verma*, Indiaphids* Basu*, Neomasonaphis* Ghosh and Raychaudhuri*, Myzakkia* Basu *and Brachyunguis* Das all other endemic genera are monotypic. All the 10 species under Anoecinae and 3 species of Taiwanaphidinae are endemic. The percentage of endemism in other subfamilies are Greenideinae (72.62%), Aphidinae (45.15%), Hormaphidinae (48.28%), Calaphidinae (66.66%), Thelaxinae (75%) and Pterocommatinae (42.85%). Only 8 endemic species are present in the Penninsular area including 2 species viz., *Eutrichosiphum dravidi* Raychaudhuri and *Paoliella nirmalae* (David) which are exclusive of this area. Greenideinae and Hormaphidnae aphids having restricted distribution are represented here by 86 and 53 species respectively. The high percentage of endemic species shows that the Himalayan areas provide congenial ecological conditions for the active speciation of aphids while the peninsular region which is a part of the Gondowana land is very old and stable landmass with distinct flora and fauna quite different from the northern parts.

Another interesting phenomenon is the occurrence of 76 gall inducing aphid species under Eriosomatinae, Hormaphidinae, Aphidinae and Calaphidinae in these areas and all of them are present in Northwest Himalaya. But Northeast Himalaya supports only 8 gall inducing species (10.52%) and most of them belong to Aphidinae. 93% of Eriosomatinae can induce galls in Northwest Himalaya.

Availability and diversification of host plants has direct influence on the diversification of aphids. The major host plant subclasses that harbour more aphids species are Rosidae, Asteridae, Dilleniidae, Colelinidae. More than 150 aphid species are found on these plants. There are many examples of special host associations in the area. The non-availability of specific primary host has influenced the life cycle patterns in many species specially under the subfamily Eriosomatinae. Many species continued anholocyclic parthenogenesis for a long time, such as species of Fordini and Hormaphidinae.

The vertical distribution of aphids in the Himalaya is related with the vertical distribution of host plants. The temperate zone which is expands from 1,500 to 3,500 meters in Northeast Himalayas and 1,400 to 3,200 meters in

Northwest Himalayas supports maximum number of aphid species. The alpine zone, however has many specific and specialized aphid species with localized distribution.

The influence of aphid fauna of Indo-Chinese and Indo-Malayan subregions of Oriental realm, and Mediterranian, Manchurian and Siberian subregions of Palaearctic realm on Indian subregion is quite common but the percentage of such species varies in different subfamilies.

Key words: Aphid, diversity, distribution, endemism, host association, Oriental realm, Indian subregion

Oral Presentation

Phylogenetic aspects of the evolution of the Saltusaphidinae

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The morphological criteria for the Saltusaphidinae and the probable course of evolution of this subfamily are elucidated. Based on detailed studies of the morphological characters of adults and embryos, an alternative systematic situation is postulated for the recent fauna of these aphids which have probably originated from *Neophyllaphis*- or *Phyllaphis*-like ancestors of which the fossil genus *Balticaphis* may be taken as an example. A striking combination of apomorphic features of the whole subfamily is the possession of multi-lensed eyes without ocular tubercle and of leaping legs which show reduction in one lineage (the Tripsaphidini), but remained relatively stable in the other lineage (the Saltusaphidini). All members of the subfamily have wishbone-shaped stiffening of the second rostral segment, and in the ovipara subsiphuncular wax gland plates. They are strictly feeding on Cyperaceae and Juncaceae. The most primitive Saltusaphidinae must have been a leaping group with leaping effectuated by all legs. Evolutionary trends in the various genera concern modifications and reduction of wax gland pore elements, development of paramedian setae on the sides of the head, change of chape of dorsal body setae from hairlike to spatulate or umbrella-shaped, fusion of tergites, siphunculi developing from poriform to short cylindrical, modification of tergite 8 from rounded to bipartite, and the scattering of accessory sensoria on the last antennal segment. In the embryonic stage two tendencies are observed, reduction of dorsal body setae from 6 longitudinal rows into a pattern with only 4 rows (protopattern) and, increase of dorsal body setae by additional setae being developed in the spinal, marginal and submarginal body zone. These changes have taken place independently in the two main lineages. In the group without leaping legs as a result of reduction (the Tripsaphidini), a split occurred giving rise to forms with setiform empodial setae and with both oblique veins in the hind wing, as compared with the normal condition of spatulate empodial setae and loss of one oblique vein in the hind wing.

A previously published hypothesis by Eastop (1958) assuming that it may be possible that the leaping habit would have arisen twice in this group of aphids is refuted. It is also mentioned that some species of the Thripsaphidini produce stalked eggs, a form of egg known from Phyllaphidinae, Greeideinae and also Aphidina Ovipara including the fossil genus *Elektraphis*.

Key words: Aphids, monophyletic lineage, evolutionary trends, leaping legs

Oral Presentation

Fossil aphids found in China (Hemiptera, Aphidomorpha) with special introduction to the oldest aphid from the Triassic*

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The results of a survey of the fossil aphids found in China are given. Up to now, 47 species and 37 genera within 11 families have been described as the impressions from the Middle Triassic, the Middle Jurassic, the Early Cretaceous and Miocene, and as inclusions in amber from the Eocene in China. Among them a species in a new family, the oldest aphid hitherto known, is described recently from the Middle Triassic in Shaanxi, China (Hong, Zhang, Guo et Heie, 2009; in press). Also a large number of species in amber from the Eocene, Northeast China, were described by Hong (2002). It is tried to place them in the classification proposed by Heie & Wegierek (in prep.) and they are referred to three superfamilies: Naibioidea, Palaeoaphidoidea and Aphidoidea. The new family is very similar to Sinojuraphididae in body features, and the two families together with Naibiidae, are placed in superfamily Naibioidea Shcherbakov, 2007.

The species of the new family is very large in body size, reaching 11 millimeters in total length. But its forewings are very short and narrow, and only 7.5 millimeters long. The fact that the new species had relatively short forewings to body length may show that the aphid was not well adapted to fly, so the movement from one host plant to another might be very difficult. The oldest aphid has unique combination of characters. The finding of the oldest aphid from the Middle Triassic gives us a new insight into the morphology of the ancestors of all aphids and the phylogeny of Aphidomorpha.

Key words: fossil aphid, Aphidomorpha, the Triassic, the Eocene, China

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Oral Presentation

Aphid fauna of arctic and subarctic regions

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Arctic and subarctic territories are characterized by severe climate. There are low temperatures, quick weather changes, shot vegetation period, etc. In spite of extreme life conditions, insipid floristic diversity and low ability of their host plant about 400 aphid species inhabit arctic and subarctic zones in Northern hemisphere.

Current level of our knowledge of aphid species composition is different for certain arctic and subarctic regions. At present the more intensively explored areas are Fennoscandia and Kola Peninsula, then arctic and subarctic territories of North America (USA: Alaska; Canada: Yukon Territory, Northwest Territories, Nunavut) and the largest islands in the Atlantic Ocean (Greenland, Iceland). Western Siberia and Magadan region of Eastern Siberia are explored poorly. Aphid fauna of Chukotka and other areas of Eastern Siberia is unknown almost completely. Nevertheless preliminary comparative analysis of species composition for listed regions revealed high level of aphid fauna originality.

Aphis fauna of arctic and subarctic areas is similar to aphid fauna of more southern ones, but the number of species declines to the North (at least, this is true for Europe). Obviously it's determined by decreasing in species richness of regional flora. Northern distribution limit of many aphid species correspond to the ones of their host plants. Another important reason for reducing the number of species is the necessity to adapt the life cycle to a short summer period. Indigenous arctic species represent only a small part of aphid fauna and their relative proportion increases only in very high latitudes where the existing climatic and floristic conditions make adaptation to more southern species very difficult. Hitherto unknown aphid species having circumpolar distribution. Recent aphid fauna of arctic and subarctic areas includes a number of invasive species. Obviously some of them have been introduced together with cultivated plants, other extended their distribution due to invasion and naturalization of synanthropic plants. A number of registered cosmopolite species are not able to overwinter [for example, *Myzus* (*Nectarosiphon*) *persicae* (Sulžer, 1776) or *Macrosiphum euphorbiae* (Thomas, 1878)] successfully outdoor.

On the northern borders of species areals aphid populations show different adaptations of their life cycles and trophic specialization. Effective adaptations to short summer period are the next:

- aphid life cycle is reduced to three generations (for example, it is true for *Aphis callunae* Theobald, 1915);
- annual life cycles are transformed into biennial with larval or imaginal hibernation (*Pemphigus bursarius* (Linnaeus, 1758) or *Muscaphis escherichi* (Börner, 1939));
- sexual generation loses, the species goes to anholocyclic (*Thecabius affinis* (Kaltenbach, 1843)).

Range of inhabiting plants may extend or aphids begin to use plants which are not preferable under the conditions of more southern regions. For example, on the North *Amphorophora rubi* (Kaltenbach, 1843) feeds on *Rubus chamaemorus* L. while in Continental Europe *Rubus caesius* L. and *Rubus fruticosus* L. are preferred host plants.

Sometimes aphids in populations from northern regions have specific differences in morphology. For example, all morphs *Megoura viciae* Buckton, 1876 possess higher number of rhinaria than ones inhabiting other parts of areal. Perhaps increased density of the receptors provides them better opportunity to find sparse host plants under the conditions of low temperatures.

Key words: Aphids, fauna, arctic and subarctic regions, zoogeography

Oral Presentation

Species diversity and fauna of Aphids in Northeast China

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Northeast China locates on the northeastern edge of Asia, and comprises Heilongjiang Province, Jilin Province, Liaoning Province and four cities of Inner Mongolia Autonomous Region. The broad region has various landforms, vegetation types and unique climate. This part of China has an important biogeographical status. It is a hinge of several faunas and they communicate frequently in this region. Thus the aphid species diversity is high and the fauna is quite complicated. On the basis of former studies, sample collections and recent large-scale surveys, the present study reported the taxonomy, species diversity and fauna in Northeast China.

Here, 297 aphid species and subspecies belonging to 111 genera in 19 subfamilies, 2 families were recorded in the region, including 8 species and 1 subspecies new to China. For aphid species diversity, the families which are cosmopolitan in the temperate zone, like Aphididae, are more common than the ones cosmopolitan in the semi-tropical zone like Hormaphididae. Aphididae has the largest number of species and genera and Phloeomyzidae has the smallest number of species. The endemic species account for 16% of known species in the region. For the host plants the aphids feeding on, Aphididae has the most diverse host plant genera and families, and Greenideidae has the least diversity. Among all families of host plants recorded in Northeast China, Salicaceae and Rosaceae have most aphid genera and families feeding on them, respectively.

The aphid fauna of Northeast China is mainly composed of the species distributed in both Palearctic realm and Oriental realm (44.44%), followed by the species distributed only in Palearctic realm (21.89%) and the species distributed in more than four realms (14.81%). Most species of Northeast China that are distributed in Palearctic realm and Oriental realm are endemic to Northeast Asia. And most of the species distributed only in Palearctic realm are endemic to Northeast China or China. The high diversity of the Palearctic+Oriental species and the endemism of Palearctic species are the obvious characters of aphid fauna in Northeast China.

Key words: Aphid, Northeast China, taxonomy, diversity, fauna

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Oral Presentation

Relationship between patterns of species richness and sampling effort: a case study of aphids in China

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Knowledge of species richness plays significant role in biodiversity conservation planning. Patterns of species richness are usually estimated by using known distributions of species. However, the estimation (especially at large scale) is sometimes affected by sampling effort. Theoretically, endemic species should be more sensitive to such sampling effect. To investigate the relationship between patterns of species richness and sampling effort, quantifying of sampling effort is a crucial step. In the present study, we used a 40-year dataset of aphids in China to examine whether spatial variation in sampling effort affects patterns of overall species richness and endemic species richness. The geographic range, fieldwork duration and sample numbers of aphid surveys in the last 40 years were quantified and allocated to grids at a resolution of 2°×2°. We then assessed the relationship between species richness estimates and measures of sampling effort by using multivariant analysis. Results indicated that relatively high species richness (especially for endemic species) for some areas with relatively low habitat heterogeneity is probably due to more sampling effort, while positive correlations between species richness and sampling effort for some hotspots with high plant diversity and habitat heterogeneity are not artifacts. Our study suggests that one should keep sampling effort in mind when explaining biodiversity patterns, and that plant diversity and habitat heterogeneity should be important factors for uncovering natural species richness patterns of phytophagous insects.

Key words: Aphids, biodiversity, multivariant analysis, sampling effort, species richness

Oral Presentation

Biodiversity and chorological outlines for Italian aphid fauna

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To date, there are 860 nominal species and subspecies of Italian aphids so being among the highest diversified ones in W-Europe. Most of these species, up to 845, are already mentioned in literature, as they are reported in the Italian fauna checklist, published in 1995, and subsequently updated during the last thirteen years, adding 15 more unrecorded species. The reason for such high biodiversity compared to other European aphids, is believed to be related either to Italy's position and orography, being in the centre of the Mediterranean basin and having very different ecological habitats. These include the Northern Alpine mountains, which orographycally continues through the Apennines along the peninsula, changing into subarid lands in Southern areas.

As a consequence, Italy has a highly diversified flora,including no less than 6000 taxa of specific and subspecific level including many floristic endemisms. All this facilitates the settlement of many aphid species, promoting the development of their different life cycles.

The Italian aphid fauna includes all the highest level taxonomic groups (i.e. families, subfamilies and tribes) known in Europe within this superfamily of Sternorryncha. The oviparous *Adelgidae* and *Phylloxeridae* have both been well investigated and include all known European species. Within the ovoviviparous *Aphididae* s.l., the most representative in terms of species biodiversity are the *Eriosomatinae* (53 species), *Calaphidinae* (71 species), *Chaitophorinae* (37 species), *Lachninae* (78 species) and mainly *Aphidinae* (540 species, including 166 *Aphidini* and 374 *Macrosiphini* species).

More than half of the twenty Italian administrative Regions have been more or less accurately surveyed for aphid species recognition. Nevertheless, several need to be better investigated as the number of species detected there remain rather low at present. Species distribution shows no peculiar divergences among the different regional areas. Nevertheless, Northern regions seem to exhibit a richer and more diversified aphid fauna compared to the Central-Southern ones, including the two main islands (Sardinia and Sicily). At present, the number of aphid species thus far recorded per region range from 104 species (Latium) up to 436 taxa (Sicily). Divergence in numbers of species among regions is very probably linked to insufficiently numerous collections.

Most Italian aphid fauna is autochthonous in origin, represented by nearly 93% of W-Palaearctic species, including those with a Mediterranean distribution. The remainder (about 7%), is composed of species with a different biogeographical origin, such as Nearctic, E-Palaearctic or Oriental species and some believed to be of intertropical origin.

In terms of chorological categories currently used in biogeography and considering the actual distribution of species independently of their centre of origin, Italian aphids can be subdivided into different groups. A large percentage of species (44.4%) show a very wide geographic distribution, consisting of cosmopolitan or sub-cosmopolitan (24.9%) and Holarctic (19.5%) taxa. Those classified as Palaearctic are about 17% of species, while the larger group (30.6%) is represented by aphids with a wide European distribution. Further subdivisions can lead to smaller groups of taxa with prevailing distributions like W-, E-, or S-European with variable percentages among them. But perhaps the most interesting is a further small group of orophilous or boreo-alpine species (18 species or about 2% of the total) which has a restricted and disjunctive distribution in mountainous systems and can be regarded as a relict from the Pleistocene. Finally, 4.1% are species with a typical Mediterranean distribution, while a further small percentage (3.9%) are represented by those species at present only known in Italy. Among the latter, there are a number of species with insufficiently known distribution or even of doubtful taxonomic validity, in addition to a few taxa which can be regarded as typical Italian faunistic endemisms.

Key words: aphid biodiversity, aphid biogeography, Italy

Oral Presentation

Aphids (Hemiptera: Aphidoidea) associated with trees in the Maltese Islands (Central Mediterranean): A preliminary check-list

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The Maltese Islands are a group of low lying islands situated in the Central Mediterranean Basin. Although the total surface area is small (c. 316 km^2) when compared to other islands/territories in the Mediterranean, and despite the high population density (about 1,200 persons/km^2) and negative impacts of the tourist industry on the natural environment, the islands still harbour various arrays of natural habitats. Due to the strategic position of the Maltese Islands, several groups of organisms have been studies and compared to those found in nearby territories. This is however not the case with the aphidofauna of Malta. Despite the fact that aphids represent a group of insects of economic significance, local studies are poorly documented. The check-list of aphids recorded for Malta produced for the Fauna Europaea includes only two species probably owing to the limited diffusion of old published data.

Reference to Maltese aphids is to be found in two main publications: the work of Caruana Gatto (1926) on plant galls entitled "*Primo contributo alla conoscenza dei Zoocecidii delle Isole Maltesi*" (Archivum melitensis 7 (3):105-124), where among other species, reference to fourteen aphid species which cause plant galls on trees are included, and the work of Saliba (1963) entitled "*Insect pests of Crop plants in the Maltese Islands*" (Dept. of Information, Malta, 35pp.) where seven species of aphids are mentioned as occurring on economically important trees. Borg (1922) in his book entitled "*Cultivation and diseases of fruit trees in the Maltese Islands*" (Malta Gov. Print Office, vii+622pp.) does mention aphids but it is often not clear whether these records are authentically Maltese. The records of Saliba (1963) lack taxonomic background and most likely are based on Borg's (1922) records.

In these last 15 years, collections were made in order to investigate the species of aphids found in Malta. For convenience aphid studies in Malta are being divided into three main parts, aphids on trees, aphids on crop plants and aphids on herbaceous plants and shrubs.

Not much species of aphids associated with trees are expected to occur in Malta primarily due to the scarcity of trees. However, results obtained during this study revealed the presence of several species previously undocumented. Most of the earlier reports have been confirmed and additional species were found to be new for Malta. Some of the new records include: *Aphis gossypii* on *Citrus*; *A. spiraecola* on *Malus*; *A. craccivora* on *Ceratonia siliqua*; *Brachyunguis tamaricis* on *Tamarix africana*; *Chaitophorus populialbae* on *Populus alba*; *Cinara palaestinensis* on *Pinus halepensis*; *Pterochloroides persicae* on *Prunus*; *Eulachnus rileyi* and *E. tuberculostemmatus* on *Pinus*; *Myzocallis schreiberi* on *Quercus ilex* and *Tuberolachnus salignus* on *Salix pedicellata*.

Several species of aphids are localized and rare in the Maltese Islands and this is mainly due to the scarcity of their host plants. These include: *Eriosoma lanuginosum* and *Tetraneura ulmi* on *Ulmus*; *C. populialbae* on *Populus*; *M. schreiberi* on *Quercus* and *T. salignus* on *Salix*.

The most recently introduced and established species of aphid associated with trees in Malta is *Greenidea ficicola* which was found to affect ornamental *Ficus* which are widely used in tree planting programmes, especially along road sides and roundabouts.

More investigations are surely needed in order to have a more complete list of aphids associated with trees occurring in the Maltese Islands.

Key words: aphid biodiversity, trees aphids, Maltese Islands

Oral Presentation

Describing cryptic species – is the game worth candles?

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Cryptic, or sibling, species are "species that fail to acquire conspicuous morphological differences during the process of speciation" (Mayr, 1969: 183). Such species are morphologically indistinguishable to the researcher thereof one of their names. Such a feature of cryptic species generates problems for taxonomists, because processing a diagnosis of the new species actually means finding morphological differences between the new species described and its closest relatives. In the case when the new species is really cryptic (it has yet failed to acquire conspicuous morphological differences in the process of separation from her allies), certain problems occur when publishing the description of such new species. History of description of *Aphis (Bursaphis) holoenotherae* Rakauskas, 2007 might serve as an example illustrating the problem.

Until now, only one *Aphis* species - *A. (Bursaphis) oenotherae* – has been reported to be specifically associated in his life cycle with *Oenothera* herbs in Europe. It was supposed to be an anholocyclic derivate of its Nearctic counterpart by having lost the connection with his primary hosts, currants and gooseberries (*Ribes* spp.) following its introduction to Europe at the end of the last century (Müller, 1974). Later on, holocyclic clones of *A. oenotherae* monoecious on *Oenothera* spp. have been reported from Poland and Lithuania (Rakauskas, 2006). Subsequently, partial sequences of mitochondrial COI and nuclear EFα1 genes (Turčinavičienė *et al*. in preparation) of the available European and one Korean samples appeared closely similar and grouped apart from the Nearctic samples of *A. oenotherae* (Turčinavičienė *et al*. in preparation). This justified separation of the European populations as a separate species, *Aphis (Bursaphis) holoenotherae* (Rakauskas, 2007). Morphological differences between the Nearctic *A. oenotherae* and Palaearctic *A. holoenotherae* appeared to be unreliable, despite the canonical discrimination analysis based on twenty six morphological characters. Therefore, the Editors of the Deutsche Entomologische Zeitschrift, based on the negative opinion of two reviewers (out of three), did not accept description of the new species for publication. The main reason – insufficient diagnosis based on an unreliable character (length of processus terminalis), which is correlated with the total body length. So, is it worth of wasting time when trying to draw the attention of scientific community in describing new cryptic species, which are not easy to separate morphologically, although they have clear differences in their life cycles, host specificity, distribution, and gene sequences? My answer is "yes". Should we suspend delimiting new cryptic species until reliable morphological characters will be found? Not necessarily. An excellent opportunity for the proper discussion and more reasoning there will appear on the 8th International Aphid Symposium in Catania and in the Symposium Materials thereafter.

Key words: Aphids, cryptic species, taxonomy

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Oral Presentation

DNA barcodes to explore diversity in Aphids

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An evolutionary tendency towards loss of taxonomically useful characters and morphological plasticity, due to host and environmental factors, complicates the identification of species and the analysis of relationships at all taxonomic levels. The presence of different morphological forms of a single species on different hosts and at different times of the year makes it difficult to consistently associate routinely collected field samples with particular species definitions.

DNA barcoding has been proposed as a standardized approach to the characterization of life forms in numerous groups of living organisms (Hajibabaei *et al.*, 2007). We have tested the effectiveness of the standard 658-bp barcode fragment from the 5' end of the mitochondrial cytochrome c oxidase 1 gene (COI) to differentiate among species of aphids and adelgids (Foottit *et al.*, 2008, 2009). This paper will present the results of an initial study on the application of DNA barcoding in which approximately 3600 specimens representing 495 species and 170 genera of most of the major subfamilies of aphids and the adelgids have been sequenced.

Most species of aphids and adelgids (>95%) are well differentiated, with low intra-species sequence variation. Despite the complex life cycles and parthenogenetic mode of reproduction, DNA barcodes are an effective tool for routine identification. Examples are provided where DNA barcoding has uncovered the existence of cryptic new taxa, has linked life stages on different hosts of adelgids and aided in the delineation of species boundaries. The impact of geographic, clonal, and host-race variation on the utility of DNA barcodes has also been investigated. The future use of these DNA barcodes for the routine detection of invasive species, the resolution of pest complexes, and the analysis of diverse faunas will be discussed.

Key words: Aphididae, Adelgidae, species identification, DNA barcodes, COI, mitochondrial DNA, life cycles, cryptic species

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Oral Presentation

Toward a molecular identification tool for European Aphididae

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Taxonomically Aphididae is a relatively well known group and Europe is one of the richest region hosting 1373 species on the 4800 known in the world (one third of the world fauna).

Identification of aphids using morphology is particularly difficult for non specialists because many species look very alike, even when they display strongly different ecology. Furthermore, identification is hampered by a considerable intraspecific variation and the range of continuous morphological variation is wider than in any other insect groups. Consequently, Aphididae is a good candidate for the development of a rapid identification tool based on barcode. This tool could enable accurate identification of pest and non pest species by non specialists and can also be of great interest to detect new invasive species.

Specimens, stored in alcohol in the collection of the National Institute of Agronomical Research (INRA), have been used to extract DNA and to prepare vouchers for morphological identification. Two hundred and seventy (270) species of European Aphididae were identified to species and sequenced for a 658 bp region of *cox1*. In order to assess the intraspecific variability, most species were represented by multiple specimens, with an overall mean of 4 specimens per species. About 1200 specimens were sequenced. Pairwise genetic distances ranged from 0 to 0.18 for the global data set. Intraspecific pairwise genetic distance ranged from 0 to 0.064 with a mean of 0.006. The great majority (90%) of intraspecific distance was under 1% and only four taxa on the 270 considered have intraspecific p-distance above 0.03%. This exception could be due to lack of taxonomic study in this group, to the existence of morphological cryptic species or to mitochondrial introgression by a species not present in our data set. Mean interspecific p-distance was 0.062 for intrageneric pairwise and 0.099 for intergeneric pairwise. These values were more than ten times higher than intraspecific values. Only few taxa had null interspecific pairwise p-distance and so could not be assigned. Most of them are known as species with ambiguous taxonomic status. An overlap of interspecific pairwise p-distance with intraspecific distribution was noted. For the majority of them, generally with interspecific p-distance value above 0.01 the number of pb difference was low but enough to recover the species delineation and to authorize species assignment of sample. However, the assignment was not possible for few cases including some species belonging to the species complex of the subgenera *Aphis* and *Dysaphis* and genus *Macrosiphum*. These few cases where barcoding did not distinguish aphid taxa could be due to i) recent speciation and lack of resolution of COI sequence, ii) recent hybridization events, and iii) an overused of the ecological species concept in these genus.

In conclusion, the DNA barcode resolved accurately species identities for most cases. In average molecular data were congruent with morphological data, but displayed the same limitations for difficult species complexes. The use of additional mitochondrial or nuclear genes seems necessary to resolve these complexes. Nevertheless our results are encouraging and extensive sampling of missing species is ongoing. Further work also includes to produce an identification tool for European Aphididae on the web.

Key words: Aphididae; Barcode; molecular identification; *cox1*

Oral Presentation

Preliminary results of the molecular phylogeny and morphological evaluation of the genus *Aphis* (Hemiptera: Aphididae) in North America

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World wide there are approximately 450 species in the genus *Aphis*, with approximately 98 species in the USA (Remaudiere & Remaudiere 1997, Smith and Parron 1978). Species within this genus are difficult to discriminate using only morphology. Most dichotomous keys rely on host plant association to identify species in this group. There is a need to develop new methods to accurately identify these species in order to improve pest management and biological control efforts. This work shows results from an attempt to molecularly characterize members of two species complex. **The *Aphis gossypii* complex.** This group is composed of *A. gossypii, A. monardae, A. oestlundi*, *A.glycines,* and *A. nasturtii*. *A. monardae* and *A. gossypii* are considered synonymous (Remaudiere & Remaudiere 1997, Blackman & Eastop 2006). *A. gossypii, A. monardae, and A. oestlundi* are part of this complex because they are morphologically alike especially in the alate morphs. Some members of this group are invasive exotic species namely *A. gossypii, A. nasturtii and A. glycines,* the first two are highly polyphagous and *A. glycines* is very host specific. Some species share primary or secondary hosts or both, making the process of identification more difficult. **The *A. helianthi* complex.** This group is composed of *A. helianthi*, *A. asclepiadis, A. decepta* and *A. cornifoliae*. All members of this group are native species. *A. helianthi* is polyphagous while the remaining species are restricted in their feeding preference. In addition, we have sequenced several other native aphid species such as *A. saniculae*, *A. thaspii, A. lacinariae, A. rubicola, A.rubifolii, A. vernoniae* and *A. hyperici* previously included within the genus *Brachysiphum* (Stroyan 1984). This is the first attempt to determine the taxonomic status and evolutionary relationship of these *Aphis* species using molecular phylogenetic methods.

Taxa studied: Aphids were collected from their primary and/or secondary hosts from different sites within the USA. Three specimens per species were sequenced from each locality. *Iowana frisoni* (Aphidinae: Aphidini), *Rhopalosiphum maidis* (Aphidinae: Aphidini) and *Uroleucon* sp. (Aphidinae: Macrosiphini) were selected as outgroups.

Molecular Data and Phylogenetic Analysis: DNA was extracted from single individual aphids using the QIAamp DNA microkit. PCR products were generated using Ready to go PCR beads and cleaned using the QIAquick PCR purification kit. Sequencing reactions were conducted using ABI Big Dye v3.1, and cleaned with Performa® DTR Ultra 96-Well Plate Kit, and run on an ABI3730 at the Keck Center (University of Illinois at U-C). We amplified and sequenced the mitochondrial gene COI (1291bp), the nuclear gene elongation factor-1-α (926 bp) and *Buchnera* 16S rDNA (primary endosymbiont) (379 bp). Raw sequence data was analyzed using Sequencher 4.7. A maximum parsimony analysis using PAUP 4.0b10 (Swofford 2002) was conducted using the heuristic search with 100 iterations with random addition. The bootstrap values were estimated using 500 replications with a heuristic search with 10 iterations of random additions.

Our results indicate that the monophyly of *Aphis* is well supported. However, it also includes *Iowana frisoni*. Blackman & Eastop (2006) and Favret (pers. com.) recognized that this genus should be re-evaluated due to of its morphological similarities to *Aphis*. The gossypii and helianthi complex form well supported clades. The gossypii complex includes *A. rubifolii* and *A. rubicola* that are morphologically atypical for members of the complex. Two species with novel combinations of characters, *Aphis sp.* 1 and *Aphis sp.* 2, are also included. Our results do not support the previous synonymy of *A. monardae* and *A. gossypii*. Our data indicate that *A. saniculi* and *A. thaspi*, previously not considered as belonging to the helianthi complex, appear to be closely related to members of this group. Morphological work is consistent with our molecular data showing the close relationship of *A. helianthi* and *A. asclepiadis*. The third well-supported clade is composed of three native species that are well defined. *A. vernoniae* and *A. lacinariae* are more closely related to each other than to *A. hyperici*, a species previously considered in *Brachysiphum*.

Key words: *Aphis*, taxonomy, phylogeny, host plant

Oral Presentation

New Additions to the Turkey Aphid (Hemiptera:Aphidoidea) Fauna*

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As a result of the study aimed to investigate to aphid species of Eastern Black Sea Region of Turkey, 15 aphid species were determined as new records for Turkey aphid fauna. These species are *Adelges pectinatae* (Cholodlovsky 1888), *Aphis cytisorum* Hartig 1841, *Aphis maculatae* Oestlund 1887, *Aphis thomasi* (Borner 1950), *Capitophorus inulae* (Passerini 1860), *Chaitophorus longisetosus* Szelegiewicz 1959, *Chaitophorus salicti* (Schrank 1801), *Kaltenbachiella carpinicola* Chakrabarti & Bhattacharya 198, *Microlophium sibiricum* (Mordvilko 1914), *Neobetulaphis pusilla* Basu 1964, *Pterocallis albida* Borner 1940, *Sitobion miscanthi* (Takahashi 1921), *Tinocallis ulmifolii* (Riley &Monell, 1879), *Tinocallis takachihoensis* Higuschi 1972, *Uroleucon siculum* Barbagallo & Stroyan 1982. There was no detailed study was organized before in study area. With these new records, the total number of aphid species in Turkey comes up to 463. Despite that, it can be considered that this number is much lower compared with records in neighbouring countries as Turkey has particular geographical, agricultural, climatic and floristic characteristics. Findings of the presented study and other recent studies showed that with the detailed study Turkey aphid fauna will be substantially increased.

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Oral Presentation

Macrosiphum on *Knautia* in Central Europe – molecular data support the synonymy of *M. silvaticum* and *M. knautiae* (Hemiptera: Aphididae)

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Three species of the genus *Macrosiphum* (Hemiptera: Aphididae) have been reported as inhabiting plant hosts of the genus *Knautia* (Dipsacales: Dipsacaceae) in Europe (Holman 1972): *M. rosae* Linnaeus, *M. knautiae* Holman and *M. silvaticum* Meier. *M. rosae* is facultatively heteroecious between *Rosa* and various Dipsacaceae, Valerianaceae and Onagraceae hosts. Hosts of the genus *Knautia* are not obligatory for this species, whilst the two remaining species, *M. knautiae* and *M. silvaticum* are holocyclic monoecious on *Knautia* spp. that was proven experimentally for *M. knautiae* in Moravia (Holman, 1972) and *M. silvaticum* in Lithuania (Rakauskas, 1985). The focus of this study was to combine DNA sequence data with the morphological and ecological information on the European *Macrosiphum* species inhabiting *Knautia* in order to resolve ambiguous taxonomy.

Twenty three field and five clonal samples collected in Lithuania, Belarus and Czech Republic in 2002-2003 were used in this study. Sampling methods were those commonly used. Host specificity and life cycle analysis of every clone were performed in the same way as described earlier (Rakauskas, 2003). Morphological identification of aphids was performed by means of the key compiled by Holman (1972) and discriminative characters given by Meier (1985). In addition, the key of Rakauskas (2003) was also used. The target sequences were 600 bp from the mitochondrial gene encoding Cytochrome Oxidase subunit I (CO-I), and 500 bp of the nuclear gene encoding Elongation Factor –1α (EF-1α).

