CONTENTS

Interactive Impacts of Rice Water Weevil and Sheath Blight Pathogen on Rice Yield
Suhas S. Vyavhare, Michael O. Way, Rebecca A. Pearson, and Donald E. Groth……363

Abundance of the Beet Leafhopper, *Circulifer tenellus*, Associated with 16SrIII-phytoplasmas in Squash at Baja California Sur, Mexico
Diana Medina-Hernández, Mayela Vargas-Salinas, and Ramón Jaime Holguín-Peña……...373

Insect Diversity in Conventional and Bt Cottons in the Comarca Lagunera, Mexico
Diversidad de Insectos en Algodoneros Bt y Convencionales en la Comarca Lagunera, México
Verónica Ávila-Rodríguez, Urbano Nava-Camberos, José L. García-Hernández, José L. Martínez-Carrillo, and Carlos A. Blanco……383

The Compromise of Intercropping: Biological Pest Control versus Competition by Crop Species
Jesús Santillano-Cázares, Aurelia Mendoza-Gómez, Juan Carlos Vázquez-Angulo, Ernesto Medina-Espinoza, Carlos Enrique Ail-Catzim, and Fidel Núñez-Ramírez……393

Walking Capacity of *Lygus hesperus* Fifth Instars in a Laboratory and Outdoors
Shimat V. Joseph and Jhalendra Rijal……...403

Physiological and Pathogenical Characterization of *Beauveria bassiana* and *Metarhizium anisopliae* Isolates for Management of Adult *Spodoptera frugiperda*
Oscar Giovanni Gutiérrez-Cárdenas, Hipolito Cortez-Madrigal, Edi A. Malo, Jaime Gómez-Ruíz, and Rineaud Nord……..409

Characterization of *Beauveria bassiana* Isolates Associated with *Euwallacea* sp. nr. *fornicatus* in *Populus* sp.
R. Montesinos-Matías, A. Gallou, A. M. Berlanga-Padilla, M. G. Serna-Domínguez, B. Laureano-Ahuelicán, M. A. Ayala-Zermeño, A. Ordáz-Hernández, J. A. López-Buenfil, and H. C. Arredondo-Bernal……423

Susceptibility of *Rhagoletis suavis* Maggots to Entomopathogenic Fungi
Muhammad J. Nisar, Robert W. Behle, Christopher A. Dunlap, Erica J. Goett, Mubashar Iqbal, and Muhammad Dildar Gogi……...431

Negative Impact of Selected Insecticides on Functional Response and Susceptibility of *Coccinella septempunctata*
Amjad Sultan, Muhammad Farhanullah Khan, Muhammad Faheem Akbar, Qurban Ali Arain, Fayyaz Nawaz Soomro, and Shagufta Siddique……437

From the Forest to the Bottle: Entomochemicals from *Pterophylla beltrani* (Bolivar and Bolivar) for the Beverage Industry
Luis Daniel García-García, Reyna Ivonne Torres-Acosta, Rodolfo Torres-de los Santos, Nohemí Niño-García, and Jorge Ariel Torres-Castillo……...449

Chemicals in the Genital Chamber of Two Mexican Species of *Phyllophaga*
A. A. Romero-López, R. Reyes-Chilpa, F. J. Pérez-Flores, G. A. Lugo-García, and J. I. Maldonado-Rodríguez……457

New State and County Records of Dung Beetles of Oklahoma
Marcela S. Gigliotti, W. Wyatt Hoback, and Justin L. Talley……..465

Sphecids of Mexico
Maximiliano Vanoye Eligio and Yazmín Guadalupe Martínez Téllez……...479

***Digonogastra kimballi* Kirkland as a Parasitoid of *Diatraea lineolata* (Walker): New Record at Tabasco, México**
Azalia Correa-Méndez, Rodolfo Osorio-Osorio, José Isaac Figueroa-de-la-Rosa, Luis Ulises Hernández-Hernández, Efraín de la Cruz-Lázaro, and Cesar Márquez-Quiroz……491

***Magnolia schiedeana* as a New Host Plant Record of Four Lepidoptera Species in a Tropical Montane Cloud Forest**
Luis Alberto Lara-Pérez, Claudia Suárez-Ramírez, Iván Oros-Ortega, Antonio Andrade-Torres, Iván Córdoba-Lara, and Luis Sáenz-Carbonell……497

First Report of *Trigona fuscipennis* and *T. nigerrima* Chewing Tissues of *Jatropha curcas* L. in Southern Chiapas, Mexico
Primer Reporte de *Trigona fuscipennis* y *T. nigerrima* Mordiendo Tejidos de *Jatropha curcas* L. en el Sur de Chiapas, México
Guillermo López-Guillén, Eduardo R. Chamé-Vázquez, Jaime Gómez Ruiz, and Juan F. Barrera……..503

Comparison of Microscope Slide-Mounting Media for Preparation of Fungus Gnats (Diptera: Mycetophilidae)
Stephen W. Taber……509

Méndez-Herrera Technique: New Clearing Technique Proposed for Immature Stages and Internal Structures of Adult Insects
N. Acevedo-Reyes, D. H. Zetina, E. Blanco-Rodríguez, J. A. López-Buenfil, and R. Martínez-Rosas……519

A Comparison of Techniques for Abdominal and Chorionic Rehydration on Species of *Emesis* Fabricius and *Lasaia* H. Bates
Una Comparación de Técnicas de Rehidratación Abdominal y Coriónica en Especies de *Emesis* Fabricius y *Lasaia* H. Bates
Jorge Llorente-Bousquets, Sandra Nieves-Uribe, Marysol Trujano-Ortega, y Arturo Arellano-Covarrubias……523

Ant Diversity in Different Ecological Regions of Durango, Mexico
Diversidad de Hormigas en Ecorregiones de Durango, México
Miguel Angel Soto-Cárdenas, Miguel Vásquez-Bolaños, Cipriano Gutiérrez-García, Miguel Mauricio Correa-Ramírez, René Torres-Ricario, Martha Celina González-Güereca, y Isaías Chairez-Hernández……539

New Records of Ant Species for Sinaloa, Mexico
Nuevos Registros de Especies de Hormigas para el Estado de Sinaloa, México
Madai Rosas-Mejía, Miguel Vásquez-Bolaños, Griselda Gaona-García, y Venancio Vanoye-Eligio……551

SCIENTIFIC NOTE

Influence of Cations on the Intestinal Proteolytic Activity of *Anticarsia gemmatalis* (Hübner) Larvae
Nohemí Niño-García, Rodolfo Torres-delosSantos, Miguel Ángel Delgado García, Ernesto Rangel-Torres, Eduardo Padrón-Torres, and Reyna Ivonne Torres-Acosta……555

MINUTES OF THE 2019 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS……559

MINUTES OF THE 2019 ANNUAL MEETING OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS……560

Secretary-Treasurer's Report for 2018……563

***Southwestern Entomologist* Editors' Report for 2018**……565

Interactive Impacts of Rice Water Weevil[1] and Sheath Blight Pathogen on Rice Yield

Suhas S. Vyavhare[1]*, Michael O. Way[2], Rebecca A. Pearson[2], and Donald E. Groth[3]

Abstract. In the southern US, rice, *Oryza sativa* L., is frequently attacked simultaneously by rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, and sheath blight pathogen, *Rhizoctonia solani* J.G. Kühn. Field studies were used to determine the impact of *L. oryzophilus* infestation on development of sheath blight and combined effects of both *L. oryzophilus* and *R. solani* on yield of rice cultivars, 'Cocodrie' and 'Jefferson'. Infestation by *L. oryzophilus* had no influence on severity of sheath blight in either Cocodrie or Jefferson. Grain yield of both cultivars was significantly less in response to infestation by *L. oryzophilus*. Infection by *R. solani* alone significantly reduced yield only in the susceptible cultivar Cocodrie. Percent yield losses in Cococdrie in response to exposure to *L. oryzophilus* and *R. solani* alone and in combination were 5.6, 23.9, and 20.0%, respectively. Similarly, percent yield losses in Jefferson in response to exposure to *L. oryzophilus* and *R. solani* alone and in combination were 7.3, 10.6, and 5.4%, respectively. Combined impact of infestation by *L. oryzophilus* and infection by *R. solani* on yield did not differ significantly from the impact of pathogen infection alone in Cocodrie. The combined impact of *R. solani* and *L. oryzophilus* on yield in Jefferson did not vary significantly from the impact of either alone. Results suggested that *L. oryzophilus* did not affect severity of sheath blight in rice, and the combined impact of *L. oryzophilus* and *R. solani* on rice yield was less than additive. Understanding the interaction between *L. oryzophilus* and *R. solani*, important pests of rice in the southern US, will help manage these pests more effectively.

Introduction

In agroecosystems, crop plants often are attacked by multiple pests, including pathogenic fungi and insect herbivores, simultaneously or sequentially. Interaction between infection by plant pathogens and insects feeding on a shared host can influence abundance of the pathogen or insect, and ultimately the yield and fitness of the mediating plant (Marquis 1992, Oerke and Dehne 2004). To defend themselves from plant pathogens or insect herbivores, plants allocate resources toward various defense mechanisms that consequently result in less plant growth and yield (Kant et al. 2015). Many studies reported how plant pests

---

Coleoptera: Curculionidae

[1]Texas A&M AgriLife Research and Extension Center, 1102 E. FM 1294, Lubbock, TX 79403
[2]Texas A&M AgriLife Research and Extension Center, 1509 Aggie Drive, Beaumont, TX 77713
[3]Louisiana State University, Rice Research Station, 1373 Caffey Rd, Rayne, LA 70578
*Corresponding author: Suhas S. Vyavhare e-mail: suhas.vyavhare@ag.tamu.edu, Phone: (806) 723-8446, FAX: (806) 723-8499

influence transmission, behavior, yield, and reproduction of other pests, and how the interaction affects shared host plants (Hatcher 1995, Stout et al. 2006, Hauser et al. 2013, Tack and Dicke 2013).

Herbivory by insects can inhibit or facilitate plant pathogens. For example, infesting rice plants, *Oryza sativa* L. with the phloem-feeding white-backed leafhopper, *Sogatella furcifera* Horváth, increased resistance of plants to rice blast disease caused by *Pyricularia grisea* Sacc., even though infestation by insects and infection by pathogens were temporally separated (Kanno and Fujita 2003). But, the leaf beetle, *Plagiodera versicolora* Laicharting, was found to systemically increase growth of the rust fungus, *Melampsora allii-fragilis* Klebahn, on willow, *Salix cuspidata* Schultz (Simon and Hilker 2003).

Rice water weevil, *Lissorhoptrus oryzophilus* Kuschel, is the most destructive pest of rice in the US (Aghaee and Godfrey 2014). Sheath blight caused by the fungal pathogen *Rhizoctonia solani* J.G. Kühn is the most important rice disease in the southern US (Lee and Rush 1983). *L. oryzophilus* adults lay eggs in rice upon application of the permanent flood when rice begins to tiller (Way 2018). *L. oryzophilus* causes damage mostly by larval feeding on roots which can inflict much yield loss through root pruning during the vegetative stage (Zou et al. 2004). Sheath blight symptoms begin to appear during late tillering to internode elongation (Groth and Castroagudin 2018). Thus, sheath blight damage follows injury by immature *L. oryzophilus*. Development of disease is most rapid in early heading and grain-filling stages of crop growth (Lee and Rush 1983). Several studies assessed the impact of *L. oryzophilus* and *R. solani* on rice yield (Marchetti and Bollich 1991, Stout et al. 2001, Groth 2008, Vyavhare et al. 2016). However, little is known about the interaction between the two pests and their combined impact, if any, on rice yield. If the two parasites do not affect abundance of one another, the combined impact on plant yield is expected to be additive (sum of individual impacts). If the two parasites interact in a mutualistic way, their combined impact on host response is expected to be synergistic (greater than the sum of individual impacts). If the two parasites interact in a competitive way, the combined impact on host yield is expected to be antagonistic (less than the sum of individual impacts). We hypothesized that *L. oryzophilus* feeding on roots might make rice more susceptible to infection by *R. solani*. Understanding interaction between *L. oryzophilus* and *R. solani,* the two most important pests of rice production in the southern US, will help manage the pests more effectively. For example, if feeding by *L. oryzophilus* makes rice more susceptible to sheath blight, the action threshold for *L. oryzophilus* could be reduced.

The objectives of the study were to determine the 1) impact of *L. oryzophilus* infestation on development of sheath blight and 2) interactive effects of *L. oryzophilus* and *R. solani* on rice yield. Rice cultivars 'Cocodrie' (PI 606331) and 'Jefferson' (PI 593892) were exposed to *L. oryzophilus* and *R. solani* in isolation and in combination. Disease development and plant yield using field experiments were quantified. The cultivars were selected because of their differential susceptibilities to *R. solani;* Cocodrie is more susceptible than Jefferson according to results of standard nursery evaluation (Zhou et al. 2014). Impact of infestation by *L. oryzophilus* on *R. solani* was measured in terms of magnitude of disease symptoms, while plant response was measured in terms of grain yield.

Materials and Methods

A 4-year field study was done at the Texas A&M AgriLife Research and Extension Center at Beaumont, TX, from 2005 to 2008. A split-split plot design was used with rice cultivars as main plots, insecticide treatments as sub plots, and *R. solani* inoculation as sub-sub plots. Table 1 lists the recommended agronomic and cultural practices done each year (Way and Cockrell 2005).

Rice cultivars 'Cocodrie' and 'Jefferson' were drill-planted at 73 and 90 kg/ha, respectively, as per local agronomic recommendations. Each plot was 5.5 m long with seven rows 18 cm apart. Fields were flushed as needed from emergence to permanent flood. Nitrogen was applied as urea at a rate of 190 kg/ha divided into three equal doses at planting, permanent flood, and 2 weeks after permanent flood. Post-emergence herbicides applied for early season weed control were propanil (Stam 80 EDF-MS [United Phosphorus, King of Prussia, PA]) at 2.40 kg/ha, sodium salt of bentazon (Basagran [Arysta, Cary, NC]) at 0.75 kg/ha, quinclorac (Facet 75DF [BASF, Research Triangle Park, NC]) at 0.25 kg/ha, and molinate (Ordram 8E [Syngenta, Greensboro, NC]) at 2.24 kg/ha. Herbicides were applied with a two-person hand-held spray boom (13-80015 nozzles, 50-mesh screens) at 112 liters/ha final spray volume.

In 2005 and 2006, selected seeds (check and *R. solani* alone treatments) were treated with ICON 6.2FS (active ingredient [a.i.] fipronil) (Bayer CropScience, Research Triangle Park, NC) at 0.06 kg a.i./ha. In 2007 and 2008, selected seeds were treated with Regent 4SC (a.i. fipronil) (BASF Corporation, Research Triangle Park, NC) at 0.06 kg a.i./ha. Seeds were treated using a slurry of insecticide and water in a plastic bag (Way et al. 2002). The contents of the bag were shaken and agitated for 10 minutes to attain maximum absorption and adsorption of the slurry. The amount of insecticide in the slurry was increased by 10% to account for remaining residue in the bag. After agitation, the seeds were allowed to air dry.

Plots not inoculated with *R. solani* (check and *L. oryzophilus* alone treatments) were sprayed twice with the fungicide, Quadris (a.i. azoxystrobin) (Syngenta Crop Protection, Greensboro, NC) at 0.22 kg a.i./ha with a hand-held, CO_2 pressurized spray boom (3-No. 2 nozzles, 50-mesh screens, 140 liters/ha) to control natural amounts of sheath blight.

Table 1. Dates of Agronomic and Cultural Practices, Treatment Applications, and Data Collection during 2005-2008

	Year			
Agronomic and cultural practice	2005	2006	2007	2008
Insecticide seed treatment	4 April	10 April	22 March	8 April
Planting	4 April	12 April	23 March	8 April
Herbicide application	4 May	12 May	23 April	28 April
Permanent flood	6 May	15 May	30 April	13 May
1st core sampling for *L. oryzophilus*	27 May	5 June	21 May	3 June
Inoculation with *R. solani*	16 June	14 June	25 May	11 June
2nd core sampling for *L. oryzophilus*	-	15 June	1 June	13 June
1st fungicide application	10 June	14 June	1 June	19 June
2nd fungicide application	28 June	28 June	21 June	26 June
Sheath blight rating	4 August	9 August	24 July	30 July
Harvest	14 August	9 August	25 July	30 July

Selected plots (*R. solani* alone and *R. solani* + *L. oryzophilus* treatments) were inoculated at panicle differentiation with a virulent isolate of *R. solani* (LR172) collected from a naturally infected rice plant (cv. 'Lebonnet') in Louisiana by Dr. M. C. Rush. Inoculum was prepared by growing *R. solani* in bacto-agar for 5 to 7 days and placing plugs of inoculum/agar on a mixture of autoclaved rice grains and hulls at a ratio of 1:4. After 3 to 5 days, the grain-hull inoculum was broken into small particles consisting of several rice grains held together by fungal mycelia and dispersed by hand into each inoculated plot at 1 kg/plot.

Numbers of *L. oryzophilus* immatures in plots were determined using a soil-root core sampler with diameter and depth each 10 cm. Five core samples were taken from each plot on each sampling date. Each core sample contained at least one rice plant with roots intact. Core samples were processed by placing them on a mesh screen sieve (0.64 x 0.64 mm) and washing soil from roots. Larvae and pupae were counted after washing soil from the roots. Plots were evaluated for development of sheath blight approximately 1 week before maturity and assigned a severity rating on a scale of 0 to 9 (where 0 = no disease to 9 = plants dead and collapsed). At maturity, each sub-sub plot was harvested with a small-plot rice combine, and grain yields recorded.

Data were pooled from 4 years and analyzed by analysis of variance (ANOVA) using a split-split plot design (PROC MIXED, SAS Institute 2010). Cultivar, insecticide treatment, and *R. solani* inoculation were considered fixed effects. Numbers of larvae/pupae in each plot on each sampling date was obtained by summing the numbers from five core samples. Separate ANOVAs of larval/pupal abundance from two sampling dates produced similar results, and the results of analysis of total larval/pupal abundance did not differ substantially from results of the analysis of individual sampling dates. Thus, only results of average larval/pupal abundance from two sampling dates were presented. Larval/pupal count data were $\sqrt{x+0.5}$ transformed to stabilize variance. Yield data were square-root transformed. Least square means (LS-means) were computed, and multiple comparisons among LS-means were determined using Bonferroni's adjustment. All statistical differences were determined using alpha 0.05 (Table 2). Percentage of yield loss for treatments was calculated using: $[(C_{yield} - U_{yield})/C_{yield}] \times 100$, where, C_{yield} = insect/pathogen controlled and U_{yield} = insect/pathogen not controlled.

Results

Both cultivars had a similar trend in regard to *L. oryzophilus* larval/pupal abundance in check plots, plots exposed only to *L. oryzophilus*, plots exposed only to *R. solani*, and plots exposed to both pests (Fig. 1). As expected, *L. oryzophilus* larval/pupal abundance was greater in check plots.

In general, more sheath blight developed in Cocodrie than Jefferson (Fig. 2, Table 2). Disease development in all four treatments was consistent across both cultivars. Mean disease rating was significantly greater in plots inoculated with *R. solani* than in check plots or plots exposed only to *L. oryzophilus*. Disease severity did not vary significantly between plots exposed only to *R. solani* and plots exposed to both *L. oryzophilus* and *R. solani*. Infestation by *L. oryzophilus* resulted in significantly less yield by either cultivar (Fig. 3, Table 2). *R. solani* had a significant negative impact on yield only in Cocodrie. In Cocodrie, the combined impact of *L. ryzophilus* and *R. solani* on yield did not vary significantly from the impact of *R.*

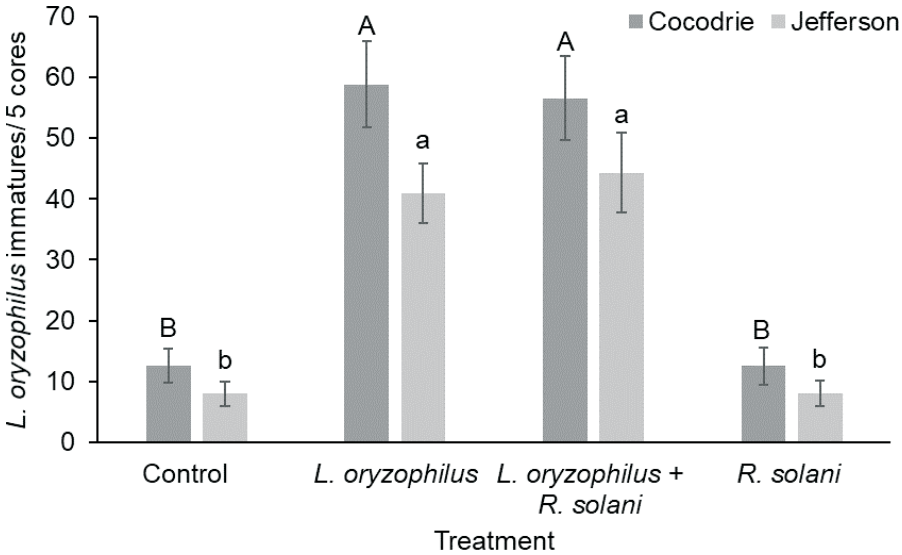

Fig. 1. Mean (± SE) *L. oryzophilus* larval/pupal abundance across treatments in Cocodrie and Jefferson rice (2005-2008). Means with different letters are significantly different (ANOVA; $P < 0.05$, alpha = 0.05).

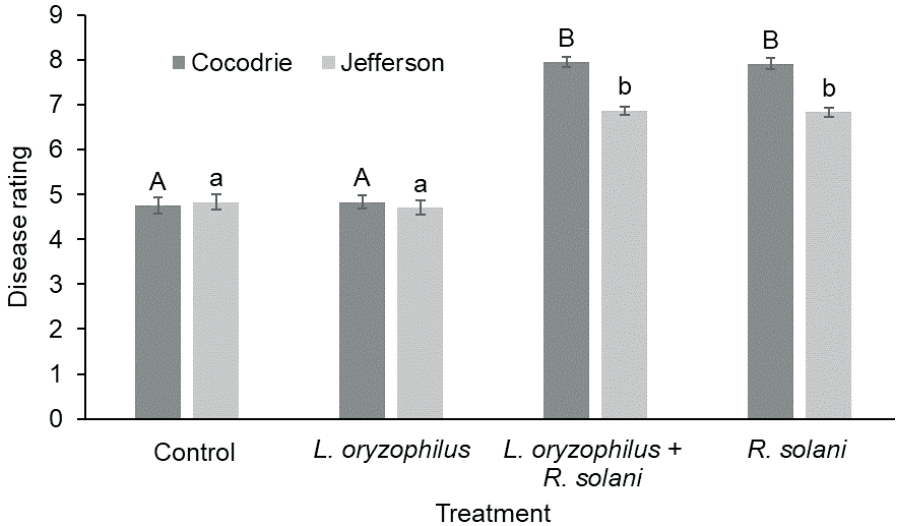

Fig. 2. Mean (± SE) disease rating (scale: 0-9, where 0 = no disease and 9 = plants dead and collapsed) in response to *L. oryzophilus* infestation and pathogen inoculation alone and in combination in Cocodrie and Jefferson rice (2005-2008). Means with different letters are significantly different (ANOVA; $P < 0.05$, alpha = 0.05).

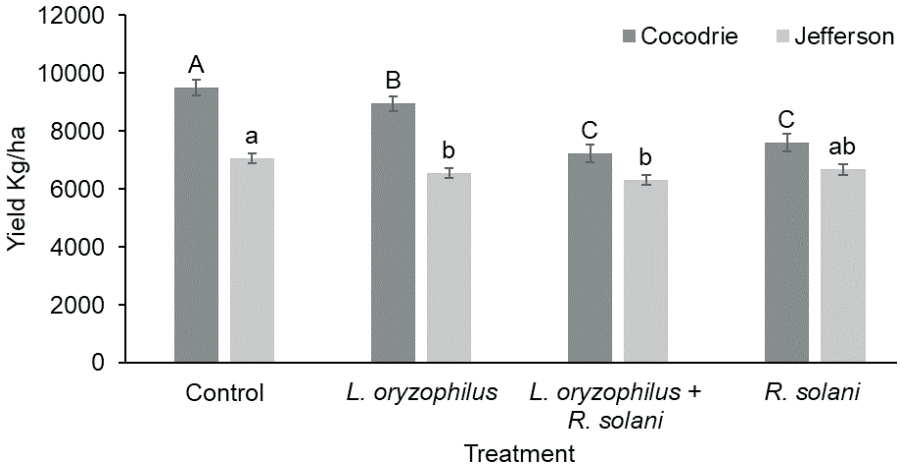

Fig. 3. Mean (± SE) grain yield in response to infestation by *L. oryzophilus* and inoculation by pathogens alone and in combination in Cocodrie and Jefferson rice (2005-2008). Means with different letters are significantly different (ANOVA; $P < 0.05$, alpha = 0.05).

Table 2. Statistical Significance of Treatment Effects of Responses to *L. oryzophilus* and *R. solani* Exposure in Rice

Trait	Effect	df 1	df 2	*F*	*p*
Disease rating	Cultivar	1	160	34.17	0.0001
	L. oryzophilus exposure	1	160	0.01	0.9123
	R. solani inoculation	1	160	766.44	0.0001
	Year	3	160	0.53	0.6615
	Cultivar* *L. oryzophilus* exposure	1	160	0.3	0.5821
	Cultivar* *R. solani* inoculation	1	160	31.64	0.0001
	L. oryzophilus exposure* *R. solani* inoculation	1	160	0.11	0.7412
	Cultivar* *L. oryzophilus* exposure**R. solani* inoculation	1	160	0.3	0.5821
	Cultivar* *L. oryzophilus* exposure**R. solani* inoculation*year	21	160	1.52	0.0763
Yield	Cultivar	1	5	299.99	0.0001
	L. oryzophilus exposure	1	150	48.96	0.0001
	R. solani inoculation	1	150	266.64	0.0001
	Year	3	150	243.18	0.0001
	Cultivar* *L. oryzophilus* exposure	1	150	0.04	0.8349
	Cultivar* *R. solani* inoculation	1	150	123.03	0.0001
	L. oryzophilus exposure* *R. solani* inoculation	1	150	1.27	0.2610
	Cultivar* *L. oryzophilus* exposure**R. solani* inoculation	1	150	0.01	0.9325
	Cultivar* *L. oryzophilus* exposure**R. solani* inoculation*year	21	150	6.82	0.0001

solani alone. In Jefferson, the combined impact of *L. oryzophilus* and *R. solani* on yield did not vary significantly from the impact of either of the pests in isolation. In both cultivars, yield loss from combined impacts of herbivore and pathogen was less than the sum of the individual impacts (Fig. 4).

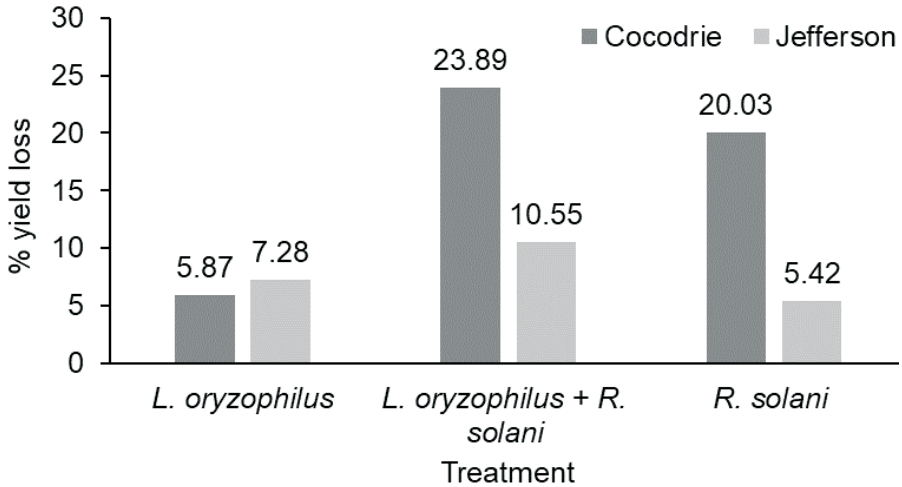

Fig. 4. Percentage of yield loss in Cocodrie and Jefferson rice in response to *L. oryzophilus* and *R. solani* exposure alone and in combination (2005-2008).

Discussion

Results showed that infestation by *L. oryzophilus* did not predispose rice to infection by *R. solani*. Infestation by *L. oryzophilus* alone reduced yield significantly in both cultivars. Infection by *R. solani* alone reduced yield significantly only in susceptible cultivar Cocodrie. In both cultivars, the combined impact of infestation by *L. oryzophilus* and infection by *R. solani* on rice yield was less than additive suggesting that rice was able to compensate for yield losses to the herbivore and pathogen.

In an agroecosystem, interaction among plant attackers is important not only because they potentially play a role determining population dynamics, but such interactions can substantially affect plant yield which is of key interest in production agriculture. When a host is attacked by an insect herbivore, pathogen, or both, the host can deploy an array of resistance mechanisms that might involve primary and secondary metabolites, morphological and physiochemical traits, and mechanisms that allow plants to tolerate attack and recruit natural enemies of attackers (Dangl and Jones 2001, Gatehouse 2002). Interactions among plant attackers, natural enemies, and the plant itself is so complex that the ultimate impact of the interactions on plant performance cannot be understood by considering each antagonist in isolation (Hatcher 1995, Stout et al. 2006). The impact of the insect herbivore *L. oryzophilus* and the plant pathogen *R. solani* alone and in combination

were determined on the yield of rice. Results showed that the combined impact of both pests on rice yield was less than additive (less than the sum of the individual impacts) which might be a result of the ability of the plant to compensate for the combined damage (Fig. 4).

Plants often compensate for direct impacts of insect feeding by changes in resource allocation, photosynthesis, and metabolism (Fornoni 2011). As a result, the combined impact of herbivore and pathogen might not always be reflected in plant yield. Results agreed with those of van Mölken et al. (2014) who evaluated the response of *Barbarea vulgaris* W.T. Aiton, a wild crucifer, to combined attack by the oomycete *Albugo* sp., causing white rust, and a flea beetle, *Phyllotreta nemorum* L. They demonstrated how plants compensate for biomass loss from single and combined attacks over time. Further studies, however, are needed to estimate the combined impacts of infestation by *L. oryzophilus* and infection by *R. solani* on other plant characteristics such as height, biomass, and time to maturity. Hauser et al. (2013), in a meta-analysis of plant-pathogen-arthropod interactions, found the combined impact of arthropods and pathogens synergistic for size and number of plant parts, additive for population growth and reproduction, and antagonistic for whole plant biomass.

Indirect interactions between phytopathogenic microorganisms and arthropod herbivores sharing the same host occur when the first attacker alters the shared host plant in a way that impacts the second attacker that might be spatially or temporally separated from the first. Several studies showed that arthropod herbivores can seriously affect plant-associated microorganisms while on the same plant, either directly or indirectly (Hatcher 1995, Stout et al. 2006, Tack and Dicke 2013). Our study did not show a significant impact of infestation by *L. oryzophilus* on the severity of sheath blight, suggesting no interaction between the *L. oryzophilus* and *R. solani* in rice. Herbivore response also can be affected either positively or negatively by phytopathogenic microorganisms feeding on the same plant. For example, Simon and Hilker (2003) observed increased mortality, decreased larval weight, and prolonged developmental time of *P. versicolora* in response to infection of willow by rust. But, faster development of *Ostrinia nubilalis* Hübner was observed on maize infected by *Colletotrichum graminicola* D.J. Politis, the causal agent of stalk rot, than on maize not infected with the pathogen (Carruthers et al. 1986). Future studies also can assess the potential impact of infection by *R. solani* on *L. oryzophilus* feeding on the same plant.