Morphologically, all samples from Belarus appeared to be *M. silvaticum*, the same as samples and clones from Lithuania, collected on *Knautia arvensis*. Lithuanian samples and clones collected from *Rosa* met the morphology of *M. rosae*. Aphid material from Czech Republic had the morphology of *M. knautiae* (sample from *Knautia sylvatica*) and *M. rosae* (samples from *K. arvensis*). Three aphid clones exploited in the present study had the morphology and biology of *M. silvaticum*, one – of *M. knautiae* and one – of *M. rosae.*

A comparison of mithochondrial and nuclear genes showed that these are strongly incongruent with each other, the nuclear gene tree being more consistent with traditional taxonomy. Central European populations of *M. knautiae* and *M. silvaticum* are identical in their mitochondrial CO-I and nuclear EF-1α sequences. In addition to earlier biological and morphological data (Rakauskas 2003), this confirms them being the same species. Splitting between *M. rosae* and *M. knautiae* seems to be rather recent, because these two species are different in their EF-1α sequences, but identical in their CO-I sequences studied. Mitochondrial DNA did not allow the differentiation of morphologically similar species. This support idea, that mitochondria are liable to cross species boundaries during occasional hybridization and cause incongruence between the gene and species tree. This justifies the need for the deeper γ-taxonomic studies of the world-wide species of the *rosae* species complex of the genus *Macrosiphum*.

Key words: Aphids, *Macrosiphum*, molecular systematics

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Oral Presentation

Molecular phylogenetics and systematics of Iberian Fordini

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The tribe Fordini (Aphididae: Eriosomatinae) comprises aphid species that induce conspicuous galls on their primary hosts and display a two-year life cycle. In the Iberian Peninsula and the Canary Islands only representatives of the subtribe Fordina are found. These species have *Pistacia* spp. as primary hosts, where they induce the galls on the leafs, and Gramineae spp. as secondary hosts, in which they develop on the roots. Many taxonomical problems still exist in this group due to the complexity of their life cycles. For example, the species *Forda marginata* and *F. riccobonii* induce quite different galls on *P. terebinthus* and *P. atlantica*, respectively, but the virginoparae living on grass roots are almost morphologically indistinguishable between both species. On the other hand, the life cycle of *Paracletus cimiciformis* is assumed to be well known, with a green flat gall being induced on *P. terebinthus*. However, the complete life cycle of *P. donisthorpei* is unknown, with no gall described for this species. Besides, Roberti (1993) suggested that there were in fact two forms of virginoparae in *P. cimiciformis*, one of them being identical to the virginoparae of *F. rotunda*, the only morph described for this species.

We carried out a molecular survey to deal with the phylogenetic relationships of Fordini species in the Iberian Peninsula. The study was complemented with a morphological analysis of some of the species aimed to investigate some of the taxonomical problems that exist in this group. First, we used nuclear (LWRh and EF1α genes) and mitochondrial (ATP6 and COI genes) data, as well as the 16S rDNA gene from the endobacterium *Buchnera aphidicola* in 10 Fordini species to infer their phylogenetic relationships, with special attention paid to the intraspecific structure of the genera *Forda* and *Paracletus*. We also performed transfer experiments from fundatrigeniae isolated from galls of *F. marginata*, *F. riccobonii* and *P. cimiciformis*, and obtained appropriate metric and meristic data from the resulting virginoparae.

The phylogenetic results supported grouping the Iberian Fordini in two groups, one including the genera *Aploneura*, *Baizongia* and *Geoica* and another including *Forda* and *Paracletus*. The position of the species *Smynthurodes betae* was conflicting among the genes analysed, but some of them highly supported grouping it with *Forda* and *Paracletus*. This topology supports a scenario for the evolution of galls towards an increase in capacity and size. The transfer experiments showed the outstanding similarity of the virginoparae of *F. marginata* and *F. riccobonii*, but the molecular data confirmed the validity of the species status of these two taxa. In *P.cimiciformis*, the transfer experiments revealed the existence of the two forms of virginoparae, as suggested by Roberti, and allowed us to propose that *F. rotunda* is a new synonym of *P. cimiciformis*. Finally, the phylogenetic analysis of the samples belonging to *P. cimiciformis* and *P. donisthorpei* showed the existence of two divergent lineages that, nevertheless, did not completely correspond to each of these species, revealing potential problems of gall morphology- based identification. Our results suggest that *P. cimiciformis* and *P. donisthorpei* probably induce indistinguishable galls on *P. terebinthus* and that a revision of the taxonomy of this genus should be undertaken.

Key words: Fordini, systematics, life cycle, galls, *Pistacia*

ROBERTI D., 1993. Gli Afidi d'Italia (Homoptera - Aphidoidea). Entomologica, 25: 3-387.

Oral Presentation

Phylogenetic position and biogeography of gall-forming aphids on *Zelkova*

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Aphids of the tribe Eriosomatini (Aphididae: Eriosomatinae) are typically associated with *Ulmus* and *Zelkova* (Urticales: Ulmaceae), on which they induce various types of galls, leaf rolls or pouch galls. Although many species of this tribe have been recorded from *Ulmus*, much fewer species have been from *Zelkova*. The genus *Ulmus*, including approximately 45 extant species, is widely distributed in the northern temperate regions. In contrast, the genus *Zelkova* is a small relict group with only six extant species, being disjunctly distributed in Sicily, Crete, the Caucasus, and East Asia. To evaluate the phylogenetic positions of the *Zelkova*-associated species, phylogenetic relationships of the tribe Eriosomatini were inferred using 36 species (29 ingroup) and 52 morphological characters. The evolution of the *Zelkova*-associated eriosomatines is discussed in relation to the resultant phylogeny and information about fossil records of *Zelkova*.

Analysis based on equal character weighting led to hundreds of most parsimonious trees, and the strict consensus of these trees poorly resolved the phylogenetic relationships. However, successive weighting method yielded three most parsimonious trees, and the strict consensus of them was well resolved. The *Zelkova*-associated species except for *Paracolopha morrisoni* branched off at the base of the Eriosomatini phylogeny, suggesting that the ancestral Eriosomatini were associated with *Zelkova*. A recent molecular phylogeny (von Dohlen & Moran, 2000) suggests that the tribe Eriosomatini may have diverged in the late Cretaceous to early Tertiary. Fossil records indicate that from the early- to mid-Tertiary the genus *Zelkova* had been a common element distributed throughout the Northern Hemisphere including localities where no extant species are present (Denk & Grimm, 2005). The ancestral stock of the Eriosomatini may have had an extensive distribution in the Northern Hemisphere during the early to middle Tertiary in association with the common and widespread occurrence of *Zelkova*. Since the late Tertiary, however, *Zelkova* had became extinct in most regions due to the arrival of cool and arid climates, and survived disjunctly in the Mediterranean and West and East Asia (Follieri *et al*., 1986). It is probable that during the late Tertiary and Quaternary, the Eriosomatine aphids associated with *Zelkova* also became extinct in many local regions where *Zelkova* was eliminated. Such frequent extinctions of *Zelkova*-associated aphids could result in the present low species diversity, disjunct distribution, and the branching of the *Zelkova*-associated group at the base of the phylogeny.

Key words: Aphid-plant associations, disjunct distribution, relicts

DENK T., GRIMM G.W., 2005. Phylogeny and biogeography of *Zelkova* (Ulmaceae sensu stricto) as inferred from leaf morphology, ITS sequence data and fossil record. Botanical Journal of the Linnean Society, 147: 129-157.
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Oral Presentation

Introduction to Aphid Species File

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Aphid Species File (ASF) is a comprehensive web-accessible aphid taxonomy database: **http://Aphid.SpeciesFile.org**. We aim it to contain all the published taxonomic and synonymic information for all Aphidomorpha (Aphidinea) taxa, extinct and extant. Along with a complete list of aphid names and a taxonomic bibliography, Aphid Species File also has capabilities for serving specimen data and publishing interactive electronic identification keys.

Approximately 10,000 aphid taxa names are listed hierarchically, each name being a hypertext link to all subordinate taxa and the full synonymic and bibliographic history of the taxon. Nomenclatural and taxonomic information served on ASF is first published elsewhere and then entered into the database, so ASF is not a publisher of primary data. However, all records are taken directly from the original sources, so errors that may have crept into the literature from publication to publication can be mitigated.

ASF also serves as an electronic bibliography of aphid taxonomic literature, with each reference individually examined before being entered into the database. Alternate names of publications and people can be recorded so as to simplify historical record keeping. Electronic copies of all aphid literature are being gathered for open accessibility and publicity, pending copyright permission.

ASF serves primary specimen data, including a full complement of text fields and images. The specimen data allow for interactive analysis of species distribution. Electronic identification keys using the Species File software can be developed and accessed from anywhere with an Internet connection. These keys can employ any type of image or text description and can be structured as dichotomous or fully interactive.

A general appeal is made for assistance in the development of this resource.

1) Provide electronic or hard copy primary literature, either reprints at the time of publication, or copies of historical literature.
2) Translate all or portions of primary literature into English: at a minimum, titles of references and publications.
3) Enter literature citations into the database, generally the references of certain taxa or authors.
4) Build electronic interactive keys that can then be referenced from the author's traditionally published works.
5) Provide other supplementary data (e.g., specimen data) that can be incorporated into ASF.
6) Catch errors and bring them to the attention of data entry personnel for correction.

Most importantly, however, a specific appeal is made to all aphidologists to use ASF and make its availability known by citing it. As a community, we can develop Aphid Species File to be a valuable and powerful resource for aphid taxonomic research in today's competitive scientific environment.

Key words: taxonomy, bibliography, database, Aphididae, Aphidomorpha

Oral Presentation

Several nomenclatural clarifications on genus-group names in the Aphididae (Hemiptera Sternorrhyncha)

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The aphidologists attending the Eighth International Symposium on Aphids, held in Fremantle (Western Australia, 2005), charged us with the preparation of a *Part* of the *List of Available Names in Zoology* devoted to the aphid genus-group names. Our work was greatly facilitated by reference to the lists and catalogues of Kirkaldy, Baker, Börner & Schilder, and especially those of Eastop & Hille Ris Lambers (*The Survey*) and Remaudière & Remaudière (*The Catalogue*). Despite the well-known completeness and quality of these works, in the course of our research we encountered a few minor mistakes as well as errors with implications for nomenclatural stability.

Referencing the International Code of Zoological Nomenclature, Fourth Edition (*The Code*), we here correct errors of nomenclatural importance for 90 genus-group names as they appeared in the last catalogues of the 20th Century, namely the *Survey* and the *Catalogue*.

1) Seven names were never actually published (*Code* articles 8.1.1. and 9.9).

2) Forty-four were published in a suppressed work and are therefore unavailable (*Code* articles 8.7.1 and 11.1).

3) Three is unavailable because it was not adopted by the first reviser (*Code* articles 32.2 and 4).

4) Two is unavailable because its subsequent spelling, attributed to the original author and date, is in prevalent usage (*Code* article 33.3.1).

5) Three are unavailable because they were established in synonymy (*Code* article 11.5).

6) Eight are unavailable because they were created after 1930 and without description (*Code* article 13.1).

7) Six are unavailable because they were created after 1930 and without type species (*Code* article 13.3).

8) One available name was omitted from the catalogues of the 20th Century.

9) Sixteen listed as unavailable in the catalogues conform to all the conditions of the *Code* (Chapter 4) and are therefore available.

Key words: Aphid genera, Unpublished names, Unavailable names, Available names, Type species

Poster S2-1

Biodiversity of the aphid fauna (Hemiptera: Aphidoidea) of Georgia

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It was investigated aphids biodiversity, theirs distribution in the landscapes and karyotypes in Imereti, Khevi and Racha-Lechkhumi floristic regions. 60 species of the aphids, which are united in 2 families and 27 genera were recorded on the above mentioned territories. Of the aphids 8 species were recorded for the first time from Transcaucasia, while 1 species was recorded for the first time from Georgia.

Karyological investigation of the species of the genus *Aphis* L. was curried out. It was established chromosome numbers of these species and modal karyotype - 2n=8.

Key words: Aphid, biodiversity, new record, karyotype

This abstract is a result of the designated project, which has been fulfilled by financial support of Georgian National Science Foundation (Grant N GNSF/ST 06/6-086).

Poster S2-2

An Eriosomatine aphid relict: *Zelkovaphis trinacriae*, living in Sicily on *Zelkova sicula*

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Zelkovaphis trinacrie is a recently described galligenous Eriosomatine aphid from Sicily, where it lives on *Zelkova sicula*. The latter is a relict species of Ulmaceae only known in Sicily where it lives in the Iblei Mounts in a restricted woodland area at 'Bosco Pisano' in the municipality of Buccheri, Syracuse Province. The plant is strictly confined to a small area of about half a hectare, at an altitude of 450 m above sea level, where a relict population of about 230 rather small shrubs are living. As a consequence, both the aphid and its host plant are at very high risk of extinction.

Galligenous Eriosomatine living on *Zelkova* are presently represented by eight species belonging to four different genera including *Byrsocryptoides*, *Colophina*, *Paracolopha* and *Zelkovaphis*; in addition on the same host plant genus live a number of Calaphidinae species (*Tinocallis* spp.) and one *Stomaphis* (mostly in the Far East), totalling about fifteen aphid species. By comparison, the aphid fauna of *Ulmus* is much richer, including eight genera of galligenous Eriosomatine and several other taxa from different taxonomic groups, totalling about seventy aphid species altogether.

The genus *Zelkovaphis* is mainly allied to *Eriosoma* and mostly to *Aphidounguis*, the latter two genera of Eriosomatine having *Ulmus* as primary host. Among the Zelkova-feeding species *Z. trinacriae* is closely related to "*Schizoneura*" *caucasica* Dzhibladze, 1960, and to "*Hemipodaphis*" *persimilis* Akimoto, 1983, which have been proposed, therefore, as belonging to the same genus. Consequently, *Zelkovaphis* shows the widest geographical distribution among the Eriosomatine genera so far known on *Zelkova*, being represented in all the three main native areas of that genus of Ulmaceae, viz. the Mediterranean, where *Z. trinacriae* lives on *Zelkova sicula*, the Caucasian-Middle East, where *Z. caucasica* lives on *Z. carpinifolia*, and, finally, the Far East, where *Z. persimilis* lives on *Zelkova serrata*. This being the genus distribution, *Zelkovaphis* shows the widest geonemy compared to the other genera of Zelkova-feeding Eriosomatines and might have been differentiated earlier. In fact, *Byrsocryptoides*, which includes two species, is only known in the Caucasus living on *Z. carpinifolia*; *Colophina, which* includes two described species, and *Paracolopha*, with the sole species *P. morrisoni*, are both known in the E-Palaearctic area (Japan), where they live on *Z. serrata* as primary host (apart from anholocyclic populations of the latter species which live on bamboo roots in N. America).

Z. trinacriae performs a dioic life-cycle, with *Z. sicula* as primary host; nevertheless, its secondary host plant remains as yet unknown. Among its allied species, *Z. caucasica* has some *Carex* species as secondary host plant, on which roots the summer generation develop. Fundatrix of *Z. trinacriae* on *Z. sicula*, is born from overwintering eggs between the end of March to the first week of April and takes about 20 days to reach the adult stage., During a period of 20 days, each fundatrix lays on average 200-300 nymphs inside its gall. All nymphs develop into alate emigrants, which fly out of the gall usually between the end of April to the second or third ten days of May (exceptionally up to the beginning of June). The gall produced by the aphid is of the open clustering type (rosette-like gall) and involves the leaves of an entire shoot, which become dried by the end of the aphid flying period. The newly produced gall is purple-reddish at the beginning of its formation, turning gradually greenish once it reaches definitive development. Galls of *Z. trinacriea* are relatively common at the investigated site; the percentage of infested *Z. sicula* shrubs in five years of observation varies between 14-33%. Each infested shrub usually showed 1-12 (exceptionally up to 50) galls per single plant, corresponding to an average of 12.5-14 galls/plant. Nevertheless, damage to infested plants appears negligible because, in practical terms, the loss of secondary shoots does not significantly affect vegetative development.

Key words: *Zelkovaphis trinacriae*, *Zelkova sicula*, Eriosomatinae aphids, Sicily

Poster S2-3

Zelkova-feeding Eriosomatinae from Georgia (Hemiptera: Aphidoidea)

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Transfer experiments were carried out to confirm heteroecious life cycles of the *Zelkova*-feeding *Byrsocryptoides zelkovae* Dzhibladze, 1960 and *Zelkovaphis caucasica* (Dzhibladze, 1960). The migrants of these species were transferred from the leaf galls of *Zelkova carpinifolia* to *Carex capitata* in the laboratory. The migrants of both species began feeding on the aerial parts of *Carex* and gave birth to apterous progeny, which reached to adult stage. In addition, apterous exules of the above mentioned two species were found on the aerial parts of *Carex capitata* in the Ajameti Sanctuary (Western Georgia) in 2008. Descriptions and illustrations of apterous exules and comparisons of gall-living apterous viviparous females with apterous exules of *Byrsocryptoides zelkovae* and *Zelkovaphis caucasica* are given. This is the first confirmation of the life cycles of *Byrsocryptoides zelkovae* and *Zelkovaphis caucasica*.

Key words: *Byrsocryptoides zelkovae, Zelkovaphis caucasica*, transfer experiment, secondary host plant, heteroecious life cycle

The designated investigation has been fulfilled by financial support of Georgian National Science Foundation (Grant N GNSF/PRES07/6-163).

Poster S2-4

Greenidea ficicola: is it an example of rapid colonization due to climatic changes?

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The genus *Greenidea* Schouteden (Aphididae: Greenideinae: Greenideini) is represented by about 45 described species distributed in the tropical and subtropical regions of Eastern Asia. Species of this genus are mainly found on plants belonging to the families Moraceae and Fagaceae but also Betulaceae, Juglandaceae, Myrtaceae and Theaceae. One of these species, *Greenidea ficicola* Takahashi, 1921, has been recently reported from the West Palaearctic, namely in Southern Italy (Barbagallo *et al*., 2005a), Southern Spain (Barbagallo *et al*., 2005b; Pérez Hidalgo *et al*., 2009) and Malta (Mifsud, 2008). The species has been also reported from the Afrotropical Region (Burundi) (Remaudière *et al*., 1992), the Nearctic Region (Florida) (Halbert, 2004), and the Neotropics (Brasil and Chile) (Sousa-Silva *et al*., 2005; Rubín de Celis *et al*., 2006). In its native range, *G. ficicola* feeds on different plants of the families Moraceae, Mirtaceae and Litraceae (Blackman & Eastop, 2000) whereas in Europe the species has been found on several species of mainly ornamental *Ficus* L. (such as *F. microcarpa* L. f., *F. rubiginosa* Desf., *F. australis* Willd., *F. sycomorus* L., *F. glomerata* Roxb., *F. benjamina* L. and *F. carica* L.). *Ficus carica*, a widely cultivated tree in the Mediterranean Region, has been mentioned in the literature as a host plant of *G. ficicola* both in natural and under laboratory conditions. However, the presence of this aphid in very small colonies on the mentioned host plant has been rarely observed in both Sicily (Bella & Mazzeo, 2009) and Malta. The modality of the accidental introduction of this species in Europe remain unknown, but the international trade of its ornamental host plants and flight capabilities of alate morphs may have played a significant role in the establishment of this aphid in new geographical areas. This aphid is a termophilous anholocyclic species and its rapid spreading in Mediterranean countries and other temperate areas is also possibly favoured by global warming.

Key words: *Greenidea ficicola*, aphid biodiversity, climatic changes.

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Poster S2-5

Aphid fauna of Bhutan and their host association

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The Royal Kingdom of Bhutan is situated entirely in the Eastern Himalayas between India in the south and China in the north. It extends between latitude $20^0$41' and $28^0$20' North and longitude of $88^0$50' and $92^0$10' East. It has the highest altitude of 7554m having valleys in the central Bhutan and a narrow Duars plain in the north bordering India. Dense vegetation in the north is a characteristic of this country. The vegetation diversity is very high which is temperate type in the mountains while a subtropical vegetation is noticed in the Duars areas.

In spite of rich vegetation, this country has been poorly investigated for its aphid diversity. Ghosh, A.K. et al. (1971), Ghosh, L.K. (1972), Dutta and Raychaudhuri (1981) studied the aphid fauna of this country from some restricted areas. Of late, Chakrabarti and Das (2008) described 3 new species of *Pterocomma* Buckton from here. Through the above works a total of 63 species distributed over 43 genera under 6 subfamilies are known so far. Recently, several surveys were made in different localities of Bhutan and 21 hitherto not known species and a few new species have been recorded. Thus, a total of 84 species are now known from Bhutan.

An analysis of the information available shows that the highest numbers of 38 species have been recorded under the subfamily Aphidinae which infest more than 40 host plants. Plants under the family Asteraceae followed by Rosaceae and Poaceae are the major host plant families of these aphids. Largest number of aphids has been collected from the altitudes between 2001 and 2500 meters.

Key words: Bhutan, aphids, faunal diversity, host association, altitudinal distribution

Poster S2-6

The European inventory of Alien aphids

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This study was developed as a part of the Delivering Alien Invasive Species In Europe (DAISIE) project funded by the sixth framework programme of the European Commission. The general objectives of DAISIE are (i) To create an inventory of invasive species that threaten European terrestrial, fresh-water and marine environments, (ii) To structure the inventory to provide the basis for prevention and control of biological invasions through the understanding of the environmental, social, economic and other factors involved (iii) To assess and summarise the ecological, economic and health risks and impacts of the most widespread and/or noxious invasive species (iv) To use distribution data and the experiences of the individual Member States as a framework for considering indicators for early warning.

The goal of our study is to provide a comprehensive list of alien Aphididae for Europe. We consider only species alien to Europe. Aphid species which originate from one European country and are introduced in another one as *Diuraphis noxia* (Kurdjumov, 1913) or *Brachycorynella asparagi* (Mordvilko, 1929) are not considered in this work even if this species has the status of an invasive species in its introduced area. We used the list of European Aphididae by Fauna Europaea (Nieto Nafría et al., 2007) to define all species present in Europe. We compiled information about each species from published sources and experts to define their origin, European *vs* non European. When a first list of alien aphids was defined, we sought additional informations, e.g. the date of first occurrence in Europe. June 2008 is cut-of date for the literature survey.

A total of 98 species originating from outside Europe have been established so far, to which we add 4 cosmopolitan species of uncertain origin (cryptogenic). Therefore, about 7% of the European aphid fauna is considered as alien. The 102 alien species of Aphididae established in Europe belong to 12 different subfamilies, most of which are already present in the native entomofauna, whereas three (Greenideinae, Lizerinae and Neophyllaphidinae) have not been known from Europe before their introduction. Five subfamilies contribute to the alien fauna by more than 5 species. The subfamily Aphidinae dominates and represents 59 % of the alien Aphididae, followed by Calaphidinae (16%), Lachninae (5.8%), Eriosomatinae (4.8%) and Chaitophorinae (4.8%). Most alien aphid species originate from temperate regions of the world. Asia and North America have contributed the most (each 44%). Aphids originated from Africa are present with only 4 species. No alien aphid species was introduced from Australasia or South America to Europe so far. The proportion of geographical origins in the alien aphid fauna of Europe is relatively constant over time. Alien aphid species are relatively well distributed across Europe, 58% are present in at least 5 European countries and 38% occur in more than 10 countries or regions. The dates of the first record in Europe is more or less precisely known for 93 of the 102 alien aphid species. The precise date of arrival is not known for most species because introduction happened unintentionally and there may be considerable differences between the date of introduction and the date of recording. Keeping in mind these biases and considering the first record as a proxy of the date of introduction, the average introduction rate since 1800 is 0.5 species per year. The mean number of new records per year increased from 0.3-0.4 before 1950 to more than 1.4 per year between 1975 and 1999. The mean number decreased since 2000, but this pattern may change in the following years.

Introductions of alien Aphididae in Europe will probably continue due to expanding markets, globalization, increasing amount of transported goods and transporting agents and climate changes.

Key words: Aphididae, alien aphid, Europe

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Poster S2-7

An attempt to make a phylogenetic classification all aphids, both extinct and extant taxa (Hemiptera)

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In the poster some information, which could not be given in the oral lecture on classification or in the paper entitled "A classification of the Aphidomorpha (Hemiptera: Sternorrhyncha) under consideration of the fossil taxa" to be published later.

All aphids are called Aphidomorpha and subdivided into several superfamilies. The oldest two from the Triassic and have characters, which probably were shared by the ancestor of all other superfamilies, but no superfamily except Phylloxeroidea, Adelgoidea and the only one with siphunculi or siphuncular pores Aphidoidea left recent representatives. Under the name of all taxa, superfamilies, families, subfamilies, tribes and subtribes, diagnoses are given.

Furthermore some drawings are shown, namely some phylogenetic trees showing the assumed relationships between the superfamilies, between the families within Apohidoidea and between the subfamilies within the Drepanosiphidae.

Poster S2-8

Distribution pattern of Aphids in Northeast China

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Northeast China locates on the northeastern edge of Asia, and comprises Heilongjiang Province, Jilin Province, Liaoning Province and four cities of Inner Mongolia Autonomous Region. Its diverse habitats are beneficial to aphids. Aphid species in this region always have large populations and do harm to a wide range of host plants. In total, 297 aphid species and subspecies belonging to 111 genera in 19 subfamilies, 2 families were recorded and the host plants they feeding on are included in 150 genera, 51 families. The aphid fauna of Northeast China is mainly composed with 5 types, the most species distributing in both Palearctic realm and Oriental realm (44.44%), followed with the ones distributing only in Palearctic realm. Besides the species diversity and complicated fauna characters, the distribution pattern was also influenced by the broad area, various landforms, vegetation types, unique climate and special biogeographical status.

Based on the distributional data of 2908 samples of 297 aphid species and subspecies in Northeast China, geographical distribution patterns of aphids in Northeast China was studied by using GIS mapping, on administrative province level and Chinese zoogeographical region level respectively. The landform and vegetation information of the region were combined into the analysis to explain the distribution patterns of aphids. The results indicated that most species distribute along the main mountains of this region. The southern part of the region has more species and distribution records than the northern part, and the southwestern part has the largest number of species and the most distribution records. The current distribution patterns of aphids in this region were probably influenced by climate, landform, geological history, activities of human beings as well as the vegetation. The distribution ranges of host plants limit the distribution of aphids directly.

Key words: Aphid, Northeast China, distribution pattern

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Poster S2-9

Morphometric discrimination of host-adapted populations of *Brachycaudus helichrysi* (Kaltenbach) (Hem.: Aphididae)

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Brachycaudus helichrysi is a world-wide polyphagous pest which has economic importance. It has a wide range of various host plants and can transmit some virus diseases. Several lines of studies have shown that most aphid taxa encounter morphological divergence associated with different host plants which resulted in formation of different forms or biotypes. Morphometrics involves the quantitative analysis of forms, using measurements of morphological structures. Morphometric techniques can be used to identify and separate morphologically similar groups of organisms when no single diagnostic character is available. The aim of this study was to investigate morphological variation in *B. helichrysi* populations associated with different host plants using multivariate morphometric analysis.

The study was based on field samples of *B. helichrysi* collected from different host plants. Since environmental conditions can affect the morphology of aphids, all of the studied samples were collected from an area which has similar climatic conditions in Kerman (South-East of Iran). Twenty two morphological characters of microscopic slide mounted apterous viviparous females were measured. The selected characters have been previously used in the taxonomy of Aphididae which is standard practice in traditional morphometric study on this group of insects.

The aphid populations were separated using Canonical variates analysis (CVA). Based on this analysis the aphid individuals belonging to *Pulicaria dysenterica* (Asteraceae) completely separated from other populations. Individuals associated with *P. dysenterica* constitute a homogenous group and showed more morphological similarity. In addition individuals belonging to *Crepis sancta* (Asteraceae) showed considerable morphological divergence and separated from other populations. The results demonstrated that morphology of *B. helichrysi* strictly has been affected by host plant. The result of CVA indicated that the characters "processus terminalis length" and "siphunculus length" show the highest magnitudes (1.041 and -0.824 respectively) at function 1. This function contributes most to the separation between populations which presented 85.2% of the total variation. The second function accounted for 12.1% of the total variation in the data. In populations associated with *P. dysenterica* and *C. sancta,* 100% of individuals were reclassified correctly into their original groups. In total, 84.8% of original grouped cases were correctly classified. Although the separation was not highly complete among some groups but our morphometric data clearly separated *B. helichrysi* individuals living on *P. dysenterica* from other aphid individuals on CV1 and represented distinct morphological entity. Morphological differences among aphid populations associated with different host plants is mostly because of their adaptation to host plant characteristics such as morphology, physiological and ecological conditions of the host plant. The observed morphological differences among aphid individuals associated with *P. dysenterica* is possibly due to the fact that the plant has aromatic compounds while other host plants under study do not show this characteristic. The presence of aromatic compounds can change the chemical components of the plant so that this could affect the morphological characters of related aphids. The overall conclusion is that morphological divergence has been occurred in *B. helichrysi* populations associated with different host plants. However more investigations based on molecular tools such as mitochondrial DNA sequencing and microsatellite DNA genotyping is needed before exact conclusions can be drawn.

Key words: *Brachycaudus helichrysi;* Morphometric; morphological variation; host-adapted populations

Poster S2-10

Aphids from Argentine Northwest (*NOA*)

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Argentine Northwest (in Spanish "*Noroeste Argentino*", abbreviated *NOA*) is a geopolitical region comprising the provinces of Catamarca, Jujuy, Salta, Santiago del Estero and Tucuman. It is situated approximately between the meridian 62ºW and the border with Chile and between 22ºS and 30ºS parallels, with a surface of almost 470000 km^2. The orography, climate and vegetation in the area are very heterogeneous. Different types of landscape are found there, the most important being, (1) the high-altitude mountains, which mark the border with Chile, (2) the middle-altitude mountains ("*sierras*" and "*quebradas*") lying in an arid north-south strip; (3) the puna, an arid plateau situated at over 3000 m altitude, without native trees, (3) the yunga, a rainforest at between 500 and 3000 m, and (4) low altitude plains with very varied characteristics, used for farming.

When this study began (in 2006) 73 of the 220 aphid species (including subspecies) known in continental Argentina had been reported in *NOA*. By province: 54 species in Tucuman, 30 in Jujuy, 16 in Salta, 11 in Catamarca and 8 in Santiago del Estero. Only *Acyrthosiphon pisum* and *Aphis gossypii* have been reported in all five provinces. Only 8 of the 73 species recorded in *NOA* (10.9%) are native of South America; most of the introduced species are linked to cultivated plants.

This study was carried out to increase our knowledge of aphid fauna in this extensive area and in particular to find out more about the distribution of autochtonous South American species. It was financed by the Regional Government of *Castilla y León*, Spain.

The aphids were collected in November 2006, during the austral spring, mainly in the puna plateau and also in the "*sierras*" and "*quebradas*". Samples were collected in 34 localities in 5 provinces in *NOA*; only one of these localities, situated in a protected area, had characteristics similar to those of the yunga forests.

Aphid fauna in arid or semi-arid areas in *NOA* were very scarce, particularly in comparison with areas of similar characteristics situated further south, in Cuyo (provinces of La Rioja, San Juan and Mendoza) and Patagonia (provinces of Chubut and Rio Negro).

Fifty-two species were identified, including the unknown species *Neophyllaphis iuiuyensis* Mier Durante and Ortego, recently described, and another 8 which are also probably unknown (seven species of *Aphis* and one species from a potentially new genus).

As a result of this study, the number of species, including *N. iuiuyensis*, now known in the region and in each province is: 92 in *NOA* (+19), 58 in Tucuman, 41 in Jujuy, 30 in Salta, 23 in Catamarca and 12 in Santiago del Estero.

Eight autochtonous species were cited for the first time in *NOA*, 6 of which had only previously been reported much further south; whereas the others were recorded further north, one in Chile and the other in Bolivia. These new records, along with *N. iuiuyensis*, bring the number of autocthonous species in *NOA* to 17 (+9), 18.5% of the recorded species (+7.6%).

Comments are made on alocthonous species, and also on those found most frequently in the localities in the area, they are the alocthonous species *Aphis gossypii* and *Myzus persicae*, and one autocthonous *Uroleucon* species.

Key words: Aphids, Argentina, Argentine Northwest, NOA

Poster S2-11

Combined nuclear and mitochondrial molecular data support the existence of three main lineages in the phylogeny of aphids

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Aphid systematics has been controversial to the point that almost as many classifications as taxonomists of the group have been proposed. The difficulties in the classification and the phylogenetics of these group of insects based on morphological data were also reflected in the first molecular studies that dealt with the phylogeny of the whole family Aphididae. These first reports bumped into a high lack of phylogenetic structure for levels higher than tribe, with only subfamilies Aphidinae and Lachninae appearing as monophyletic clades. The inability of finding well supported relationships among aphid subfamilies was explained as a consequence of the rapid adaptive radiation at the tribe level that these insects went through at the end of the Cretaceous, connected to a transition of feeding from gymnosperms to angiosperms. Despite its importance for the research about key features of aphid biology like the evolution of life cycles and feeding or the cospeciation with the endobacterium *Buchnera aphidicola*, a robust picture of the phylogeny of this family has not been obtained yet from molecular data. Nevertheless, a previous study using the LWRh and ATP6 genes proved that the compilation and combination of molecular data can eventually lead to a precise understanding of the phylogenetic relationships among the main groups of aphids.

In the present study, the phylogenetic relationships of aphid species belonging to 11 subfamilies were inferred by the independent and combined analyses of nuclear (LWRh and EF1α genes) and mitochondrial (ATP6 and COII) data. Each of the genes was analysed independently by maximum-likelihood, while two combined data sets were analysed using maximum-likelihood, maximum-parsimony, bayesian inference and distance-based methods. The first combined data set was built by joining all non saturated codon positions of the LWRh, EF1α and ATP6 genes from 34 aphid species. A second combined data set was made by joining all codon positions of the four genes from 20 species.

The lack of resolution for the deepest nodes of the phylogenetic tree of the family Aphididae that characterised the first molecular studies was also observed in most of the topologies resulting from the independent analyses of the genes. Nevertheless, the analysis of the LWRh gene and most of the combined analyses highly supported the existence of three main lineages in which the subfamilies analysed were grouped. We named these lineages as: A+D (containing the representatives of Aphidinae, Calaphidinae, Chaitophorinae, Drepanosiphinae and Pterocommatinae); E+T (including Anoeciinae, Eriosomatinae, Hormaphidinae, Mindarinae and Thelaxinae) and L (composed of the species of the subfamily Lachninae). Furthermore, some of the phylogenetic reconstructions suggested a basal position for the subfamily Lachninae in the phylogeny of aphids. The fact that an ancestral feeding on conifers has been proposed for this subfamily suggests that feeding on gymnosperms might also be primitive in the family Aphididae, as it is assumed by most aphid specialists. On the other hand, the lack of a well supported structure inside the lineage E+T makes it difficult to know how many origins of host-alternating cycles might have occurred in the evolution of aphids. However, the phylogenetic results obtained might support an evolutionary scenario with just two origins of host alternation for the subfamilies analysed.

Key words: Aphids, phylogeny, combined analysis, host alternation

Poster S2-12

To the problem of the genus *Uroleucon* Mordv. in Europe

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The genus *Uroleucon* Mordvilko, 1914 comprises about 180 know species in the World (Blackman & Eastop, 2006). These taxa are separated within 5 subgenera: *Uroleucon* Mordvilko, 1914, *Uromelan* Mordvilko, 1914, *Belochilum* Börner, 1932, *Satula* Olive, 1963 and *Lambersius* Olive, 1965 (Remaudière & Remaudière, 1997). Four of these subgenera: *Uroleucon*, *Uromelan Lambersius* and *Belochilum*, comprising 54 species are reported from Europe. These aphids are trophically related to *Asteraceae* and *Campanulaceae*. Most of European taxa (57 species and subspecies) are related to *Asteraceae* (Remaudière & Remaudière, 1997; Blackman & Eastop, 2006). Recent molecular results (Moran *et al*., 1999) have put into question monophyly of subgenera within *Uroleucon*. Only *Lambersius* mostly from *Asteraceae* in America seems to be monophyletic (Eastop, 1985), while Nearctic species placed in *Uroleucon* form a clade nested within a clade comprising European species in subgenera *Uroleucon+Uromelan* (Moran *et al*. 1999). *Uroleucon* has been subdivided primarily on the basis of pigmentation of the cauda, siphunculi and coloration in life (Hille Ris Lambers, 1939; Olive, 1965; Heie, 1995). As coloration is homoplasious feature, and other morphological features suggest non-monophyly of subgenera new set of morphological characters is needed to establish monophyletic units within the genus. The genera *Lambersius* and *Belochilum*, green in colour and with very long ultimate rostral segment, are subgeneric groupings of convenience, but have been used as full genera by some authors (Eastop, 1985).

Based on host plant relationships most European species placed in the subgenus *Uroleucon* are related to *Asteraceae* tribe *Lactuceae*. European species placed in *Uromelan* are feeding on host plants of tribes *Cardueae* and *Astereae*. A few species of *Uromelan* are given from tribe Lactuceae. Monophagous aphid species feeding on *Asteraceae* seem to be more restricted to plants with milky sap (*Lactuceae*), while polyphagous species of aphids seem not to feed on *Lactuceae* (Osiadacz & Wojciechowski, 2005). Preliminary results suggest that the other division of species within monophyletic subgenera could be presented on the basis of host plant relationships and morphology. Therefore the main aims for the investigations in the nearest future are: identification of the monophyletic units within genus *Uroleucon* on the basis of morphological research, checking host plant – aphid relationships data in respect to search of monophyletic units and preparation of a key to identification of European (and worldwide) species of the genus *Uroleucon*.

Key words: Aphids, *Uroleucon*, *Asteraceae*, taxonomy, host plants

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Poster S2-13

Biodiversity of aphids in Costa Rica

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It is generally accepted that there is less aphid diversity in tropical regions in the world than temperate or temperate-cold ones, especially in Laurasic territory. This affirmation is based on studies that consider the diversity like the number of aphid species in relation to the surface of the territory and/or the number of species of phanerogams present there. Although this is true, it cannot be denied that the low level of diversity could also be partly due to limited knowledge of aphid fauna in various parts of the world, as is the case of Central America.

105 aphid species are known in Central America: 72 in Panama (unpublished data), 67 in Costa Rica, 44 in Honduras, 26 in Nicaragua, 10 in Guatemala and 9 in El Salvador and Belize. An overall analysis reveals that (1) the number of autochthonous species is minimum, (2) most reported species originate mainly from Palaearctic, Nearctic and Asian territories; and (3) many species, including those most commonly found, are linked with cultivated plants.

The present study of Costa Rican aphid fauna was carried out as part of the training of experts in aphid taxonomy, financed by the "Agencia Española de Cooperación Internacional".

The material was collected in February and December, 2008 in localities situated throughout Costa Rican territory at very varied altitudes. 660 samples were collected on their host plants, as well as the ants attending them. The study enabled nine species to be reported for the first time in Central America and eighteen in Costa Rica.

Comments are made on plant/aphid and aphid/ant relationships and on the origin of the introduced species.

Key words: Aphids, Ants, Costa Rica, Central America

Poster S2-14

Morphology and morphometry of the development instars in *Phloeomyzus passerinii*, the poplar woolly aphid (Aphididae: Phloeomyzinae)

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Aphid life cycle involves a succession of morphologically different morphs of the species. Each of these forms passes through a number of larval instars, which could be difficult to differentiate. Within Aphididae, the subfamily Phloeomyzinae includes only one species *Phloeomyzus passerinii* Signoret (1875), the woolly poplar aphid, which colonizes *Populus* trunks. It usually develops anholocyclically through populations of apterous virginoparous females covered with white wax wool. Only alates are sexuals but oviposition is ascertained and considered as an exceptional event. This species, unique in morphology, has been poorly investigated (Arzone & Vidano, 1984) and instars identification need to be detailed as they will be useful for further life history and population dynamic studies.

The aim of this work was to perform a detailed morphological and biometrical description of the developmental apterous and alate morphs composing the complete lifecycle of *P. passerinii*: 4 apterous larval instars, 1 apterous virginoparous adult, 2 nymphs with wing buds, and male and female alates. Such an analysis should provide an identification key at the intraspecific level.

Aphid collection. The different instars were obtained by an age-grading method under controlled conditions (20°C; LD 16:8h) from a strain collected in France. Apterous females were placed during 24h on poplar cuttings of the I214 cultivar until they produced 10 larvae per cutting. The development of each larva was then checked daily and exuviae were removed after each moult. Concerning nymphs with wing buds and alates, we collected them from the same generation directly from the rearing strain. Thirty individuals per instar were preserved in 70% ethanol until analyses.

Sample preparation. The collected aphids were processed for permanent preparations using a method slightly modified from Martin's (1983). Photos of slides were taken with a camera integrated into a microscope and measurements were performed on photos after calibration.

Parameters. Morphological variables were body, eye and leg colours, as well as the number of antennal segments. Morphometrical analyses included body length, body width, width of rostrum, length of last antennal segment, length of each hing leg segment and ratio between a few parameters (e.g. antennal last segment versus body length).

While the morphological observations did not clearly differentiate most larval instars, the biometrical studies allowed drawing the main characters defining each instar. Moreover, we noticed the existence of two morphs of apterous virginoparous females within the same strain, one with five, another with six antennal segments. Finally, an identification key of immature instars is proposed.

From the first larval instar, developmental changes are gradual in shape, size and a number of characters. These changes should be sufficiently distinct to allow differentiating each of the life stages, irrespective of the season. Nevertheless, the present observations need to be corroborated with additional data from a pool of field collected aphids belonging to the same strain.

Key words: Aphididae, *Phloeomyzus passerinii*, morphometrics, morphs, immature instar key

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Poster S2-15

Siphini Mordvilko, 1928 (Aphidoidea, Chaitophorinae) – taxonomy, bionomy and distribution

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The tribe Siphini Mordvilko, 1928 which belongs to the subfamily Chaitophorinae (Hemiptera, Aphidoidea) comprises now 5 genera, 25 species (*Atheroides brevicornis, A. doncasteri, A. hirtellus, A. karakumi, A. persianus, A. serrulatus, Caricosipha paniculatae, Chaetosiphella berlesei, Ch. longirostris, Ch. massagetica, Ch. stipae, Ch. tshernavini, Laingia psammae, Sipha (Sipha) agropyronensis, S. (S.) flava, S. (S.) glyceriae, S. (S.) littoralis, S. (Rungsia) arenarii, S. (R.) burakowskii, S. (R.) elegans, S. (R.) maydis, S. (R.) praecocis, S. (R.) taurica, S. (R.) uvarovi*) and 1 subspecies (*Chaetosiphella stipae setosa*).

Aphids belonging to Siphini are known to be monoecious and holocyclic with excepction of *S. (S.) flava* which is anholocyclic wherever winter temperatures permit.

They are monophagous or strictly oligophagous connected with 90 genera and 212 species of Poacea, 6 genera and 36 species of Cyperaceae, 2 genera and 7 species of Juncaceae and 1 genus and 1 species of Typhaceae. *S. (S.) flava* is connected with 48 genera of host plants whereas 7 species of Siphini live on 1 species of host plant. The species live, usually singly or in small colonies, on the overground parts of their host plants and most of them are visited by ants.

The most distributed species represent the genus *Sipha* (*S. (S.) flava, S. (S.) glyceriae, S. (R.) elegans, S. (R.) maydis)*; 7 species are very local, known only from a single or a few localities. The most numerous group constitute the species which belong to the Palaearctic (8 species) and European (5 species) elements.

Siphini, with the commonly accepted view that aphids in their development moved from phylogenetically older host plants (arborescent) to plants which were phylogenetically younger (herbaceous plants, grasses and sedges) are regarded as the youngest branch of Chaitophorinae. They are characterized by the body elongate, narrow or pear-shaped, usually hairy (setae may be long or short, thorn-like, pointed or with apices modified in various ways); the reduced number of antennal segments to 5 or 4, sclerotisation of abdominal tergites, pore-shaped or slightly elevated siphunculi without reticulation, broadly rounded or knobbed cauda and broadly rounded anal plate.

Key words: aphids, Siphini, taxonomy, distribution

Session 3

Aphid biology and ecology

Plenary Lecture

Effects of inbreeding and outbreeding on aphid biology

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Cyclic parthenogenesis in aphids leads to the peculiar mode of population structures and adaptations to the host plants. One outcome of this reproductive mode is the frequent incidence of inbreeding; male and female members of the same clone can mate to produce fertilized eggs (Helden & Dixon, 1997). This mode of mating is comparable to selfing, or self-fertilization in plants but cannot be found in other animals. Aphid populations often consist of sexual, asexual, and intermediate clones in temperate regions. In such mixed populations, the effects of inbreeding may be restricted. However, in holocyclic populations, a high level of inbreeding may have influenced the local adaptations and the evolution of sex ratios and mating systems. The present study reviews the literature and my own study on Eriosomatine aphids by focusing on the effects of inbreeding and outbreeding on the aphid traits, mating systems, and sex ratios.

The extent of inbreeding is affected by the life cycle and taxonomic group of the aphid species. As in other insects, inbreeding is expected to occur frequently in species with low migratory ability (Thornhill, 1993). In this respect, aphids species without host alternation are likely to inbreed (e.g., Komatsu & Akimoto, 1995). Furthermore, in the Eriosomatinae and Hormaphidinae, inbreeding may arise easily because the autumnal alates (sexuparae) of these groups have fully grown male and female embryos in their abdomen (Dixon, 1998); the sexuals are dwarfish, stay on the host trunk after birth, and mate without feeding. Actually, when males and females of the same clone are confined in a small cage, they mate readily, producing selfed eggs (Akimoto, 2006).

The effect of inbreeding has been estimated by comparing the hatch rates of selfed and outbred eggs (Via, 1992; Helden & Dixon, 1997; Akimoto, 2006). These studies indicate that eggs from intra-clonal mating (selfed eggs) hatch less successfully than do eggs from inter-clonal mating, suggesting inbreeding depression. However, the impact of inbreeding depression varied largely among aphid species. In general, the effect of inbreeding depression appears to be weak in species that usually experience inbreeding.

The direct effects of inbreeding on aphid traits were studied using the Eriosomatine species *Prociphilus oriens,* for which a certain level of inbreeding has been expected (Akimoto, 2006). Enforced selfing led to a large variation in the hatching time and morphology of first instars. On average, larvae from selfed eggs hatched later and had longer tibiae and antennae than did larvae from outbred eggs. In contrast, when selfing was enforced, the gonad of larvae was much reduced in size. Statistical analysis based on families detected a genetic trade-off between tibia length and gonad size. Thus, this study shows that reduced gonad size in *P. oriens* can be ascribed to the excessive development of larval tibiae due to deleterious alleles and a side effect through the trade-off. First instars hatched from eggs in the field were also affected by inbreeding. Larvae that hatched later from eggs in the field tended to have a smaller amount of gonad, whereas this tendency was not observed in larvae from outbred eggs.

The occurrence of inbreeding in the field was verified by inter-population crosses. Sexuals from two adjacent local populations, 11 km from each other, were crossed in the laboratory, and the first instars derived from inter-population crosses were compared morphologically with those from intra-population crosses. Enforced outbreeding led to an increase in general body size. Although no difference was found in gonad size, the increased body size appears to represent heterosis, or hybrid vigor. These results indicate that inbreeding actually arises in local populations of *P. oriens*.

The incidence of inbreeding may have influenced aphid sex ratios and mating systems. *P. oriens* sexuparae have female-biased sex ratios (Yamaguchi, 1985). Sex ratios in aphids have been well studied (Moran, 1993; Foster, 2002), and local mate competition has been postulated to be responsible for female-biased sex ratios. In the situation where local mate competition occurs, inbreeding is also expected. However, previous studies have overlooked the presence of sexuparae producing females only. *P. oriens* sexuparae consist of two types; one type produces males and females simultaneously in the abdomen ("M+F" type), while the other type produces females only ("F" type). Long-term observations show that the proportions of the "M +F" and "F" types varied from year to year, and accordingly that the sex ratio also varied greatly. It is possible that "F" type sexuparae have the advantage of avoiding inbreeding. For understanding the evolution of aphid mating systems, it is necessary to focus on the incidence of inbreeding in the field.

Key words: selfing, life cycle, parthenogenesis, mating, Eriosomatinae

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Plenary Lecture

Quantifying the role of predation in the seasonal dynamics of mealy plum aphid populations in California

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The role of predation in the seasonal dynamics of aphid populations has proved to be quite difficult to quantify as any change in aphid density results from a balance between two opposing processes, aphid recruitment through reproduction and aphid mortality through predator consumption. In general, inferences regarding the role of predation in the dynamics of aphid populations have been derived either from field observations of correlations between predator and aphid abundance (e.g., Minarro *et al.*, 2005) or from comparisons of aphid densities in cages that are open to natural enemies with those from cages that exclude natural enemies (e.g., Costamanga *et al.*, 2008). While widely used, both of these approaches need to be treated with caution, as firstly, correlations are not cause and effect, and secondly, cages can exaggerate the apparent effects of natural enemies by increasing aphid recruitment in response to amelioration of microclimatic conditions and prevention of aphid emigration. Our goal was to quantify aphid predation using a mechanistic approach that incorporates the balance between recruitment and mortality, and to determine the degree to which naturally-occurring densities of aphidophagous predators are responsible for observed seasonal changes in aphid densities, using the mealy plum aphid, *Hylaopterus pruni*, as a model system.

Our approach was to compare two quantities: *expected aphid mortality* and *observed predator consumption. Expected aphid mortality* makes use of a simple model of aphid population growth and predation to estimate aphid mortality from observed aphid densities on successive sample dates in prune orchards, given an independent field estimate of the seasonal change in aphid population growth rates in the absence of predators. Aphid population growth rate was estimated in the field by enclosing colonies of mixed-instar aphids on prune tree shoots over successive 7-day periods from mid April to the end of June. The 7-day period was chosen to be sufficient to monitor changes in aphid abundance while minimizing the effects of enclosure. *Observed predator consumption* was estimated by combining field data on predator densities in prune orchards with stage- and species-specific per capita predation rates estimated from field observations. The predator assemblage monitored included *Harmonia axyridis* and *Hippodamia convergens* (Col.: Coccinellidae), *Chrysopa nigricornis* and *Chrysoperla carnea* (Neur.: Chrysopidae), *Leucopis* sp. (Dip.: Chamaemyiidae), and *Aphidoletes aphidimyza* (Dip.: Cecidomyiidae).

Aphid densities peaked in May to early June and predator densities increased through spring but lagged behind those of the aphids. While observed predator consumption may have reduced the rate at which mealy plum aphid populations increased early in the season, it was not sufficient to either prevent high aphid densities in early June, or to accelerate the rate of decline as aphids migrated to their summer host plants. Moreover, predator densities on prune trees were not sufficient to drive the directional changes in mealy plum aphid densities at any point through the season.

This study provides an effective approach to quantify the role of predation in the dynamics of aphid populations, and the contribution of predation to the management of aphid populations in agricultural ecosystems. It further highlights the value of using simple population models to resolve the contributions of recruitment and predator consumption to observed changes in aphid densities in field populations.

Key words: *Chrysopa nigricornis*, direct observation, *Harmonia axyridis*, population growth rate

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Oral Presentation

Metabolic requirements in essential amino acids in parthenogenetic pea aphid embryos

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Aphids are renowned for their phenomenal reproductive output and almost all aphid species rely on symbiotic bacteria belonging to the *Buchnera* genus for development and reproduction. Several metabolic studies, led on artificial media in controlled conditions, showed that *Buchnera* supplies the aphid host with amino acids essential for its larval development when these amino acids are absent from the aphid diet. Asexually reproducing aphids contain symbiotic bacteria in two distinct compartments, either within maternal bacteriocytes or developing embryos. Embryos are re-infected by a very small number of *Buchnera* very early in development, which then undergo rapid growth to re-establish a functional symbiosis. This raises the possibility that the nutritional basis of this association may vary both between the compartments and over the course of embryonic development. Preliminary studies on embryos suggested that the demand in essential amino acids of embryonic tissues (all stages included) is more important than those of adult and larval tissues, but, at the moment, little is known about the nutritional physiology that drives aphid embryogenesis. By coupling biochemical methods and embryo culture approaches, the aim of this study was to determine the metabolic requirements of pea aphid embryos during development.

Immediately after dissection, embryos were divided into three groups based on size and distinct morphological characteristics representing early, intermediate and late stages of development. In the first part of the study, the amino acid pools of these different embryo groups were analyzed and compared to maternal tissues. The analysis of hydrolysed total protein revealed no difference in the relative contribution of individual amino acids to protein between whole adult and any of the embryo groups, reflecting the conserved nature of aphid protein composition. However, the key result of our work, was that free amino acid profiles were highly divergent among different embryonic stages. More particularly, the proportion of histidine, arginine, methionine, phenylalanine and lysine was significantly reduced in the early and intermediate embryos compared to the late and adult stages, suggesting a high need of these amino acids during the first stages of development. An interesting case was the accumulation of tyrosine in late embryos compared to all the other stages. The nonessential amino acids aspartate and glutamate were generally elevated in embryo tissues, but particularly in the early and intermediate groups. All these data indicate that metabolic requirements of pea aphid embryos change during development and underline the importance to work separately on different embryonic stages.

In the second part of our work, we developed short-term culture systems adapted to pea aphid embryos. The culture conditions for embryos at early, intermediate and late stages of development were determined comparing their respective growth rates after 72 hours of culture in media containing different composition in amino acids. At present, we are using this culture system to estimate the effect of essential amino acids depletions on embryo development, which offers us the opportunity to analyse the metabolic contribution of embryonic *Buchnera* separately from that of maternal tissues.

Key words: *Acyrthosiphon pisum*, *Buchnera*, symbiosis, amino acids metabolism, embryos

Oral Presentation

Plant penetration variables; how to use them?

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Electrical recording of plant penetration by aphids using the electrical penetration graph (EPG) technique results in a time sequence of probing behavioural events (qualified as waveforms), each representing both, aphid activities and tissue locations of the stylet tips. After analyzing the primary data a large number of variables can be quantified and these have been used to categorise plant suitability and in more detail, the tissue location of factors of plant suitability and resistance. Some activities also play an important role in virus transmission and can be used to screen plant material suitable for plant breeding to reduce the vector's success.

An overview of a large number of variables will be given as well as a strength and weakness evaluation. Variable definitions and calculation problems will be discussed and several computerised data processing routines developed by different scientific groups in Europe will be discussed.

Oral Presentation

Complete trophic signature reversal by aphid parasitism

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Studies of predator-prey relationships by stable isotope analysis indicate that the ratio of $^{15}N/^{14}N$ increases with trophic level. These studies provide the basis for the universal assumption that ^{15}N concentration increases through ecosystems unidirectionally. Some systems however such as parasite-host systems do not show this typical pattern of ^{15}N enrichment with trophic level.

Using isotope ratio mass spectrometry, we examined the interaction between the aphid *Myzus persicae* and two of its hosts in the family Brassicaceae and found that aphids are consistently depleted in ^{15}N relative to their host and surprisingly increase the $\delta^{15}N$ of their host under crowded conditions. Isotopic labeling experiments demonstrated that this unusual pattern of nitrogen use is not the result of isotopic mass balance. That is, aphids are not retaining ^{14}N and excreting heavier ^{15}N back into the host. Rather, we find that host plants with aphids have significantly elevated nitrate reductase activity and infer that the increase in $\delta^{15}N$ of aphid parasitized host plants results from nitrate substrate limitation.

Our observed pattern of nitrogen isotope use represents a complete trophic signature reversal, thus cautioning the widespread application of stable isotopes for trophic level assignment in food chains that include parasites and plant sap and exudate feeding insects.

In summary, we find that failure of nitrogen isotope fractionation during nitrogen assimilation in host plants heavily parasitized by aphids increases the ^{15}N composition of hosts resulting in complete trophic signature reversal.

Oral Presentation

Potato viral infections affect the biology and behavior of aphid vectors

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Viral infections can affect host nutritional quality by altering amino acids concentration and soluble carbohydrates, which influence aphid vectors performance. Heterologous viruses such as *Potato virus Y* (Potyviridae: *Potyvirus*) (PVY) and *Potato leafroll virus* (Luteoviridae: *Polerovirus*) (PLRV) are a regular occurrence in Idaho's potato cropping systems. PLRV arrests its vectors longer to encourage sustained feeding, whereas PVY supports shorter feeding periods (probes) by its vector sufficient to successfully transmit the virus. An increased number of plant samples from Idaho's potato fields over the last years has serologically tested positive for both PVY and PLRV via double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) and exhibited more severe symptoms than singly-infected plants (PVY or PLRV). Several authors have extensively studied the mixed infection phenomenon but to the best of our knowledge none have examined the effect of such infections on vector biology and preference.

In order to determine the potential effects of mixed-viral (PVY-PLRV) infection on the biology of aphid vectors, laboratory studies were conducted to examine the fecundity and preference of two of the most efficient PVY and PLRV vectors, the green peach aphid, *Myzus persicae* (Sulzer), and the potato aphid, *Macrosiphum euphorbiae* (Thomas), (Homoptera: Aphididae). *Myzus persicae* and *M. euphorbiae* fecundity was significantly higher on mixed-infected plants than on singly-infected plants or non-infected plants. Both alatae and apterae *M. persicae* and *M. euphorbiae* preferentially settled on PVY-PLRV infected plants than on singly-infected plants (PVY or PLRV) or non-infected plants. Mixed infections have resulted in synergistic interactions, drastically altering plant viral-susceptibility and physiological status, which severely altered host plant symptom expression patterns as well as vector-biology and preference behavior. Thus these interactions could potentially affect the virus disease epidemiology in potato fields.

Key words Potato, Mixed-viral infections, Fecundity, Host preference

Oral Presentation

Role of temperature on the development and fecundity of the emergent species *Phloeomyzus passerinii* (Aphididae: Phloeomyzinae)

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The poplar woolly aphid *Phloeomyzus passerinii* (Signoret) (Homoptera: Aphididae) has been an important emergent pest of poplar plantations in France since 1995. Now its damage are extending northward from the Southern half of France, especially in the stands of one of the most planted cultivars I214, which can be destroyed within one year. This aphid species develops on the trunks and at the base of branches of above six-year-old and about 90 cm circumference trees. It reproduces mainly by parthenogenetic apterous morphs, but it can also produce sexual alates in autumn. The apterous morph of *P. passerinii* developed through four larval instars.

Basic life history attributes including aphid life table statistics were investigated in order to obtain information about abiotic factors driving aphid development. Life history parameters of all stages were followed everyday at four constant temperatures 10°C, 15°C, 20°C, and 25°C, under controlled conditions of day length (16L : 8D) and relative humidity (65-75%). For each temperature, 30 replicates of potted poplar cuttings of I214 were infested, each of them with four randomly selected apterous females.

First, the development and longevity of only 5 larvae born within 24h were followed daily through every stage until final ecdysis and then removed to keep only one adult female. Secondly, this adult was left *in situ* to measure the time at which it began to reproduce and to record daily the offspring of this adult until its death.

Results are interpreted to assess the effect of temperature on developmental time of immature stages, adult longevity and fecundity. Development thresholds are estimated as well as the number of degree-days required to complete the development for each stage and from birth to final ecdysis. Pre-reproductive delay and mean fecundity are used to evaluate the intrinsic rate of increase and the generation time for each temperature. Results are discussed and compared with those from other tree-dwelling aphids.

Key words: *Phloeomyzus passerinii*, poplar, temperature, development, fecundity, population increase

Oral Presentation

Thermal tolerance and resource partitioning in aphids

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In a recent article Ole Heie raises 6 questions he is still unable to answer after fifty years of studying aphids. The first is what he calls "Riddles concerning the choice of host plants". Under this heading he raises several issues one of which also intrigues me. "How is that several species can coexist and form very large colonies at the same time on a particular host?" This is well illustrated by the aphid fauna associated with certain trees like birch, oak and sycamore.

In attempting to answer this question I first looked for patterns and then for the processes underlying the patterns. Plants that host aphids tend to be abundant and widely distributed. Just how many species of aphids these plants host is partly associated with their structural complexity, with each species of aphid adapted to feed on a particular part of a plant. However, several species may exploit the same structure, e.g., the leaves of trees. Interestingly, although these species co-occur on the host plant for most of the vegetative period they differ in when during that period they actively grow and reproduce. The aim of this study was to determine the process underlying this temporal pattern in reproductive activity.

For example, several species of aphids can feed and coexist on the leaves of certain trees. Of these species some only actively grow and reproduce in spring and autumn, and others only in summer. This temporal patterning in reproductive activity is a well documented and common feature. In the past the summer reproductive diapause in the sycamore aphid has been attributed to the low quality of the food available to this aphid during summer (Mordwilko, 1928). However, the fact that another species belonging to the same genus feeds on the same leaves and is reproductively active only during summer indicates that it is unlikely that reproductive diapause in the sycamore aphid is a response to poor food quality. Evidence will be presented that indicates that the temporal patterns in reproductive activity of species coexisting on the same host plant are associated with differences in their thermal tolerances. The thermal tolerance range of each species is approximately 20^{o}C, but some species are better adapted to and therefore active over a lower range of temperatures than other species and *vice versa*. In addition, temperate species of aphid do better at low temperatures than subtropical and tropical species and *vice versa.* Thus thermal tolerance is possibly an important factor determining both the seasonal development and distribution of aphids.

Key words: aphids, distribution, resource partitioning, seasonal development, thermal tolerance

Oral Presentation

Symbiont-mediated coevolution in aphid host-parasitoid systems

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The present understanding of host-parasite coevolution is largely derived from models based on interaction loci, i.e. polymorphic genes in host and parasite that interact to determine the outcome of infection. In aphids, however, much of the variation in resistance to parasitoids and pathogens is due to endosymbiotic bacteria rather than genetic differences among hosts. We provide evidence (1) that the symbiont's ability to protect hosts against parasitoids evolves readily as it occurs in even more bacteria than previously thought, (2) that parasitoid populations exhibit genetic variation in the ability to overcome symbiont-conferred resistance, and (3) that the benefits of harbouring defensive symbionts may be reduced by high induced costs of symbiont-derived defenses. Based on these findings, we argue that aphid-parasitoid systems represent a case of symbiont-mediated coevolution that can only be understood by accounting for the complex interactions among host, symbiont and parasitoid genotypes, and we highlight promising avenues of future research towards this goal.

Key words: antagonistic coevolution, cost of resistance, genetic variation, parasitoids, symbiosis

Oral Presentation

Artificial diet for aphids – thirty years' experience

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After nearly a decade of failure with Mittler & Dadd's (1962) fully defined diet for *Myzus persicae* (Sulzer), we established a clone of the aphid on 9th February 1976 which outlasted other clones started around the same time and has proved that the diet is capable of sustaining the insect for over 30 years. Initially I reported (van Emden, 1988) that we had found a genotype of *M. persicae* which could cope with the diet, but we can now rear other genotypes successfully and we have clearly made some unknown modification to the technique which has transformed failure into success. We believe that our early success depended on the quality of the particular water still we used, but this constraint has now been removed by the availability of nanopure water. The other technical essentials for success are to dissolve each ingredient completely (however long that takes) before adding the next, to ensure that the ingredients are dissolved in a prescribed order, and to change the diet every 2-3 days to prevent microbial contamination and the loss of vitamins (particularly ascorbic acid). The full recipe and technical details can be found in Douglas & van Emden (2007).

In spite of its ability to sustain aphid growth and reproduction, the diet is still clearly an inferior substrate to plant (Brussels sprout) leaves. Development to adult is extended from about 8 to 10.5 days, and on diet there is then an additional 4-day prereproductive phase almost missing in plant-reared aphids. Aphids on diet also have a much reduced fecundity (the mean per aphid is only 17.6 compared with 51.6 on plants).

The early diet literature of the 1960s suggests that the volume of fluid uptake by aphids from diet is only about $^1/_8$th of that from plants. One obvious difference between diet and plants is the high pressure of the phloem sap which effectively 'force-feeds' the aphids. However work with *Aphis fabae* Scopoli, putting a pressure of 2 kg/cm^2 behind the diet, made very little difference, suggesting that the poor liquid uptake from diet by the aphids had a different explanation. A short interval when *A. fabae* were given water before being returned to diet was followed by a 2-3 fold increase in honeydew production for 5-6 hours. Later experiments alternating 17 h on diet with 7 h of either water, ½ strength diet, full strength diet apart from ½ strength amino acids and full strength diet with ½ strength sucrose all gave heavier *A. fabae* than a 7h move to continued full strength diet. It is therefore concluded that aphids require some fluctuation in the composition of their diet (as happens continually – and certainly diurnally – in plant phloem) to maintain a high intake rate of food.

What research use does such a diet have? Although originally I got involved in diet work to study aphid nutrition, it has not proved useful for this. The diet has been developed to maximise the growth and reproduction of the aphids and so, perhaps not surprisingly, any change in any direction is deleterious. It is, however, useful for looking at compounds affecting aphid behaviour. Even here there is the problem that the low uptake from diet makes it hard to equate concentrations of allelochemicals with those in the plant and, in any case, most such experiments require such a short timescale that 15% sucrose can replace the complex diet. The diet is clearly useful for introducing chemicals into aphids, such as antibiotics for work on aphid symbionts and for creating aphid mummies with no or defined secondary plant chemical cues in studies on parasitoid behaviour. Finally, we have found the diet useful for keeping many clones of *M. persicae* separately in a small space, and for a few months in a refrigerator without changing the diet.

Key words: Aphids, artificial diet, *Myzus persicae*, *Aphis fabae*

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Oral Presentation

Defensive behavior of four *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) clones during a parasitoid attack

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Myzus persicae is considered one of the principal aphid pests in greenhouse pepper. There are several natural enemies (parasitoids and predators) which are used to control this aphid under Integrated Pest Management (IPM) and biological control strategies. One of the most used and successful parasitoid is *Aphidius colemani* (Viereck) (Hymenoptera: Braconidae), many companies commercialize this prasitoid to manage *M. persicae* populations.

The parasitoid behavior attack against aphids has been well studied; however aphid defensive behavior is not well determined. When a parasitoid attacks a pest is well known that there is a chemical communication between individuals. These chemical communications are different between species. Clonal variation is common in aphids, and we hypothesize that might be an intraspecific variation in their defensive behavior response. The main aim of this work was to study, describe and compare the defensive behavior of *M. persicae* clones

Four different clones, which present genetical and phenotipycal differences were video analyzed during a parasitoid attack. Dispersion was very similar in the four clones analyzed. Red clones presented very active defensive behaviors (when ones were stung the others start to execute a fast movement) which imply a very strong communication between colony individuals in order to communicate the attack. Dark green clone was less stung by parasitoid.

This work confirms the intraspecific variation between *M. persicae* clones and establishes different defense strategies inside the same species. There are also evidences that involve a different chemical communication between individuals of the same clones. These conclusions must be taken in consideration to design a biological control strategy.