In conclusion, the study provided information on the effect of infestation by *L. oryzophilus* on development of sheath blight in rice and interactive impacts of both the insect herbivore and plant pathogen on rice yield. Results indicated that infestation by *L. oryzophilus* and resulting root damage did not influence development of sheath blight in rice. The combined impact of *L. oryzophilus* and *R. solani* on yield was less than additive, which might result from compensation by rice. Findings from this study suggested that infestation by *L. oryzophilus* would not impact severity of sheath blight in rice.

Acknowledgment

We thank the Texas Rice Research Foundation for funding this research. We also thank LSU AgCenter Rice Research Station at Crowley, LA, for assistance with field studies.

References Cited

Aghaee, M. A., and L. D. Godfrey. 2014. A century of rice water weevil (Coleoptera: Curculionidae): a history of research and management with an emphasis on the United States. J. Integrated Pest Management 5: D1-D14.

Carruthers, R. I., G. C. Bergstrom, and P. A. Haynes. 1986. Accelerated development of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), induced by interactions with *Colletotrichum graminicola* (Melanconiales: Melanconiaceae), the causal fungus of maize anthracnose. Ann. Entomol. Soc. Am. 79: 385-389.

Dangl, J. L., and J. D. G. Jones. 2001. Plant pathogens and integrated defence responses to infection. Nature 411: 826-833.

Fornoni, J. 2011. Ecological and evolutionary implications of plant tolerance to herbivory. Funct. Ecol. 25: 399-407.

Gatehouse, J. A. 2002. Plant resistance towards insect herbivores: a dynamic interaction. New Phytologist 156: 145-169.

Groth, D. E. 2008. Effects of cultivar resistance and single fungicide application on rice sheath blight, yield, and quality. Crop Protection 27: 1125-1130.

Groth, D. E., and V. Castroagudin. 2018. Sheath blight, pp. 37-40. *In* Compendium of Rice Diseases and Pests, 2nd ed. APS Press.

Hatcher, P. E. 1995. 3-way interactions between plant-pathogenic fungi, herbivorous insects and their host plants. Biol. Rev. 70: 639-694.

Hauser, T. P., S. Christensen, C. Heimes, and L. P. Kiær. 2013. Combined effects of arthropod herbivores and phytopathogens on plant performance. Funct. Ecol. 27: 623-632.

Kanno, H., and Y. Fujita. 2003. Induced systemic resistance to rice blast fungus in rice plants infested by white-backed planthopper. Entomol. Exp. Applic. 107: 155-158.

Kant, M. R., W. Jonckheere, B. Knegt, F. Lemos, J. Liu, B. C. J. Schimmel, C. A. Villarroel, L. M. S. Ataide, W. Dermauw, J. J. Glas, M. Egas, A. Janssen, T. Van Leeuwen, R. C. Schuurink, M. W. Sabelis, and J. M. Alba. 2015. Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities. Ann. Bot. 115: 1015-1051.

Lee, F. N., and M. Rush. 1983. Rice sheath blight: a major rice disease. Plant Dis. 67: 829-833.

Marchetti, M. A., and C. N. Bollich. 1991. Quantification of the relationship between sheath blight severity and yield loss in rice. Plant Dis. 75: 773-775.

Marquis, R. J. 1992. The selective impact of herbivores, pp. 301-325. *In* Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics. University of Chicago Press, Chicago.

Oerke, E. C., and H. W. Dehne. 2004. Safeguarding production -- losses in major crops and the role of crop protection. Crop Protection 23: 275-285.

SAS Institute. 2010. SAS/STAT User's Guide. Version 9.4. SAS Institute, Cary, NC.

Simon, M., and M. Hilker. 2003. Herbivores and pathogens on willow: do they affect each other? Agric. For. Entomol. 5: 275-284.

Stout, M. J., W. C. Rice, S. D. Linscombe, and P. K. Bollich. 2001. Identification of rice cultivars resistant to *Lissorhoptrus oryzophilus* (Coleoptera: Curculionidae), and their use in an integrated management program. J. Econ. Entomol. 94: 963-970.

Stout, M. J., J. S. Thaler, and B. P. H. J. Thomma. 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annu. Rev. Entomol. 51: 663-689.

Tack, A. J. M., and M. Dicke. 2013. Plant pathogens structure arthropod communities across multiple spatial and temporal scales. Funct. Ecol. 27: 633-645.

van Mölken, T., V. Kuzina, K. Munk, C. Olsen, T. Sundelin, N. van Dam, and T. Hauser. 2014. Consequences of combined herbivore feeding and pathogen infection for fitness of *Barbarea vulgaris* plants. Oecologia 175: 589-600.

Vyavhare, S. S., D. R. Gealy, M. O. Way, R. E. Tabien, and R. A. Pearson. 2016. Evaluation of host-plant resistance of selected rice genotypes to the rice water weevil (Coleoptera: Curculionidae). Environ. Entomol. 45: 1439-1444.

Way, M. O. 2018. Arthropod pests, pp. 90-97. *In* Compendium of Rice Diseases and Pests. 2nd ed. APS Press.

Way, M. O., and J. Cockrell. 2005. 2005 Texas Rice Production Guidelines. https://beaumont.tamu.edu/eLibrary/Bulletins/2005_Rice_Production_Guidelines.pdf Accessed 20 September 2017.

Way, M. O., R. G. Wallace, M. S. Nunez, and G. N. McCauley. 2002. Evaluation of Icon 6.2 FS, Karate Z and Dimilin 2 l for rice water weevil control, 2001. Arthropod Management Tests 27: F85.

Zhou, X. G., and Y.-K Jo. 2014. Disease management, pp. 44-57. *In* 2014 Texas Rice Production Guidelines. Texas A&M AgriLife Research and Extension B-6131.

Zou, L., M. J. Stout, and D. R. Ring. 2004. Density-yield relationships for rice water weevil on rice for different varieties and under different water management regimes. Crop Protection 23: 543-550.

Abundance of the Beet Leafhopper, *Circulifer tenellus*, Associated with 16SrIII-phytoplasmas in Squash at Baja California Sur, Mexico

Diana Medina-Hernández, Mayela Vargas-Salinas, and
Ramón Jaime Holguín-Peña*

Centro de Investigaciones Biológicas del Noroeste, Instituto Politécnico Nacional 195, Colonia Playa Palo de Santa Rita Sur, 23096, La Paz, Baja California Sur, México

Abstract. During 2016-2017, growers at San Juan de Los Planes, El Carrizal, and El Pescadero at Baja California Sur, Mexico experienced serious outbreaks of phytoplasma squash yellowing disease, causing significant economic loss, mostly from low yield and quality of squash, *Cucurbita pepo* L., for export. In addition, abundant beet leafhoppers, *Circulifer tenellus* (Baker), presumed to transmit the disease, were observed in each field. Using nested-polymerase chain reaction (PCR) amplification of the 16S rRNA gene and comparing *in silico* patterns, positive Western X-disease phytoplasma of the 16SrIII group was detected. Scanning electron microscopy showed phytoplasmatic particles in sieve tubes of infected plants and insects. Phytoplasma risk based on combined data of insect abundance, disease incidence, and economic threshold was analyzed to evaluate the impact of the disease over time. Only at Los Planes with 128 adult insects per trap and 75.5% incidence of disease was risk of phytoplasma high, while at El Carrizal and El Pescadero, risk was moderate. This is the first evidence of beet leafhoppers transmitting phytoplasma to squash at Baja California Sur. The information will be useful for managing disease in the region and where squash is grown worldwide.

Introduction

Squash, *Cucurbita pepo* L., is one of the most important horticultural crops worldwide. Mexico contributed an average of 200,000 tons per year during the last three decades, which is equivalent to 33.4% of the squash production in the world (Geisler 2014). The Italian variety, also known as summer squash or zucchini, is one of the most commercialized in Mexico, with Baja California Sur the state exporting the most (approximately 5,600 tons of 84% of total fresh production) to US markets (ASERCA 1999). Profits during the last 10 years were equivalent to those of tomato, *Solanum esculentum* L., with the same cross-border marketing channels (ATA 2015). However, despite this promising scenario, loss because of phytoplasma squash yellowing disease increased during the last 5 years, mostly in field crops. Coincidentally, abundant beet leafhoppers and other species of the family Cicadellidae were observed (Vargas-Salinas 2017). Among families most commonly confirmed as vectors of phytoplasma, vectors are confined to this family and Fulgoromorpha: Psillideae (Weintraub and Beanland 2006).

*Correspondence: jholguin04@cibnor.mx, Tel: 612 123 84 72

Phytoplasmas are bacteria without cell walls but with small genomes ranging from 530 to 1,350 kb capable of self-replication (Christensen et al. 2005). The bacteria cannot be cultured in vitro. Taxonomists assigned them to the genus "*Candidatus* Phytoplasma" (IRPCM 2004, Lee et al. 2010). Based on analysis of the 16S ribosomal gene (16Sr RNA) and restriction-fragment length polymorphisms (RFLP), they were classified in 28 groups (I to XXVIII) with 51 well-defined strains with distinctive features of biodiversity and other evolutionary processes (Wei et al. 2007, Zhao et al. 2009, Jomantiene et al. 2011).

Diversity of phytoplasma at Baja California Sur was determined in a limited number of hosts, mostly crops such as tomato, chiltepin and bell pepper, *Capsicum annuum* L., and papaya, *Carica papaya* L. (Poghosyan et al. 2004, Lebsky and Poghosyan 2007, Lebsky et al. 2011). Less frequently, the 16SrIII-B phytoplasma was found in ornamentals such as periwinkle, *Catharanthus roseus* G. Don (Holguin-Peña et al. 2007) and kumquat, *Citrus japonica* Thunb. (Poghosyan et al. 2015). Only the smoke tree sharpshooter, *Homalodisca litura* Ball (Cicadellidae), found associated with native hosts such as wild plum, *Cyrtocarpa edulis* [Brandegee] Standl, and gandul bean, *Cajanus cajan* L. Huth, (Servín-Villegas et al. 2018), was reported to transmit phytoplasmas in the region. But, association of phytoplasmas with crops has not been determined.

The role of beet leafhoppers in transmitting phytoplasma is widely known (Crosslin et al. 2005, Weintraub and Beanland 2006, Munyaneza 2010, Bertaccini et al. 2014, Perez-Lopez et al. 2016). However, at Baja California Sur, only two cases of leafhoppers have been associated with crops or wild plants; potato psyllid, *Bactericera cockerelli* (Sulc) (Psyllidae), was in green tomato, bean, pepper, tomato, and basil, *Ocimum basilicum* L. (Servín-Villegas et al. 2008), and citrus psyllid, *Diaphorina citri* Kuwayama, was in phytoplasma-infected kumquat (Poghosyan et al. 2015). However, in neither case was the role of the insect as a transmitter of phytoplasma conclusive. Historically, most research on phytoplasmas focused on the effect on plants rather than on the insect (Weintraub and Beanland 2006). Detection of the pathogen in both hosts is crucial to understanding the epidemiological basis in dissemination of disease.

The objectives of the study were to detect phytoplasma in naturally infected squash plants and individual leafhoppers to elucidate the role both play in transmission of phytoplasma squash yellowing disease. Insect abundance, disease incidence, and economic threshold were combined to analyze risk.

Materials and Methods

During the cropping seasons of 2016-2017, localities with most squash cultivation and where phytoplasma disease symptoms previously were observed in the La Paz Valley were surveyed. Diseased and healthy plants and adult leafhoppers were collected to estimate incidence of disease and insect abundance. Localities selected were San Juan de Los Planes, El Carrizal, and El Pescadero, within 100 km of each other. To characterize phytoplasma squash yellowing disease, symptoms of phytoplasma diseases in squash and other cucurbitaceous crops were examined and compared (Salehi et al. 2015, Martini et al. 2018). Insects collected by vacuum sampling methods using a leaf blower were preserved in 70% alcohol and stored at room temperature until analyzed by scanning electron microscopy (SEM) and polymerase chain reaction (PCR). Plants sampled were processed and stored

according to standard procedures for SEM techniques (Lebsky et al. 2011) and molecular probes (Crosslin et al. 2005).

SEM was used for preliminary analysis to detect phytoplasma in diseased plants and insects. Samples fixed in glutaraldehyde (2.5%) in a buffer sodium solution of 0.2 M (pH 7.2-7.4) were incubated at 4°C for 24 hours. After gradual dehydration (30, 50, 70, 96, and 100%) in alcohol, samples were washed in 100% acetone for 20 minutes and dried at critical point in carbon dioxide (Samdri PVT-3B). Imaging was by electron microscope (Hitachi-S-3000N, Tokyo, Japan) according to procedures of the manufacturer.

Total DNA was extracted from individual insects as described by Crosslin et al. (2005). Nested-PCR was done with a set of primers. The first reaction was done with P1/P7 primers and the nested reaction with R16F2n/R16R2 primers that amplified the 16Sr RNA ribosomal region (Gundersen and Lee 1996). For group and sub-group determination, BLAST was used to compare 16Sr DNA nucleotide (nt) sequences with a database (NCBI, GenBank) and by *in silico* analysis of electrophoretic patterns (Zhao and Davis 2016).

Carlos Palacios-Cardiel at the Entomology and Acarology Laboratory of Centro de Investigaciones Biológicas del Noroeste (CIBNOR) identified the leafhoppers. Voucher specimens of leafhoppers were deposited in the arachnological and entomological collection (CAECIB) at CIBNOR.

Adult leafhoppers on 10 x 20-cm yellow sticky traps (Olsen Products, Medina, OH) were counted directly according to standard procedures (Munyaneza et al. 2008). Collection was every 10 (± 2) days. Intensity of the disease was assessed as the incidence by using the formula: $I (\%) = \Sigma A/N \times 100$, where A = total number of infected and N = total number of plants. Symptomatic plants for estimation of disease incidence were corroborated by PCR.

To estimate the potential damage of the disease, we estimate a phytoplasma risk analysis (PRA), based on the spatial distribution of the insects in infected fields (LI) (number of leafhoppers/plant) and by the occurrence of phytoplasma disease (INC), estimated on the incidence and in the aster yellow index (AYI) (Madden et al. 1995). The different quadrants of the PRA, resulting from the combination of the Y (INC) and X-axis (LI) were typified as; low (when LI and INC values are less than ET), medium (when LI or INC are within the range of ET), and high (when LI and INC are greater than ET).

Results and Discussion

Incidence of phytoplasma-like diseases has increased at Baja California Sur, south of the Baja California Peninsula, mostly in open fields of squash crops where four phytoplasma species were reported (Holguín-Peña et al. 2007, Lebsky et al. 2011, Poghosyan et al. 2015, Servín-Villegas et al. 2018). Growers in the Valley of La Paz experienced serious outbreaks of yellow-type diseases that caused significant economic loss because of yield reduction and decrease in quality of exported squash.