Key words: *Myzus persicae*, parasitoids, defensive behavior, intraspecific variation

Oral Presentation

Pea aphids drop off the plant to evade incidental predation by mammalian herbivores

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Insect herbivores that live and feed on plants may be subjected to incidental predation by large mammalian herbivores. We studied the escape of pea aphids (*Acyrthosiphon pisum*) from the danger of being consumed by a large herbivore along with their host plant. Most studies focused on aphids' defense behavior against arthropods (predators and parasitoids). It is well documented that when a predator or parasitoid approaches an aphid, the aphid may respond in several ways, including twitching, walking away or dropping sporadically off the host plant.

Here we report an immediate, massive dropping of most of a pea aphid colony, triggered by mammalian herbivore exhalation. We hypothesize that the massive colony dropping, as opposed to the predator induced sporadic dropping, is a pre-encounter evasive behavior that reduces the chance of being eaten by mammalian herbivores. When we subjected pea aphid colonies to goat grazing, an average of 65±27% of mature aphids in the colony rescued themselves by immediately dropping to the ground.

We constructed an artificial breath apparatus, which allowed us to control for the different attributes of an air stream, blown at individual aphids, and used it to examine the effect of the main components of ruminant exhalation on aphid dropping behavior. We found that only when the air stream was both warm (36^{o}C) and humid (>90% RH), it induced massive dropping (87% of adult aphids). Heat and humidity have a striking synergistic effect on dropping behavior, as they reliably indicate the close proximity of a mammal's mouth. When we exposed the aphids to an air stream that contained several chemicals that are present in ruminant exhalation (Acetone, Acetic acid, Octenol, Isopropanol, Nonanal and Decanal), none of the aphids dropped. CO_2 at a concentration of 5%, which resembles the concentration of CO_2 exhaled by a mammal, had no effect either. Pea aphids also moderately drop (26±5% of the colony) in response to the vibrations caused by a leaf-picking device.

Incidental predation of arthropods by large mammalian herbivores is probably common, yet it is a poorly studied phenomenon. The magnitude of aphid dropping in response to mammalian breath, despite the inherent hazards (host loss, desiccation and ground predation), emphasizes the strength of the selection pressure that large herbivores impose on aphids and other arthropods that live on edible plants.

Key words: Escape behavior, intraguild predation, mammalian breath

Oral Presentation

Aphid alarm pheromone: costs and benefits

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Recent advances in chemical ecology have unravelled a vast array of chemical signals in plant-plant, plant-herbivore, herbivore-predator and plant-predator interactions. While most of these infochemicals are investigated in the context of a specific interaction between two particular species, the evolution of such a signal takes place in a network of biotic interactions. Consequently, the same chemical compound can have different roles depending on the organisms involved. The relative costs and benefits of the signal in its different roles will affect not only whether the signal may evolve at all, but will also influence the quantities emitted and its chemical identity.

Using the example of aphid alarm pheromone I will investigate how top-down and and bottom-up forces affect the evolution of this intraspecific signal. When attacked by a predator or parasitoid aphids may emit this alarm pheromone, in most cases (E)- β-Farnesene (EBF), to warn conspecifics of the presence of a natural enemy. In addition to this function as a pheromone, EBF may be perceived by a variety of natural enemies. I will present evidence that the costs associated with EBF's role as a kairomone may be substantial. EBF also alerts ants, and may act as kairomone or synomone depending on the interaction between aphid and ant. Finally, there is increasing evidence that aphids have to counter-act attempts of manipulation by the host plant. Thus, in the network of interactions between plants, aphids, natural enemies and ants, EBF can take all roles as pheromone, allomone, synomone and kairomone which has consequences for alarm pheromone evolution and emission dynamics.

Oral Presentation

Do plants affect the population dynamic of aphids?

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There are many studies describing the effects of abiotic factors on the population dynamics of herbivores, especially aphids. In addition, the effects of natural enemies on aphid abundance are frequently discussed. Here we focus on the effects of different features of host plants on the performance of aphids and hence on their population dynamics. A host plant is not continuously present and not all its parts are suitable for aphids as during the development of a plant there are both morphological and physiological changes. This is especially important if the time available for aphid development is restricted at certain times of a year as is the case in host alternating aphids. The probability of oviparous larvae completing their development and reaching the adult stage depends on when their primary host sheds its leaves in autumn. We will demonstrate that the variation in the proportion of *Aphis fabae* overwintering on *Viburnum opulus* compared to its other primary host plants is determined by when this plant sheds its leaves in autumn.
In other host alternating aphids that have only one species of primary host plant there is a strong selection pressure on the gynoparae to produce their offspring sufficiently in advance of leaf fall for them to mature and lay eggs. In the case of *Rhopalosiphum padi* this resulted in the production of gynoparae, which differ in their flight activity, feeding behaviour and reproductive strategy from those of host alternating species with several primary hosts. For practical reasons this is also of interest to epidemiologists studying the spread of plant viruses, because gynoparae of *R. padi* do not act as vectors of BYDV. There are two methods of discriminating between gynoparae and winged summer morphs. Both are limited in their application, one is only suitable for freshly caught aphids and the other requires that the aphids in question are mounted on slides. We describe a fast method, which is related to their respective ecologies (e.g. function of both morphs), for separating these morphs.

Key words: host plants, aphids, Aphis fabae, Rhopalosiphum padi, population dynamic

Oral Presentation

Testing the assumptions of cage exclusion experiments in field conditions

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Cage exclusion experiments are widely used for assessment of the degree to which predators are able to affect aphid population dynamics by suppressing their abundance. The growth rates and peak densities of aphid populations within cages that exclude natural enemies are usually larger than those in uncaged populations. However, cages change the microenvironment, especially temperature, which is thought to be important in determining the outcome of predator-prey interactions. Even more importantly, cages prevent aphids from emigrating, which is their usual response to high density. Interestingly, when only polythene enclosures, 60 cm high, buried to a depth of 30 cm, and not cages, were used, which do not affect the microenvironment of the manipulated plots, allow aphids to emigrate, but exclude ground predators, there was no difference in the number of grain aphids in control plots and those where the number of ground predators were reduced. Cage exclusion experiments are based on two crucial assumptions:

(A1) The cages exclude predators, which means that in the cages there are either no predators at all, or significantly less predators per plant than outside the cages.

(A2) All other effects caused by the cages (change of microclimate, prevention of migration of the pests – aphids in our case – from the cages) are negligible, compared with the effect of predator exclusion.

However, even if many papers claim that predators were excluded in the cages and their presence outside the cages caused a significant decrease of aphid growth rates and/or aphid numbers (e.g., Schmidt et al., 2003, 2004), these assumptions have – at least to our knowledge - never been rigorously tested. Here we test the assumptions (A1) and (A2), using a large data set collected in the frame of the Agripopes project within the ESF EuroDiversity program. Our experimental plots were located in 8 wheat fields belonging to different conventional farms in the region of České Budějovice, Czech Republic. In each field, 8 circular plots with a diameter of 1 m, resulting in a plot area of 0.79 m^2 were set. Four treatments were used as described in Schmidt *et al*. (2003) and Schmidt *et al.* (2004), with 2 replicates per field: (i) control (open plot), (ii) a 40 cm high plastic barrier buried 10 cm into the soil along the perimeter of the plot to exclude ground-living predators, (iii) circular wire cages with 8 mm mesh to avoid changes in microclimate, covered with sticky glue to deter or capture flying arthropods; the cages were 1m high and 1 m in diameter to exclude flying predators, (iv) a combination of (ii) and (iii) (cage + barrier). Aphids and all flying natural enemies were counted visually in all treatments on 100 wheat shoots when the plots and cages were set up at the time of wheat flowering (first sampling date), and three weeks later, when wheat was in milk ripening stage, after the cages had been removed (second date).

The numbers of flying predators inside the cages did not differ significantly from those outside the cages. More tillers were infested by aphids in the cages, compared with outside the cages. A larger percentage of adult aphids were winged inside the cages, compared with the plots without cages. A larger proportion of larvae of all aphids was found inside the cages, compared with the control plots without cages. All these results were independent of whether the barrier was present or not. We conclude that there is a strong evidence that neither (A1), nor (A2) are satisfied, when the type of cages described by Schmidt *et al.* (2003) and Schmidt *et al.* (2004) are used.

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Key words: Cage exclusion experiments, predator efficiency, biological control

SCHMIDT M.H., LAUER A., PURTAUF T., THIES C., SCHAEFER M., TSCHARNTKE T., 2003. Relative importance of predators and parasitoids for cereal aphid control. Proc. R. Soc. Lond. B, 270: 1905–1909.
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Oral Presentation

The true role of predators in man-made ecosystems

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Understanding the true value of predators in ecosystems is necessary for proper evaluation of ecosystem services. Insect predators are often assumed to significantly contribute to the regulation of insect pests in agroecosystems. The generation time ratio hypothesis, however, predicts that long-lived predators should not considerably affect the dynamics of their short-lived prey (Kindlmann & Dixon, 1999, 2001). This prediction is supported by the almost complete failure of artificially released predators in reducing numbers of their prey in hundreds of cases archived in the BIOCAT database (CAB International). Manipulative hand-removal of predators did not result in an increase in peak pest numbers either, as reported in some studies (Kindlmann *et al*., 2005). On the other hand, prey numbers in cage exclusion experiments were larger in cages, compared with uncaged plots, which the authors attributed to the effect of predators.

Ameixa & Kindlmann (this symposium) have shown, using a large data set, that some types of cages do not reduce the numbers of predators significantly and therefore the larger prey numbers in cages must be attributed to other factors than lacking predation. A detailed analysis revealed that the impossibility for prey to emigrate from cages might account for the larger prey numbers inside the cages. Thus there is no proof of predator large efficiency in controlling insect pests by means of cage exclusion experiments.

Thus the results from exclusion experiments, using cages, must be interpreted with caution. Before any conclusions are made, it must be carefully checked, whether or not the cages really exclude predators and whether their absence is the only factor causing the larger number of aphids inside the cages, compared with uncaged control plots. The results presented here and in Ameixa & Kindlmann (this symposium) lend a strong support to the hypothesis that the main reason, why there are more aphids inside the cages, compared with uncaged control plots is that aphids stay in the cages because they are prevented from emigration, while they are free to emigrate from the uncaged plots. Therefore, as cage exclusion experiments cannot be considered as a proof that predators suppress aphid numbers, no empirical evidence exists (at least to our knowledge) that predators suppress aphid numbers significantly, which supports the generation time ratio hypothesis.

The research reported here was funded in the Agripopes project within the ESF EuroDiversity program through the Grant Agency of the Czech Republic, grant number DIV-06-E013.

Key words: predator efficiency, generation time ratio hypothesis, biological control

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Oral Presentation

The community composition and influence on aphid performance of the bacteria associated with the cabbage aphid (*Brevicoryne brassicae*)

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Aphids harbour symbiotic bacteria that can have positive, negative or neutral effects on their survival and performance. These bacteria are split into two groups: the primary obligate endosymbiont, *Buchnera aphidicola*, and the secondary symbionts. In pea aphid (*Acyrthosiphon pisum*) the secondary symbionts have been shown to influence various fitness traits in their aphid hosts including, susceptibility to natural enemies and host plant specificity (Ferrari *et al.* 2004), but very little is known about their fitness effects in other aphid species. To investigate whether bacterial composition influences trophic interactions in other aphid species we have used molecular methods to characterise the bacteria associated with a Scottish arable pest, the cabbage aphid (*Brevicoryne brassicae*), and we are testing their impact on aphid-parasitoid interactions.

Molecular cloning and sequencing and Real-time (Taqman®) PCR were used to analyse the bacterial community composition of several different cabbage aphid lines. Based on copy number of the *GroEL* gene we found a higher copy number of *Buchnera* in pea aphid than cabbage aphid with relatively little variation in the abundance of *Buchnera* within and between cabbage aphid lines. The relative abundance of bacterial types other than *Buchnera* on the other hand, did vary between cabbage aphid lines. Molecular cloning and sequencing and a 16S based Real-time PCR assay indicated there are at least three different community types in cabbage aphid: (1) aphid lines dominated by one bacterial type; (2) aphid lines dominated by a second bacterial type; and (3) aphid lines in which no bacteria other than *Buchnera* have been detected.

Based on the molecular results we have devised aphid performance experiments to test the influence of bacterial composition on trophic interactions, with a particular focus on the success of hymenopteran parasitism by the wasp *Diaeretiella rapae*. The truly multitrophic nature of cabbage aphid population dynamics in arable systems is highlighted by this study.

Key words: *Brevicoryne brassicae*, endosymbiont, Real-time PCR, *Diaeretiella rapae*

FERRARI J., DARBY A.C., DANIELL T.J., CHARLES H., GODFRAY J., DOUGLAS A.E. (2004) Linking the bacterial community in pea aphids with host-plant use and natural enemy resistance. Ecological Entomology, 29, 60-65.

Oral Presentation

Potato plant acceptance of apterous and alate morphs of the aphids, *Myzus persicae* and *Macrosiphum euphorbiae*

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During summer, aphids present a parthenogenetic reproduction producing apterous and alate females. The alates had to migrate to find a new host plant and settle a new colony. Host finding is performed on the basis of perceived volatiles, and after landing by assessing physical and chemical components of plant surface. Finally, the last step leading to plant acceptance or rejection relies on brief epidermis and mesophyll intracellular punctures. In contrast, apterous individuals present a gregarious behaviour and stay on the infested plant to develop the colony. However, some apterous morphs can also spread walking from a plant to an adjoining one.

Because alate migration implies a tropism reversal which does not occur in apterous aphids we question about potato plant acceptance by alates and apterous females of two potato-colonizing aphid species, *Myzus persicae* and *Macrosiphum euphorbiae*.

Potato plant (*Solanum tuberosum*) acceptance by *Myzus persicae* and *Macrosiphum euphorbiae* alates and apterous morphs was investigated performing non-choice bioassays and electrical penetration graph (EPG) experiments.

We showed that *M. euphorbiae* accepted and probed the potato leaves more rapidly than M. persicae. Comparing morphs whatever species, we observed an increased xylem consumption associated with a reduced phloem ingestion by alates females. These results are discussed regarding biological characteristics of these two aphid species and their co-evolutive history with the *Solanum* genus.

Key words: *Myzus persicae*, *Macrosiphum euphorbiae*, Potato, Host-plant acceptance, morphs

Oral Presentation

Life-cycle evolution and host plant use in the genus *Brachycaudus*: insight from a molecular phylogeny

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The genus *Brachycaudus* comprises about fifty species. About 14 species live on or alternate from *Prunus* species. There are species groups associated with secondary host belonging to Caryophyllaceae, Polygonaceae, and Scrophulariaceae, and two of the most common species (*B. helichrysi* and *B. cardui*) alternate mainly from *Prunus* to Compositae. Other species are monoecious, living either on Rosales or on herbaceous plants belonging to Compositae, Ranunculaceae, Polygonaceae, Scrophulariaceae, Caryophyllaceae or some other families. This diversity in terms of host plant association and life cycle makes the genus *Brachycaudus* an ideal candidate for studying life cycle evolution and host plant association. Phylogenetic relationships among members of the Aphid genus *Brachycaudus* were inferred from two mitochondrial DNA fragments (Cyt B and COI) and two nuclear DNA fragments (ITS2 and Aph). Three *Buchnera aphidicola* DNA fragments were also sequenced and after testing the congruence of the phylogenies obtained with *Buchnera* markers and aphid's DNA markers, we built a total evidence phylogenetic tree of the *Brachycaudus* genus. Twenty nine species, with several specimens per species, were included, representing all the historically recognized species-groups and subgenera used in the genus. To avoid a prioris, based on host-plant association, on species definition in *Brachycaudus*, we first conducted DNA-based methods of species delimitations. We show that *Brachycaudus* species do not always correspond to phylogenetic clusters and some species delineation are highly dependent on host plant associations. Using these results we reconstructed the history of host-plant association and life cycle evolution. We show that heteroecy evolved several times in the genus and discuss the role of host-plant use in diversification scenario.

Key words: phylogeny, host-plant evolution, life cycle

Oral Presentation

A synthetic overview of *Sitobion avenae* population functioning in France

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More than 30 years of work including population dynamics and genetics studies allow giving a general scheme of the functioning of the grain aphid *Sitobion avenae* in France, at 3 different spatial scales.
1) At country scale on cereals:
- Clonal reproduction and parthenogenetic overwintering is dominant everywhere in France, but there is a trend toward increasing sexuality northward.
- The weak genetic differentiation of regional populations from cereals and the presence of many identical genotypes in most sampled regions confirm the high dispersal abilities of *S. avenae*.
- The high occurrence of widespread genotypes in multiple copies, belonging to the same genetic pool, persistent over several years and also detected in other countries (Llewellyn et al., 2003), indicates a homogenising effect of selection by millions of hectares of cereals over Europe
2) At landscape scale on different Poaceae:
- Populations on wheat, maize and barley are not genetically differentiated. Conversely, populations from weed margins and pastures (mostly Poeae) are clearly differentiated from populations on cereals, indicating a low level of gene flow between the 'uncultivated' system (mostly perennial) and the cultivated one (annual). Consequently weeds or pastured grasses are probably weak *S. avenae* reservoirs for cereal fields' further infestation by alates.
-The role of surrounding crops and weeds on wheat contamination was assessed by stable isotopic ratios (C_{13}/C_{12} and N_{15}/N_{14}) and by population genetic tools. In autumn, most *S. avenae* landing on wheat originated from maize till beginning October and from cereal volunteers after this period. In spring the contribution of surrounding cereal volunteers is variable with the year, but uncultivated Poeae play a minor role.
3) At field scale on wheat
- In April and May fields are primarily infested by a quantity of alates ranging from 30-40 to 100-200 per square meter and are till July continuously re-infested by other ones, as previously established colonies experienced high extinction rates. This leads to a progressive spatial homogenisation of the populations and to their lack of genetic structure in space and time, and highlights the role of spring immigrants in the field dynamics of *S. avenae*.
We are synthesising these results in a model of spatial invasion of wheat crops by *S. avenae* at the scale of France

Key words: genetic structure, over-wintering, Poaceae, geographic scales, aphid flight

LLEWELLYN K.S., LOXDALE H.D., HARRINGTON R., BROOKES C.P., CLARK S.J., SUNNUCKS P., 2003 Migration and genetic structure of the grain aphid (*Sitobion avenae*) in Britain related to climate and clonal fluctuation as revealed using microsatellites. Molecular Ecology, 12: 21-34.

Oral Presentation

The GAMes aphids play

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Aphid populations are highly variable in space and time. Many methods have been used to characterise their dynamics and understand factors controlling them, but prediction remains a challenge. Here we use generalised additive models (GAMs) to summarise the historical spatial and temporal trends of the grain aphid *Sitobion avenae* using Rothamsted Insect Survey data. Our objective is to identify significant upturns or downturns in the population time series whilst also accounting for variation over space. Ultimately we plan to group species with similar responses and investigate the determinants of the turning points. The annual abundances of *S. avenae* were recorded for 11 suction traps (3 in Scotland, 8 in England) over a 30 year period (1976-2005). These data were used to construct a generalised linear model (GLM) that captured the natural fluctuation in abundances between sites over time. The GLM assumes that the aphid counts are Poisson distributed and as such are independent. The GLM was then modified to include an additive component (a non-linear function) to the fitted year effect which allowed for the shape of the smoothing function to be determined by the data. This statistical model with a non-linear function is termed a GAM. The GAM retains some of the core properties of the GLM, notably the Poisson error distribution and a logarithmic link function, but the predictor (year) is subject to the properties of a smoothing function and is no longer a simple site + year model. At this stage the GAM is still incomplete because it is desirable to know whether the rate of change in the non-linear trend is significant between years. A relatively new development is to examine the change points as discussed by Fewster et al. (2000) who pin-pointed significant upturns or downturns in the population time series using second derivatives ($f''(x)$) with bootstrapped confidence intervals. We found that the dynamics of *S. avenae* population were modelled adequately using GAMs even when the dataset was perturbed by cross validation. For example, when one or more sites were dropped and the subset reanalysed, the location of the change points remained relatively stable. Further, varying the degrees of freedom of the smoothing function from parsimonious to optimal did not dramatically change the interpretation of the trend. We conclude by discussing how GAMs with change points show great promise. In practical terms, GAMs allow for the further simplification of the non-linear trend by coding change points as interval data in a square matrix and then grouping those species that show a common response. In principle, GAMs could be used for analysing and comparing a range of disparate taxa with the only restriction being that time series must overlap. This would allow the dynamics of taxa with sparse data to be predicted from those for which there were sufficient data for robust analysis.

Key words: Generalized additive models, *Sitobion avenae*, grain aphid, population dynamics

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Poster S3-1

Diversity of aphids *vis-a-vis* aphidophagous predators in Northwest and Western Himalayas, India

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Northwest and Western Himalayas lie between the Nanga Parbat in west and the River Kali on the Indo-Napal border in east of the Greater Himalayas, and they are demarcated by the River Sutlej. These parts of the Himalayas are rich in floral diversity so also the aphid faunal diversity. As many as 444 aphid species distributed under 146 genera and 12 subfamilies are known from these areas (Chakrabarti, 2006 and 2009). The species composition under different subfamilies are: Aphidinae (275), Chaitophorinae (23), Drepanosiphinae (31), Eriosomatinae (40), Greenideinae (23), Lachninae (23), Pterocommatinae (4), others (23). As many as 231 species are endemic to the Himalayas where as 118 species are endemic to Northwest and Western Himalayas.

The predatory species of ladybirds (Coccinellidae, Coleoptera), hoverflies (Syrphidae, Diptera), antlions and lacewings (Neuroptera), and anthocorid bugs (Anthocoridae, Heteroptera) are associated with aphid colonies and they play an important role in the regulation and control of aphid population. In addition, some miridid and nabid bugs (Heteroptera), and some other dipteran flies are also of minor importance.

Coccinellids (Coleoptera) are diverse aphid predators having 35 species under 24 genera in the area. Except *Harmonia* (3), *Balia* (2), *Coleophora* (3), *Exochomus* (2) and *Oenopia* (5) all other genera are represented by a sole species in the region. Maximum prey aphid species are recorded with *Coccinella septempunctata* (43), *Hippodamia (Adonia) variegata* (21), *Oenopia sauzeti* (28), *Oenopia kirby* (23) and *Scymnus* sp. (20). Although 30 coccinellid species are associated with aphids under the subfamily Aphidinae, only 18 species are with Eriosomatinae, 6 species with Greenideinae, 4 with Lachninae, 2 each with Chaitophorinae and Drepanosiphinae. *C. septempunctata, Halyzia sanscrita, Harmonia (Leis) dimidiata, O. kirby* and *O. sauzeti* are associated with aphids under different subfamilies.

Twenty-nine species of aphidophagous neuropteran species under 12 genera are known from this region. Neuropterans are more hosts specific than other aphidophagous predators. Fifteen species feed on aphid species under the subfamily Aphidinae, 7 each under Eriosomatinae and Drepanosiphinae, 6 under Chaitophorinae, 5 under Greenideinae and 4 under Lachninae. *Chrysoperla carnea* and *Retipenna jubingensis* have wide host ranges.

Aphidophagous hoverflies constitute 17 species under 11 genera. Only the genus *Metasyrphus* contains 4 species. All syrphid species are associated with aphidine species but 6 are with greenideine, 3 with lachnids and only 1 with chaitophorine aphid species. Host specificity is less common in case of syrphyid species.

Anthocorid bugs have only 8 species under 3 genera in the region. The genus *Anthocoris* represented by 4 species. The dominating species are *Anthocoris minki pistaceae, A. confuses* and *Orius niger.*

The species diversity, host association, geographical, seasonal and altitudinal distributions of the above aphidophagous insects are discussed.

Key words: Aphids, northwest and western Himalayas, predators, association, distribution

Poster S3-2

Stylet penetration of *Adelges laricis* (Vallot) on its secondary host *Larix decidua* Mill.

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Adelgids (Hemiptera: Aphidoidea: Adelgidae) form a small group of 70 described species that occur in boreal and temperate environments of Northern Hemisphere. Adelgids are cyclically parthenogenetic and exhibit multigeneration complex life cycles. Adelgids are highly host specific and they feed only on certain genera in the Pinaceae.

Larch wooly adelgid *Adelges laricis* Mill. is a holocyclic and host alternating species and it needs two years to complete the life cycle. *A. laricis* alternates between spruce *Picea* spp. (primary host) and larch *Larix decidua* Mill. (secondary host). On primary host, *A. laricis* causes the development of cone-shaped galls. After overwintering and completing the development to winged females, adelgids migrate to larch trees where they reproduce parthenogenetically by laying eggs on needles. Hatched larvae feed on larch needles through late autumn. They overwinter in bark crevices or on shoots. In spring they become wingless females after short feeding. Females lay eggs on needles and the hatched larvae feed on needles in their middle part. A portion of larvae develops into black winged aphids (sexupara) that move to a spruce where they give a bisexual generation (sexulantes). Another portion of larvae stays on a larch and develops into wingless females that parthenogenetically produce 2-3 generations of aphids.

The knowledge on the probing behavior of non-Aphididae members of Aphidoidea is very scarce. We are going to present preliminary results on the EPG (Electrical Penetration Graph) recording of the probing behavior of *A. laricis* morphs that develop on its secondary host – *L. decidua*. We EPG-recorded females at the dwarf stem base, early nymphs on needles, and advanced nymphs or adults ('wooly' instars) on needles.

The EPG waveforms were divided into two groups according to the voltage potential of the signal: extra- and intracellular level. The extracellular level patterns included the so called 'pathway' (CA0, CA1, CA2, GA). The intracellular level patterns were named EA1, EA2, EA3. A is for Adelgidae. The CA0 patterns occurred in all recorded instars with approximately equal frequency. The peaks and waves were irregular. The suggested meaning was pathway without intracellular punctures. The CA1 waveform was similar to the former but it included short potential drops and it was hypothesized to show a pathway with intracellular punctures. The CA2 pattern occurred only in advanced nymphs or adults and it included long 10s potential drops that might reflect probing in vascular tissues. The GA waveform had regular peaks (0.4-2 per second) and it occurred in all studied morphs. EA1 occurred at intracellular level and it consisted of regular peaks repeated at frequency of 1-4 per second. The shape of the peaks resembled those correlated with salivation into sieve elements in aphids. EA2 was recorded in all adelgid stages. It resembled the E2 pattern of passive phloem sap ingestion in aphids. EA3 resembled the pattern G in aphids but it occurs at intracellular level. We assume that it may represent active phloem sap ingestion activity. In the presentation, all patterns will be discussed and confronted with evidence from electron microscopy studies.

Poster S3-3

Reactive oxygen and antioxidants modulate the interaction between *Myzus persicae* (Sulzer) and plant hosts

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Reactive oxygen species (ROS) play important roles in the initial stage of plant – aphid interaction as implied by microarray studies showing that transcripts coding for enzymes involved in detoxification of ROS are some of the first to be up regulated following aphid infestation (Kuśnierczyk *et al.*, 2008). ROS could serve a number of functions in plant-aphid interactions. The high reactivity of ROS makes them directly toxic to insects or alternatively, they can generate secondary free radicals that may also serve a defence function (Miles & Oertli, 1993). In addition, ROS are now widely recognised as plant signalling compounds inducing the expression of a number of defence genes. The aim of the present study was to examine the role of ROS and plant redox buffers (ascorbic acid and glutathione) in the interaction between the generalist aphid *Myzus persicae* and several of its plant hosts.

Aphid fecundity was modulated by the redox status of its host plant with colonies cultured on potato leaves manipulated to contain high levels of ascorbic acid (AsA) outperforming those cultured on potato leaves containing lower levels of AsA. On the contrary, no differences were observed in colony expansion rates when cultured on *Arabidopsis thaliana* wild-type plants or *vtc* mutants impaired in their capacity to synthesise AsA and containing levels as low as 25% of wild-type. Ongoing work is examining the impact of reduced glutathione on aphid colony expansion.

Time course experiments revealed significant changes in the levels of H_2O_2, AsA and glutathione within 4 h of aphid infestation of oil seed rape (*Brassica napus*) seedlings supporting the hypothesis that ROS and plant antioxidants play significant roles in signalling and/or defence in plant-aphid interactions. Significant changes were also observed in the activities of several enzymes of the ascorbate-glutathione cycle.

Further analysis of the role of ROS in plant signalling will examine the spatial and temporal dynamics of ROS through the use of redox sensitive fluorescent stains. Downstream signalling events will be monitored through analysis of the cellular partitioning of an NPR1-GFP fusion protein. The redox regulated nuclear localisation of NPR1 is required for the expression of several key pathogenesis regulated proteins.

A direct defensive role for ROS will be assessed by comparison of levels of oxidative stress markers (e.g. lipid peroxides, protein carbonyls) in aphids cultured on leaves containing low or elevated levels of AsA.

Key words: hydrogen peroxide, plant defence, plant signalling, pathogenesis related proteins

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Poster S3-4

Feeding site dependant probing behavior of the pea aphid *Acyrthosiphon pisum* on two species of lupines *Lupinus* sp.

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There is a growing interest in the cultivation of lupines *Lupinus* spp. (Fabaceae: Genisteae) in Europe and all over the world due to their features that are desirable in agronomy and animal nutrition. In Poland, many varieties of cultivated lupines belong to three species: white lupine *L. albus* L., yellow lupine *L. luteus* L., and narrow-leaved lupine *L. angustifolius* L. At present, Sitona weevils (*Sitona* spp., Coleoptera: Curculionidae) and the pea aphid *Acyrthosiphon pisum* Harris are the major insect pests of wild and cultivated lupines in Poland. Although the pea aphid is usually considered the major pest of peas, faba beans, alfalfa, and clover, it is becoming an increasingly important herbivore on lupines due to the rising economic importance of this crop, especially the sweet low-alkaloid varieties. The present strategy of insect control requires the maximum application of natural control measures, including the use of naturally occurring mechanisms of plant resistance.

The aim of the present work was to study the hitherto unknown aspects of trophic relationships of the *A. pisum*, i. e. the behavioural background of the feeding site selection on lupines. We studied the pea aphid probing behaviour on stems and leaves of *L. albus* and *L. angustifolius* and compared it to the behaviour on the most suitable host plant of the genus - *L. luteus*.

The behavior of was feeding site selection by the pea aphid was studied using the technique of electronic registration of aphid stylet penetration in plant tissues referred to as EPG (Electronic Penetration Graph). In this experimental set-up, aphid and plant are made parts of an electric circuit, which is completed when the aphid inserts its stylets into the plant. Weak voltage is supplied in the circuit, and all changing electric properties are recorded as EPG waveforms that can be correlated with aphid activities and stylet position in plant tissues.

The electronic registration (EPG) of the pea aphid probing behavior on white, yellow, and narrow-leaved lupines revealed waveform C that represents probing in mesophyll and waveforms E1 and E2 indicating salivation in phloem vessels and ingestion of sap, respectively. Occasionally, patterns F and G occurred that reflected difficulties in stylet penetration and ingestion of xylem sap, respectively. On all studied varieties of the three lupine species there occurred differences in the duration, frequency, and proportion of various behavioral activities between aphids on stems and leaves. However, the differences depended on the lupine variety. *A. pisum* was the most feeding site-sensitive on white lupine, var. 'Butan': the leaves were the preferred site and the stems were clearly rejected. The preference was manifested mainly in longer duration of probing and phloem sap ingestion. The results will be discussed in the aspect of plant secondary metabolite content of the plants.

Poster S3-5

Wound repair and regeneration of gall tissue by soldier aphids in a social aphid, *Nipponaphis monzeni*

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In a social aphid *Nipponaphis monzeni*, gall repair is among important tasks of soldier nymphs for colony defense (Kurosu *et al*., 2003; Kutsukake *et al*., 2009; Kutsukake *et al*., this symposium). When a hole is bored on a spring immature gall, monomorphic first-instar soldier nymphs discharge a large amount of body fluid from their cornicles on the breach, which solidifies and plugs the hole. In this study, we experimentally investigated subsequent fate of the repaired galls. In the field, we bored a small hole on the galls of *N. monzeni* and examined them after one month. The repaired galls survived significantly better than non-repaired galls. In the survived galls, the plant tissue proliferated and sealed up the hole. Histological analysis revealed that the plant tissue around the hole grew and extended towards the center, and then covered the wound completely. A number of soldier nymphs were localized at the wounded area in the regenerating galls, suggesting that they may stimulate and induce the proliferation of the plant tissue. The gall regeneration did not occur when inhabiting aphids were killed by insecticides. These results indicated that the inhabiting aphids are needed for the gall regeneration, wherein soldier nymphs probably, play a major role. In the novel phenomenon, the insects heal the damaged plant tissue through two processes: rapid "scab" formation derived from aphid body fluid, and subsequent plant tissue regeneration induced by inhabiting aphids. The insect-mediated wound healing processes of gall tissue are, at least superficially, reminiscent of the wound healing processes of body surface breach found in vertebrates and invertebrates.

Keywords: social aphid, soldier nymph, gall repair and regeneration, wound sealing and healing

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Poster S3-6

Intraspecific variation between *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) clones in development, longevity, fecundity and other biological parameters

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Myzus persicae is considered one of the main aphid pests in Spanish southeastern greenhouse pepper and elsewhere. This aphid is well managed by chemical control but during the last years this has been replaced by Integrated Pest Management (IPM) and biological control. For this reason new control strategies must be researched to control this aphid.

Because of its characteristic life cycle (holocyclic or anholocyclic), several phenotypes or clones are found in sweet pepper cultivations and in the surrounding area (primary hosts and other herbaceous cultures). This entails a high intraspecific diversity. This diversity might be the cause of the different skin colorations observed by growers and agronomist technicians. Apparently, *M. persicae* with red and dark green coloration have produced higher damages on pepper cultivations.