Based on nested-PCR techniques, the 16SrIII-phytoplasma was positive in the X-disease group amplifying the expected fragment of 1.2 kb size (Fig. 1A). The closest relationship in BLAST analysis was with the Western X-disease phytoplasma (GenBank L04682). The phytoplasma group also was corroborated *in silico,* observing the expected electrophoretic pattern for the 16SrIII group (Fig. 1B) (Wei et al. 2007, Zhao et al. 2009). The same phytoplasma group was consistently observed in diseased plants and individual beet leafhoppers identified as *Circulifer tenellus*

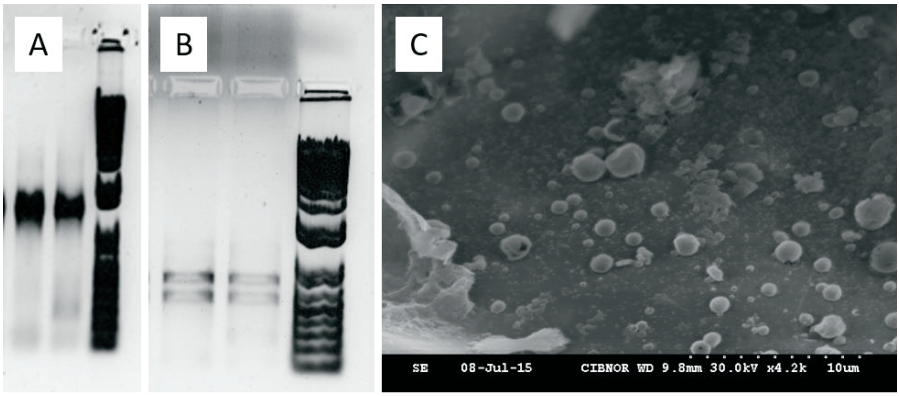

Fig. 1. Detection of 16SrIII-group phytoplasma in infected squash fields at Baja California Sur, Mexico. (A) Agarose gel electrophoresis of nested-polymerase chain reaction products showing of ~1.2 kb size fragment with primers R16F2n/R16R2; (B) Electrophoretic pattern of nested-PCR products generated by restriction fragment length polymorphisms (RFLP), showing profiles similar to peach X-disease phtyoplasma with *Eco*R1 products showing of ~800 and 600 kb size; (C) Scanning electron microscopy images of phytoplasmas in tube sieves of phloem parenchyma in symptomatic squash plants at Los Planes, Baja California Sur, México.

(Baker) (Hemiptera: Cicadellidae). SEM also was used to show phytoplasmas in sieve tubes of naturally infected squash plants (Fig. 1C). This fact and evidence that the disease had spread to all sites separated by 100 km, suggested that both hosts actively participated in dispersion and epidemiology of the disease.

At Baja California Sur, the appearance of phytoplasmas dates back 15 years when SEM was used to show a phytoplasma disease in papaya (Poghosyan et al. 2004). Three years later, by using molecular techniques, a strain of '*Ca*. P. asteris' (subgroup 16SrI-B) was found associated with yellow-type disease in tomato in the northern part of the peninsula (Holguín-Peña et al. 2007). Diversity of phytoplasmas was later reported in horticultural crops, ornamental plants, and weeds surrounding the cultivated areas (Table 1). In some cases, determination of sub-groups to which the phytoplasmas belonged also was inconclusive. For example, 16SrIII was detected in pepper but without determining the sub-group or proving the role of an insect in transmitting the phytoplasma disease (Lebsky et al. 2011). Some phytoplasmas (16SrI-B) also have been reported in ornamentals such as periwinkle and kumquat and presumably associated with *D. citri*, but the insect role was not determined (Poghosyan et al. 2015).

The 16SrIII group detected in this work is related to the Western X-disease phytoplasma (WXDPh) and has two sub-groups (16SrIII-A and B) in a variety of hosts, including pepper and tomato at Baja California Sur (Zhao and Davis 2016). The 16SrIII-A (Western X-disease phytoplasma) also was reported in peach, *Prunus persica* (L.) Batsch (Davis et al. 2013) and cassava, *Manihot esculenta* Crantz (Souza et al. 2014). The 16SrIII-B (clover yellow edge phytoplasma), apparently the most diversified, has been reported infected strawberry, *Fragaria x ananassa* Duchesne (Jomantiene et al. 1999), hazelnut (*Corylus avellana* L.) (Jomantiene et al. 2000), and clover, *Trifolium* L. (Staniulis et al. 2000).

Table 1. Phytoplasma-related Groups Associated with Cultivated and Ornamental Crops and Insects at Baja California Peninsula, Mexico

Phytoplasma	Group[1]	Host	Insect vector	Reference
In this study:				
´*Ca*. P. pruni´	16SrIII	Squash (*Cucurbita pepo* L)	*Circullifer tenellus*	
In literature:				
´*Ca*. P. asteris´	16SrI-B	Tomato (*Solanum esculentum* Mill) and periwinkle (*Catharantus roseus* L)	ND	Holguin-Peña et al. 2007
Phytoplasma X disease	16SrIII	Pepper (*Capsicum annuum* L)	ND	Lebsky et al. 2011
´*Ca*. P. asteris´	16SrI-B	Kumquat (*Citrus japonica* Swingle)	*Diaphorina citri*[2]	Poghosyan et al. 2015
´*Ca*. P. hispanicum´	16SrXIII-D	Wild plum (*Cyrtocarpa edulis* Brandegee [Standl]) and pigeon pea (*Cajanus cajan* L [Huth])	*Homalodisca liturata*	Servín-Villegas et al. 2018

[1]ND = not determined.
[2]Presumably associated with the disease, but not proven as a vector or transmitter of phytoplasma disease.

A wide range of phytoplasma strains has been associated with diverse beet leafhoppers, mostly *C. tenellus* with wide distribution in the USA (Munyaneza and Upton 2005, Munyaneza et al. 2010). Beet leafhopper was reported to cause several phytoplasma diseases in several vegetable crops, mostly potato, *Solanum tuberosum* L. (Munyaneza et al. 2008) and pepper (Creamer et al. 2003). The species caused serious problems as phytoplasma transmitters in several regions of northern Mexico (Abrajan-del Rio et al. 2014, Davila-Berumen et al. 2014, López-Romo et al. 2014. *C. tenellus* also transmitted other phloem-diseases such as curtovirus (Geminiviridae) (Chen and Gilbertson 2016, Reveles-Torres et al. 2017) or mixed infection (pyhtoplasma/ curtovirus) in pepper (Velazquez-Valle and Reveles-Torres 2014). At Baja California Sur, the only insect mentioned as a transmitter was smoke-tree sharpshooter associated with ´*Ca*. P. hispanicum´ (16SrXIII-D) but not associated with any crop host (Servín-Villegas et al. 2018).

Risk analysis data combined with economic thresholds (ET) are useful for implementing disease management strategies. Assessment of risk of phytoplasma disease by combining incidence of disease and insect abundance at each site evaluated in 2016-2017 was medium to high (Fig. 2). Most phytoplasma risk was at Los Planes where combination of disease incidence (75.5%) and infestation by leafhoppers (125 ± 5 per trap) were related to greatest risk in the first quadrant. Results agreed with those of Munyaneza et al. (2008) who concluded that abundance was greatest at 192.2 ± 19.3 leafhoppers per trap-week (Fig. 2). At El Pescadero and Carrizal, risk was medium with 70% incidence and 80 and 90 insects per trap, respectively. At all sites, only at the beginning of the cropping season (10 days after transplant) was risk low. Considering risk levels in this study, <100 leafhoppers per trap and 50% incidence of plants diseased were proposed as the economic threshold.

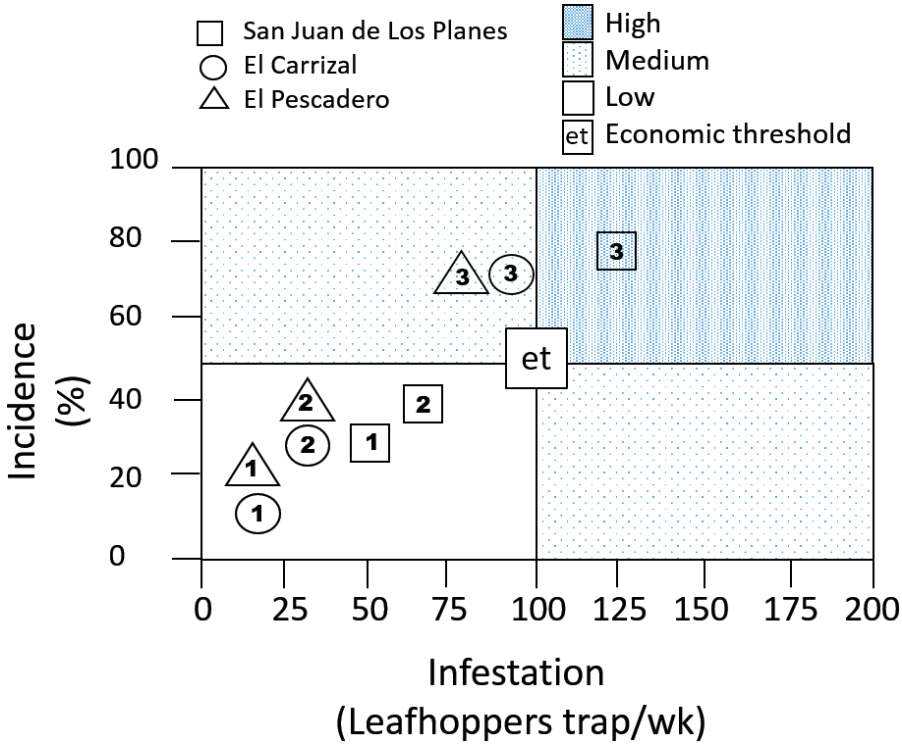

Fig. 2. Phytoplasma risk analysis (PRA) of the disease at three localities at Baja California Sur, Mexico. Quadrants represent the risk level based on combination of phytoplasma incidence and infestation by leafhoppers. The number in the symbols (□, Δ, o) assigned to each locality represent days after planting: 1 = 10, 2 = 20, and 3 = 30. Insect abundance (infestation) on the X-axis represents the average of four traps per hectare per week. Incidence on the Y-axis represents the average number of phytoplasma-positive plants.

Greater phytoplasma risk analysis values implicated immediate implementation of control, and low values represented prevention.

To our knowledge, this is the first report of a 16SrIII phytoplasma group associated with the leafhopper *C. tenellus* as a transmitter to squash at Baja California Sur, Mexico. The information will be relevant to authorities and producers for implementing safety measures to reduce risk of dissemination of infectious diseases caused by phytoplasmas.

In conclusion, a phytoplasma that belonged to the 16SrIII group was detected in symptomatic squash plants and in individual *C. tenellus* leafhoppers at Baja California Sur. Squash plants could serve as the primary host and leafhoppers participate in transmission. Based on results of evaluating incidence and abundance of infective insects for 2 years, further management strategies should be implemented to prevent high risk of disseminating phytoplasma diseases to other areas of Baja California Sur and beyond.

Acknowledgment

The authors thank CIBNOR technical staff Carlos Palacios-Cardiel of the Laboratory of Entomology and Acarology for support in insect identification, M. Aguilar G. and S. Ramos G. for assistance in the Molecular Phytopathology Laboratory, and D. Fischer for editorial assistance with English.

References Cited

Abrajan-del Rio, X., L. R. Reveles-Torres, R. Velázquez-Valle, M. Reveles-Hernández, and J. A. Cid-Ríos. 2014. Presence of phytoplasma in the cicadellid *Circulifer tenellus* in the state of Zacatecas, Mexico. Agrofaz 14: 9-23.

ASERCA (Apoyos y Servicios a la Comercialización Agropecuaria). 1999. La calabaza y la calabacita en el mercado norteamericano. Revista Claridades Agropecuarias 1-36. http://www.infoaserca.gob.mx/claridades/revistas/076/ca076.pdf.

ATA (Agenda Técnica Agrícola de Baja California Sur). 2015. Paquetes tecnológicos, calabacita (Technological packages, squash). SAGARPA. pp. 1-252. www./http/03_BajaCaliforniaSur_2015_SIN%20(1).pdf.

Bertaccini, A., B. Duduk, S. Paltrinieri, and N. Contaldo. 2014. Phytoplasmas and phytoplasma diseases: a severe threat to agriculture. Am. J. Plant Sci. 5: 1763-1788.

Chen, L. F., and R. L. Gilbertson. 2016. Transmission of curtoviruses (beet curly top virus) by the beet leafhopper (*Circulifer tenellus*); vector-mediated transmission of plant pathogens. Chapter 17. APS Books. pp. 243-262.

Christensen, N. M., K. B. Axelsen, M. Nicolaisen, and A. Schulz. 2005. Phytoplasmas and their interactions with hosts. Trends Plant Sci. 10: 526-535.

Creamer, R., J. Carpenter, and J. Rascon. 2003. Incidence of the beet leafhopper, *Circulifer tenellus* (Homoptera: Cicadellidae), in New Mexico. Report 12. Available via DIALOG. http://www.chiletaskforce.org

Crosslin, J. M., J. E. Munyaneza, A. Jensen, and P. B. Hamm. 2005. Association of beet leafhopper (Hemiptera: Cicadellidae) with a clover proliferation group phytoplasma in Columbia Basin of Washington and Oregon. J. Econ. Entomol. 98: 279-283.

Davila-Berumen, F., R. Velázquez-Valle, L. R. Reveles-Torres, and J. A. Mauricio-Castillo. 2014. Detection of phytoplasma in populations of *Dalbulus, Empoasca, Graminella* and *Aceratagallia* from the state of Zacatecas, Mexico. Agrofaz 14: 39-45.

Davis, R. E., Y. Zhao, E. L. Dally, I. M. Lee, R. Jomantiene, and S. M. Douglas. 2013. '*Candidatus* Phytoplasma pruni', a novel taxon associated with X-disease of stone fruits, *Prunus* spp.: multilocus characterization based on 16S rRNA, secY, and ribosomal protein genes. Int. J. Syst. Evol. Micr. 63: 766-776.

Geisler, M. 2014. Squash. A national information resource for value-added agriculture. Agricultural Marketing Resource Center, Iowa State University. https://www.agmrc.org/commodities-products/vegetables/squash.

Gundersen, D. E., and I. M. Lee. 1996. Ultrasensitive detection of phytoplasmas by nested-PCR assays using two universal primer pairs. Phytopathol. Mediterr. 35: 144-151.

Holguín-Peña, R. J., R. C. Vázquez-Juárez, and J. P. Martínez-Soriano. 2007. First report of a 16SrI-B group phytoplasma associated with a yellows-type disease affecting tomato plants in the Baja California Peninsula of Mexico. Plant Dis. 91: 328.

IRPCM. 2004. The Phytoplasma/Spiroplasma Working Team – Phytoplasma taxonomy group. 'Candidatus Phytoplasma', a taxon for the wall-less, non-helical prokaryotes that colonize plant phloem and insects. Int. J. Syst. Evol. Micr. 54: 1243-1255.

Jomantiene, R., J. L. Maas, E. L. Dally, and R. E. Davis. 1999. First report of clover yellow edge and STRAWB2 phytoplasmas in strawberry in Maryland. Plant Dis. 83: 1072.

Jomantiene, R., J. D. Postman, H. G. Montano, J. L. Maas, R. E. Davis, and K. B. Johnson. 2000. First report of clover yellow edge phytoplasma in *Corylus* (hazelnut). Plant Dis. 84: 102.

Jomantiene, R., Y. Zhao, I. M. Lee, and R. E. Davis. 2011. Phytoplasmas infecting sour cherry and lilac represent two distinct lineages having close evolutionary affinities with clover phyllody phytoplasma. Eur. J. Plant Pathol. 130: 97.

Lebsky, V., and A. Poghosyan. 2007. Phytoplasma associated diseases in tomato and pepper in the state of BCS, Mexico: a brief overview. Bull. Insectology 60: 131-132.

Lebsky, V., J. Hernandez-Gonzalez, G. Arguello-Astorga, Y. Cárdenas-Conejo, and A. Poghosyan. 2011. Detection of phytoplasmas in mixed infection with begomoviruses: a case study of tomato and pepper in Mexico. Bull. Insectology 64 Suppl. S55-S56.