To contrast possible differences between clones, the traditional green peach (green), red and dark green clones of *M.persicae* were collected during 2006, and reared under controlled climatic conditions. A genetic characterization was carried out to confirm genotype differences. Development, longevity, fecundity and other common biological parameters, of the three possible clones, were determined at five temperatures (from 10 to 30ºC) to elucitate if there is a real intraspecific variation.

The developmental time was similar in the three clones. However the clones differed in other parameters. Thus, the red clone had a higher survival index at high temperatures. The green clone presented a lower intrinsic growth rates. Moreover, the daily offspring of the red clone was different at high temperatures and dark green clone was different at low temperatures.

In conclusion there is a clear intraspecific variation between *M. persicae* clones collected from sweet pepper. Apparently, the red clone is better adapted to high temperatures whereas the green clone at low temperatures, these differences may explain the high damages on sweet pepper plants observed and they must be taken in consideration to design a good control strategy.

Key words: *Myzus persicae*, development, biological parameters, intraspecific variation

Poster S3-7

Side effect of bird cherry tree on development of *R. padi* local colonies

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The bird cherry-oat aphid, *Rhopalosiphum padi* (L.) (Hemiptera: Aphididae) is one of the most important cereal aphid in the Northern and Central Europe. It is an oligophagous species that alternates between wooden and herbaceous host-plants. The primary host is the bird cherry, *Prunus padus* (L.) and secondary ones belong to grasses. Life cycle of the bird cherry-oat aphid was quite extensively studied, and its spring host alternation is pretty well known. The bird cherry-oat aphid hatches from winter eggs in the end of March on the bird cherry trees. During the next few weeks its population is growing up on the primary host, and in the middle of May winged migrants are produced and leave for the herbaceous plants. While occurring on the primary host, many colonies of the bird cherry-oat aphid are formed on a single tree. Our primary observations showed that such colonies had a various number of insects at different part of the trees. In the present paper we report on side effect of the bird cherry tree on growth and development of the bird cherry-oat aphid. In addition, exposition of the local aphid colonies on illumination, temperature and humidity at the feeding sides was determined.

The observations were performed on a single bird cherry tree located at Aleksandria Park in Siedlce, middle-east part of Poland. Five observation points, located around of the tree, about 1.5 m above the ground were selected. Distribution of the bird cherry tree sectors was as follows: 1) northern side of the tree (N), 2) north-eastern side of the tree (NE), 3) east-southern side of the tree (ES), 4) southern side of the tree, and 5) south-western side of the tree (SW). Development of the local aphid populations in the studied tree sectors was monitored weakly on 10 marked green shoots at each tree sector. In addition, during the observations, illumination, temperature and humidity at each tree sector was measured.

Obtained results showed that the bird cherry-oat aphid formed the biggest populations at the southern and south-western sectors of the bird cherry tree. The other sides of the tree were less suitable for *R. padi* growth and development. Temperature and humidity at the studied sectors of the bird cherry tree only slightly varied between the aphid feeding sides. The great difference was found in case of illumination of the sectors of the bird cherry tree. The highest illumination was recorded between 10am and 1 pm at southern and the south-western sectors of the bird cherry tree. Usually it was about twice higher than at the other studied sides of the tree.

Importance of side effect of the bird cherry tree and the studied abiotic factors on plant metabolites and development of the bird cherry-oat aphid population is discussed.

Key words: *Rhopalosiphum padi*, bird cherry, illumination, temperature, humidity

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Poster S3-8

Morphology of Aphid salivary glands

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Salivary glands of aphids of three taxonomic families were studied, Phylloxeridae, Adelgidae and Aphididae, and from Aphididae two subfamilies, Aphidinae and Mindarinae. The principle gland morphology suggests de novo secretory activity whereas the accessory gland cells - by showing a haemolymph directed microvillar system – suggest absorption of material from the haemolymph, respectively. The epithelial cells of the ducts from both salivary glands show extensive microvillar systems but these are duct directed, suggesting a resorption from the duct fluid into the cells and back into the haemolymph.

Poster S3-9

Accumulation of putrescine within triticale attacked by grain aphid, *Sitobion avenae* (F.)

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Amino acids are the group metabolites that decide about nutritive value of plants to herbivorous insects. Thus their metabolic transformations often limit feeding, growth, development and of the cereal aphids. Plant polyamines are synthesized by decarboxylation of aliphatic alkaline amino acids. The first step in the polyamine biosynthesis is connected with formation of putrescine after decarboxylation of ornithine. Thus the ornithine decarboxylase (ODC; EC 4.1.1.17) is a key enzyme in biosynthesis of the plant polyamines and formation of their hydroxycinnamic acid amide (HCAA) derivatives. Polyamines are common components of plant cells, when its content is in range hundred micromolars to a few millimolars and is tightly regulated (Kusano *et al.*, 2008). In higher concentration, these compounds are toxic to cells by induction of programmed cell death (PCD). It is well now, that polyamines and their HCAAs participated in plant responses to pathogens (Walters, 2003), but there is only few data about their importance for insect-plant interactions (Sempruch & Ciepiela, 2005). HCAAs derivatives induced paralysis of numerous insects by binding to quisqualte type glutamate receptors on the exoskeletal muscles and blocking synaptic transmission (Klose *et al.,* 2002). Such abilities suggest that plant derived phenolic polyamines might serve as natural bioinsecticides.

Our previous studies showed that the ODC activity was changed within triticale tissues in response to the grain aphid feeding (Sempruch *et al.*, 2008). The present paper reports on changes in ornithine and putrescine content and ODC activity within two winter triticale cultivars varied in acceptance by the grain aphid (*Sitobion avenae* F.).

The grain aphid feeding decreased the ornithine content, and increased the putrescine level and ODC activity within ears of less accepted Fidelio cv. In the ear tissues of more susceptible Lamberto cv the increase of the putrescine content, and decrease of the ODC activity were observed. Obtained results suggest that changes in the ornithine and putrescine level within ears of the Fidelio cv were related to induction of the ODC activity. However, decrease of the enzyme activity suggest that increase in putrescine level within ears of the Lamberto cv resulted rather from changes in pool of its bounded or/and conjugated forms, i.e. HCAA derivatives. The importance of the polyamines and their HCAAs in the induce defensive of the triticale towards the grain aphid is discussed.

Key words: *Sitobion avenae*, triticale, putrescine, ornithine, insect-plants interactions

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Poster S3-10

On the biological differences of *Schizaphis graminum* Rond. (Homoptera: Aphididae) in wheat varieties: A comparative survey of 2 equations

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Many characteristics of host can affect development, growth, fecundity, and survivorship of herbivorous insects. The knowledge of host effects on the life table of pests is essential in pest management. r_m; the intrinsic rate of natural increase is a measure of performance to assess the level of plant resistance to aphids. It should be note that using of r_m as a tool for comparison is depended on the precision of its calculation. So, we estimated the r_m of greenbug; *Schizaphis graminum* on various wheat varieties by Euler-Lotka equation in comparison with those estimated using Wyatt and White equation under experimental condition of 22±1˚C, 70±10% and 16:8 L:D. There was no significant difference between the length of pre-reproductive, reproductive and fecundity on different wheat varieties. Based on jackknife re-sampling method, r_m stimated 0.312, 0.292, 0.276 and 0.279 on Shiraz, Pishtaz, Mahdavi and Marvdasht respectively. Feeding on Mahdavi and Marvdasht reduced the reproduction and survival of greenbug compared with others. Statistical analysis of Wyatt and White output showed that there was no significant difference between r_m on different varieties and r_m were significantly higher than that of Euler-Lotka equation. Although researcher mostly use Wyatt and White equation to compare aphid performance under different treatments, this equation could not reflect effect of host on r_m in our study since it uses only a part of fecundity data for r_m and many null replicates may be omitted while measuring the logarithmic calculations. Hence, this method is not valid enough to use in comparative surveys.

Key words *Schizaphis graminum*, Variety, Life table, Euler-Lotka equation, Wyatt and White equation, Jackknife

Poster S3-11

The pea aphid; *Acyrthosiphon pisum* (Harris) (Aphididae: Homoptera) from developmental to reproductive aspects on broad bean

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It is believed that pea aphid, *Acyrthosiphon pisum* (Harris), as an important pest of leguminous crops, may cause up to 30 percent losses. In comparison to many other aphid species that are entirely host specific, this pest is found on a few different families of plants as well as alfalfa, clover, and field beans. Broad bean; *Faba vulgaris* Moench is one of the most common host of pea aphid that have been poorly studied from the point of biological parameters of mentioned destructive pest. The fertility life table parameters of pea aphid were evaluated in laboratory under controlled circumstances of 23±1˚C, 70±10% and 16:8 L:D on broad bean. Aphids had been reared for a variety of generations on this host plant through non-shifting method. The result of the investigation of biology indicated that the duration of nymphal period and aphid fecundity rate were 7.98 days and 48.62 nymphs on broad bean. Net reproductive rate (R_0); mean generation time (T), doubling time (DT), and finite rate of increase (λ) were measured as well. Based on Birch method, gross reproductive rate (GRR) was 83.50. r_m, R_0, λ, T, and DT were 0.25, 49.42, 1.29, 15.49 and 2.75 respectively. Additionally, r_m was calculated by means of Wyatt and White method that was 0.32. The recent method is a quick, simple and imprecise way of calculation of r_m, commonly used by many biologists. In fact, the results show that broad bean is a suitable host for rearing the aphid in different laboratory studies.

Key words Pea aphid, *Acyrthosiphon pisum*, Fertility life table parameters, Birch, Wyatt and White

Poster S3-12

Effect of lectin PHA on feeding behavior of grain aphid

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Plant lectins are class of entomotoxins considered as potential biopesticides that might be used against aphids. Thus, it is a few important to understand how these molecules affect on the herbivorous insects. It has been proposed that modes of the lectin insecticidal activity such as: (1) changing behavior of the insects; (2) affecting on target tissues of the insects; (3) influencing of physiology and metabolism of the insects (Sauvion *et al*., 2003). In this paper, the effect of the lectin PHA on the grain aphid *Sitobion avenae* F. feeding behavior was examined.

The effect of PHA lectin on the grain aphid feeding behavior was investigated *in vitro*, using sucrose-agarose gels. The probing behavior of adult apterous aphids was recorded for 4 hr using the DC EPG system (Tjallingii, 1988). The experimental setup was recorded as follows; single adult apterous aphid was placed onto each gel and EPG recordings were made for 10 aphids placed on 10 control gels (without PHA lectin) and on 10 gels contained 10, 50, 250, 500, 750, 1000, 1250, or 1500 $\mu g \cdot cm^{-3}$ concentrations of the lectin PHA.

Obtained results showed that the lectin PHA clearly affected the feeding behavior of *S. avenae.* The grain aphid probing behaviour on the control gels revealed all probing activities that are performed while feeding in plant tissues. All characteristic waveforms were also present when the aphids probed into the gels with lower concentrations of the lectin (10, 50, 250 µg·cm-3). The aphids exposed to higher concentrations of PHA lectin (500, 750, 1000, 1250 and 1500 µg·cm-3) did not show any typical phloem activity. Moreover, the aphids exposed to the lectin prolonged activity corresponded to penetration of the plant epidermis and mesophyll. Total number of the gel penetrations by the grain aphid was reduced and duration of the pathways was prolonged by higher concentrations of the lectin PHA.

Presented results support the hypothesis that the mechanism of the PHA lectin toxicity is dependent on its deleterious effect on feeding behavior of the grain aphid. Possible role of the lectin PHA in controlling the grain aphid population is disscused.

Key words: grain aphid, EPG, artificial diet, protein toxicity, lectin PHA

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Poster S3-13

Do aphid galls provide good nutrients for the aphids?: comparisons of amino acid concentrations in galls among *Tetraneura* species (Aphididae: Eriosomatinae)

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Some aphid species induce leaf galls, in which the fundatrix parthenogenetically produces many nymphs. In order to ensure high performance, galls have to provide the aphids with sufficient nutrients, in particular, amino acids as a nitrogen source. We tested this hypothesis using six *Tetraneura* aphid species that induce closed galls. We extracted free amino acids from the whole gall tissues of unit weight and quantified the concentration of each amino acid. There were large differences in the total amino acid concentrations among galls of the *Tetraneura* species. Galls of *T. yezoensis*, *T. fusiformis* and one undescribed species (*T.* sp. O) had significantly higher concentrations of the total amino acids than *T. sorini* and *T. radicicola* galls or intact leaves, and the concentration of the essential amino acids exhibited a similar tendency. Of the amino acids found in the plant tissues, asparagine varied most greatly in the concentration among galls of *Tetraneura* species and leaves. Asparagine accounted for more than 70% of the total amino acids for *T. yezoensis*, *T. fusiformis* and *T.* sp. O galls but about 30% for *T. sorini, T. radicicola* and *T. triangula* galls. This percentage was still higher than the values for control leaves (3%). The asparagine concentration in *T. yezoensis* and *T.* sp. O galls was 480 times and 372 times as high as that in intact leaves, respectively. Usual leaf tissues were characterized by a high percentage of glutamic acid (24% of the total amino acid), instead of asparagine. *Tetraneura* species in which higher concentrations of total amino acids were found in the gall tended to produce larger numbers of offspring. The high concentrations of glutamine, instead of asparagine, observed in leaf tissues show that despite the same origin of the tissues, galling aphids are able to modify the chemical components of the host tissues. In aphids, asparagine and glutamine are absorbed by the bacteriocyte, in which the intracellular symbiot *Buchnera* converted them into essential amino acids for supply to the host aphid. Thus, it is concluded that high asparagine concentrations in galls of some *Tetraneura* species are induced through aphid manipulation as a result of their host adaptation.

Key words: Asparagine, Essential amino acids, Glutamine, Nutrition hypothesis, *Ulmus*

Poster S3-14

Colony defense by post-reproductive adults in a social aphid

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Theoretical studies suggest that extended post-reproductive lifespan will evolve when indirect (kin-selected) fitness benefits from a post-reproductive altruistic behavior are greater than the direct fitness benefits from continuing her own reproduction. Post-reproductive altruism is expected to occur in social aphids because there is no kin-selected conflict over reproduction within the clones. However, in social aphids, younger nymphs generally perform altruistic behavior and post-reproductive altruism has been unknown.

Here we show the evidence of a post-reproductive altruism in the Japanese gall-forming aphid *Quadrartus yoshinomiyai*, which forms conspicuous large galls on its primary host plant *Distylium racemosum*, and produces non-specialized, monomorphic first-instar defenders. A closed gall was formed by the fundatrix (gall founder). In the gall, two to three generations occurred until April in the second year. The mature gall contained about 50-200 wingless adults and 500-2000 winged or pre-winged individuals. After the gall opened, winged adults escaped through the exit holes and emigrated to their secondary host plants to found colonies of the secondary host generation. Thus, the number of the escaping winged adults would represent the reproductive success of the aphid clone.

Wingless adults of *Q. yoshinomiyai* discharged adhesive waxy droplets from their cornicles when disturbed. In the field, we found that some wingless adults attached themselves to the body surface of natural predators with the waxy droplets, whereby the mobility of the predators was impeded. Wingless adults generally clustered around the exit holes of the galls. Removal of the wingless adults had a significant negative effect on the probability of successfully repelling the predators from the galls. We also found that the adults adhering to predators contained no mature embryo in their abdomen. These results suggest that the wingless adults altruistically perform colony defense that contributes to their indirect fitness benefits. Wingless adults survived extended periods within the mature open galls, and most of them contained no mature embryo. Histological analysis showed that the abdomen of the wingless adults was filled with the waxy droplets. These results suggest that the wingless adults terminate reproduction before galls open and then accumulate waxy substance for colony defense in preparation for increased predation pressure after galls open.

This study provides the first evidence that post-reproductive adults show altruistic behavior in aphids.

Key words: social aphid, post-reproductive adults, cornicle droplets, colony defense

Poster S3-15

Changing the host selection responses of aphids through a short experience of a novel secondary plant compound

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The role of secondary plant compounds in host selection by aphids has recently been reviewed by Pettersson *et al.* (2007) and, apart from the different responses of *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L.) to glucosinolates, little is known except for the obvious association of many hosr-specific aphids with plants sharing taxon-characteristic secondary compounds.

We have tested how far it is possible to change the host selection responses of *M. persicae* as a polyphagous species, *B. brassicae* as oligophagous and *Macrosiphum albifrons* Essig (lupin aphid) as monophagous, by rearing them on Brussels sprout or lupin (for *M. albifrons*) and then testing their host plant after they had spent 24 h on a fully defined artificial diet (based on Mittler & Dadd, 1962) with or without the addition of 0.05% tomatine, a secondary compound characteristic of tomato.

Myzus persicae from Brussels sprouts did not discriminate between leaves of normal host plants (sprouts and potato) and tomato in a simple arena after 24 h on normal diet, but preference clearly shifted towards tomato after time on diet containing tomatine. A technique involving systemic insecticide to detect feeding from the phloem region (van Emden *et al.,* 1991) confirmed this shift in host preference.

The same two experiments were carried out with *B. brassicae*, but here sprout or cabbage and Chinese cabbage represented normal hosts. In the arena experiment, experience of diet with tomatine again caused a clear shift in host preference towards tomato, which was rejected by aphids after 24 h on normal diet. In the pair-wise systemic insecticide experiment, a shift in preference towards tomato did not occur in the comparison with sprouts, but was observed in the comparison with Chinese cabbage. The oligophagous *B. brassicae* was therefore less positively influenced by experience of tomatine than the polyphagous *M. persicae.*

With the monophagous *M. albifrons* offered lupin or tomato in the arena, 24h experience of tomatine caused a slight but statistically insignificant shift towards tomato, but such experience did not change the far higher proportion of aphids feeding from lupin than tomato plants in the systemic insecticide experiment.

We conclude that the degree by which a 24h experience of a novel secondary compound on subsequent host selection behaviour is greatest for the polyphagous *M .persicae* and virtually zero for the monophagous *M. albifrons*. The most interesting result is that *B. brassicae*, which is a specialist on plants containing glucosinolates, shows a more positive response to a quite unrelated plant (tomato) after a brief experience of the relevant secondary compound (the glycoalkaloid tomatine).

Key words: Aphids, host selection, *Myzus persicae*, *Brevicoryne brassicae, Macrosiphum albifrons*, secondary compounds, tomatine

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Poster S3-16

Variation of alarm aphid pheromone production: impact of the social environment

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In most aphid species, the volatile sesquiterpene (E)-β-farnesene (Eβf) is released as an alarm pheromone in response to predation and is also emitted continuously at low levels. Some aphid predators use Eβf as a foraging cue, suggesting that the benefits to aphids of signaling via Eβf must be weighed against the cost of increasing apparency to natural enemies. To determine whether aphids vary Eβf production in response to features of their social environment, we compared the production of Eβf by *Acyrthosiphon pisum* (Harris) individuals reared in isolation to that of individuals reared among conspecifics or among individuals of a different aphid species, *Myzus persicae*. Production of EβF by *A. pisum* reared in isolation was significantly lower than that of aphids reared among conspecifics or among *M. persicae* individuals. When we reared *A. pisum* individuals in isolation but exposed them to odors from an aphid colony, EβF production was similar to that of aphids reared among conspecifics, suggesting that aphids use a volatile cue to assess their social environment and regulate their production of alarm pheromone. It is likely that this cue is EβF itself, which was the only volatile compound found in headspace collections. Finally, we examined the attraction of a predatory hoverfly, which uses EβF as a foraging cue, to groups of aphids reared in isolation or among conspecifics and found that groups comprising individuals reared in isolation were significantly less attractive to the predator, suggesting that the observed variation in Eβf production may be ecologically relevant.

Poster S3-17

Morphological and ultrastructural investigations of the male reproductive system in aphids: observations of *Tuberculatus (Tuberculoides) eggleri* Börner (Hemiptera: Aphidoidea)

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Investigations into the reproductive mechanisms of amphigonic forms and generations in species belonging to the Superfamily Aphidoidea, of which little in literature is known, can surely provide useful feedback on aphid reproductive biology. However, a preliminary ultrastructural study of the reproductive system, of which almost nothing is known in literature, is doubtless necessary. Hence, the aim of our investigation is to extend the knowledge of testes and spermducts in aphids by observing several species from an ultra-structural point of view.

In this study, *Tuberculatus (Tuberculoides) eggleri* Börner, species belonging to the subfamily Calaphidine, is used to describe the general characteristics of the male reproductive system in aphids. After dissection, male reproductive tracts were observed *in toto* by the stereomicroscope or processed for scanning (SEM) or transmission electron microscope (TEM) observations. The histological investigations were carried out by optical microscope (OM) on semithin sections.

The results of our observations showed that testes, each consisting of three large follicles, appear to constitute a single body. A spermduct, uniform in diameter except for its enlarged distal tract, arises from each testis and merges, below the gut, into an ejaculatory duct that leads to the genital opening. A club-shaped accessory gland is annexed to each spermduct and merges into the ejaculatory duct as well. Each follicle, in which meiotic processes have not been identified, is subdivided into several cysts, arranged in order of maturation, increasing from the distal to the proximal tract of the follicle. In each cyst, gametes are at the same maturation stage. In more proximal cysts, mature spermatids are aligned to form bundles (spermatodesms); in each bundle, gametes seem to be kept together by a loosely structured 'cap' interposed between the sperms for most of their length. Moreover, the wall cell cytoplasm is poor in organules and with numerous lamellar bodies. The spermduct consists of a mono-stratified epithelium, lying on a basal lamina surrounded by a muscle-connective sheath. The epithelial cells display lateral membrane interdigitations with extensive septate junction systems. Furthermore, granules with heterogeneous content of different electron density are observed in the cytoplasm of these cells involved in the secretory activity. From our investigations anatomo-morphological characteristics similar to those described in the literature (Wieczorek, 2006) were found. From an ultra-structural point of view, the male reproductive tracts display a very simple organization resembling that observed by Wieczorek and Swiatek (2008). The absence of seminal vesicles morphologically distinct from the spermduct should be highlighted; however, it is possible that their function could be carried out by the spermduct tract proximal to the testis, in agreement with Blackman's hypothesis (1987). Finally, our data would seem to suggest that the 'cap' derives from the material released during spermatid maturation and cyst-wall cell degeneration.

The results so far, although still limited, represent a promising starting point for further research, to be extended to female genital tracts, to understand the role of aphid male genital structures during reproduction; moreover, such research can provide a valuable contribution to improving both systematics and applicative studies.

Key Words: Aphididae; male reproductive system; ultrastructure; spermatodesms

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Session 4

Aphids in agriculture, horticulture and forestry

Plenary Lecture

Fungal endophytes in temperate grasses: important or insignificant mediators of host plant resistance to aphids?

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The plant kingdom contains a wide diversity of chemicals that are involved in defence against attack by aphids and other arthropods. For example, several chemicals (jasmonic acid, salicylic acid, ethylene, abscisic acid, giberellic acid, nitric oxide) are involved in plant responses to aphid-inflicted tissue damage. Also, nitrogen compounds (alkaloids), terpenoids, and phenolics are some of the best known plant defensive chemicals (Smith & Boyko, 2007 and references therein). Moreover, symbiotic fungi can enhance the defensive chemical composition of plants, exemplified by secondary metabolite production by *Neotyphodium* (Clavicipitaceae) fungal endophytes of temperate grasses. These metabolites are ergot alkaloids (particularly ergovaline), lolitrems (indolediterpenoids), pyrrolizidine lolines, and peramine. The most widely-studied endophyte–grass associations synthesising these alkaloids for host grass resistance to insects (including aphids) and grazing livestock are *Neotyphodium*-infected (E+) tall fescue (TF) (*Lolium arundinaceum* (Schreber) S.J. Darbyshire (= *Festuca arundinacea* Schreber)) producing ergot, loline, and perimine alkaloids; E+ perennial ryegrass (PR) (*Lolium perenne* L.) producing lolitrem, peramine, and ergot alkaloids; E+ meadow fescue (MF) (*Lolium pratense* (Hudson) Darbyshire (= *Festuca pratensis* Hudson)) producing lolines; and E+ wild barley (*Hordeum* spp.) producing loline and ergot-type alkaloids. A clear link between ergot and lolitrem production by E+ TF and PR and livestock toxicity has been demonstrated, whereas peramine and lolines, which do not effect vertebrates, are widely considered to have good anti-insect activity (Lane *et al*., 2000).

Although many studies have shown that over 40 species of insects are adversely affected by E+ grasses, it is important to note that endophyte infection does not always confer temperate grass resistance to insect herbivores (Clement *et al.*, 1994). In this presentation for the 8th International Symposium on Aphids, I address the diversity of responses by plant feeding insects to E+ grasses by reviewing the results of research involving different grass-endophyte associations and five species of pest aphids, namely *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), *Metopolophium dirhodum* (Walker), *Diuraphis noxia* (Mordvilko), and *Aploneura lentisci* (Passerini). Additionally, I address the growing deployment of 'non-toxic *Neotyphodium* strains' in forage grass cultivars for agricultural environments, as well as the potential for developing E+ cereal crops for aphid resistance. Non-toxic strains (sometimes referred to as 'friendly' or 'novel' strains) do not produce the ergot and lolitrem alkaloids linked to poor livestock health, but produce the necessary metabolites (loline and peramine alklaloids) for insect resistance and other ecological benefits (Latch, 1997).

Entomologists and ecologists employ laboratory, glasshouse, and field studies with E+ and E- grass plants or clones to quantify the type of aphid resistance (antixenosis, antibiosis) mediated by *Neotyphodium* infection. These *in planta* studies show that endophyte effects on aphids are highly variable and highly dependent upon the host grass species/genotype, endophyte strain and alkaloid profile, and aphid species involved in the interaction. Examples below illustrate the variable nature of these interactions.

Numerous studies show that *R. padi* survival and reproduction is reduced on E+ TF and MF containing lolines, compared with aphids on E- plants and E+ plants with no detectable lolines. However, the magnitude of *R. padi* resistance on E+ grasses can be influenced by genetic differences in both the host and fungus, as illustrated by interactions involving this aphid and different wild E+ TF accessions from Tunisia. In this study, some endophyte strains imparted high levels of resistance to *R. padi*, whereas other Tunisian TF-endophyte associations conferred little or no resistance to this aphid (Clement *et al*., 2007). These variable *R. padi* densities were likely related to differences in alkaloid types and concentrations produced by the different TF–endophyte associations. *Schizaphis graminum* survival is reduced on E+ *Festuca* and *Poa* plants producing peramine only and both peramine and loline alkaloids. *Schizaphis graminum* is also sensitive to E+ PR, which produces peramine and mammalian toxins, but no loline alkaloids (Siegel *et al.,* 1990). Although peramine alone has not been directly linked with resistance to *R. padi* and *M. dirhodum*, this alkaloid has been implicated in E+ PR resistance to these aphids (Meister *et al*., 2006).

Highly variable effects of endophytes are revealed by other research with *M. dirhodum* and published research involving *D. noxia*. In the *M. dirhodum* study, mortality was accelerated on E+ plants of wild TF from Sardinia, compared to E- plants; however, the aphid survival was uniformly reduced on both E+ and E- plants of a TF accession from Morocco (Clement, unpublished). Endophyte infection of two wild barley accessions reduced the survival of *D. noxia*, whereas infection of two other wild barley accessions from Central Asia did not impart resistance to this aphid (Clement *et al*., 2005).

Although scientists have much to learn about grass–endophyte–insect interactions and the absolute function of the alkaloids produced by endophytes, researchers and seed companies have forged ahead and marketed E+ grasses for better stand persistence and insect resistance. Through the development and commercial release of MaxP™ tall fescue with a non-toxic endophyte, pastures in Australia and New Zealand are protected from attack by root aphid (*A. lentisci*) (Jensen & Popay, 2007). A new use for endophytes in agriculture could emerge because *R. padi*, an important vector of *Barley yellow dwarf virus* (BYDV), is deterred from feeding on some E+ grasses. Thus, plantings of E+ perennial grasses in agroecosystems might reduce the overall incidence of BYDV in these environments, thereby reducing yield losses in nearby cereal crops (Lehtonen *et al*., 2006). Finally, the discovery of *Neotyphodium* fungi in cereal relatives suggests these fungi could protect cereal crops from aphids. However, the development of E+ cereals for aphid resistance is fraught with problems, including the potential accumulation in seeds of metabolites toxic to humans and possible incompatibility between modern cereal cultivars and natural or genetically modified endophyte strains (Clement *et al*., 1994, 2005).

In conclusion, grass endophytes may or may not be mediators of host resistance to aphids. Despite the complex nature of grass–endophyte–aphid interactions, early efforts to commercialise endophyte strains for grass resistance to aphids have been impressive, with potential for more exploitation of diverse strains for crop protection.

Key words: Aphids, temperate grasses, fungal endophytes, host-plant resistance

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Oral Presentation

Host plant resistance to aphids: a new strategy

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We recently showed that the Protein C002 from pea aphid salivary glands is absolutely essential for survival on the host plant (Mutti *et al*., 2006,2008). So far, all nine aphid species examined have had the *c002* transcript encoding this protein, but it appears in no database of any other species of insect, and none of the species we tested. While RNAi based host plant resistance has been recently reported for coleopteran and lepidopteran pests, as well as nematodes, it has not yet been used for aphids. However, use of *coo2* as a target has huge potential as a highly specific (aphid pests only), highly effective (100% of aphids in which *c002* was silenced died within 8 days of treatment) new strategy for host plant resistance.

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Oral Presentation

The pea aphid and *Medicago truncatula*: a wealth of interactions

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The pea aphid (*Acyrthosiphon pisum* (Harris)) is well known to show extensive genetic divergence associated with host-plant adaptation. In Europe, several host races exist, showing remarkably consistent fidelity to particular plant species. Several studies have examined this host-based adaptation by comparing aphid performance on different plant species, but less information is available on responses of pea aphid clones to different plant genotypes within a single host species. We are currently investigating the extent and biological basis of aphid adaptation to different genotypes of the model legume *Medicago truncatula* Gaertner. This plant species shows extensive genetic variation, both within and between populations. Twenty-two lines of *Medicago*, representing extensive polymorphism, were tested against eight clones of pea aphid. Four aphid clones were originally collected from alfalfa (*M. sativa* L.) and therefore might be expected to show relatively good performance on *M. truncatula*. A further four clones originated from other plants species: one each from *Trifolium pratense* L., *Pisum sativum* L., *Vicia faba* L. and *Lathyrus pratensis* L..

The matrix of 176 aphid-plant interactions showed extensive variation in both plant resistance and aphid virulence. Each plant line showed a spectrum of interactions, from resistant to susceptible, depending on aphid genotype. Each aphid clone was able to survive and reproduce on at least one plant genotype, but the majority also showed at least one incompatible (resistant) interaction. Surprisingly, the number of incompatible interactions showed no clear relationship with original collection plant: some clones from *M. sativa* showed a similar extent of incompatibility as the clones from other plant species.

Two interactions showing particularly extreme contrast were selected for an investigation of the genetic basis of plant–aphid interactions. The aphid clone PS01, originally collected from *V. faba*, performs extremely well on the *M. truncatula* line DZA315.16, but a second plant line (Jemalong A17) is highly resistant to this clone. These two plant lines have been crossed to establish a mapping population based on recombinant inbred lines (RILs). A screen of the RILs population for resistance to PS01, followed by QTL analysis, identified a major locus (*Resistance to A. pisum 1*; *RAP1*) controlling aphid resistance and located on chromosome 3 of *M. truncatula*. Further work has confirmed that *RAP1* segregates as a single dominant gene and explains the full Jemalong resistance phenotype. The presentation will discuss the potential role of such *R*-genes in determining host specificity in aphid-plant interactions.

Key words: Pea aphid, *Acyrthosiphon pisum, Medicago truncatula,* aphid-plant interactions

Oral Presentation

Clone-specific resistance in *Medicago truncatula* against the pea aphid

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The exploitation of natural sources of plant resistance against aphid pests offers potential for crop improvement and reduced reliance on harmful pesticides. The availability of model species such as the pea aphid, *Acyrthosiphon pisum* (Harris), and the barrel medic, *Medicago truncatula* Gaertner, presents exciting opportunities with which to identify plant resistance to aphids and explore plant mechanisms of defence. Using this system, we assessed survival of several pea aphid clones on two lines of *M. truncatula*, Jemalong A17 and DZA315.16. This identified a pair of aphid clones that both survived well on DZA315.16, but differed dramatically in performance on Jemalong A17. Clone PS01 nymphs died rapidly (within two days) on Jemalong A17, whereas all clone LL01 nymphs survived. An observation of necrotic lesions only in the resistant interaction provoked investigation of this as a potential mechanism of resistance. After extensive studies (not discussed here) this hypothesis was rejected, and various other aspects of the resistance have been explored. This included EPG analysis of stylet behaviours during incompatible and compatible interactions, effect of plant extracts in artificial diet on aphid survival, the requirement of an intact plant for full aphid resistance and the possibility of systemic acquired resistance. Results from this work indicated that ingestion from the phloem does not occur on resistant plants which, together with a lack of a toxic effect of plant extracts on aphid clone PS01, implies aphids die from starvation. When aphid survival was compared on intact versus detached leaves, it seemed resistance requires an intact plant, but any systemic action of defence is unclear. The specific mechanisms that act to prevent aphid survival in this system as well as other reported cases remain unknown but with the tools attributed to the pea aphid as well as *M. truncatula*, future work in this area is likely to be profitable.

Key words: Plant resistance, defence mechanisms, electrical penetration graph technique

Oral Presentation

Effects of genotypes of inter/border crops on aphid incidence and their natural enemies in a rain fed cotton ecosystem

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Analysis was made of data on the effect of different crops and their various genotypes raised in three replications under field conditions in the rain fed season. The aim was to study the incidence of pests and natural enemies with a view to suggesting the ideal trap crops, inter- and border crops and pollen-yielding plants in a cropping system approach to cotton pest management. The following numbers of crops and genotypes were included in the study.

Cowpea	- 4
Lablab	- 2
Soyabean	- 3
Bhendi	- 5
Maize	- 3

Cowpea: Cowpea was found to be an ideal intercrop to attract the legume aphid, *Aphis craccivora* Koch in large numbers, which could in turn attract predatory insects such as the ladybird beetle, *Cheilomenes sexmaculata* (F.). While the legume aphids cannot shift to cotton and cause damage, the predatory beetle could move to cotton and feed on cotton aphid and other sucking pests. Among the genotypes, cowpea CO 2 and CO 4 harboured the highest population of legume aphids. The ladybird and spider predators were feeding in larger numbers on the aphids on all the three varieties other than CO 6.

Lablab: The *A. craccivora* population was higher on CO 13 than on CO 12 lablab variety which enhanced the coccinellid predator population.

Soyabean: No aphids were recorded on any of the three genotypes.

Bhendi: *Aphis gossypii* Glover is the most important insect pest of bhendi attracting large numbers of predators such as the ladybird, *C. sexmeculatus,* the hoverfly, *Syrphus indicus* Doleschall and the green lacewing, *Chrysoperla carnea* (Stephens), and parasitoids, *Aphidius platensis*. MDU 1 bhendi variety had the highest aphid population of 120 per plant unit of three leaves in one week. This cultivar seems to be the most suitable trap crop and for enhancing natural enemy population. The variety Parbhani Krandi also attracted many aphids though not to the level of MDU 1.

Maize: All the three cultivars of maize were more or less equally susceptible to the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) and leaf aphid, *Longiunguis sacchari* Zehntner attracting the ladybird *C. sexmaculata*.

Key words: Aphids, natural enemies, border crops, cotton

Oral Presentation

Host selection of the giant willow aphid (*Tuberolachnus salignus*)

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Willow (*Salix* spp) grown as short rotation coppice (SRC) is one of the main biomass crops in the UK and has been identified as appropriate for the production of biomass energy in the Palearctic and North America due to its potential for rapid growth in temperate climates. Willows serve as hosts for several insect species, many of which are potential pests. The latter include the giant willow aphid (*Tuberolachnus salignus* (Gmelin)), which often occurs at high density. Many aspects of the ecology of this aphid species and its interactions with host plants are poorly understood and, consequently, the development of effective management strategies is not yet possible. The predicted expansion of land under SRC willow, and the possibility of springs and summers in the UK becoming warmer, have increased concern that aphids may become a serious pest on this crop.

Host selection studies of the giant willow aphid (*T. salignus*) on short rotation coppice (SRC) willow varieties focus on the hypotheses that a) aphid behaviour is affected by chemical cues from the host and b) differences in host/aphid interactions have a basis in the genetics of the host. This work has been done using laboratory bioassays, electrophysiological responses of *T. salignus* to willow volatiles and field experiments studying the infestation pattern of *T. salignus* on different willow varieties.

Laboratory olfactometry tests were done using the Linear Track Olfactometer (LTO), and ten willow genotypes were initially chosen for use in the bioassays. *Tuberolachnus salignus* showed significant responses to certain SRC willow varieties, but not to others. No willow variety proved to be repellent to the aphids. Bioassays done at different times of year (May vs. October) yielded different results, suggesting a seasonal effect of *T. salignus* responses to willows. *Tuberolachnus salignus* attraction to conspecifics was also tested in the LTO. The alatae did not show an attraction to the apterous aphids.

As the olfactometer tests provided some evidence of host preferences, two willow varieties for which the aphids showed opposite behaviour in the LTO were chosen for further experiments on behaviourally active compounds in their volatile profile. Volatiles were isolated from the willow varieties using air entrainments, and coupled Gas Chromatography - Electroantennography (GC-EAG) was used to look at *T. salignus* responses to volatiles from entrainment samples, which were then tentatively identified by GC-Mass Spectrometry. No previous Electroantennography (EAG) or GC–EAG work has been done on *T. salignus*. *Tuberolachnus salignus* responded to 12 compounds from each of the two willow variety entrainment samples, but only three compounds were common to both varieties.

Field trials during 2007 and 2008 have shown that *T. salignus* infestation levels vary significantly on different SRC willow varieties when infestation is at its peak density, matching the laboratory bioassays. There was also a significant difference in infestation levels between years, with infestation peaking a month earlier in 2007 than in 2008.

This work on host selection of *T. salignus* has shown that the aphids respond differently to different willow varieties. Knowledge of which SRC willow varieties are preferred by *T. salignus* will allow the inclusion of resistant genotypes in willow breeding programmes.

Key words: Willow, aphid, *Tuberolachnus salignus*, chemical ecology, host selection

Oral Presentation

Co-operation between plant enemies – do raspberry viruses attract more aphid vectors?

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Plants can be simultaneously attacked by a range of organisms, including both insect herbivores and viral pathogens. Recent evidence suggests that plant pathogens can alter their hosts in such a way as to recruit more insect vectors (Eigenbrode *et al*., 2002), thereby facilitating further transmission. The large raspberry aphid, *Amphorophora idaei* (Börner) is a highly mobile and effective vector of at least four viral pathogens commonly found in raspberry plantations in the United Kingdom and wider Europe (McMenemy *et al*., 2009). These viruses include *Black raspberry necrosis virus* (BRNV) and *Raspberry leaf spot virus* (RLSV). A better understanding of the ecology of *A. idaei*, including interactions with viral pathogens and other insect herbivores could be crucial for the development of new control strategies for this economically important pest.

Key words: *Amphorophora idaei,* viral pathogen, vector, insect herbivore

EIGENBRODE S.D., DING H., SHEIL P, BERGER P.H., 2002. Volatiles from potato plants infected with potato leafroll virus attract and arrest the virus vector, *Myzus persicae* (Homoptera: Aphididae). Proceedings of the Royal Society of London: B, 269: 455-460.
MCMENEMY L.S., MITCHELL C., JOHNSON S.N., 2009. Biology of the European large raspberry aphid (*Amphorophora idaei*): its role in virus transmission and resistance breakdown in red raspberry. Agricultural and Forest Entomology, 11: 61-71.

Oral Presentation

Aphid saliva effects on plant defense

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Acceptance of *Solanum tuberosum* L. plants by aphids decreased when the plant was initially infested by conspecific aphids or ones belonging to another species (Dugravot *et al.*, 2007). The results suggest the induction of systemic resistance during the first infestation involving recognition of elicitors. Aphids produce two types of saliva during the progression of the aphid stylet between the cell wall layers to reach the phloem: one a liquid saliva injected into cells or phloem and the other a solid saliva that makes a sheath around the stylets. With the aim of identifying salivary elicitors, we characterised local potato responses against *Macrosiphum euphorbiae* (Thomas) at macroscopic, microscopic and molecular levels. Our results revealed a set of plant responses induced by aphid infestation at the infection site. The deposition of "brown spots", associated with the salivary sheath, cell death and the induction of defence genes appeared after infestation. Callose deposition appeared around the damaged plant cells and in the phloem, but interestingly this response was highly reduced during when aphids were present and salivating. Some of these responses like cell death can be induced on foliar discs by applying aphid saliva alone. These results suggest that aphid saliva is able to both induce and inhibit plant defence responses. The characterisation of *M. euphorbiae* saliva components is being initiated by protein analysis in order to identify elicitors or plant defence inhibitors produced by phloem-feeding insects.

Key words: *Macrosiphum euphorbiae,* potato, saliva, plant response

DUGRAVOT S.L., BRUNISSEN L., LETOCARD E., TJALLINGI W.F., VINCENT C., GIORDANENGO P., CHERQUI A., 2007. Local and systemic responses induced by aphids in *Solanum tuberosum* plants. Entomologia Experimentalis et Applicata, 115: 107-115.

Oral Presentation

Complementary methods for monitoring sugar beet aphids to improve risk management of virus yellows

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There is rarely an undisputedly perfect way of doing anything. For many years, we have been funded by the British Beet Research Organisation (BBRO) to provide early season forecasts of the flight timings of aphid vectors of sugar beet viruses, particularly *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas), and information on their distribution, abundance, insecticide resistance status (e.g. MACE and kdr) and virus content (e.g. *Beet mild yellowing virus*) during the critical growing period in which the crop is especially susceptible to viruses.

One tool available is the network of suction traps that has been operated since the mid 1960s and provides daily aphid data. These data, in conjunction with meteorological records, can provide predictions and assessments of aphid activity, and information on insecticide resistance status and virus content of aphids at a regional (e.g. 80 km radius) scale. The disadvantage of such data is that there will be much field to field variation within a region according to local factors.

The network of suction traps is complemented by a network of yellow water pan traps which are strategically positioned within sugar beet crops and used to monitor aphids from April to July each year. Whilst these provide information relevant to specific fields, they are not representative of a wide area and so, if used alone, can only provide useful information to the farms close to where they are sited.

A combination of the two trapping methods provides regional information combined with an indication of the range of variability experienced at the field scale and will eventually allow an assessment of the field characteristics that determine how regional data translate into field incidence.

We present results from each methodology and highlight the relationships that exist between the two data sets. Exploitation of the information by the BBRO and UK sugar beet growers will be discussed.

Key words: monitoring, forecasting, sugar beet, viruses, insecticide resistance

Oral Presentation

Do non-colonising potato aphids exhibit behaviour which facilitates non-persistent virus transmission?

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Aphids are the most common vectors of plant viruses which can cause important economic damages in several annual cropping systems. In Europe, *Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas) are the most serious virus vectors on potato crops. Their transmission of non-persistent potyviruses is achieved during superficial brief probes.

However, virus epidemics do not appear directly related to potato colonising aphids as high rates of infection have been reported despite the lack of developing colonies. Therefore we hypothesised that non-colonising potato aphids can transmit non-persistent potyviruses.

As aphids are required to vector those viruses, and because a large number of aphid species can transmit them, aphid behaviour appears to be a key element in virus epidemics. So we compared potato plant acceptance and probing activity between non-colonising potato aphids and the potato aphids *M. persicae* and *M. euphorbiae*.

Petri dish tests were performed in the laboratory to evaluate residence time and probing activity on potato leaflets. The electrical penetration graph (EPG) technique was used to investigate probing behaviour within plant tissues, especially intracellular penetration associated with non-persistent virus acquisition and inoculation.

Results showed that some non-colonising aphid species exhibited behaviour highly favourable to virus transmission in the potato crop. Combining those laboratory results with aphid population densities evaluated by field trapping, the involvement of these aphids in non-persistent virus epidemics in potato plots is discussed.

Key words: Aphids, potato, PVY spread

Oral Presentation

Towards the characterization of the functional role of the common duct in aphid stylets

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Nearly all plant viruses use specific vectors to spread from one host to another, the most common vectors being insects, especially aphids. The predominant strategy for virus–vector interaction is the so-called non-circulative transmission, in which the virus is taken up by a vector on an infected plant, attached to unknown receptors somewhere in the feeding apparatus, and subsequently released to inoculate a new host plant.

The most precise data on these enigmatic binding sites in insect mouthparts came from a recent pioneering study using *Cauliflower mosaic virus* (CaMV), a non-circulative virus, to search for receptor molecules in the aphid vectors. A novel *in vitro* system allowed rapid visualization of the interaction between dissected aphid stylets and the CaMV ligand protein. This provided the first direct evidence for the existence, the precise localization and the chemical nature of a true receptor molecule used by a plant virus in the vector's mouthparts. The receptor molecules are concentrated exclusively in a tiny area located in the common duct at the extreme distal tip of the aphid maxillary stylets, and are non-glycosylated proteins strongly linked to and deeply embedded into the chitin matrix (Uzest *et al.*, 2007).

Using transmission and scanning electron microscopy for further investigating the ultra-structure of this area, we uncovered an intriguing anatomical zone, lining the bed of the common duct in each maxillary stylet, that had thus far been overlooked. This area appears as a swelling of the cuticle surface that perfectly matches the area where the CaMV is specifically binding. It is present at all developmental stages of the aphid vectors, and also found in aphid species that do not transmit CaMV. We are currently investigating the protein contents, the ultrastructure, the biochemical and biological properties of this peculiar structure, in order ultimately to elucidate its physiological function, presumably in the feeding process of aphids or in their relationship with the host plant.

Key words: virus-transmission, aphid, maxillary stylets, common duct, cuticular proteins

UZEST M., GARGANI M., DRUCKER M., HEBRARD E., GARZO E., CANDRESSE T., FERERES A., BLANC S., 2007. A protein key to plant virus transmission at the tip of the insect vector stylet. PNAS USA, 104: 17959-17964.

Oral Presentation

A plant virus transmission-blocking peptide

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The transmission of the more than 300 plant viruses that are vectored by aphids involves specific molecular interactions between the virus and its vector: For aphid transmission of luteoviruses, the virus must bind to a receptor in the gut for uptake into the haemocoel, where the virus circulates. A second receptor is involved in movement of virus from the haemocoel into the accessory salivary gland, from which the virus is delivered with the aphid saliva into the phloem of the plant. The molecular mechanisms of recognition and uptake of plant viruses into their aphid vectors are poorly understood. By screening a phage display library for peptides that bind in the gut of the pea aphid *Acyrthosiphon pisum* (Harris), we identified a peptide, GBP3.1 that binds to the aphid gut. Binding of this peptide blocks uptake of the plant virus, *Pea enation mosaic virus* (PEMV) into the aphid haemocoel. This peptide provides a unique tool for characterisation of the aphid receptor involved in PEMV binding in the aphid gut and for development of PEMV transmission-blocking transgenic plants.

Use of GBP3.1 in combination with antibiotic host plant resistance, on which aphids are restless and may move more between plants, is likely to be beneficial.

Key words: pea aphid, luteoviruses, virus transmission-blocking peptide, *Pea enation mosaic virus*

Oral Presentation

A concept for management of virus vectors and insecticide resistance in *Myzus persicae* on potatoes

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Aphids spread of *Potato leafroll* virus (PLRV), *Potato virus A* (PVA) and *Potato virus Y* (PVY). In Europe, potato crops are often treated with multiple foliar applications of insecticides from neonicotinoid, carbamate, pyrethroid and other chemical groups to control aphids and thereby limit infection of potatoes by these viruses. This practice, along with insecticide applications to other crops containing aphids, has lead to widespread resistance to carbamates and pyrethroids in the most important virus vector, peach–potato aphid (*Myzus persicae* (Sulzer)). This resistance is conferred by three genetically distinct mechanisms. Elevated carboxylesterases, resulting from amplification of genes encoding E4 and FE4 carboxylesterases, confer resistance of various degrees (related to the amount of enzyme produced) to organophosphates and to a lesser extent to carbamates and pyrethroids. Modified acetylcholinesterase (MACE) results from a serine to phenylalanine S431F substitution within the active site of the enzyme, and specifically confers resistance to the di-methyl carbamates. Knockdown (kdr) confers resistance to pyrethroids. It involves a mutation in a voltage-gated sodium channel gene from leucine to phenylalanine L1014F, and sometimes a second mutation from methionine to threonine M918T (super-kdr or s-kdr) that enhances the resistance phenotype. Judicial use of insecticides requires their application only when aphids flying into the crop are infected with the potato viruses, and from chemical classes to which the aphids are susceptible (Parker *et al.*, 2006). This requires the availability of a rapid diagnostic tool for detecting the viruses and the frequency of resistance mechanisms to particular insecticide classes within a field population of *M. persicae*. RT-PCR has been developed to detect potato viruses (e.g. Singh *et al.*, 1995), and quantative PCR to detect the type of insecticide resistance in populations of *M. persicae* (Williamson *et al.*, 2008), but no procedure combines both tests.

We have developed such a procedure and in using this as a tool, we propose a concept for sustainable management of aphid-vectored viruses. When potatoes emerge in spring, aphids are collected from the foliage by hand, or separated from traps placed in the crop, using a water-based flume specially developed for separation of aphids from other insects. The aphids are tested in bulk until virus or insecticide resistance mutations are detected in the sample, whereupon it will be necessary to test for the frequency of virus and insecticide resistance in individual aphids. Nucleic acid is extracted from aphid samples using TriPure Isolation Reagent (Roche). The viral RNA and aphid esterase genes are amplified with primers for PLRV, PVA, PVY and esterase in a RT-PCR reaction. PVA and PVY have an additional round of PCR amplification with nested primers to increase the sensitivity of detection. Primers specific to MACE and kdr are multiplexed in a PCR reaction. The presence of a MACE allele can be detected as a heteroduplex band. The kdr is detected following restriction digestion of sodium channel PCR products. The wild type allele is cleaved and produces two smaller bands whereas the mutated allele remains as an uncleaved product. A PCR product from the microsatellite locus MS86 is also used to identify key *M. persicae* genotypes. All products are run on polyacrylamide gels. Bands of specific sizes represent the presence/absence of the viruses and insecticide resistance alleles.

The information can used to advise growers when to apply an appropriate insecticide best suited for control of the aphids present. This concept will be field evaluated in the 2009-10 potato growing season for its impact on reducing insecticide applications without compromising seed quality.

Key words: potato, viruses, aphids, diagnostics, management

ANSTEAD J.A., WILLIAMSON M.S., DENHOLM I., 2008. New methods for the detection of insecticide resistant *Myzus persicae* in the U.K. suction trap network. Agricultural and Forest Entomology, 10: 291-295.

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SINGH R.P., KURZ J., BOITEAU G., BERNARD G., 1995. Detection of potato leafroll virus in single aphids by the reverse transcription of polymerase chain reaction and its potential epidemiological application. Journal of Virological Methods, 55: 133-143.

Oral Presentation

Impairment of xylem-feeding behaviour in response to a sublethal dose of thiamethoxam is associated with dehydration and reduced performance in the bird cherry-oat aphid (*Rhopalosiphum padi*)

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Many aphid species are major pests of agricultural crops, causing damage from the transmission of plant viruses and direct feeding damage. Control of these pests is primarily through the application of insecticides. As aphids feed predominantly from the phloem, there has been a great deal of interest in the development of phloem-mobile insecticides (Hsu & Kleier, 1996). However, the majority of recently developed systemic aphicides are xylem-mobile, with translocation occurring via an apoplastic pathway. For example, the xylem-mobile neonicotinoid thiamethoxam (marketed as Actara® (for foliar and soil treatment) and Cruiser® (for seed treatment) by Syngenta Crop Protection) is an extremely effective aphicide (Maienfisch *et al.*, 2001).

In addition to their lethal neurotoxic effects, many insecticides have been found to affect insect behaviour at low, sublethal levels (Haynes, 1988). By virtue of these behaviour-modifying effects, sublethal doses of insecticides may prove to be useful tools for determining the physiological significance of insect behaviours. Using the electrical penetration graph (EPG) technique, this study reports the novel observation that a sublethal dose of thiamethoxam causes a reversible impairment of xylem-feeding behaviour in starved apterous *Rhopalosiphum padi* (L.). The reversible nature of this impairment in xylem-feeding behaviour suggests an antifeedant effect, specifically anti-xylem feeding.

Since the active ingestion of xylem sap is hypothesised to be an important mechanism for rehydration (Spiller *et al.*, 1990; Powell & Hardie, 2002), the effects of the sublethal dose of thiamethoxam on aphid water content, honeydew excretion, growth and fecundity were investigated. Body water contents of starved *R. padi* feeding on wheat treated with thiamethoxam were significantly reduced compared to aphids feeding on wheat treated with distilled water (74.5±0.23 and 75.6±0.18%, respectively). In addition, the volumes of honeydew drops excreted by *R. padi* feeding on wheat treated with thiamethoxam were also reduced. In association with the reduction in xylem feeding, severe detrimental effects on aphid performance were observed. At reproductive maturity, aphids that had been born on wheat treated with thiamethoxam were significantly smaller (as measured by body plan area; 1.07±0.03 mm^2), lighter (0.31±0.04 mg) and less fecund (2.85±0.36 nymphs/day) than aphids born on wheat treated with distilled water (1.87±0.02 mm^2, 0.72±0.03 mg, 11.28±0.58 nymphs/day, respectively).

Regardless of whether the observed impairment of xylem feeding is due to a neurotoxic or an antifeedant effect, these results have important implications for commercial crop protection as the behaviour-modifying effects of the sublethal dose of thiamethoxam may change the efficacy of this pesticide throughout the growing season. In addition, these data may provide further evidence for the role of xylem-feeding behaviour as a mechanism for rehydration in aphids.

Key words: Aphid, dehydration, electrical penetration graph (EPG), thiamethoxam, xylem-feeding

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Oral Presentation

Characterisation of neonicotinoid resistance in the peach–potato aphid, *Myzus persicae* (Hemiptera: Aphididae)

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The neonicotinoid insecticides are a relatively new class of insecticides that target the insect nicotinic acetylcholine receptor (nAChR). In the UK, and many other parts of the world, they now play a central role in the control of pests such as the peach –potato aphid, *Myzus persicae* (Sulzer), that have developed broad-spectrum resistance to a range of other insecticide classes, particularly carbamates and pyrethroids. Fortunately, the novel mode of action of neonicotinoids has left them unaffected by existing resistance mechanisms and failed treatments at recommended field-doses have not been reported to date. Nevertheless, the increased reliance on this class of insecticides raises concerns that development of field resistance to these compounds may be difficult to avoid.

We have been surveying for reduced sensitivity to imidacloprid in European *M. persicae* populations over a number of years and have observed a gradual increase in the resistance factors for selected populations, particularly from Southern Europe. At present, our most resistant clone (5191A) gives a resistance factor of around 50 fold compared to a standard susceptible laboratory clone (4106A) in topical bioassays where imidacloprid is applied directly to adult aphids. 5191A shows substantial cross resistance to other neonicotinoids and is only partially syngergised by pretreatment with piperonyl butoxide (PBO), suggesting that additional mechanism(s) to monooxygenase detoxification are likely to be present.

To investigate the possibility of a target site alteration in the 5191A clone, [^{3}H]-imidacloprid binding studies were performed on isolated membranes from the susceptible and resistant clones. These did not indicate any significant difference in ligand affinity (Kd), but the total binding (Bmax) was significantly reduced for the resistant clone. Binding assays on the same membranes with [N-methyl-3H] scopolamine (a muscarinic acetylcholine receptor ligand) showed no significant difference between the two clones, thereby validating the integrity of the membrane preparations.

We are currently investigating the possibility that mutation(s) leading to amino acid changes in the ligand-binding domains of one or more nAChR subunits and/or changes in the relative expression levels of these subunits may be responsible for the resistance and altered binding characteristics of the 5191A clone. Comparative sequencing studies and qPCR analysis of at least 6 *M. persicae* nAChR subunits (α1, α2, α3, α4, α7 and β1) will be presented and the possible role of any amino acid substitutions and/or changes in subunit expression discussed. We believe that this early characterisation of resistance in *M. persicae,* before field rates are compromised, could prove critical in developing future management strategies aimed at delaying the onset of more severe field resistance.

Key words: imidacloprid, neonicotinoids, *Myzus persicae*, insecticide resistance, nAChR

Oral Presentation

Morphological and molecular identification of apple pests *Aphis spiraecola* and *Aphis pomi* in Serbia

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In apple orchards of Germany and Switzerland, the presence of spirea aphid *Aphis spiraecola* Patch was noticed (Thieme & Eggers-Schumacher, 2003). The first appearance of spirea aphid in Serbia was recorded in spring 2007 on decorative ornamental plants *Spiraea vanhouttei* (Briot) Zabel and *Chaenomeles speciosa* (Sweet) Nakai. Recently in apple orchards of Serbia green apple aphids (*Aphis pomi* de Geer) have shown insecticide resistance. This raised a suspicion that *A. spiraecola* could be present in mixed population with green apple aphid in apple orchards of Serbia. In order to determine the presence of *A. spiraecola* as a pest of apple specimens were subjected to both morphological and molecular analyses.

Aphid samples were collected during 2007 and 2008 in apple orchards on variety of localities in Serbia. Collected specimens were preserved in 75% ethanol for morphometric analysis and in 96% ethanol for molecular analysis. Morphological characters were counted and measured and the results were compared with available keys for separation these two sibling species. Counted characters were: number of lateral tubercles on abdominal segments II – V and number of caudal hairs. Character measured was the length of ultimate rostral segment. The following keys were used: Blackman & Eastop (2000) and Halbert & Voegtlin (1992). For molecular analyses specimens of *A. pomi* from *Malus domestica* Borkhauser, and *A. spiraecola* from *M. domestica, S. vanhouttei* and *C. speciosa* were used. After the DNA extraction, aphids were slide-mounted and morphologically analyzed. For molecular identification of these species COI marker was tested by means of RFLP and sequencing.

More than 200 apterous specimens from *M. domestica* were slide-mounted. About 36% of collected specimens were without tubercles with number of caudal hairs ranging from 6 to 11 and a length of ultimate rostral segment from 0.12mm to 0.135mm. Presence of 2 tubercles was recorded in 17% of collected specimens with range of caudal hairs from 6 to 12 and a length of ultimate rostral segment from 0.125mm to 0.135mm. About 22% of specimens were with 4 tubercles with 8 to 15 caudal hairs and a length of ultimate rostral segment from 0.130mm to 0.140mm. Presence of 6 tubercles was recorded in 23% of collected specimens with caudal hairs from 9 to 18 and a length of ultimate rostral segment from 0.130mm to 0.147mm. 2% of specimens were with 8 tubercles with caudal hairs from 13 to 16 and a length of ultimate rostral segment from 0.135mm to 0.147mm.

Morphometric analysis has shown obvious overlaps in number of caudal hairs and length of ultimate rostral segment between specimens. Nonetheless about 36% of specimens had morphology typical of *A. spiraecola.* Analysis of COI mitochondrial gene marker with *Hinf*I and *Rsa*I restriction enzymes has shown distinct RFLP patterns for these two aphid species. Sequencing has confirmed 4.7% of genetic divergence on COI gene, unlike morphological characters which are not sufficiently distinguishable between these species. As a result, for proper identification of these two apple pests combined morphological and molecular analyses are required.

Key words: apple, *Aphis spiraecola*, *Aphis pomi*, key, molecular analyses

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Oral Presentation

Efficacy of *Lysiphlebus testaceipes* in control of *Aphis gossypii* on pepper

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In Tunisia, the most important regions of production of greenhouses crops are badly damaged by *Aphis gossypii* Glover and *Myzus persicae* (Sulzer) (Ben Halima-Kamel, 1991; Ben Halima-Kamel & Ben Hammouda, 1993,1998). These are considered the most dangerous pests to pepper because of their biology and biotic potential (Blackman & Eastop, 1984; Ben Halima-Kamel, 1991). Controlling this pest with several strategies has become an urgent priority in Tunisia. Chemical control presents more and more disadvantages compared with its benefits (Sawicki, 1982).By contrast, aphids on protected crops are known to have many naturally-occurring enemies: *Aphidius matricariae* Haliday, *Lysiphlebus fabarum* (Marshall), *Aphidoletes aphidimyza* (Rondani) and *Episyrphus balteatus* (De Geer) (Ben Halima-Kamel & Ben Hammouda, 1998). Subsequently, in relation to biological control on peppers in glasshouses, it was imperative to evaluate the importance of *Lysiphlebus testaceipes* (Cresson) against *A. gossypii.* This parasitoid has been introduced into the Mediterranean area (Carver & Franzmann, 2001) and we observed it in Tunisia in 1999. The importance of this parasitoid in the biological control of *A. gossypii* was indicated by Lopes *et al*. (2007).

Thus, we studied the different factors affecting the establishment of a successful biological control programme involving the introduction of this parasitoid.

Especially, we identified *in vitro* the appropriate dose of *L. testaceipes* relative to the initial density of *A. gossypii* required to increase its efficiency.

In situ bioassays were conducted to compare the time course of aphid population growth in plants with and without the parasitoid.

Key words: *Lysiphlebus testaceipes,* pepper, *Aphis gossypii*, biocontrol, greenhouses

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Oral Presentation

Aphids on ragweed

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Common ragweed (*Ambrosia artemisiifolia* L.) is native in North America. It was introduced into Europe in the 19th century. It has spread to the whole of Europe and to middle-east and far-east Asia (Reznik, 2008). The most heavily infested regions in Europe are the Carpathian basin, south-east France and the Po valley (Bohren, 2006). Ragweed is an aggressively invasive weed species whose pollen is highly allergenic, causing seasonal allergic rhinitis in an increasing proportion of the human population. Under favourable climatic and environmental conditions it has become the most frequent weed species, present on 5 million ha of the 6.5 million ha arable crop area in Hungary (Tóth *et al.*, 2004).

Surveys were conducted for indigenous insects associated with the invasive common ragweed in Hungary. Among mostly polyphagous and univoltine insects, three aphid species were found feeding on common ragweed. Among these, *Brachycaudus helichrysi* (Kaltenbach) caused chlorotic spots and leaf distortion on infested plants. On rare occasions, *Aphis fabae* (Scopoli) formed dense colonies on the stems and *Myzus persicae* (Sulzer) was found on the underside of the fully developed leaves without causing any visible symptoms.

To study the effect of these aphid species on the development of ragweed, potted ragweed plants were grown in a greenhouse and artificially infested at the 4-leaf stage with 5 apterous individuals of *A. fabae, B. helichrysi* and *M. persicae*. Feeding by all three aphid species over a 5-week period significantly reduced plant height, length of flower spikes, plant dry mass, the number of male inflorescences and pollen emission. Colony growth rate of *B. helichrysi* was the highest, followed by *M. persicae* and *A. fabae*.

In a host plant choice test, *B. helichrysi* showed significant preference for ragweed over sunflower, whereas *A. fabae* preferred sunflower. *Myzus persicae* did not show any preference.

In a field cage experiment, the growth rate of *A. fabae* on caged ragweed plants was similar to that in the greenhouse, but final numbers of the other two species, *B. helichrysi* and *M. persicae,* were much lower (after 30 days 10 and 7 times respectively lower than in greenhouse). Under field conditions the development of ragweed was more dynamic, and no aphid species significantly affected the height or dry weight of either caged or exposed plants during a 30-day period. On exposed field plants, *B. helichrysi* was significantly more abundant compared to the other two species. During longer exposures of 83 and 112 days, however, the exposed plants suffered more from aphid feeding and a significant plant height and dry mass decrease resulted regardless of the aphid species. But statistical significance is not necessarily the same as biological significance. Naturally occurring aphids can enhance the ability of native vegetation to compete with the weed but the effect of the aphid is not strong enough to reduce the number of this invasive species.

Key words: Ragweed, aphid damage, *Brachycaudus helichrysi, Aphis fabae, Myzus persicae*

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Poster S4-1

Effect of certain botanicals and entomopathogenic fungi against cotton aphids

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A laboratory study was conducted to show whether there is any systemic action of the non-edible oils (NEO) 3% neem oil (NO) and 3% pungam oil (PO) in combination with 5 and 10% dimethyl sulphoxide (DMSO) against cotton aphid (*Aphis gossypii* Glover). Cotton aphids were collected from cotton fields, which were not sprayed with pesticides, and maintained on the cotton variety SVPR-2. Twenty aphid nymphs were introduced onto the top leaves of each plant after starvation for 30 min. Each plant was considered as one replication. The non-edible oils and DMSO treatments were prepared by using 0.1% teepol as a wetting and spreading agent and sprayed onto the bottom leaves and also at the base of the shoot system to study the systemic action, if any, of the plant products on the nymphs on the top leaves. Finally, the plants were caged in mylar film cages and mortality of the aphids was recorded after 1, 3, 5 and 7 days after treatment. The results indicated that the reduction in the aphid population was minimum in the case of NEO + DMSO treatments on the first and third days. However, the efficacy was enhanced on the fifth and seventh days after treatment. A cumulative reduction of 37 to 50% was observed in the case of NO and PO combined with DMSO, either at 5 or 10%. But the reductions were not significantly different from those achieved by NO and PO alone.

A field study was conducted to show the combined effect of entomopathogenic fungi such as *Beauveria bassiana* Vuillemin, *Metarhizium anisopliae* Sorokin and *Nomuraea rileyi* Samson with leaf extract of *Vitex negundo* L. against the cotton aphid *Aphis gossypii* Glover. The aphid population was recorded 24, 48 and 72 hours after treatment. The white muscardine fungus *B. bassiana* alone was effective and caused 47 to 68% mortality over the period of observation. *Beauveria bassiana* with *V. negundo* caused mortality ranging from 61 to 87%. *Metarhizium anisopliae* with *V. negundo* was next most effective with mortality ranging from 56 to 76%, followed by *N. rileyi* with *V. negundo*. The treatments differed significantly from each other. Dimethoate was effective with complete mortality, and in an untreated control natural mortality was recorded in the range of 2 to 4%.

Key words: aphids, cotton, dimethyl sulphoxide, entomopathogenic fungi

Poster S4-2

Molecular identification of the *Myzus persicae* voltage gated sodium channel

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Myzus.persicae (Sulzer), the peach–potato aphid, is a major agricultural pest. Not only do aphid infestations cause primary damage to such staple crops as cabbage, sugarbeet, lettuce and potato, but they are also known vectors of over a hundred plant viruses including *Potato leafroll virus*. This last can reduce potato yield by 40-70% (Mowry, 2005). It is thus of agricultural and economic importance to control the *M. persicae* population. Pesticidal control has been made more difficult by the evolution of resistance to one of the principal insecticidal chemistries – the pyrethroids and their derivatives. Pyrethroids bind to the voltage gated sodium channel locking it in an open position which leads firstly to overstimulation of the nerve and eventually paralysis known as 'knock-down'. The resistance phenotype has thus been termed knock-down and super knockdown resistance (kdr and s-kdr).