Lee, I. M., K. D. Bottner, Y. Zhao, R. E. Davis, and N. A. Harrison. 2010. Phylogenetic analysis and delineation of phytoplasmas based on secY gene sequences. Int. J. Syst. Evol. Micr. 60: 2887-2897.

López-Romo, G., L. R. Reveles-Torres, R. Velázquez-Valle, and S. Salas-Muñoz. 2014. Phytoplasma transmission by the vector *Circulifer tenellus* in different plant hosts. Agrofaz 14: 53-58.

Madden, L. V., L. R. Nault, D. J. Murral, and M. R. Apelt. 1995. Spatial pattern analysis of the incidence of aster yellows disease in lettuce. Res. Popul. Ecol. 37: 279-289.

Martini, M., D. Delic, L. Liefting, and H. Montano. 2018. Phytoplasmas infecting vegetable, pulse and oil crops, pp. 31-65. *In* G. Rao, A. Bertaccini, N. Fiore, and L. Liefting [eds.], Phytoplasmas: Plant Pathogenic Bacteria - I.

Munyaneza, J. E. 2010. Psyllids as vectors of emerging bacterial diseases of annual crops. Southwest. Entomol. 35: 471-477.

Munyaneza, J. E., and J. E. Upton. 2005. Beet leafhopper (Hemiptera: Cicadellidae) settling behavior, survival, and reproduction on selected host plants. J. Econ. Entomol 6: 1824-1830.

Munyaneza, J. E., A. S. Jensen, and P. B. Hamm. 2008. Seasonal occurrence and abundance of beet leafhopper in the potato-growing region of Washington and Oregon Columbia Basin and Yakima valley. Am. J. Potato Res. 85: 77-84.

Munyaneza, J. E., J. M. Crosslin, J. E. Upton, and J. L. Buchman. 2010. Incidence of the beet leafhopper-transmitted virescence agent phytoplasma in local populations of the beet leafhopper, *Circulifer tenellus*, in Washington State. J. Insect Sci. 10: 18.

Pérez-López, E., M. Luna-Rodríguez, C. Y. Olivier, and T. J. Dumonceaux. 2016. The underestimated diversity of phytoplasma in Latin America. Int. J. Syst. Evol. Micr. 66: 492-513.

Poghosyan, A., V. K. Lebsky, M. Arce-Montoya, and L. Landa. 2004. Possible phytoplasma disease in papaya (*Carica papaya* L.) from Baja California Sur: diagnosis by scanning electron microscopy. J. Phytopathol. 152: 321-382.

Poghosyan, A., J. Hernández-Gonzalez, and V. Lebsky. 2015. Phytoplasmas associated with naturally infected *Catharanthus roseus* in La Paz, Baja California Sur, Mexico. Phytopathogenic Mollicutes 5 (1-Suppl.) S25-S26.

Reveles-Torres, L. R., S. Salas-Muñoz, J. Mena-Covarrubias, R. Velásquez-Valle, and J. A. Mauricio-Castillo. 2017. Curtovirus como agentes fitopatógenos en el norte centro de México. Folleto Técnico 84. Campo Experimental Zacatecas. CIRNOC – INIFAP 31

Salehi, M., M. Siampour, S. A. Esmailzadeh Hosseini, and A. Bertaccini. 2015. Characterization and vector identification of phytoplasmas associated with cucumber and squash phyllody in Iran. Bull. Insectology 68: 311-319.

Servín-Villegas, R., A. Tejas, and A. Cota. 2008. Primer reporte de *Bactericera cockerelli* Sulc, en plantaciones hortícolas de Baja California Sur, México. Southwest. Entomol. 33: 239-241.

Servín-Villegas, R., M. G. Caamal-Chan, A. Chávez-Medina, A. Loera-Muro, A. Barraza, D. Medina-Hernández, and R. J. Holguín-Peña. 2018. Identification of a '*Candidatus* Phytoplasma hispanicum'-related strain, associated with yellows-type diseases, in smoke-tree sharpshooter (*Homalodisca liturata* Ball). Int. J. Syst. Evol. Micr. 68: 2093-2101.

Souza de, A. N., F. N. da Silva, I. P. Bedendo, and C. M. Carvalho. 2014. A phytoplasma belonging to a 16SrIII-A subgroup and dsRNA virus associated with cassava frogskin disease in Brazil. Plant Dis. 98: 771-779.

Staniulis, J. B., R. E. Davis, R. Jomantiene, A. Kalvelyte, and E. L. Dally. 2000. Single and mixed phytoplasma infections in phyllody- and dwarf-diseased clover plants in Lithuania. Plant Dis. 84: 1061-1066.

Vargas-Salinas, M. 2017. Caracterización molecular de begomovirus y fitoplasmas asociados a una infección mixta en calabacita *Cucurbita pepo* L. en B.C.S., México. Master dissertation, Centro de Investigaciones Biológicas del Noroeste, México.

Velázquez-Valle, R., and L. R. Reveles-Torres. 2014. Síntomas asociados con la infección por curtovirus y fitoplasmas en chile para secado. Actualidades y desafíos de la investigación en recursos bióticos de zonas áridas. INIFAP Zacatecas 567-576. www. sinInfCutFitoChile.pdf

Wei, W., R. E. Davis, I. M. Lee, and Y. C. Zhao. 2007. Computer-simulater RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. Int. J. Syst. Evol. Micr. 57: 1855-1867.

Weintraub, P., and L. Beanland. 2006. Insect vectors of phytoplasmas. Annu. Rev. Entomol. 51: 91-111.

Zhao, Y., and R. E. Davis. 2016. Criteria for phytoplasma 16Sr group/subgroup delineation and the need of a platform for proper registration of new groups and subgroups. Int. J. Syst. Evol. Micr. 66: 2121-2123.

Zhao, Y., W. Wei, I. M. Lee, J. Shao, X. Suo, and R. E. Davis. 2009. Construction of an interactive online phytoplasma classification tool, iPhyClassifier, and its application in analysis of the peach X-disease phytoplasma group (16SrIII). Int. J. Syst. Evol. Micr. 59: 2582-93.

Insect Diversity in Conventional and Bt Cottons in the Comarca Lagunera, Mexico

Diversidad de Insectos en Algodoneros Bt y Convencionales en la Comarca Lagunera, México

Verónica Ávila-Rodríguez[1], Urbano Nava-Camberos[2], José L. García-Hernández[3]*, José L. Martínez-Carrillo[4], and Carlos A. Blanco[5]

Abstract. Since the introduction of genetically modified cotton (GM or Bt cotton) to Mexico in 1996, less insecticide is used, and cotton yields have increased significantly. Considering the controversy over potential environmental impact of Bt cotton, and particularly the lack of information on its effects on diversity of entomofauna, the objective of this study was to determine insect diversity in non-Bt and Bt cottons in the Comarca Lagunera, Mexico. Insects collected in both types of cotton were in the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Thysanoptera. Insects in the order Hemiptera were most abundant, followed by Diptera and Thysanoptera. Insects belonged to 40 families, with most abundant being Aleyrodidae, Anthocoridae, Thripidae, Noctuidae, Chrysopidae, Agromyzidae, Syrphidae, Coccinellidae, Aphididae, and Lygaeidae. In total, 35 species of insects were identified, of which 18 and 17 were entomophagous and phytophagous, respectively. The most abundant species were sweetpotato whitefly, *Bemisia tabaci* (Gennadius); minute pirate bug, *Orius tristicolor* (White); onion thrips, *Thrips tabaci* Lindeman; western flower thrips, *Frankliniella occidentalis* (Pergande); beet armyworm, *Spodoptera exigua* (Hübner); pirate bug, *Orius laevigatus* (Fieber); serpentine leafminer, *Liriomyza trifolii* (Burgess); *Allograpta obliqua*; convergent lady beetle, *Hippodamia convergens* Guérin-Méneville; and green lacewing, *Chrysoperla carnea* (Stephens). The total numbers of entomophagous and phytophagous insects were similar in both types of cotton. Only bollworm, *Helicoverpa zea* (Boddie), and beet armyworm were more abundant in non-Bt than in Bt cotton. The 33 other species were similar in abundance in both types of cotton. Based on Margalef, Simpson and Shannon-Wiener indices, insect diversity did not different between Bt and non-Bt cottons. Minute pirate bug, *O. laevigatus*, and to some extent insidious flower bug, *Orius insidiosus* (Say), might be species to use for risk assessment and management of

[1]Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México.
[2]Facultad de Agricultura y Zootecnia/Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México.
[3]Facultad de Agricultura y Zootecnia, Universidad Juárez del Estado de Durango, Gómez Palacio, Durango, México. luis_garher@hotmail.com
[4]Dirección de Recursos Naturales, Instituto Tecnológico de Sonora, 5 de Febrero 818 Sur, Colonia Centro Cd., Obregón, C.P. 85000, Sonora, México.
[5]The University of New Mexico.
*Author of correspondence.

Bt cotton in the region, because of their abundance and presence during most of the cotton-growing season and in adjacent crops.

Resumen. Desde la introducción del algodonero genéticamente modificado que expresa toxinas Cry de la bacteria *Bacillus thuringiensis* (algodonero Bt o transgénico) a México en 1996, el uso de insecticidas en ese cultivo se ha reducido y el rendimiento se ha incrementado significativamente. A pesar de estas ventajas, se han señalado posibles efectos negativos ambientales por su siembra, como efectos negativos de las proteínas Cry en los organismos no blanco. Se realizó el presente estudio con el objetivo de determinar la diversidad entomofaunística en algodonero no Bt y Bt en la Comarca Lagunera. Los insectos colectados en ambos tipos de algodonero pertenecieron a los órdenes Hemiptera, Thysanoptera, Neuroptera, Coleoptera, Diptera, Lepidoptera, e Hymenoptera. El orden Hemiptera fue el más abundante, seguido por Thysanoptera y Diptera. Los insectos pertenecieron a 40 familias, siendo las más abundantes Aleyrodidae, Anthocoridae, Thripidae, Noctuidae, Chrysopidae, Agromyzidae, Syrphidae, Coccinellidae, Aphididae, y Lygaeidae. Se identificaron 35 especies, de las cuales 18 y 17 fueron entomófagas y fitófagas. Las especies más abundantes fueron la mosca blanca *Bemisia tabaci* (Gennadius); la chinche pirata, *Orius tristicolor* (White); el trips de la cebolla, *Thrips tabaci* Lindeman; el trips de las flores, *Frankliniella occidentalis* (Pergande); el gusano soldado, *Spodoptera exigua* (Hübner); la chinche pirata, *Orius laevigatus* (Fieber); el minador de la hoja, *Liriomyza trifolii* (Burgess); *Allograpta obliqua*; la catarinita convergente, *Hippodamia convergens* Guérin-Méneville; y la crisopa verde, *Chrysoperla carnea* (Stephens). El número total de insectos entomófagos y fitófagos fue similar en ambos tipos de algodonero. Solo el gusano bellotero *Helicoverpa zea* (Boddie), y el gusano soldado fueron más abundantes en algodonero no Bt. Las 33 especies restantes presentaron una abundancia similar en ambos tipos de algodonero. Con base en los valores de los índices de diversidad de Margalef y Shannon-Wiener se encontró que la diversidad de insectos no fue diferente entre algodonero convencional y Bt. *Orius laevigatus*, *O. tristicolor,* y en algún grado *O. insidiosus* pueden ser las especies a utilizar en la evaluación y manejo de riesgos por la introducción de algodonero Bt en una región, debido a su abundancia y presencia durante la mayor parte del ciclo de desarrollo del algodonero, así como su presencia en cultivos adyacentes.

Introduction

La Comarca Lagunera (also named La Laguna) was a well-known cotton-producing area for more than two centuries. It was one of the first places where cotton cultivars expressing *Bacillus thuringiensis* Berliner proteins (Bt cotton) were planted in 1997. Since then, the agricultural technology has been adopted in 96% of the planted area (Nava et al. 2001, Traxler et al. 2003, Martínez 2004, Gutiérrez et al. 2015, Rocha-Munive et al. 2018). The main purpose for planting Bt cotton was control of pink bollworm, *Pectinophora gossypiella* Saunders; and tobacco budworm, *Chloridea virescens* (Fabricus), pests now almost absent from the region, and for moderate control of bollworm, *Helicoverpa zea* (Boddie); and beet armyworm, *Spodoptera exigua* (Hübner). However, other important pests such as boll weevil, *Anthonomus grandis* Boheman; conchuela, *Chlorochroa ligata* (Say); and sweetpotato whitefly, *Bemisia tabaci* (Gennadius) biotype B (= *B. argentifolii*

Bellows & Perring) for which Bt cotton cultivars are not effective, are common at La Laguna (Nava et al. 2001).

Adoption of Bt cotton (more than 90% since 2010) reduced use of insecticide ~66% on 15,000 ha of cotton at La Laguna (Rocha-Munive et al. 2018, Nava-Camberos unpublished). Besides reduction, insecticides less toxic to non-target animals have been used since ~2010, which might have had less detrimental effects on non-target species in cotton fields (Nava-Camberos unpublished). Cotton production in the region presents a unique opportunity to evaluate current differences in insect diversity and abundance after the transformation that Bt cotton brought in cultivation of the crop. This intensively managed area that annually received as many as 20 applications of broad-spectrum insecticide on conventional cotton a few years ago (Rocha-Munive et al. 2018, Nava-Camberos unpublished) is an agricultural mosaic of maize, *Zea mays* L.; sorghum, *Sorghum bicolor* (L.) Moench; alfalfa, *Medicago sativa* L.; cantaloupes, *Cucumis melo* L.; watermelon, *Citrullus lanatus* (Thunb.) Matsum. and Makai; and pepper, *Capsicum annuum* L., fields infested by common pests such as bollworm, tobacco budworm, beet armyworm, sweetpotato whitefly, conchuela, cotton aphid, *Aphis gossypii* Glover, onion thrips, *Thrips tabaci* Lindeman, and western flower thrips, *Frankliniella occidentalis* (Pergande), and common natural enemies.

Materials and Methods

Four commercial conventional, and four commercial Bt cotton fields were sampled in 2016 at La Comarca Lagunera (Table 1), a region in parts of Coahuila and Durango states of Mexico. Insects were obtained with 20 sweeps of a net at five random locations in each field on 30 June-4 July, 18-22 July, 4-8 August, and 25-27 August. Insects collected were bagged, sorted, and identified at the Facultad de Ciencias Biológicas de la Universidad Juárez del Estado de Durango by using taxonomic guides (Domínguez 1999a,b,c, Triplehorn et al. 2005; Bahena 2008; Nájera and Souza 2010; Bohmfalk et al. 2011; Catchot et al. 2014).

Table 1. Commercial Cotton Fields at Comarca Lagunera, Mexico, Where Samples of Insects Were Obtained in 2016

Field	Cotton type	Variety	Area (ha)	Planting date	Insecticide applications[a]					
					Ag	*Se-Hz*	*Cl*	*Bt*	Tr	Total
P. P. La Esperanza	Non Bt	DP 167	8.0	15 April	8	3	0	4	0	15
	Bt	FM 1740	17.5	15 April	8	0	0	5	0	13
P. P. San Patricio	Non Bt	DP 167	16.5	2-3 April	5	4	1	0	0	10
	Bt	FM 1740	12.7	2-3 April	5	0	1	1	0	7
Ej. Horizonte	Non Bt	FM 989	2.0	18 April	8	1	0	0	1	10
	Bt	FM 1740	10.0	18 April	8	1	0	0	1	10
Ej. Rosas	Non Bt	DP 167, DP 1441	7.5	10 April	5	5	0	0	0	10
	Bt	DP 912	7.5	10 April	6	0	1	0	0	7

[a]Insecticides: malathion to control *Anthonomus grandis* (*Ag*); monocrotophos, chlorpyrifos, and emamectin benzoate to control *Spodoptera exigua* (*Se*), and *Helicoverpa zea* (*Hz*);, cypermethrin to control *Chlorochroa ligata* (*Cl*); imidacloprid and bifenthrin to control *Bemisia tabaci* (*Bt*); dimethoate to control thrips (Tr).

Margalef species richness index (D_{Mg}) and Shannon-Wiener diversity (H´) tests (Villarreal et al. 2004) of the identified species were done using the program Past 3.20 (Hammer et al. 2001). Numbers of orders, families, and species, their abundance data, and species diversity indices were transformed with the natural logarithm (x+1) and analyzed by a two-factorial completely randomized analysis of variance, considering cotton types and sample dates as factors and cotton fields as replications (Ott 1988). The least significant difference (LSD) test ($P < 0.05$) was used to compare means (SAS Institute 2002).

Results and Discussion

Diversity of Insect Orders. Insects of the orders Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Neuroptera, and Thysanoptera were found. Hemiptera, including an abundant number of predators was the most abundant order -- 76.7% in conventional cotton and 87.4% in Bt cotton, followed by 7.8 and 5.2% Thysanoptera, and 6.2 and 2.6% Diptera. Hemipterans and neuropterans were more abundant at the end of the sampling period, and the opposite trend – less abundance – was observed for the other five orders on those sampling dates (Fig. 1). The number of insect orders did not differ significantly between the types of cotton, and neither did the total numbers of insects. Although Hemiptera were more abundant at the end of the sampling period, particularly on Bt cotton, it was not significantly different from conventional cotton. Lepidoptera were more abundant in conventional cotton fields ($F = 4.79$, df = 1, $P = 0.04$), but no significant differences in abundance were found with the other orders ($F = 0.17$-0.79, df = 1, $P = 0.38$-0.68).

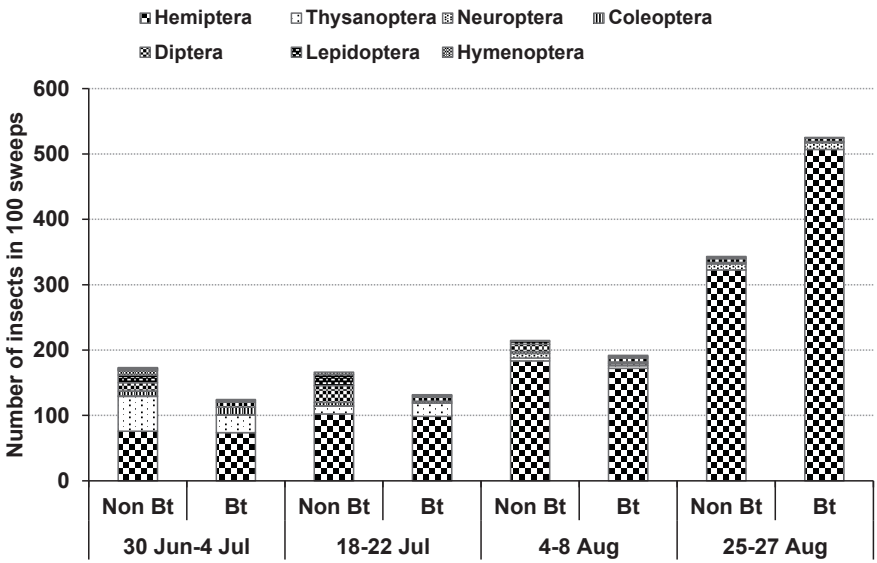

Fig. 1. Abundance of insect orders in conventional and Bt cotton during 2016 in the Comarca Lagunera, Mexico.

Diversity of Insect Families. The entire collection of insects were in a total of 40 families, of which 38 occurred in conventional and 35 in Bt cotton fields. Families were Hemiptera: Aleyrodidae, Anthocoridae, Aphididae, Aradidae, Cicadellidae, Lygaeidae, Miridae, Nabidae, Pentatomidae, and Reduviidae; Coleoptera: Carabidae, Chrysomelidae, Coccinellidae, Curculionidae, Latridiidae, Melyridae, Scarabaeidae, and Tenebrionidae; Diptera: Agromyzidae, Asteiidae, Culicidae, Muscidae, Piophilidae, Sciaridae, Syrphidae, Tachinidae, and Tephritidae; Hymenoptera: Aphelinidae, Apidae, Brachonidae, Encyrtidae, Eulophidae, Platygastridae, Pteromalidae, Scelionidae, Torymidae, and Vespidae; Thysanoptera: Thripidae; Neuroptera: Chrysopidae, and Lepidoptera: Noctuidae.

The 10 most abundant families in conventional and Bt cotton fields were: Aleyrodidae (61.7 and 73.5%), Anthocoridae (11.8 and 9.6%), Thripidae (7.8 and 5.2%), Noctuidae (3.9 and 0.6%), Chrysopidae (2.0 and 1.5%), Agromyzidae (1.7 and 1.2%), Syrphidae (1.4 and 0.4%), Coccinellidae (0.6 and 0.7%), Aphididae (0.2 and 0.9%), and Lygaeidae (0.1 and 1.0%) (Fig. 2). The 10 families comprised 91.2 and 94.6% of all the insects collected from conventional and Bt cotton fields. Few insects (4.1 and 3.2%) were obtained from the other 30 families. Key families differed in abundance. For example, insects belonging to Aleyrodidae and Chrysopidae became more abundant at the end of the sampling period, while the other families, especially Noctuidae and Thripidae pests, showed the opposite trend in abundance. The number of insect families did not differ significantly between cotton types at the four sampling dates. Whiteflies (Aleyrodidae) particularly were apparently but not significantly more abundant on Bt cotton, especially at the end of the sampling period. Only bollworms and beet armyworms (Noctuidae) were significantly more abundant in conventional cotton fields (F = 4.79, df = 1, P = 0.04). Abundance of the other insect families did not differ between the two types of cottons (F = 0-3.26, df = 1, P = 0.1-1.0).

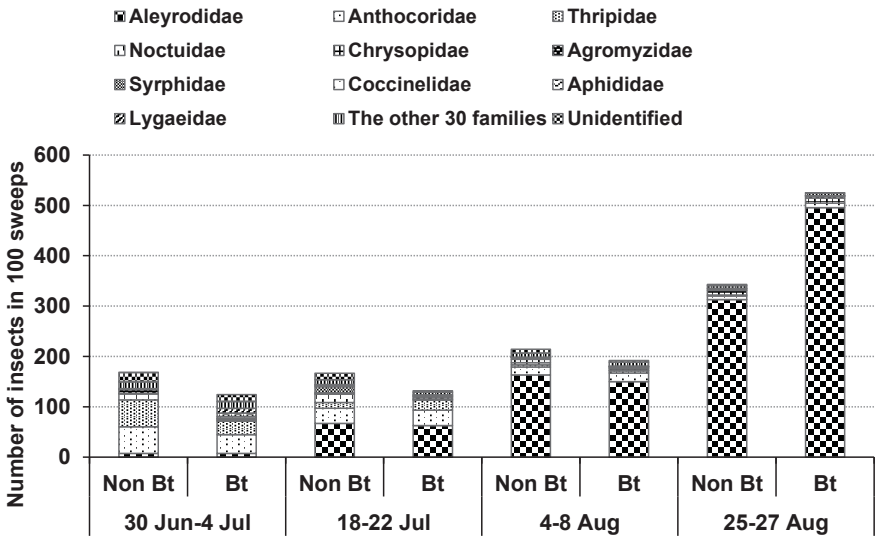

Fig. 2. Abundance of insect families in conventional and Bt cotton during 2016 in the Comarca Lagunera, Mexico.

Diversity of Insect Genera and Species. Of the 35 insect species identified, 18 were entomophagous (beneficials, predators, and parasitoids) and 17 were phytophagous (pests). The number of beneficial insect and pest species as well as abundance were not significantly different between the types of cotton. Ten were the most abundant species in conventional and Bt cotton fields: the pests sweetpotato whitefly (67.4 and 75.2%), onion thrips (4.4 and 3.2%), western flower thrips (4.1 and 3.1%), beet armyworm (3.2 and 0.4%), American serpentine leafminer (1.3 and 1.2%) and the predators minute pirate bug (10.8 and 9.2%), *O. laevigatus* (1.7 and 1.9%), *A. obliqua* (1.5 and 0.4%), convergent lady beetle (0.7 and 0.8%), and green lacewing, *Chrysoperla carnea* (Stephens) (0.5 and 0.7%), totaling 95.5 and 96.0% of all insect species collected in conventional and Bt cotton fields. The other 25 species comprised 4.5 and 4.0% of the species collected in conventional and Bt cotton fields (Tables 2 and 3). Numbers of the most abundant pest, sweetpotato whitefly were not significantly different between cotton types, but other abundant pests such as bollworm and beet armyworm, only partially controlled by Bt cotton, were significantly more abundant in conventional cotton fields (F = 4.24, df = 1, P = 0.05; F = 4.44, df = 1, P = 0.046). Abundance of the other 14 pests (F = 0-1.93, df = 1, P = 0.18-1.00) and 18 beneficial insect species (F = 0-1.00, df = 1, P = 0.08-1.00) did not differ significantly between the two types of cottons. All the insect species were abundant in other important crops that grew simultaneously or not with cotton at La Laguna.

Table 2. Abundance of Entomophagous Insect Species in Conventional and Bt Cotton During 2016 in the Comarca Lagunera, Mexico

	30 Jun-4 Jul		18-22 Jul		4-8 Aug		25-27 Aug		Average	
Species (Genus)	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt
Orius leavigatus	3.8[a]	2.3	1.8	6.3	2.0	2.8	1.0	0.5	2.1	2.9
O. tristicolor	27.3	21.0	16.8	24.0	7.8	7.5	3.5	5.0	13.8	14.4
O. insidiosus	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.1
Zelus sp.	0.3	0.3	0.0	0.0	1.0	0.0	0.8	0.3	0.5	0.1
Nabis sp.	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.1	0.1
Chrysoperla carnea	0.5	0.5	0.3	0.5	1.3	0.5	0.5	2.8	0.6	1.1
C. comanche	0.0	0.0	0.3	0.0	0.0	0.0	3.0	1.5	0.8	0.4
Chrysoperla sp.	0.0	0.0	0.0	0.0	2.8	0.8	1.0	0.8	0.9	0.4
C. nigricornis	0.0	0.0	0.0	0.0	0.3	0.3	1.3	1.3	0.4	0.4
Hippodmia convergens	0.8	2.0	1.8	2.0	0.3	0.5	0.8	0.5	0.9	1.3
Collops vitatus	1.3	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.4	0.1
C. quadrimaculatus	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
A. obliqua	0.0	0.8	7.0	1.3	0.5	0.5	0.0	0.3	1.9	0.7
Coccophagus sp.	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Cotesia sp.	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Trissolcus sp.	0.0	0.0	0.3	0.0	0.0	0.3	0.0	0.0	0.1	0.1
Bracon sp.	0.0	0.0	0.0	0.0	0.3	0.5	0.8	0.0	0.3	0.1
Chelonus sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1
Total	34.0	28.0	28.5	34.3	16.3	14.0	12.5	13.0	22.8	22.3

[a]Values are number of insects in 100 sweeps.

Table 3. Abundance of Phytophagous Insect Species in Conventional and Bt Cotton During 2016 in the Comarca Lagunera, Mexico

	30 Jun-4 Jul		18-22 Jul		4-8 Aug		25-27 Aug		Average	
Species (Genus)	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt	Non Bt	Bt
B. tabaci	4.5	4.5	42.0	62.8	102.0	93.8	195.5	309.8	86.0	117.7
Nysius sp.	0.3	5.8	0.0	0.0	0.3	0.0	0.0	0.0	0.1	1.4
C. ligata	0.0	0.3	0.0	0.5	0.3	0.0	0.0	0.0	0.1	0.2
A. gossypii	0.3	2.5	0.8	1.0	0.0	0.0	0.0	0.3	0.3	0.9
Lygus sp.	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1
Macrolophus sp.	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1
F. occidentalis	14.8	10.5	3.8	7.0	2.5	2.0	0.0	0.0	5.3	4.9
T. tabaci	18.5	6.3	3.5	13.0	0.3	0.5	0.0	0.0	5.6	4.9
D. undecimpunctata	0.3	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.2	0.0
D. balteata	0.0	0.0	0.0	0.0	1.0	2.3	0.0	0.0	0.3	0.6
A. grandis	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.8	0.1	0.2
L. trifolii	2.5	1.0	1.3	4.0	1.0	1.0	1.8	1.3	1.6	1.8
L. brionidae	0.0	0.0	0.0	0.0	0.0	0.5	0.0	1.0	0.0	0.4
Chaetopsis sp.	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.1
S. exigua	5.3	1.3	7.5	1.0	2.8	0.5	1.0	0.0	4.1	0.7
H. zea	2.0	0.0	2.5	0.8	0.3	0.3	0.3	0.0	1.3	0.3
S. frugiperda	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.1	0.1
Total	48.5	32.0	61.8	91.0	110.3	100.8	198.8	313.0	104.8	134.2

[a]Values are number of insects in 100 sweeps.

A significant increase (F = 13.35, df = 3, P < 0.0001; F = 3.16, df = 3, P = 0.04; F = 3.40, df = 3, P = 0.03) in numbers of sweetpotato whitefly, Comanche lacewing, *Chrysoperla comanche* (Banks), and *Chrysopa nigricornis* Burmeister was noticed at the end of the sampling period, indicating a density-dependent relationship between prey and predators. Numbers of onion thrips and *C. vittatus* decreased significantly (F = 7.87, df = 3, P = 0.0008, and F = 4.86, df = 3, P = 0.009) at the end of the season. Less abundant onion thrips might have had the same effect on abundance of minute pirate bug and *O. laevigatus* because of the density-dependent relationship between prey and predators. Of particular importance was an increase in abundance of sweetpotato whitefly in both types of cottons, forcing growers to apply broad-spectrum insecticide such as malathion, monocrotophos, chlorpyrifos, dimethoate, or cypermethrin, and insecticides described in Table 1 -- all were especially toxic to cotton pests and their predators. Lack of good control of the whitefly species caused a serious problem at La Laguna, because abundance of the pest reduced yields and lowered quality of cotton fiber.

Similar results showing no difference in abundance of cotton insects were reported from the region. Marquez-Hernández et al. (2014) reported much diversity of insects (192 morphospecies of 57 families and 10 orders), of which Hemiptera, Hymenoptera, and Coleoptera were most abundant in Bt cotton fields at La Laguna. Santana-Espinosa et al. (2015) at other cotton-growing regions of Mexico (Baja California, Chihuahua, Coahuila, Durango, and Sonora) found 29 morphospecies of 26 families and nine orders. Elsewhere, Men et al. (2003), and Cattaneo et al. (2006) found more diverse and abundant arthropods on Bt cotton treated with insecticide than in treated conventional cotton. No differences between cottons

were observed in India (Dhillon et al. 2011) or the United States (Sisterson et al. 2004, Naranjo 2005, Whitehouse et al. 2005, Wolfenbarger et al. 2008). Some differences in the studies were attributed to the effect Bt cotton had on Lepidoptera, reducing pest abundance and decreasing the availability of prey for predators.

Other than absolute values such as the number of families or species, the data were analyzed by different indices. The Margalef index of species richness varied between 1.520 and 1.951 in conventional cotton for the different evaluation dates, and from 0.937 to 1.925 in Bt cotton, but was not significantly different between the cotton types. The Shannon-Wiener index of species diversity varied from 0.718 to 1.430 in conventional, and 0.370 to 1.427 in Bt cotton, which was not significantly different between the cotton types, and decreased at the end of the evaluation period ($F = 7.63$, df = 3, $P = 0.0009$).