The voltage gated sodium channel is a highly conserved protein found in the membrane of excitable cells where it is responsible for the rising phase of the action potential. Sodium channels comprise four domains each containing six membrane spanning sections. There is significant homology between voltage gated sodium and potassium channels which has been exploited to further our structural understanding of the sodium channel since the publication of the Kv1.2 channel structure (Long *et al.*, 2005). The selectivity filter in sodium channels, like that in K^+ channels, is located in the P-loops between the S5 and S6 helices of each domain. A critical ring of residues (DEKA), one from each P-loop, provides the Na^+ selectivity of the channel. The S4 helices of each domain form the voltage sensors and are characterised by a number of Arg and Lys residues distributed along the helix.

In the large genomes of mammals multiple isoforms of the voltage gated sodium channel are found, for example the human genome contains nine isoforms. In contrast the *Drosophila melanogaster (*Meigen) genome yielded a single voltage gated sodium channel gene 'para' which gains sequence and thus presumably functional diversity by containing a number of alternative and optional exons.

We have utilised the recently completed *.Acyrthosiphon pisum* (Harris) genome sequence to identify the aphid homologue of the 'para' voltage activated sodium channel by homology blast search. Subsequent cloning of the putative voltage gated sodium channel from *A. pisum* and *M. persicae* was accomplished by a PCR based method using degenerate primers designed against the predicted *A. pisum* gene. The exonic sequence is predicted to be ~6.2kbp in length and encodes some but not all of the optional exons identified in *D. melanogaster*. The putative 'para' homologues of *A. pisum* and *M. persicae* are >95% identical. Homology between the aphid genes and 'para' itself is 69% while there is still >40% between the *A. pisum* putative sodium channel and the 9 human isoforms. Surprisingly the DEKA filter in both putative aphid sodium channels is not fully conserved being predicted to be DENS. We would expect this to impact profoundly on channel function, since a DENS filter has been found in the mammalian Na_x channel which is thought to function as a sodium 'sensor'. Comparative sequencing of cDNA obtained from clonal lines of *M. persicae* exhibiting various levels of knockdown resistance was performed. Sequence variation found as a result of the varying kdr phenotypes of the *M. persicae* clones will be related to the model of the insecticide binding pocket developed by O'Reilly (O'Reilly *et al.*, 2006).

Key words: *Myzus persicae,* pyrethroids, resistance, sodium channel, kdr, s-kdr

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Poster S4-3

Aphids as pests of fruit- and berry-producing plants in Byelorussia

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Aphids (Rhynchota: Aphidoidea) are a numerous and economically important group of pests damaging fruit- and berry-producing plants under the conditions of Byelorussia. These crops may be subdivided into three groups: 1) berry-producing plants growing in natural stands such as platyphyllous, coniferous and mixed forests, moss bogs, etc.; 2) traditional for East European region gardening cultures; 3) not traditional and little-known berry-producing cultures, introduced in Byelorussia or actively planted only in the last few years.

Research on some aspects of aphid damage to fruit- and berry-producing plants has been carried out since 1985. As a result, it is possible to compile a list of aphid species damaging these crops under the conditions of Byelorussia.

Apple (*Malus* x *domestica* Borkh. hort. cv., *Malus sylvestris* Mill., *Malus praecox* (Pall.) Borkh.) – *Aphis pomi* (de Geer, 1773), *Rhopalosiphum insertum* (Walker, 1849), *Dysaphis anthrisci* Börner, 1950, *Dysaphis brancoi* (Börner, 1950), *Dysaphis devecta* (Walker, 1849), *Dysaphis plantaginea* (Passerini, 1860).

Pearleaf crab (*Malus prunifolia* (Willd.) Borkh.) – *A. pomi*, *Rh. insertum*.

Pear (*Pyrus communis* L., *P. communis* hort. cv.) – *Eriosoma lanuginosum* (Hartig, 1839), *A. pomi*, *Rh. insertum*, *Anuraphis subterranea* (Walker, 1852), *Anuraphis farfarae* (Koch, 1854).

Egriot, sour cherry (*Cerasus* x *vulgaris* Mill.) – *Myzus cerasi cerasi* (Fabricius, 1775).

Sweet (crab) cherry (*Cerasus avium* (L.) Moench. hort. cv.) – *Myzus cerasi pruniavium* Börner, 1926.

Plum (*Prunus* x *domestica* L.) – *Hyalopterus pruni* (Geoffroy, 1762), *Rhopalosiphum nymphaeae* (Linnaeus, 1761), *Brachycaudus cardui* (Linnaeus, 1758), *Brachycaudus helichrysi* (Kaltenbach,1843), *Brachycaudus prunicola* (Kaltenbach, 1843), *Phorodon humuli* (Schrank, 1801).

Black currant (*Ribes nigrum* L., *R. nigrum* hort. cv.) – *Eriosoma ulmi* (Linnaeus, 1758), *Aphis grossulariae* Kaltenbach, 1843, *Aphis schneideri* Börner, 1940, *Aphis triglochinis* (Theobald, 1926), *Hyperomyzus lactucae* (Linnaeus, 1758), *Hyperomyzus rhinanthi* (Schouteden, 1903), *Cryptomyzus galeopsidis* (Kaltenbach, 1843), *Nasonovia ribisnigri* (Mosley, 1841).

Red currant (*Ribes rubrum* L.hort. cv.) – *A. grossulariae*, *Cryptomyzus ribis* (Linnaeus, 1758).

Garden gooseberry (*Grossularia reclinata* (L.) Mill. hort. cv.) – *A. grossulariae*, *Hyperomyzus pallidus* Hille Ris Lambers, 1935.

Red raspberry (*Rubus idaeus* L., *R. idaeus* hort. cv.) – *Aphis idaei* van der Goot, 1912, *Amphorophora idaei* (Börner, 1939).

Forest blackberry (*Rubus caesius* L.) – *Aphis ruborum* (Börner, 1932), *Amphorophora rubi* (Kaltenbach, 1843), *Macrosiphum funestum* (Macchiati, 1885), *Sitobion fragariae* (Walker, 1848).

European blackberry (*Rubus nessensis* W. Hall) – *Aulacorthum cylactis* Börner, 1942.

Bird cherry (*Padus avium* Mill.) – *Rhopalosiphum padi* (Linnaeus, 1758), *Myzus padellus* Hille Ris Lambers et Rogerson, 1946.

Rowan tree (*Sorbus aucuparia* L.) – *A. pomi*, *Rh. insertum*, *Dysaphis sorbi* (Kaltenbach, 1843).

Elder (*Viburnum opulus* L., *V. opulus* hort. cv.) – *Aphis fabae* Scopoli, 1763, *Aphis viburni* Scopoli, 1763, *Ceruraphis eriophori* (Walker, 1848).

Bog bilberry, bog whortleberry (*Vaccinium uliginosum* L.) – *Aphis vaccinii* (Börner, 1940), *Aulacorthum flavum* F.P. Müller, 1958, *Acyrthosiphon knechteli* (Börner, 1950), *Macrosiphum nasonovi* Mordvilko, 1919, *Sitobion paludum* F.P. Müller, 1982.

Whortleberry (*Vaccinium myrtillus* L.) – *A. vaccinii*.

Cowberry (*Vaccinium* (*Rhodococcum*) *vitis-idaea* L., *V. vitis-idaea* hort. cv.) – *A. vaccinii*, *A. knechteli Wahlgreniella vaccinii* (Theobald, 1924).

European cranberry (*Oxycoccus palustris* Pers.) – *A. vaccinii*, *M. nasonovi.*

Sea buckthorn (*Hippophaë rhamnoides* L., *H. rhamnoides* hort. cv.) – *Capitophorus elaeagni* (del Guercio, 1894), *Capitophorus hippophaes* (Walker, 1852).

Key words: Aphids, pests, fruit-producing plants, berry-producing plants, Byelorussia

Poster S4-4

Plant resistance management strategies for greenbug (*Schizaphis graminum*) in wheat-sorghum cropping systems

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Strategies for deploying crop cultivars with durable resistance to insects have for years been the focus of considerable debate and speculation. Interpretation of elaborate simulation models used to predict durability of resistance has led to the generally accepted paradigm that the widespread use of an insect-resistant cultivar with a single, major gene for resistance will be selective for new, virulent biotypes. Our research has shown that this breeding tenet does not hold for the greenbug.

We define a greenbug biotype as an infraspecific population, independent of geographic distribution, that is able to injure a plant containing specific resistant gene(s) that are resistant to other infraspecific populations. Moreover, there is no presumption of the genetic basis within the greenbug for the ability to cause injury, nor is any evolutionary or taxonomic status implied. Obviously, there are genetic differences among greenbug biotypes, which affect feeding behaviour and the phenotypic response of the plant. However, the term biotype does not describe those differences, nor does it require knowledge of the biotype-specific traits that induce the damage symptoms. Thus, we conclude that a greenbug biotype is merely a plant phenotypic expression elicited by an indefinite number of genotypes.

We also conclude that greenbug virulence on crops does not coincide with greenbug fitness, and the use of plant resistance has not selected for more virulent biotypes. Instead, the greenbug species complex is made up of host-adapted races that have diverged on non-cultivated grass species well before the advent of modern agriculture, and biotypes are comprised of genetically diverse individuals among different host races that merely share similar virulence genes.

Plant resistance to greenbugs will continue to be an important strategy in pest management; however, we question the tenet that places an emphasis on releasing tolerant, multigenic cultivars and de-emphasises antibiotic, simply inherited greenbug resistance.

Key words: Aphid, greenbug, *Schizaphis graminum*, biotypes, host races, phenotype

Poster S4-5

Insecticide resistance in Italian populations of the peach potato aphid *Myzus persicae* (Hemiptera: Aphididae)

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Our research group began studies on insecticide resistance in *Myzus persicae* (Sulzer) in the early 90s of the last century. We started using bioassays (leaf-dip test) on wild populations collected in peach orchards, mainly from the Emilia–Romagna region, checking the susceptibility to commonly used aphicides. Indications of reduced activity of these insecticides were found and, comparing populations with different phytosanitary history, an increase of LC_{50}s (even if not statistically significant), was observed (Cravedi *et al.*, 1991). The next step was to set up a collection of *M. persicae* populations collected in many Italian regions, from several hosts (though peach orchards were always preferred) and with different phytosanitary history. So, starting in 1995, about 100 populations have been collected and their continuous rearing in the laboratory was started, transferring them from the natural host to pea seedlings (*Pisum sativum* L.). Meanwhile the resistance to di-methylcarbamates was studied in most of the populations, using a filter paper bioassay and a discriminating dose of pirimicarb. Moreover a programme to characterise them biochemically was started. A few characterised strains were obtained from colleagues in various European countries (UK and Germany) and maintained in the laboratory for further reference. Biochemical analysis showed that esterase-based resistance was widely distributed, while modified acetylcholinesterase was present in less than 25% of the strains (Mazzoni & Cravedi, 2002). Introducing also a biomolecular approach led to: a) the development of a diagnostic test to identify mutations responsible for "MACE" and "kdr" in a single PCR – RLFP assay (Cassanelli *et al.*, 2005); b) the assessment of esterase activity, the type and the amplification levels of the corresponding genes (E4 or FE4) in reference laboratory strains and field populations (Bizzaro et al., 2005). During a molecular screening aimed at providing a deeper insight of the main resistance mechanisms developed in Italian strains collected also from herbaceous hosts, a new mutation (F979S) increasing resistance to pyrethroid insecticides was discovered (Cassanelli *et al.*, 2005; Criniti *et al.*, 2008). Further characterisation of Italian *M. persicae* strains with different kdr and s-kdr genotypes is underway. Also modified acetylcholinesterase was investigated, and preliminary data considering molecular, kinetic and thermal characteristics of this enzyme in some Italian populations have been collected (Mazzoni *et al.*, 2007).

Key words: bioassays, esterases, acetylcholinesterase, kdr, s-kdr

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Poster S4-6

Field efficacy of plant extracts and microbial insecticides against aphid (*Aphis gossypii*) infesting okra (*Abelmoschus esculentus*)

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Okra (*Abelmoschus esculentus* (L.) Moench) is an annual crop belonging to the Family Malvaceae and one of the most important vegetable crops grown in various parts of the tropical and sub-tropical areas of the globe. The crop is susceptible to various insect pests of which aphids (*Aphis gossypii* Glover) are found to cause heavy damage. It is very difficult to control this pest as the fruit are harvested at frequent intervals and consumed after little cooking so that there is every possibility of health hazards arising from toxic residues remaining in the fruit. Studies were made to evaluate the efficacy of extracts from plants such as *Polygonum hydropiper* L. and *Pongamia pinnata* (L.), microbial insecticides like spinosad 45 SC (*Saccharopolyspora spinosa* Mertz & Yao) and *Beauveria bassiana* Vuillemin against aphids infesting okra under field conditions of the sub-Himalayan region of north-east India during the post-kharif season. Methanol was used as the solvent for extracting from the plant parts of *Polygonum* and leaves of *Pongamia*. Imidacloprid 17.8% SL was used as a control. Plant extracts, microbial insecticides and imidacloprid were sprayed four times at 12-day intervals. Total aphid numbers per leaf were counted at 3, 7 and 11 days after treatment (DAT). The data thus obtained were converted to the per cent reduction of the aphid population and analysed statistically. Significant differences were found in the relative efficacy of different treatments in reducing the aphid population and their persistence at different DAT. Imidacloprid was found the most effective treatment for controlling aphids, followed by the microbial insecticide spinosad. It was observed that extracts of *Polygonum* plants and *Pongamia* leaves at a concentration of 5% and the microbial insecticide spinosad gave satisfactory aphid control, giving more than 50% mortality. The extract of *Polygonum* at 5% was found very effective against aphids, achieving more than 60% mortality at 3 and 7 DAT. Plant extracts and microbial insecticides are of biological origin (biopesticides), having less or no hazardous effects on human health and the environment. Thus they can be incorporated in IPM programmes and organic farming in vegetable cultivation.

Key words: spinosad, *Polygonum*, *Pongamia*, *Beauveria,* vegetables, IPM, organic farming, biopesticides

Poster S4-7

Does plant resistance to cereal aphids increase under a bi-cropping regime?

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Previous work has shown that the growing of *Trifolium repens* L. (white clover) as a bicrop with wheat significantly decreases the landing rates and population growth of cereal-aphid species. We aimed to test experimentally if there was a link between the nutritional status and the increased protection of wheat grown in a bicrop system, compared to that of conventionally grown wheat. A randomised block design experiment of wheat plots with and without a clover under storey was set up, each receiving one of four nitrogen treatments (0Kg, 100Kg, 150Kg & 200Kg [standard] N/ha). Over three seasons, weekly collections of aphids were taken to assess aphid population response. Results showed no statistical difference between N-treatments or between clover treatments. We are exploring this apparent plasticity on the different treatments by analysing aphid feeding behaviour, phloem sap nutritional quality and bacterial populations in the aphids.

This presentation will highlight preliminary data that offer mechanistic explanations for the apparent resistance of cereals grown under the bi-cropping regime, including data from electronic penetration graph (EPG) studies and bacterial ribosomal intergenic spacer analysis (RISA).

Poster S4-8

Variation in the abundance of *Rhopalosiphum padi* in Finland

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The bird cherry–oat aphid, *Rhopalosiphum padi* (L.), is the major aphid pest in spring cereals in Finland. It is also the vector of *Barley yellow dwarf virus* (BYDV) which is the most serious virus disease of cereals. The epidemics of BYDV have clearly been connected with outbreaks of *R. padi*. Generally, outbreaks of aphids appear periodically, every 3-7 years, but are very irregular. BYDV problems have occurred in the whole cropping area of spring cereals, mainly in oats and barley.

In Finland, *R. padi* is holocyclic and requires to host-alternate. It overwinters as a winter egg on the primary woody host, *Prunus padus* L. The mortality rate of winter eggs ranges between 10 and 45 % depending on the region. In the spring and early summer, new migrants colonise spring cereal crops. During the late summer, perennial grasslands are the main habitats of *R. padi*, before their migration back to the primary host.

Changes in cultivation practices and climate change may affect the risk of *R. padi* and BYDV. During the last decades, the proportion of the cropping area sown to spring cereals has increased and was over 50% of the arable area in 2008. At the same time, the percentage of winter cereals has been low, and the proportion of grassland has decreased, especially in southern Finland. New cultivation practices, such as direct drilling and no-tillage, have become very popular in cereal production. All these changes may affect the survival of *R. padi* and change the epidemiology of BYDV. Weather conditions above all affect both the population biology of vectors and the growth of host plants. The synchronisation of aphid phenology with the susceptible growth stage of the host plant is important for virus epidemiology and its spread.

The Finnish forecast of *R. padi* is based on egg counts on the winter host. The winter egg count in *P. padus* gives an estimate of the abundance of overwintering native aphids and takes regional risk differences into consideration. However, the long term migration of aphids by southern winds is also a recurrent but unpredictable phenomenon. The numbers of migrating aphids in spring and autumn are monitored with a suction trap at three locations in Finland. Furthermore, the numbers of *R. padi* attacking cereal crops are observed with yellow sticky traps in the field experiment plots and commercial farms.

In Finland, the most severe outbreak of *R. padi* was in 1988. After that, the numbers of *R. padi* have been above the threshold for chemical control in 1992, 1999 and 2002. In these years, winter egg counts also forecasted high aphid risk although this varied with region. However, in some years local risks based on winter egg counts have not been realised because of unfavourable weather conditions in spring and early summer. Early arrival in fields is often correlated with the later abundance of aphids and severity of damage, because the risk is greatest during the seedling stage of spring cereals.

The effects of no-tillage on aphid abundance have been variable. According to yellow sticky trap data over several years, more alate aphids have been detected, almost without exception, in ploughed rather than in no-tillage plots although the threshold for chemical control has not been exceeded. In 2002, numbers of aphids on tillers were counted in two tillage experiments and significantly more plants infested by aphids were found in ploughed than in no-tillage plots in both experiments. This can result from the 'repellent effect of stubble' in which stubble ground can decrease landing rates of aphids among crops compared to bare tilled soil.

Temporal and spatial variation in the abundance and importance of pest insects is typical in arable cropping in Finland. Because aphid problems are occasional but may cause locally severe damages, long-term and short-term forecasting and warning systems are important and field monitoring is necessary for effective control.

Key words: aphids, insect pests, plant protection, cereals, no-tillage

Poster S4-9

Study of the population fluctuations of the cabbage aphid *Brevicoryne brassicae* in Sistan (Iran)

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Four different species of aphids belonging to the Aphididae family in four genera were collected and identified in Rape-seed fields in Sistan region during 2000-2001. Among them, the cabbage aphid *Brevicoryne brassicae* (L.) was the dominant species with an abundance of about 89% in the area. The population fluctuation of *B. brassicae* was studied simultaneously in fields of the Sistan region (Zahak research station and surrounding fields). The research was conducted in half a hectare of each farm. Thirty leaves from each field were randomly selected at weekly intervals and the aphids were collected and identified to species level. *Brevicoryne brassicae* had a peak of activity in the early middle of March to April at between 22-28 °C and 45-65 R.H. Studies under controlled conditions showed that *B. brassicae* has a life cycle of 6-7 days and an adult female longevity of 20-31 days (at 25±2 °C and 75±5 R.H.). Rearing also revealed that this species can reproduce up to 18-98 nymphs under laboratory conditions.

Key words: *Brevicoryne brassicae*, population fluctuation

Poster S4-10

Potato virus Y transmitting aphids in a Finnish seed potato area

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The aphid-transmissible *Potato virus Y* (PVY) is a major problem in seed potato production (Valkonen, 2007). Therefore, areas with a low virus infection pressure are preferred, e.g. those in the far north or at high altitudes. An example is the Tyrnävä-Liminka area (64°46′N, 25°38′E) which is one of the five European High Grade Seed Potato Production zones approved by the EU. Also in this area in Finland the prevalence of PVY has shown signs of increase, possibly because aphid populations have thrived under rising temperatures due to climate change and/or because the current PVY strains are more readily transmitted. Because essential information on PVY epidemiology in this area in Finland is lacking, this study aimed to collect data on the species composition and phenology of potential PVY vectors there.

The aphid flight activity was monitored from mid-June to the end of August with a suction trap (ST) and with yellow pan traps (YPT) in 2007 and 2008. YPTs were placed on bare soil at the edge of eight seed potato fields. Vector pressure was calculated in two steps. First, aphid numbers were multiplied by a species-specific relative vector efficiency factor (de Bokx & Piron, 1990). The resulting figures were then totalled for the time of highest virus susceptibility, i.e. from crop emergence to flower opening.

The year 2007 was characterised by an overall low aphid flight activity, with a moderate peak at the end of July and the main peak at the beginning of August. In the early phase of crop development (end of June - middle of July) when plants are most susceptible to PVY, flight activity was low. In 2008, the flight activity was about 5 times higher than in 2007. The YPTs and ST revealed a moderate flight activity peak at the end of June/beginning of July and a second, more pronounced peak at the end of August, mainly caused by *Rhopalosiphum padi* (L.).

A total of 7545 winged aphid individuals was caught in the YPTs, and 644 individuals in the ST. From both trap types 97 different aphid taxa were identified (84 taxa in the YPTs, and 51 in the ST). In both years, *R. padi* was the most dominant species caught by the ST (30.3 % and 56.2 %, respectively) followed by *Metopolophium dirhodum* (Walker) in 2007 (15.2 %) and *Hyalopterus pruni* (Geoffroy) (9.2 %). Although *R. padi* also dominated the YPT catch in 2008 (24.1 %), its presence in 2007 was negligible (1.6 %). *Hayhurstia atriplicis* (L.) was the most dominant species in YPTs in 2007. In both years, *Cryptomyzus galeopsidis* (Kaltenbach) and the *Aphis fabae* Scopoli group showed a relatively high dominance in the YPTs (9.3-13.5 %). No aphids were observed to colonise potato plants. Based on the vector pressure estimations, the most important vector species in 2007 and 2008 were probably *M. dirhodum*, the *A. fabae* group, *C. galeopsidis* and *Hyperomyzus lactucae* (L.). Owing to its high numbers, *R. padi* may also be considered as a PVY vector of potential relevance, despite its late occurrence in the season.

Previous studies have concluded that potato colonising aphids are not the main vectors of PVY (e.g., Boiteau *et al.*, 1988). Since no aphids were observed to colonise potato plants in the Tyrnävä-Liminka area, this must be true also here. Also *Myzus persicae* (Sulzer), which has the highest known propensity to transmit PVY and also an ability to colonise potatoes, was virtually absent from the catch. Instead, non-colonisers like *C. galeopsidis*, *H. lactucae* and *M. dirhodum* could be considered as the main PVY vectors. They occurred in reasonable numbers at potato growth stages when the infected plants are good sources of PVY and susceptible to infection (Valkonen, 2007). Vector efficiency estimations combined with data on the actual increase of PVY incidence in potato crops during the growing season should provide a reasonable way of identifying the aphid species that cause the greatest infection pressure.

Key words: Aphids, Finland, PVY, phenology, vector

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Poster S4-11

Comparison of two types of yellow water traps for sampling alate aphids

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A regional study aimed at evaluating the risk of *Potato virus Y* (PVY) transmission in potato fields by aphids is currently in progress in the Picardy region (France). During this study, the efficiency of two types of yellow water trap was compared. The objective is to demonstrate the relative scientific efficiencies of the yellow water pan trap and the Moericke yellow water trap for sampling alate aphid populations.

In fact, the yellow water pan trap is usually used by the French Agriculture Ministry and by FREDON Picardy as the basis for issuing agricultural warnings to alert farmers in the region. The Moericke yellow trap is the reference tool for all major scientific international communications.

The final aim is a double one: technical and scientific. On one hand, the comparison will allow the recording of flights of alatae above French fields to be simplified (fewer specimens collected imply a reduction in sampling, sorting and identification times). On the other hand, it will allow the main results obtained from Moericke yellow water traps in international scientific publications to be extrapolated to the yellow water pan trap.

Total densities and total number of species are compared for each sampling date during the campaign in the year 2008. Traps of both types have the same yellow coloration (reference RAL 1028) and are positioned in the same potato field, a few metres apart. Only the capture surfaces differ between the two traps.

The results are significantly linearly correlated both for densities and for number of species. However, this correlation is not proportional to the capture surfaces.

Key words: Yellow water trap, alate aphids, potato, *Potato virus Y*

Poster S4-12

Rootstock-phylloxera interaction

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In the second part of the 18th century grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)) was introduced to Europe and led to a widespread destruction of vineyards of all indigenous European grapevine varieties (*Vitis vinifera* L.), leading to devastating economic and social consequences for winegrowers until the late 19th century. The root system of susceptible cultivars is severely damaged by the formation of gall tissues, called nodosities and tuberosities. Scientists and agronomists have circumvented the problem by grafting *V. vinifera* cultivars on tolerant rootstocks gained from North American *Vitis* species, such as e.g. *V. berlandieri* Planchon, *V. riparia* Michaux or *V. rupestris* Scheele. The bred rootstocks allow limited reproduction of phylloxera on roots and leaves, but with no economically important damage. This strategy has been working well for over 100 years, but recent reports show that tolerance is endangered by the appearance of aggressive phylloxera biotypes.

Phylloxera are sedentary feeders that require a gall to feed and reproduce. Without a successfully initiated gall no infestation will take place. The aphids induce their feeding site within the meristematic zone of the root tip, where they stay attached to the root, feeding intra- and intercellularly. Morphological studies have illustrated anatomical changes in the root during the formation of nodosities and the sugar metabolism seems to be significantly involved in the rootstock-phylloxera interaction. Nodosities were found to be strong sinks within the root system, especially for carbohydrates. Furthermore, a gradient of starch granules towards the feeding site is observed. It seems that starch accumulation is essential to provide adequate food resources.

In this project we will focus on the processes taking place in grape roots as a result of phylloxera feeding with special emphasis on nodosity development and on the nutrient supply to the aphid. All studies will be conducted with the tolerant rootstock Teleki 5C (*V. berlandieri* x *V. riparia*) and a clonal line of phylloxera which was collected near Eisenstadt (Austria) in 2007 and has since been maintained in the greenhouse.

The induction, formation and maintenanceof the root gall is the lynch pin of the rootstock-phylloxera interaction. Based on previous research, we postulate that altered starch and sugar metabolism is a key mechanism for the formation and maintenance of galls and thus accounts for host rootstock susceptibility. By employing molecular genetic methods, we aim to gain deeper insight into the basic molecular mechanisms of the physiology of nodosity, focusing on the starch and sugar metabolism of nodosity cells. One part of the project will provide detail transcriptional information on young developing nodosities, whereas the second part will focus on the sugar supply to nodosities and the feeding aphid. Using an Affymetrix GeneChip, we can gain a picture of transcriptional expression changes induced through phylloxera feeding on rootstocks. Results will be verified with quantitative PCR. Nodosities will be harvested at different developmental stages and gene expression in relation to uninfested root tips occurring simultaneously will be analysed to answer questions as following: 1. When does starch synthesis start? 2. Is there continuous or erratic synthesis of starch? 3. To what extent does starch degradation occur? The detailed temporal and quantitative gene expression during nodosity establishment will provide new information.

The second part of the project focuses on the sugar supply of young developing nodosities. Analytical and histological methods will be applied to determine which sugars are present in developing nodosities and which sugar transport mechanisms are involved. Starch and soluble sugar contents of nodosities at different developmental stages will be determined with GC/MS. Furthermore we will look in depth at whether the transport of sugars to nodosity feeding cells and starch storage cells depends on the apoplasmic or symplasmic pathway. The answer to this question is essential to understanding the feeding of phylloxera on grapevine roots and will provide knowledge of the extent to which the transport mechanisms are manipulated by the aphid.

All together, the results will give new insights into phylloxera-rootstock interactions, a still not well understood plant-pathogen system, and will provide essential knowledge for the identification of crucial physiological processes for the development of new strategies to fight aggressive phylloxera biotypes.

Key words: Grape phylloxera, transcriptome analysis, sugar and starch metabolism

Poster S4-13

Toward aphid-resistant transgenic plants

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While transgenic plants expressing *Bacillus thuringiensis* Berliner (Bt)-derived toxins have met with widespread success for management of lepidopteran and coleopteran pests, Bt-derived toxins are not effective for management of the sap-sucking insects within the order Hemiptera. Indeed in some instances, damage caused by hemipteran pest species which include aphids and plant bugs has compromised the success of the Bt-based technology. Plant viruses, which are transmitted by aphids in a persistent, circulative manner, enter the aphid haemocoel by a receptor-mediated process. We have shown that the coat protein (CP) of such a virus, *Pea enation mosaic virus* (PEMV: Luteoviridae), when fused to an effector protein, delivers the fusion protein into the aphid haemocoel. For example, a CP-P-EGFP fusion protein with a proline-rich linker derived from the virus (-P-) was delivered into the aphid haemocoel. Uptake of this fusion protein showed that the virion structure is not required for uptake of CP from the aphid gut. PEMV CP fused to the spider-derived insecticidal toxin ω-atracotoxin-Hv1a was tested for aphicidal activity by using membrane feeding assays with *Escherichia coli* (Migula)-expressed fusion proteins, and by transient expression of fusion proteins in *Nicotiana benthamian* Domin. The results of these experiments show promise for use of this approach for the production of aphid-resistant transgenic plants.

Key words: aphicidal toxin delivery, spider toxin, *Pea enation mosaic virus*

Poster S4-14

Detection of aphid migrations in Finland

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Large numbers of insects migrate to Finland during the growing season. Among these the most numerous are aphids, moths, and butterflies. The most inconvenient of them are pest species which can cause great damage to crops if they are not controlled in time. Masses of bird cherry–oat aphids, *Rhopalosiphum padi* (L.) , have regularly led to serious infestations on crops in Finland, and local populations of overwintering eggs are regularly monitored for predicting outbreaks. However, early infestations, and often the most serious ones, are caused by immigrants from far away populations. *R. padi* is one of the key species in an insect immigration warning system being developed in Finland. This work is a joined effort of MTT Agrifood Research Finland, the Finnish Meteorological Institute, and the University of Helsinki. Earlier experience of long-range migrations and a literature survey were used to develop this warning system. During the newly observed immigration episodes we collected all relevant meteorological data for further analyses and system development.

Our insect immigration warning system was built on the atmospheric dispersion model that has been used in predicting long-range transport of airborne pollen. We observed immigrations with a trap network consisting of rotating tow-nets, yellow sticky traps, and suction traps. A polarimetric weather radar in Helsinki gave us information on insect migrants in the air. This radar can differentiate between rain, migrating birds, and insects. The radar commonly detects insect migrations coming over the Gulf of Finland, and the typical distance that the insects travel above the water is 100-200 km. One of the tow-nets was operated at the radar site on the roof, but generally insect traps were put on the fields less than 60 km away from the radar. Since air currents between south-east and south-west are most likely to carry migrating insects to Finland we selected fields that were open towards the south as monitoring sites.

These traps were monitored during May and June in 2007 and 2008. The field trapping had two modes: continuous catchment and alarm catchment. Rotating tow-nets and sticky papers were used at five standard monitoring sites continuously. Airborne insects were collected at least twice a week using tow-nets and yellow sticky traps. When favourable weather conditions for insect migrations were predicted, an alarm was given by the meteorologists of the research group. During alarm catchment all standard sites were visited and the catching period was changed. In addition, yellow sticky traps were deployed in larger numbers than in continuous catchment.

All the catches were scrutinized rapidly. Most of this work was focused on identification of pest species due to their high importance in crop production and insects were identified to genus. All the sticky papers were also photographed for potential future analyses. The insect monitoring was supplemented with two suction traps, one in Jokioinen (south-western Finland) and the other at Viikki in Helsinki, both belonging to an international network of suction traps.

In 2007 the main immigration of *R. padi* occurred in Finland from May 24th to 28th. Hundreds of aphids were trapped in tow-nets during these few days. At this time the domestic population was still on the overwintering host bird cherry trees (*Prunus padus* L.) and therefore these aphids could only be immigrants. Another invasion of aphids happened just before midsummer in 2008. At this time the domestic population was very small and the invasion was so sudden that the aphids, very likely, had their origin abroad.

Based on our studies the aphids can be detected with radars when they occur in large numbers. Radar surveillance must, however, be combined with a field trap monitoring so that the insects can be identified after their aerial migration has begun. Results of this project led to a novel alarm system prototype which operated via our current web pages to inform farmers if there was an increased need for protection of their crops.

Key words: migration, *Rhopalosiphum padi*, polarimetric weather radar, alarm system

Poster S4-15

Aphids on almonds and peach: biology and life cycle in different area of Tunisia

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UR.04AGRO4: Invertébrés, Microorganismes, malherbes nuisibles:Méthodes alternatives de lutte. Institut Supérieur Agronomique (ISA), Chott-Mariem 4042. Sousse. Tunisia. e-mail: Kamonia_tn@yahoo.fr

This study was conducted in almond and peach at different sites in Tunisia where the aphid faune was identified and the biology and life cycle were analysed by various methods.