Of the 18 beneficial insect species in the study, minute pirate bug, *O. laevigatus*, and to some extent insidious flower bug, *Orius insidiosus* (Say), seemed to be indicator species for potential effect of Bt cotton in the predatory guild. The three species were abundant throughout the sampling period, and are easily distinguishable at least to genus (Bohmfalk et al. 2011). The species also are important in adjacent crops such as maize, sorghum, cantaloupe, watermelon, chili peppers, and pecans in the La Laguna region. But, all the crops also were sprayed with broad-spectrum insecticide, have acquired resistance and are present in significant numbers to aid natural control of pests, and allow evaluation of effects of the agricultural biotechnology. It is our opinion that *Orius* spp. would be an ideal species complex for risk analysis and management of genetically-engineered crops.

Acknowledgment

We thank to the Interministerial Commission of Biosafety (CIBIOGEM) of the National Council of Science and Technology (CONACyT), Sol Ortiz García and Laura E. Tovar Castillo, for financing the research project FO-CIBIOGEM 259410 “Comparación del sistema de producción de algodón (G*ossypium hirsutum*), transgénico y convencional, en los siete estados productores de México”.

References Cited

Bahena J., F. 2008. Enemigos naturales de las plagas agrícolas del maíz y otros cultivos. Libro Técnico Núm. 5. INIFAP, Campo Experimental Uruapan. Uruapan, Michoacán, México.

Bohmfalk, G. T., R. E. Frisbie, W. L. Sterling, R. B. Metzer, and A. E. Knutson. 2011. Identification, biology and sampling of cotton insects. http://www.soilcropandmore.info/crops/CottonInformation/insect/B-933/b-933.htm

Catchot, A., S. D. Stewart, J. A. MacGown, J. Gore, D. R. Cook, C. Daves, G. Andrews, F. Musser, S. Akin, G. Lorenz, R. B. Leonard, R. Jackson, C. Allen, J. Smith, L. N. Owen, and D. Dodds. 2014. Cotton Insect Identification Guide. P1640. Mississippi State University, Extension Service. https://mississippientomologicalmuseum.org.msstate.edu/museumpersonnel./Joes.published.drawings/CottonPests.pdf

Cattaneo, M. G., C. Yafuso, C. Schmidt, C-y. Huang, M. Rahman, C. Olson, C. Ellers-Kirk, B. J. Orr, S. E. Marsh, L. Antilla, P. Dutilleul, and Y. Carrière. 2006. Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield. PNAS 103: 7571-7576.

Dhillon, M. K., G. T. Gujar, and V. Kalia. 2011. Impact of Bt cotton on insect biodiversity in cotton ecosystem in India. Pak. Entomol. 33: 161-165.

Domínguez R., R. 1999a. Taxonomía 1, Protura a Homoptera, claves y diagnosis. UACH, Parasitología Agrícola. Chapingo, México.

Domínguez R., R. 1999b. Taxonomía 2, Neuroptera a Coleoptera, claves y diagnosis. UACH, Parasitología Agrícola. Chapingo, México.

Domínguez R., R. 1999c. Taxonomía 3, Stresiptera a Hymenoptera, claves y diagnosis. UACH, Parasitología Agrícola. Chapingo, México.

Gutiérrez G., D. F., R. Ruiz M., y B. Xonocostle C. 2015. Estado Actual de los Cultivos Genéticamente Modificados en México y su Contexto Internacional. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional. México, DF. http://www.conacyt.mx/cibiogem/images/cibiogem/comunicacion/publicaciones/Estado-actual-de-los-cultivos.pdf).

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4: 9. https://folk.uio.no/ohammer/past/

Márquez-Hernández, C., S. Santana, V. Ávila, J. L. García, P. Preciado, and A. Moreno. 2014. Entomofaunistic diversity in a transgenic cotton (*Gossypium hirsutum* L.) agroecosystem in Coahuila, Mexico. Southwest. Entomol. 39: 317-326.

Martínez C., J. L. 2004. Evolución del algodón transgénico en México. VII Congreso Internacional de en Ciencias Agrícolas. Universidad Autónoma de Baja California. Mexicali, Baja California.

Men, X., F. Ge, X. Liu, and E. N. Yardim. 2003. Diversity of arthropod communities in transgenic Bt cotton and nontransgenic cotton agroecosystems. Environ. Entomol. 32: 270-275.

Nájera R., M. B., and B. Souza. 2010. Insectos benéficos, guía para su identificación. INIFAP, Campo Experimental Uruapan. Uruapan, Michoacán, México. https://docs.google.com/viewerng/viewer?url=https://www.socla.co/wp-content/uploads/2014/INSECTOS_BENEFICOSNajeraySouza.pdf.

Naranjo, S. E. 2005. Long-term assessment of the effects of transgenic *Bt* cotton on the abundance of nontarget arthropod natural enemies. Environ. Entomol. 34: 1193-1210.

Nava C., U., M. Ramírez D., y J. L. Martínez C. 2001. Manejo integrado de plagas del algodonero convencional y transgénico, pp. 51-92. *En* D. Teliz [ed.], El Manejo Integrado de Plagas. Simposio. Congreso Anual Sociedad Mexicana de Entomología (XXXVI) y Sociedad Mexicana de Fitopatología (XXVIII). Querétaro, México.

Ott, L. 1988. An Introduction to Statistical Methods and Data Analysis. 3rd ed. PWS-Kent Publishing Co., Boston, MA.

Rocha-Munive, M. G., L. E. Eguiarte, M. Soberón, S. Castañeda, E. Niaves-Nava, E. Scheinvar, D. Mota-Sanchez, E. Rosales-Robles, U. Nava-Camberos, C. A Blanco, J. L. Martinez-Carrillo, A. Bravo, and V. Souza. 2018. Evaluation of the impact of genetically modified cotton after 20 years of cultivation in Mexico. Front. Bioeng. Biotechnol. 6: 82.

Santana-Espinoza, S., V. Ávila-Rodríguez, G. Castañeda-Gaytan, E. de La Cruz-Lázaro, C. García-de La Peña, U. Romero-Méndez, and C. Márquez-Hernández. 2015. Entomofauna presente en algodonero (*Gossypium hirsutum* L.) genéticamente modificado en zonas productoras de México. Southwest. Entomol. 40: 151-160.

SAS Institute. 2002. SAS User´s Guide. Version 9.1. SAS Institute, Inc. Cary, NC.

Sisterson, M. S., R. W. Biggs, C. Olson, Y. Carrière, T. J. Dennehy, and B. E. Tabashnik. 2004. Arthropod abundance and diversity in Bt and non-Bt cotton fields. Environ. Entomol. 33: 921-929.

Traxler, G., S. Godoy-Avila., J. Falck-Zepeda, and J. de J. Espinoza-Arellano. 2003. Transgenic Cotton in Mexico: economic and environmental impacts, pp. 183-202. *In* N. Kalaitzandonakes [ed.]. The Economics and Environmental Impacts of Agbiotech. Springer, NY. https://www.researchgate.net/publication/ 228584807_Transgenic_Cotton_in_Mexico_Economic_and_Environmental_Impacts.

Triplehorn, C. A., N. F. Johnson, and D. J. Borror. 2005. Introduction to the Study of Insects. 7th ed. Thompson Brooks/Cole, Belmont, CA.

Villarreal, H., M. Álvarez, S. Córdoba, F. Escobar, G. Fagua, F. Gast, H. Mendoza, M. Ospina, y A. M. Umaña. 2004. Manual de Métodos para el Desarrollo de Inventarios de Biodiversidad. Programa de Inventarios de Biodiversidad. Instituto de Investigación de Recursos Biológicos Alexander von Humboldt. Bogotá, Colombia. https://www.zin.ru/Animalia/Coleoptera/pdf/villareal_et_al_ 2004.pdf

Whitehouse, M. E. A., L. J. Wilson, and G. P. Fitt. 2005. A comparison of arthropod communities in transgenic *Bt* and conventional cotton in Australia. Environ. Entomol. 34: 1224-1241.

Wolfenbarger, L. L., S. E. Naranjo, J. G. Lundgren, R. J. Bitzer, and L.S. Watrud. 2008. Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLoS 3: e2118.

The Compromise of Intercropping: Biological Pest Control versus Competition by Crop Species

Jesús Santillano-Cázares[1], Aurelia Mendoza-Gómez[2],
Juan Carlos Vázquez-Angulo[2], Ernesto Medina-Espinoza[3],
Carlos Enrique Ail-Catzim[1], and Fidel Núñez-Ramírez[1]*

Abstract. Leek, *Allium ampeloprasum* L., is susceptible to attack by onion thrips, *Thrips tabaci* Lindeman, that reduce yield and quality. Intercropping has been proposed as a strategy for reducing negative effects of pests on one or all crops in a system. The objective of the study was to assess the effect of intercropping maize, *Zea mays* L., and leek on abundance of thrips and production and morphology of both crops, but emphasizing effects on leek. The experiment was done during spring 2016 in northwestern Mexico, under extreme climatic conditions characteristic of desert environments and high soil salinity. Two maize varieties (sweet and field) at zero, three, five, and 10 plants per square meter were intercropped with a fixed number of 10 leek plants per square meter. The response variables were thrips abundance and plant yields and morphology characteristics. Maize reduced abundance of thrips on leek, with 5.6, 2.6, 2.2, and 1.5 thrips for 0, 3, 5, and 10 maize plants m^{-2}, respectively; i.e., the closer together the maize plants, the less abundant were thrips on leek, but leek yields were less with more maize plants (plant competition) per meter, with 12,075, 4,875, 4,581, and 4,159 kg ha^{-1}, respectively. Leeks intercropped showed morphological adjustments compared with a leek monoculture, indicating negative effects of competition with maize, instead of benefiting from the microenvironment (cooler leek canopy temperatures) created by maize. Maize was effective at reducing abundance of thrips but severely out-competed leeks. Further testing is needed on less competitive secondary crop species in main crops in a region to reduce pest abundance naturally without decreasing yield potential of associated crops.

Introduction

Because wheat, *Triticum aestivum* L., the main crop in the Valley of Mexicali, Baja California, Mexico, is faced with issues of decreasing profitability and production, local government is advising farmers to change, or at least, diversify their crops (through local newspapers). Production of vegetables is slowly gaining popularity among farmers in this part of Mexico. Leek, *Allium ampeloprasum* var.

[1]Instituto de Ciencias Agrícolas, Universidad Autónoma de Baja California, Ejido Nuevo León, Mexicali, B. C.-210705, México.
[2]Facultad de Ingeniería y Negocios San Quintín, Universidad Autónoma de Baja California (ICA-UABC), Carretera Transpeninsular Km. 180.2, Ejido Padre Kino, San Quintín, Baja California, México.
[3]Universidad Veracruzana. Facultad de Ciencias Biológicas y Agropecuarias, Carretera Tuxpan-Tampico Km. 7,5 Col. Universitaria C.P. 92850, Tuxpan, Veracruz, México.

porrum L., is becoming a popular crop in Baja California. According to the Secretariat of Agriculture, Livestock, Rural Development, Fisheries and Food in Mexico (SAGARPA), almost 10,000 tons of leeks valued at more than $6 million were produced in 2013, with the State of Baja California producing 70% of the total. Leek, in the same family as onions (*Allium cepa* L.) and garlic (*Allium sativum* L.) (Gorai et al. 2016), has long been known for its taste. Recently, benefits of leeks for human health have been documented. Najda et al. (2016) reported that amino acids, secondary metabolites, and antioxidant compounds were greater in leek than in garlic.

Leek and other members of the genus *Allium* are susceptible to pests, especially onion thrips, *Thrips tabaci* Lindemann, that reduce yield and quality (Theunissen and Schelling 1997, Kucharczyk and Legutowska 2002, Bosco and Tavella 2010). According to Theunissen and Schelling (1997) and Gombač and Trdan (2014), onion thrips are difficult to control with insecticide because the insect is physically protected during its life cycle; an integrated approach for pest management is advised. According to Andrews and Kassam (1976), intercropping involves growing two or more crops at the same time and place and was proposed as a management practice for reducing negative effects of pests on one or all crops growing together. Åsman et al. (2001) hypothesized that intercropping could help reduce pest damage to a crop by inducing plant diversity, thus resulting in less attack by pests than in monocultures. However, Begg et al. (2017) said research results on effectiveness of intercropping to control pests have been inconclusive.

Maize, *Zea mays* L., is a common component in most intercropping systems (Seran and Brintha 2010), and is attacked by thrips, as is leek. Maize is the primary crop in intercropping studies; i.e., other crops have been included in intercropping systems to reduce insect damage to maize (Manandhar and Wright 2016, Bereś et al. 2017). In contrast, maize has been included in intercropping systems worldwide as a crop to assess its potential for reducing pest damage to associated primary crops. In this research, leek was the primary (or target) crop, and maize was the secondary crop. Sweet maize (*Zea mays* var. *saccharata* [Sturtev] L.H. Bailey) and field maize (*Zea mays* L.) might respond differently to thrips and associated crops in an intercropping system, as shown with biological control of European corn borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae), by egg parasitoids of *Trichogramma* spp. (Wright et al. 2002). Other differences between sweet and field maize that might help (or damage) leeks in an intercropping system might be leaf architecture (upright versus horizontal) reported by Begna et al. (2001) and Sankula et al. (2004), plant height and associated shading effects (Seran and Brintha 2010), and growth rates of all plant species in an intercropping system (Mushagalusa et al. 2008, Seran and Brintha 2010, Huang et al. 2017).

El-Fakharany et al. (2012) studied effects on abundance of pests and associated predators on sugar beets, *Beta vulgaris* L., when intercropped with maize or other crops or using organic or inorganic insecticide. They found the rate of infestation was greater in sugar beet plants alone than in those intercropped with faba bean, *Vicia faba* L.; maize; or cabbage, *Brassica oleracea* L. El-Fakharany et al. also found that intercropping with maize led to more beneficial (predators of insect pests) *Chrysoperla carnea* (Stephens), *Paederus alfierii* Koch, and *Scymnus* spp. Pitan and Filani (2014) investigated the effectiveness of intercropping maize at different times in reducing abundance of four insect pests on two varieties of cucumber, *Cucumis sativus* L. They found that independent of the variety of cucumber, pest abundance was significantly (more than 50%) less with a

cucumber-maize intercrop, compared with cucumber alone. Seran and Brintha (2010) cited several research studies where intercropped maize helped reduce pests on associated crops compared to monoculture.

Intercropping exerts microclimatic effects on the crops in the system (Azam-Ali et al. 1990, Jiao et al. 2008), and outcome in terms of crop productivity depends on the degree of competition/cooperation from species interaction. Williams and Gordon (1995) found that differences in crop development affected the environment of intercropped species in many ways; differences in crop height resulted in wind patterns among crops, affecting air temperature and vapor pressure deficit in summer. Differences in crop height also had minor effects on light in summer, but differences in light levels among the crops affected soil temperature later in the growing season. Using a prediction model, Knörzer et al. (2011) studied competitive relationships in a wheat-maize intercropped system with regard to solar radiation and microclimatic effects, and found wheat border rows were exposed to faster wind speed until mid-June and slower wind speed thereafter. As a result, solar radiation, soil temperature, and wind speed differed between single crops and intercropping, thus influencing crop yields. Intercropped maize yielded as much as a monoculture of maize, and grain yield of intercropped wheat increased as much as 32%. Yang et al. (2014) said intercropping could modify the morphology of species in systems, indicating that intercropping maize-soybean (*Glycine max* L.) could induce changes in microclimate in a crop canopy. Stem diameter, root length, aboveground biomass, total root biomass, and root-shoot ratio of intercropped soybean were significantly less, while seedling height increased. To our knowledge, no research has been published about intercropping maize with leek. The objective of the study was to assess the effect of intercropping maize and leek on thrips abundance and crop morphology and yield, emphasizing effects on leek.