This survey confirmed the presence of three Aphidinae: *Myzus persicae* (Sulzer), *Hyalopterus pruni* (Geoffroy), *Brachycaudus amygdalinus* (Schouteden) and *Pterochloroides persicae* (Cholodkovsky) (Lachninae) (Blackman & Eastop, 1984). The biology of these aphids was studied in order to discover their cycle under Tunisian conditions. Research on the eggs of Aphidinae on branches demonstrated that the laying of eggs started in December and that two types of ovoid eggs were found. One was white, collected from peach and, when kept under laboratory conditions, hatched as *M. persicae* in February. The other was green, similar to *H. pruni* eggs described by Jerraya (1996). Moreover, the density of *H. pruni* eggs per linear metre was higher on almond than on peach and especially at the north orientation. These results confirmed the holocycly of *H. pruni* and *M. persicae* under our conditions on peach and almond trees. The analysis of the spatial succession of the species on the primary hosts showed a preferential choice of *H. pruni* and *M. persicae* for almond and peach respectively, but *B. amygdalinus* showed no preference. This analysis was reflected in the results for mean relative growth rate (Leather & Dixon, 1984) where the different values obtained on almond and peach were consistent with the preferences and population development of the aphid species.

However, the survey conducted with *P. persicae* demonstrated that this species is anholocyclic and monoecious on Rosaceae (Ben Halima Kamel & Ben Hamouda, 1998, 2004, 2005). In addition, the calculation of growth rate (Stearns, 1992) of this lachnid at different temperatures demonstrated that growth rate is different on peach.

Key words: Aphids, biology, eggs, *Prunus,* life cycle

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Poster S4-16

Annotation of *Acyrthosiphon pisum* genes potentially involved in Luteoviridae transcytosis: yeast two-hybrid system to confirm interaction between aphid and virus proteins

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The Luteoviridae family is composed of single-stranded RNA plant viruses restricted to phloem tissues that are specifically transmitted by aphids in a circulative, non-propagative manner. In order to be successfully transmitted, luteovirids (members of the *Luteovirus* and *Polerovirus* genera) must cross two epithelial "barriers": (i) the midgut and/or hindgut cell layers to enter insect haemocoel and (ii) the accessory salivary gland cell layer to be inoculated to a healthy plant through saliva. Transcytosis of these cell layers relies on the presence of specific aphid components (receptors) able to recognize structural viral proteins and then to sustain virion transport (endocytic components) from one pole to the other in the cell (Gildow 1999; Brault *et al.,* 2007). Transmissible virions enter the cells following a clathrin-mediated endocytosis (CME) process and are transported across the cells enclosed in different types of vesicles (Gildow, 1993). It is believed that the virions hijack a naturally occurring mechanism which most likely enables transport of essential aphid macromolecules (Marsh & Helenius, 2006).

Acyrthosiphum pisum (Harris) is the insect vector of two luteoviriduses: *Pea enation mosaic virus* (PEMV) and *Soybean dwarf virus* (SbDV). However, the virus pathway in this aphid is not as completely described as in the case of other aphid/luteovirid combinations (Brault *et al.*, 2007) like *Myzus persicae* (Sulzer) / *Beet western yellows virus* (BWYV) for example.

Accordingly, we have annotated 45 *A. pisum* genes implicated in clathrin-mediated endocytosis, using previously identified members of the CME pathway in *Drosophila melanogaster* Meigen and occasionally in *Homo sapiens* L. or *Rattus norvegicus* Berkenhout. This finding represents a quasi-perfect correlation with *D. melanogaster* and is in good agreement with previous reports affirming the conservation of the CME network across Animalia (Schmid & McMahon, 2007).

Moreover, we have annotated some genes or families of genes previously identified as implicated in luteovirid transmission (Seddas *et al.,* 2004; Yang *et al.*, 2008): Rack-1 (Receptor for activated C kinase 1; 1 gene), Gapdh (Glyceraldehyde-3-phosphate dehydrogenase; 4 genes), luciferase (Acyl-CoA synthetase; 11 genes) and cyclophilin (peptidyl-prolyl cis-trans isomerase, 14 genes).

In order to confirm interaction between some of these candidates (Rack-1 and Gapdh), we developed a yeast 2 hybrid (Y2H) system using structural viral proteins (major and minor capsid protein) as bait and each *M. persicae* candidate as prey.

In order to identify new candidates ("membrane receptors") of structural BWYV proteins we will employ a split-ubiquitin Y2H system coupled with an accessory salivary glands library and a digestive tract cDNA library which we will make from *M. persicae.*

Key words: Aphids, luteovirus, circulative transmission, clathrin-mediated endocytosis, membrane receptor

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Poster S4-17

Autumn migration of *Rhopalosiphum padi* in the Czech Republic 1994 – 2008 and the risk of spread *Barley yellow dwarf virus* (BYDV)

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Viral diseases have been common in cereal crops in the Czech Republic of late. The most prominent damage has been to winter barley and early sown winter wheat. Crops have sometimes been ploughed in as a result. The most common virus disease is *Barley yellow dwarf virus* (BYDV) and *Rhopalosiphum padi* (L.) (bird cherry – oat aphid) is the most important vector. Flight of *R. padi* is monitored using five 12.2m high suction traps. The first suction trap was erected in the Czech Republic in 1992. Suction traps are located in Čáslav, Chrlice, Lípa, Věrovany and Žatec and are operated from the beginning of April to the end of November. Numbers of 16 species of aphid are recorded. *R. padi* has a large autumn migration but abundance varies considerably between years and sites. Climatic factors (in particular temperature) determine abundance.

The main steps to reduce BYDV are severence of a green bridge between the harvesting of one crop and emergence of the next, later sowing and use of insecticides.

Current information about aphid migration can be found at at www.srs.cz (Aphid Bulletin).

Key words: Autumn migration, *Rhopalosiphum padi*, BYDV, suction traps

Poster S4-18

The importance of the behaviour of the vector in PVY transmission

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Potato virus Y is transmitted to potato in a non-persistent manner by many aphid species, some of which do not colonise this crop. The behaviour of *Rhopalosiphum padi* (L.) on potato, a plant species that is not colonised by this aphid, was described and compared with that of the potato colonising aphid, *Myzus persicae* (Sulzer). A higher proportion of winged morphs of *R. padi* than *M. persicae* left the plant, but aphids that stayed in contact with the plant took the same mean time to initiate the first probe and it lasted the same mean time compared to *M. persicae*. The Electronic Penetration Graph (EPG) technique was used to study the probing behaviour of the aphids during PVY transmission tests. Transmission rate decreased from 29% to 8% when the acquisition time increased from 5 minutes of continuous probing to one hour with *M. persicae*, but remained low (2% and 1%) with *R. padi*. Most of the difference in transmission rate between acquisition time with *M. persicae* and between aphid species was related to the change in the time and behaviour taking place between the last cell puncture of the acquisition phase and the first cell puncture of the inoculation phase. Results presented here clearly demonstrate the importance of host-plant selection and probing behaviour in the transmission of non-persistent plant viruses. This behaviour was also studied with several species of aphid present around potato fields. The results could be used to identify aphid species having more chance of transmitting PVY. They also stress the need to consider the behaviour of the aphid in the design of laboratory tests of virus vector efficacy.

Key words: Potato virus Y, virus transmission, aphid vector

Poster S4-19

Physiological response of different poplar clones to aphid colonisation

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The influence of aphids on the most important physiological parameters for poplar was studied on the foliage of different clones. The analysed physiological parameters are important parameters of the plant's physiological status and form parts of the complex process of photosynthesis. Even the slightest changes in some of the parameters studied can significantly decrease photosynthesis. Leaf samples (with and without the presence of aphid colonies) were taken randomly from different poplar clones belonging to the group *Populus deltoides* Marshall*, Populus x euramericana* (Dode) and *Populus alba* L.

The research shows that by their presence, aphids decrease the host plant's photosynthesis to some extent, and that they also somewhat increase the respiration. The per cent diffusion resistance of the stomata was greatly increased in foliage attacked by aphids. The increase in diffusion resistance of the stomata differed between clones.

Although in the poplar clones studied the influence of aphids on the content of photosynthetic pigments was inconsistent, it can nevertheless be concluded that aphids do have a significant influence on the content of photosynthetic pigments in the leaves. The results show that aphids on poplar foliage have a significant impact on the content of photosynthetic pigments in the leaves and that there are differences among the clones studied. In one group of clones, there was an increased content of photosynthetic pigments, while in another group the content was decreased.

Key words: Aphids, Aphididae, poplar, physiological parameters

Poster S4-20

Poplar aphids in Serbia

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A very fast and intensive production of poplars has been brought about by favourable ecological conditions. However, there is a whole series of factors with adverse effects, and among them insect pests are of great significance. By their density, a multitude of species and feeding habits, and by the damage caused to the production of nursery material and to the cultivation of artificial plantations (especially in the first years after planting), aphids have a special position. As they have been insufficiently researched in our country in the sphere of poplar research, it was necessary to study them in more detail, i.e. to carry out a systematic census of aphids on poplars in the region of Serbia, to identify them, to study their basic characteristics and the most important stages of their life cycles. The aphid species present were collected by tree inspection.

The aphid species differed in their preferences for various poplar species and clones. The most endangered clones were those of autochthonous black poplar and white poplar. During the research over several years, 16 species of aphids in five families (Aphididae, Chaitophorinae, Pemphiginae, Phleomyzinae and Pterocomatinae) were identified on poplars in Serbia. As the result of our research, the following aphid species were identified: *Chaitophorus leucomelas* Koch, *Chaitophorus longisetosus* Szelegiewicz*, C. nassonowi* Mordvilko*, C. populeti* (Panzer), *C. populialbae* (Boyer de Fonscolombe), *C. populifolii* (Essig), *C. tremulae* Koch, *Pemphigus bursarius* (L.), *P. immunis* Buckton*, P. passeki* Börner, *P. phenax* (Börner et Blunck), *P. populi* Courchet, *P. protospirae* Lichtenstein, *P. spirothecae* Passerini, *P. trehernei* Foster, *Pachypappa warsharensis* Nasonov, *Thecabius affinis* (Kaltenbach), *T. lysimachiae* Börner, *Phloeomyzus passerinii* (Signoret), *Pterocomma populeum* (Kaltenbach), *Aulacorthum solani* (Kaltenbach), *Hyadaphis polonica,* Szelegiewicz,

Five of these species are new to the entomofauna of Serbia. The new species are *Pachypappa warsharensis*, *Pemphigus passeki*, *Pemphigus populi, Chaitophorus populifolii* and *Hyadaphis polonica*. With these findings, the number of aphid species recorded on poplars in Serbia has increased to 22.

Key words: aphids, poplar, Aphididae, Serbia

Poster S4-21

Physiological impact of xylem consumption on *Macrosiphum euphorbiae*

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Aphids mainly consume phloem sap in which they find their required nutrients. Xylem sap is a diluted water solution compared to phloem sap and is also consumed by aphids. The xylem consumption behaviour is thought to represent a reaction to dehydration. Dehydration naturally occurs in alatae after their final ecdysis or after flying, but xylem consumption has been monitored in both apterous and alate aphids after an artificial starvation period. The objective of this study was to document xylem sap consumption behaviour during the adult life of *Macrosiphum euphorbiae* (Thomas) and to monitor relationships between xylem consumption and life-history traits.

As a function of aphid age after the final moult, feeding behaviour was monitored with EPG. Flight capability and water content of the aphids were recorded. Individual daily fecundity was measured using clip cages.

Phloem consumption did not significantly change as a function of aphid age. In alatae, the proportion of xylem consumption in the total sap consumption was high until 4 days after the final ecdysis and from 8 days until the end of the experiment (12 days), and was almost nil in between. In apterae, the proportion of xylem consumption was very low until 9 days after the final moult, and then increased until the end of the aptera experiment (16 days). The proportion of xylem consumption was negatively correlated with fecundity in both alatae and apterae. Water content was not correlated with xylem-consumption proportion except for the first day. Flight capability was negatively correlated with fecundity for the first 4 days.

Our results suggest that xylem consumption behaviour is not exclusively triggered by dehydration. Xylem sap is composed of amino acids and minerals that enable leafhoppers to live by feeding on it exclusively. A mixture of diets has been shown to affect the fecundity of certain grasshoppers. The negative correlation between fecundity and the xylem-consumption proportion suggests that xylem sap constituents negatively affect aphid fecundity. Our results confirm the presence of a trade-off between fecundity and flight capability in alate aphids. This trade-off might be modulated by xylem consumption. Both aphid morphs consumed more xylem at the end of their life while their fecundity is low. Post-reproductive aphids have been suspected as helping to direct the phloem nutrients to the colony from other parts of the plant. Although speculative, we suspect that a trade-off between fecundity and life-span is modulated by xylem consumption.

Key words: Aphids, feeding behaviour, xylem, phloem, trade-off

Poster S4-22

Monitoring aphids in urban green areas: a simple method for evaluating aphid damage

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Monitoring aphids in urban green areas has important limitations. Insects can be directly measured by counting the number of individuals on a part of the plant; however it is tedious, time consuming and damage caused by aphid difficult to assess. An easier and less time consuming method, particularly when aphids become numerous, is to measure the actual problem through monitoring their honeydew production with yellow water-sensitive cards. This method also has some limitations. We have developed an alternative sampling method for estimating aphid damage based on the use of aphid abundance classes. The method is very simple, allows a good estimation of honeydew damage and eliminates some of the limitations inherent in the two alternative methodologies. The new methodology has been tested in the following aphid –plant associations: *Eucallipterus tiliae* (L.) – *Tilia* sp., *Hoplocallis pictus* (Ferrari) – *Quercus rubra* L., *Periphyllus hirticornis* (Walker) – *Acer campestre* L., *Drepanaphis acerifoliae* (Thomas) – *A. saccharinum* Marshall , *Monelliopsis caryae* (Monell) – *Juglans nigra* L., *Chaitophorus populialbae* (de Fonscolombe) – *Populus alba* L. and *Myzocallis* (*Lineomyzocallis*) *walshii* (Monell) – *Q. rubra*. Our method provides a new tool to obtain quantitative information that can be used in developing integrated pest management programs in urban green areas.

Key words: Aphid, monitoring, ornamental trees, honeydew, comfort damages

Poster S4-23

Karyotype variation and insecticide resistance in Italian populations of the peach–potato aphid *Myzus persicae* (Hemiptera: Aphididae)

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In aphids, chromosomal rearrangements within species are largely diffused because a) they possess holocentric chromosomes so that, if a chromosome breaks, all fragments can move into the daughter cells and be maintained through the following cell divisions, b) they can reproduce parthenogenetically, thus avoiding the meiotic constraints which need homologous pairing. Moreover, there are distinct indications that fragmentations and translocations may be associated with new environmental tolerances. The best known example is the association of organophosphate insecticide resistance with a chromosomal translocation in *Myzus persicae* (Sulzer). In this species, the esterase E4 amplified form is widespread in North Europe populations and it is correlated with a translocation between autosomes 1 and 3, whereas the FE4 form, which is largely diffused in Mediterranean regions, is not associated with chromosomal rearrangements (Blackman *et al.*, 1999). In this study we present cytogenetic and molecular data attesting the identification of an Italian *M. persicae* strain possessing an A 1,3 chromosomal translocation and we analyse the relationships between this chromosomal rearrangement and insecticide resistance by means of biochemical and DNA-based diagnostic assays.

Myzus persicae populations analysed in the present study were collected in different Italian areas and maintained as colonies of parthenogenetic females on pea-seedlings (*Pisum sativum* L. cv "Meraviglia d'Italia") under constant conditions: 21°C, 16h light : 8h dark photoperiod.

In accordance with literature data (Bizzaro *et al.*, 2005), all populations analysed had amplified esterase FE4 genes only. Chromosome spreads of embryo cells obtained from parthenogenetic females revealed that most of the populations possessed the normal karyotype (n=12) but, a strain collected in a peach orchard near Chieti (Mp64) showed a chromosome complement characterised by a heterozygous 1, 3 autosomal translocation. When analysing the resistance pattern of this population, biochemical assays aimed to evaluate total esterase activity showed that it can be classified as R1 (*sensu* Devonshire, 1992) whereas the PCR-based diagnostic test which allows the identification of both MACE and KDR phenotypes in a single amplification experiment showed that Mp64 did not possess these target-site resistance mechanisms.

The data collected as a whole allow us to conclude that Mp 64 is the first *M. persicae* strain possessing an A 1, 3 chromosomal translocation linked with FE4 and only slightly resistant towards organophosphate, carbamate and pyrethroid insecticides as a consequence of moderate esterase activity.

Key words: *Myzus persicae*, Chromosomal rearrangements, Insecticide resistance, E4 and FE4 gene amplification

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Poster S4-24

Aphids on cereals and wild grasses in different environments

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The aim of the research is to reveal differences in aphid fauna on winter wheat, winter barley, maize and wild grasses in two climatically different regions of Poland (Wielkopolska and Podlasie) which are also different in terms of land use.

The region of Wielkopolska is characterised by a mild climate compared with Podlasie which is the coldest one throughout Poland. Land use differs in Wielkopolska and Podlasie in that the former has a large overall area of cereal crops, a large size of those fields used for monoculture and a more intensive method of cultivation.

The region of Podlasie, especially near Białowieża, represents the last primeval virgin forest with a unique ecosystem characterised by differently structured landscapes, extensive plant diversity and by the small fields with a variety of crops (Baranouski, 1994).

The observations of the aphid fauna were carried out in July and November 2008 on winter wheat cv. 'Zyta', winter barley cv. 'Tiffany', maize cv. 'Reduta', and on the grasses *Agropyron repens* (L.) and *Carex fusca* Alljoni (= *Carex fusca* (L.) Reichard). In each region the aphids were counted on 300 plants, with 100 plants from each of three sites.

In the Wielkopolska region *Rhopalosiphum padi* (L.) was the clearly dominant species on all cereals. On winter wheat this species constituted 75% of the aphid fauna, 90% on winter barley. and 91% on maize. On grasses it was rare, but was the only species recorded.

In the Podlasie region *Metopolophium dirhodum* (Walker) was the most numerous species on all cereals. On winter wheat this species constituted 30% of aphid fauna, 37% on winter barley, and 42% on maize. *Rhopalosiphum padi* constituted 21% of aphid fauna on winter wheat, 22% on winter barley, 37% on maize and 10% on grasses. Grasses in the Podlasie region were colonised by five aphid species. The most numerous was *Subsaltusaphis flava* (Hille Ris Lambers) on *C. fusca*. This species constituted 78% of aphid fauna on this plant, 14% were *R. padi*, 7% *Sitobion avenae* (F.), 0.6% *M. dirhodum* and 0.2% *Rhopalosiphum insertum* (Walker).

In the Wielkopolska region anholocyclic forms of *R. padi* were recorded on winter cereals in autumn, whereas in Podlasie no such specimens were found.

On the basis of these observations, one can claim that the aphid species dominant in numbers colonising cereals tend to prefer crops with large areas and force out the less numerous species. Monocultures with a large area (Wielkopolska) impoverish the diversity of the aphid fauna on cereals and wild grasses, and clearly favour the numbers of *R. padi*. In the Podlasie region extensive diversity of aphid fauna results from greater diversity of plants.

In the Podlasie region, *Barley yellow dwarf virus* (BYDV) transmitted mainly by anholocyclic forms of *R. padi* has not as yet been recorded, whereas in the Wielkopolska region this virus disease attacks an increasing area of the crops. It has become a new problem for plant protection in Poland.

Key words: aphids, different environments, cereals, grasses

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Poster S4-25

Evaluation of plant genetic resources of wheat and barley for aphid resistance

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Aphids are serious pests of cereals. Their feeding on the plants reduces the grain yield and as vectors they transmit important viruses, such as *Cereal yellow dwarf virus* (CYDV) and *Barley yellow dwarf virus* (BYDV). In Germany the bird cherry–oat aphid *Rhopalosiphum padi* (L.), the grain aphid *Sitobion avenae* (F.) and the rose–grain aphid *Metopolophium dirhodum* (Walker) are the most important cereal aphids. The corn leaf aphid *Rhopalosiphum maidis* (Fitch) has only local importance in some years. Other aphid species, e.g. the Russian wheat aphid *Diuraphis noxia* (Kurdjumov) or species of the genus *Schizaphis* or *Sipha* are not present or of minor importance.

Applications of insecticides are necessary to prevent yield losses caused by virus infection and aphid feeding. The enhancement of aphid resistance by breeding could reduce the number of insecticide applications, pre-supposing that effective resistance genes are detected and available for the breeding process. Germplasm collections conserve worldwide a great number of cultivated plants and their relatives and may be a valuable source of different types of resistance. Therefore the evaluation of the plant genetic resources is an important step for the exploitation of resistance for the breeding process.

The German gene bank of the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben comprises 21,244 accessions of barley (*Hordeum* spp.) and 28,191 accessions of wheat (*Triticum* spp.). Up to now, only a small number of these accessions have evaluated for resistance against plant diseases and pests.

For this reason a standard evaluation method was developed with a sequence of different trials. The first step was the screening of selected accessions in microplots (2 rows, 1m long) by scoring the natural aphid infestation at different times. The scores are transformed by an exponentially function into aphid numbers, the area under the aphid development curve is calculated and the average ordinate (AO as aphids/d) for this area is estimated as follows:

$$\mathrm{AO} = \frac{1}{D} * \sum_{i=1}^{t-1} \frac{1}{2}(n_i + n_{i+1}) * d_i$$

where n = number of aphids, d = difference between the scoring dates (number of days) and D = total number of days between first and last scoring.

The accessions with the lowest AO (to a maximum of 100 accessions) were harvested for further evaluation.

As the next step, the accessions without or with only low natural infestation in the screening were evaluated in an exact field test. This trial was a randomised block design with 3 replications. The plots consisted of 3 rows and after every 10th plot a plot with a susceptible standard was included. The aphid number was counted every 7 to 10 days on 10 plants per plot (one stalk/plant), separately for each aphid species and for alate and apterous individuals, until the the population crash. The area under the aphid population development curve was estimated for every plot and aphid species and the AO calculated. The mean AO of the accessions was compared with the susceptible standard and the differences were analysed for significance. Accessions with a significant lower AO are selected for testing in a controlled environment chamber trial. Young plants growing in containers (24 pots with 7 cm wide) are colonised with 3 aphids per plant at the 2-leaf-stage. The plants are kept in the chamber at 22°C and a 16h light (approximately 10 klx) regime. After 14 days the total number of aphids and the number of winged aphids is counted. The tests are repeated 3 times for every aphid species with 10 plants/accession and every test includes the susceptible standard. The total number of aphids is compared with the standard and differences tested for significance (ANOVA). The whole data are stored in an Access-database and linked with the passport-data of the gene bank. Up to now 1027 accessions of barley and 2941 accessions of wheat have been evaluated in the field for aphid resistance.

Key words: cereal aphids, resistance, evaluation, *Rhopalosiphum padi*, *Sitobion avenae*, *Metopolophium dirhodum*

Poster S4-26

The carbohydrate-binding activity of the elderberry protein SNA-I is a determining factor for its insecticidal activity

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In recent years, different classes of plant proteins have been reported to promote entomotoxic effects. Type-2 ribosome-inactivating proteins (RIPs) are a group of chimeric proteins built up of an A-chain with RNA N-glycosidase activity and a B-chain with lectin activity. These proteins are thought to play a role in plant protection. *Sambucus nigra* agglutinin I (SNA-I) is a type-2 RIP, isolated from the bark of elderberry (*Sambucus nigra* L.). This study demonstrated the insecticidal potency of SNA-I on two hemipteran insect species using two different methods. An artificial diet supplemented with different concentrations of the purified RIP reduced survival and fecundity of pea aphids *Acyrthosiphon pisum* (Harris). In addition, feeding of tobacco aphids *Myzus persicae nicotianae* Blackman on leaves from transfected plants constitutively expressing SNA-I, resulted in a delayed development and reduced adult survival and also the fertility parameters of the surviving aphids were reduced, suggesting that a population of aphids would build up significantly slower on plants expressing SNA-I. Finally, a series of experiments with transgenic lines expressing a mutant RIP, revealed that the carbohydrate-binding activity of SNA-I is necessary for its insecticidal activity. The data support the possible application of RIPs for a role in the integrated management of pest insects.

Key words: *Acyrthosiphon pisum, Myzus persicae nicotianae,* elderberry, *Sambucus nigra,* agglutinin

Poster S4-27

Analysis of the behaviour of virus transmitting Peach–potato Aphid, *Myzus persicae,* feeding on wild potato, *Solanum tarnii*, interspecific somatic hybrids and their progeny

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Aphids are the most important vectors of more than 50 viruses of potato, which seriously reduce the yield of potato in the field. Improving the resistance of potato to one of the main viruses, *Potato virus Y* (PVY), would greatly decrease the quantity of insecticide applied. In addition to the major genes that confer PVY resistance in wild potato species *Solanum demissum* Lindley, *S. hougasii* Correll, *S. stoloniferum* Schlechtendal & Bouché, *S. tuberosum* L. ssp. *andigena* (Juzepczuk & Bukasov) and *S. chacoense* Bitter, potato breeders are interested in finding linked resistance in the pool of exotic wild *Solanum* species, which are reproductively isolated from tetraploid *S. tuberosum* L. These wild species are difficult to cross with potato and sexual hybrids with potato are unknown. *S. tarnii* Hawkes & Hjerting is such a diploid wild tuber-bearing Mexican species (2n=2x=24), belonging to the series *Pinnatisecta* section *Petota.* The accession GLKS 32870 from Genebank External Branch 'North', Gross Lüsewitz, of the Leibniz Institute of Plant Genetics and Crop Plant Research, Germany was used to characterize resistance to viruses. There is evidence that *S. tarnii* is extremely resistant to PVY. Because of sexual barriers somatic hybridization by cell fusion was used to produce hybrids between *S. tarnii* and potato cv. Delikat, which is susceptible to PVY. The hybrids showed no symptoms of viral infection after mechanical inoculation in a greenhouse with six isolates of the most important virus strains and growing on in the field (Thieme *et al*., 2008). This indicates that PVY resistance was transferred from sexually incompatible wild species into cultivated potatoes by protoplast fusion. After backcrossing somatic hybrids with the susceptible cv. Delikat, all BC_1 lines tested expressed no PVY incidence when exposed to different strains of this virus. In the BC_2 lines resistant and susceptible genotypes were identified.

To determine if this kind of resistance is correlated with the feeding behaviour of aphids the electrical penetration graph (EPG) technique (Tjallingii, 1988) was used. EPG monitoring of aphid feeding behaviour was used to search for sources of resistance to *M. persicae* in the wild species *S. tarnii*, somatic hybrids and sexual progeny. The EPG signals, analyzed as waveform patterns, indicate the stylet position and feeding activity of an aphid (Tjallingii & Hogen Esch, 1993)**.** The features indicating resistance to aphids are time to first probe, number of probes shorter than 3 min (test probes) before phloem phase, time to first E1 and time to the first sustained E2 period of longer than 10 minutes indicating 'committed phloem ingestion', and a potential drop is an important indicator of the potential for acquisition and transmission of PVY. Feeding traces on *S. tarnii*, somatic hybrids and BC_1 clones reveal that there are no significant differences in the time to first penetration, duration of first probe, number of potential drops, time to first E1 and duration of all E1 (with E2), indicating that resistance to the non-persistent PVY is not correlated with the feeding activities of *M. persicae*. Nevertheless, aphids on leaves of the wild species, hybrids and BC clones penetrated the phloem but ingested significantly less sap (E2 phase), which indicates aphid resistance. Additionally the higher mortality and lower MRGR (Mean Relative Growth Rate) of aphids feeding on the leaves of these genotypes compared to cv. Delikat indicate that *S. tarnii*, somatic hybrids and BC clones can be characterized as unsuitable hosts for potato aphids.

Key words: Wild potato, EPG, virus transmission, resistance to PVY

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Poster S4-28

Diuraphis noxia overwintering strategy can affect its performance on resistant and susceptible wheat

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The Russian wheat aphid (RWA), *Diuraphis noxia* (Mordvilko), is a pest of cereals throughout the world, except in Australia where it tops the list of insect biosecurity concerns. In its native range, where it is not considered as a pest on wheat, *D. noxia* is primarily holocyclic, producing overwintering eggs, but in the southern distribution of its native range it can be anholocyclic. Invasive populations are mainly anholocyclic, overwintering as viviparous females, but after a time, sexual populations can appear. It is not known whether the anholocyclic condition influences the amount of plant damage caused. This study was designed to investigate whether investing in overwintering eggs (holocycly) influences the damage caused by RWA.

Two *D. noxia* colonies of Hungarian origin were used: one maintained under conditions conducive to anholocycly and the other by holding viviparae under conditions to maintain holocyclic aphids for two years. Two wheat lines were used: PI 220127 (a resistant line) and Arminda (a susceptible line). The experiment consisted of a randomised block design with a total of 300 plants. Each experimental plant was infested with two fourth-instar aphids, either anholocyclic or holocyclic. Twenty-one days after initial infestation, all plants were cut, scored for plant damage, and dried for biomass assessment.

Significant differences were found between the aphid colonies and the wheat lines in terms of both plant damage and biomass loss. The Arminda plants showed greater damage symptoms and lost more biomass than PI220127 plants. Aphids from the anholocyclic colony caused more damage and a greater loss of biomass on both wheat lines compared to those from the holocyclic colonies.

Here, we demonstrate that the same population of aphids caused greater damage and biomass loss on both resistant and susceptible plants when derived from viviparous (anholocyclic colony) rather than oviparous (holocyclic colony) females. Others reasons for the differences in plant damage, such as fecundity and the toxic components in the aphid saliva, are discussed.

Key words: *Diuraphis noxia*, reproductive modes, plant damage

Poster S4-29

The occurrence and control of cucumber aphid (*Aphis gossypii*) in Liaoning province, China

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Aphids are serious pests of cucumber in Liaoning province, China, especially in June and July. To control them, farmers apply high doses of pesticides. The overuse of pesticides has resulted in resistance. Environmental residues are also a concern, because consumers demand pesticide-free food. Thus, many countries are trying to reduce their use of pesticides. Biological control and non-chemical control are alternative control methods. Using compost helps to produce healthy plants that become resistant to attacks. Yellow sticky traps can be used to control aphids. Silver-gray plastic film can be used to keep aphids off cucumber plants.

Cucumber aphids can be killed by spraying detergent powder solution (1 part powder to 500 parts water) twice at an interval of 3 days. Potash based soft soap that is used for washing dishes should be used rather than modern washing powders that contain caustic soda which will harm plants. Farmers may plant leek between cucumber growing rows so that some cucumber aphids are repelled.

Environments for predators and parasites of aphids should be encouraged by maintaining natural surroundings with plenty of breeding places for them. Botanical pesticides should be used sparingly and in conjunction with other non-chemical control methods to decrease the development of resistance.

Key words: Cucumber aphid, multiple control methods

Poster S4-30

Control of cucumber aphid in greenhouse with biological methods in early spring

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This paper reports on the control of cucumber aphid (*Aphis gossypii* Glover) using the parasite wasp (*Aphidius gifuensis* Ashmead) and multicolored Asian lady beetle (*Harmonia axyridis* (Pallas)) in Liaoning province, China. Wasps are reared by planting Chinese cabbage seedlings, that peach–potato aphid (*Myzus persicae* (Sulzer)) infests, under cucumber plants in the greenhouse. Because the occurrence of *M. persicae* is earlier than *A. gossypii*, and *M. persicae* does not feed on cucumber plants, some wasps that emerge from *M. persicae* parasitise *A. gossypii*.

When the number of *A. gossypii* increases, growers can collect multicolored Asian lady beetle eggs and larvae and release them into the greenhouse. Thus *A. gossypii* can be controlled without using chemical insecticides.

Key words: Cucumber aphid, biological control, greenhouse

Poster S4-31

Control of *Myzus malisuctus* depending on natural enemies

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Over 30 years, the frequency of application of insecticides in apple orchards in China has increased in order to maintain satisfactory pest control. This has been considered perhaps to be due to the appearance of strains of *Myzus malisuctus* Matsumura resistant to insecticides and, in certain cases, due to an adverse effect of insecticides on natural enemy populations. This leads to a more intensive use of insecticides; as a result, apple growers are generally applying a complex schedule of sprays. It is necessary to develop additional information on the direct and indirect effects of the different number of spray applications on the structure of arthropod communities to be able to use fewer insecticides so that the apples will not be polluted, in order to satisfy consumer demand. To control *M. malisuctus*, apple growers should adopt methods which protect harlequin ladybirds and parasitoids by planting soybean or red clover, and not spraying insecticides between apple tree rows.

Key words: *Myzus malisuctus*, apple, natural enemies, control

Poster S4-32

Status of cotton bollworm and cotton-melon aphid resistance to insecticides and a pesticide management strategy in China

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The cotton bollworm, *Helicoverpa armigera* (Hübner) and the cotton-melon aphid, *Aphis gossipii* Glover, have been the most important pests of cotton in China. Outbreaks of *H. armigera* often occurred in China in the 1990s. Biologically it is one of the most successful pests due to its high fecundity, great migration potential, wide host range, and diapausing behaviour to overcome unfavourable environmental conditions. Cotton-melon aphid has been an insect pest that is mainly managed by chemical insecticides. Cotton aphid became more serious on cotton after the introduction of trans-Bt-gene cotton.

This paper introduces resistance of cotton pests, mechanisms of *H. armigera* and *Aphis gossypii* resistance to insecticide management of cotton pest resistance in China.

Key words: cotton bollworm, cotton-melon aphid, resistance; insecticides, management strategy

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