Materials and Methods

The experiment was done in spring 2016 at the Instituto de Ciencias Agricolas, Universidad Autonoma de Baja California, at Ejido Nuevo Leon, Mexicali, BC, Mexico (latitude 32° 24’ 26.38” N, longitude 115° 11’ 55.43” O, elevation 14 m above sea level). The soil at the site was aquic haplotorrert smectitic, hyperthermic, haplic vertisol (calcaric, endogleyic) with 7.8 pH. The climate is desertic, warm, and arid, with scarce precipitation in winter (BW [h’] hs [x´] [e’]) (García, 1988). Temperatures are extreme, with mean annual 22.3°C temperature, ranging from 50°C during the summer to -7°C during the winter. Mean annual precipitation is only 58 mm. Minimum and maximum temperatures during the experiment were recorded at a weather station 380 m from the site (Fig. 1).

Seedling ‘Avigator’ leeks were transplanted at 100,000 plants per hectare on 15 February 2016. Sweet maize (yellow) (XR20A Ceres®) or field maize (white) (XR66 Ceres®) were seeded on the same date the leek was transplanted. Planting was in raised beds 0.8 m wide and 1.0 m apart, with polyethylene drip tapes with drippers every 20 cm (4.1 liters per meter per hour). Irrigation was applied based on availability of soil water when soil water tension reached 25 kPa, as indicated by tensiometers 30 cm deep in the soil. The tensiometers were distributed across the entire experimental area. The plants were fertilized with a total of 220 kg N ha^{-1} of nitrogen as urea and 22 kg ha^{-1} of phosphorus as phosphoric acid. Fertilizers were split applied through an irrigation system weekly throughout the duration of the

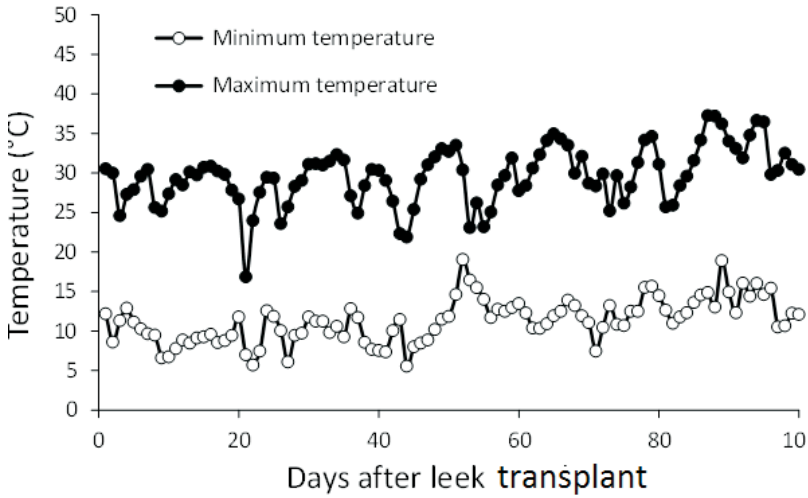

Fig. 1. Minimum and maximum temperatures at the experimental site where the effects of intercropping maize with leek were assessed in 2016 in the Valley of Mexicali, Baja California, Mexico.

experiment. On 12 April, dimethoate at 600 ml per hectare was applied to the entire experimental area.

The treatment was a two-by-four factorial, arranged in a randomized complete block design, with four replications. One factor consisted of two maize types (sweet and field), and the other factor was four maize densities (zero, three, five, and 10 plants per square meter) intercropped with leek. The number of leek plants per area was constant at 10, independent of the number of maize plants.

Variables in the study were the number of thrips, yields, plant morphology characteristics, and microclimate in both leek and maize intercropped. Thrips on leek and maize were counted on the most recently mature leaf at approximately 0800 and 1000 hours on four sampling dates 57, 65, 79, and 93 days after transplanting. When leek was mature enough to harvest for commercial sale (25 May), yields were determined from the two central beds and expressed as fresh weigh per area. Leek plant height, diameter, and number of leaves were determined 23, 44, 58, 72, and 85 days after transplanting. Morphology was measured in all treatments with maize (except zero plants) at 44, 58, 72, and 85 days after maize was planted. The temperature of the most recently mature leek leaf was measured by a digital thermohygrometer (CEM DT-172 model, Twin Light Instruments, Monterrey, México) 62, 78, and 92 days after transplanting.

Despite all response variables measured in the study, varying number of times in the growing cycles of both crops intercropped, data were pooled and means of all sampling dates or only final measurements were analyzed. The approach was used because results varied little among sampling dates. Numbers of thrips and leaf temperatures were analyzed for each sampling date. Yields and plant morphology variables (height, diameter, and leaf number) were analyzed using data from only the final sampling date. When differences were detected by analysis of variance (ANOVA), a least significant difference (LSD) test at ≤0.05 was used to compare differences among means.

Results

The only significant ($P < 0.0001$) factor affecting thrips abundance on the most recently mature leek leaf was maize plant density; thrips were more abundant when maize plants were farther apart (Table 1). The mean number of thrips was 0.68 on the most recently mature maize leaf, and thrips on maize were not affected by any source of variation ($P > 0.05$).

Interaction of maize type × maize plant density was not significant ($P = 0.320$), but leek yields were significantly ($P < 0.0001$) influenced by density of maize plants. Leek yield was greater when maize plants were farther apart (Table 1). No statistical difference ($P > 0.050$) was found among treatments when excluding the zero maize pant density from the comparison. A trend ($P = 0.083$) was found for maize type, with leek yields of 7,008 kg ha^{-1} when intercropped with sweet maize and 5,838 kg ha^{-1} when intercropped with field maize (Fig. 2). No maize grain yields were recorded, regardless of the treatment. A lack of maize production was explained mostly by soil salinity, with electrical conductivity as much as 16 dS/m.

Table 1. Abundance of Thrips on Leek and Maize Plants and Leek Yield Affected by Maize Type and Density in Leek-maize Intercropped in Spring 2016 in the Valley of Mexicali, Baja California, Mexico

Maize densities (plants m^{-2})	Leek thrips number leaf^{-1}		Maize thrips number leaf^{-1}		Leek yields†	
0	5.6	a‡	-		12075	a
3	2.6	b	0.7		4875	b
5	2.2	bc	0.7		4581	b
10	1.5	c	0.6		4159	b
n	*20*		*20*		*8*	
Mean	*3.0*		*0.7*		*6423*	
LSD	*1.0*		*0.3*		*1892*	

†kg ha^{-1} fresh weight

‡In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test

A significant interaction ($P < 0.0001$) of maize type × maize plant density was recorded for the final height of leek plants. The interaction occurred because at zero maize plants, leek heights were similar between maize types, while at greatest densities, leek intercropped with sweet maize was taller than when intercropped with field maize (Fig. 2a). A decreasing trend in leek plant height was observed as maize density increased. Similarly, the final maize plant height was significantly ($P = 0.048$) affected by the interaction of maize type × plant density. Interaction occurred because with three plants per square meter, maize plant density and height were similar across both maize types, while at five or 10 maize plants, sweet maize grew taller than field maize (Fig. 2b).

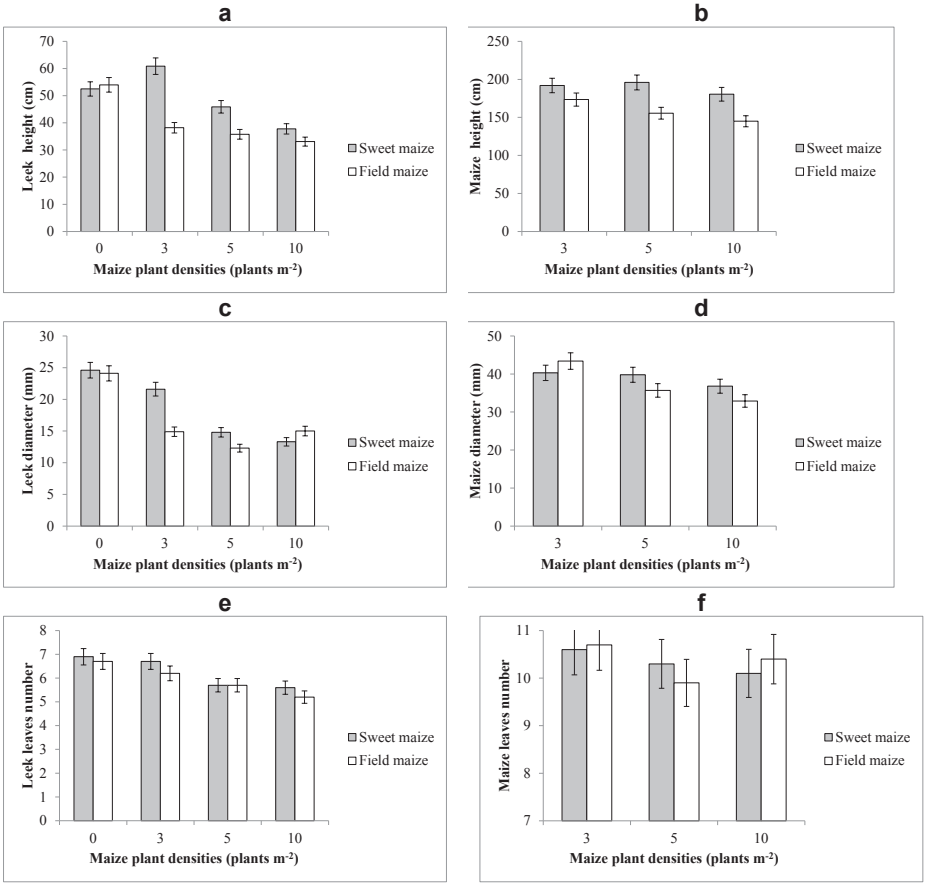

Fig. 2. Leek and maize height (a and b), diameter (c and d), and leaves (e and f), as affected by maize type and plant density in leek-maize intercropped during spring 2016 in the Valley of Mexicali, Baja California, Mexico.

The final diameter of leek stems was significantly ($P < 0.0001$) influenced by the maize type × maize plant density interaction. Interaction is explained by zero and 10 maize plant densities, with similar leek diameters across both maize types, while at three and five maize plants, the diameter of leek stems was greater when intercropped with sweet than with field maize (Fig. 2c). The final maize stalk diameter had a very significant ($P < 0.0001$) maize type × maize plant density interaction. Interaction was explained by the similarity of maize stalk diameters for both maize types planted farthest apart (Fig. 2d), unlike what was observed for greater maize densities when sweet maize had larger stalk diameters than did field maize.

Only the final leek leaf number per plant was significantly ($P < 0.0001$) affected by maize plant density. Densities of zero and three maize plants per

square meter resulted in more leek leaves, while densities of five or 10 maize plants per square meter resulted in fewer leek leaves per plant (Fig. 2e). Similarly, maize leaf number per plant was only significantly (P = 0.044) influenced by plant density (Fig. 2f).

The mean temperature of leek leaves was significantly related to maize type (P < 0.0001) and maize plant density (P < 0.0001), but not with maize type × maize plant density interaction (P = 0.137). However, of the two main factors, maize type explained twice the variability in leek mean temperature than did maize density, as found by corresponding mean squares (data not shown). The mean temperature of leek leaves growing under field maize was 23.2°C compared with 19.9°C with sweet maize, while temperatures of leek leaves were 24.6, 21.2, 20.9, and 19.4°C with zero, three, five, and 10 maize plants, respectively.

Discussion

From observed abundance of thrips on the two intercropped plant species, it was clear that maize reduced thrips on leek, because the more maize plants, the less thrips on leek. The findings coincided with those of Theunissen and Schelling (1997) who found the number of thrips per leek plant caused less damage to leek when intercropped with subterranean clover, *Trifolium subterraneum* L., than on leek without intercropping, because of reduction in abundance of thrips. The maize plants might have been able to prevent thrips from increasing in abundance by causing physical and/or chemical confusion and/or by hosting predators. The possibility of maize inducing confusion or repelling thrips from the microenvironment with intercropping coincides with the disruptive theory (Vandermeer 1989, Theunissen and Schelling 1997). The disruptive hypothesis suggested that plant diversity (intercropping) can affect insect abundance by 1) the insect being less likely to find a host plant because of some degree of confusion (physical or chemical), and 2) the insect, after finding a host plant, being more likely to leave a multi-crop system because of frequent encounters with natural enemies living in another host plant species in the ecosystem. According to Shiberu and Mahammed (2014), other intercropping systems proven to significantly reduce abundance of thrips on leek include leek with clover (Den Belder et al. 2000); leek with carrot, *Glaucus carota*; and clover with French bean, (Kucharczyk and Legutowska 2002).

From a production perspective, the problem of achieving less pest abundance on the main crop (crop of greatest economic interest) through intercropping is that the main crop might yield less because of competition with a secondary crop. The effect was observed, because yield increased as maize plant density (plant competition) decreased and, similarly, abundance of thrips increased as maize plant density decreased. There was positive correlation (r = 0.83, P < 0.001) between yield and number of thrips. However, a positive relationship was logically explained only by plant competition. Plant competition in the study might have overridden the effect of abundant thrips on leek yield despite thrips being abundant enough to affect yield. Theunissen and Schelling (1997) estimated economic threshold between five or six thrips larvae per leek plant. Based on this reference, numbers of (adult) thrips in the study (1.5 to 5.6 per leaf, with a mean of six leaves per plant) were a risk to leek production, although thrips did not cause any reduction in the yield of leek.

Several indices have been developed to assess plant competition/facilitation in intercropping systems (Zhang et al. 2011, Doubi et al. 2016, Jalilian et al. 2017, Yang et al. 2017). Land equivalent ratio (LER) is an index of intercropping advantage that indicates the amount of interspecific competition or facilitation in intercropping systems. Calculation of land equivalent ratio takes into account yields of all crops in plant association, both in monoculture and intercropping. Because maize yield was zero in the experiment, the resulting land equivalent ratio (dividing yield of intercropped leeks by yield in a monoculture) for the three maize plant densities was 0.4 or less. According to Jalilian et al. (2017), a land equivalent ratio less than 1.0 indicated interspecific competition instead of interspecific facilitation. Morphological modifications by the leek crop confirm effects of aggressive competition with maize, because leek plant height and stem diameter were similar and greatest with no competition (zero maize plants), decreased slightly with least competition (three maize plants per square meter), and definitely decreased with the greatest amount of competition (five or 10 maize plants per square meter) (Fig. 2), when the competitiveness of maize over leek was more evident. The temperature of leek leaves also confirmed that the greater the competition, the cooler the leek canopy, which could be indicative of restricted photosynthetic rate by less than full photosynthetically active radiation reaching leek leaves. In conclusion, intercropping maize with leek successfully decreased thrips abundance in leek, but maize crop competed too aggressively with the leek crop and drastically reduced yield compared with leek in monoculture. In the future, it would be worthwhile to test a shorter associated crop with the hope of successfully reducing abundance of thrips and avoiding aggressive competition with leek (or other vegetable cash crops) that is increasing in importance in northwestern Mexico, in substitution for currently low profit and extensively grown crops such as wheat.

References Cited

Andrews, D. J., and A. H. Kassam. 1976. The importance of multiple cropping in increasing world food supplies. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America.

Åsman, K., B. Ekbom, and B. Rämert. 2001. Effect of Intercropping on oviposition and emigration behavior of the leek moth (Lepidoptera: Acrolepiidae) and the diamondback moth (Lepidoptera: Plutellidae). Environ Entomol. 288.

Azam-Ali, S. N., R. B. Matthews, J. H. Williams, and J. M. Peacock. 1990. Light use, water uptake and performance of individual components of a sorghum/ groundnut intercrop. Exp Agric. 26: 413-427.

Begg, G. S., S. M. Cook, R. Dye, M. Ferrante, P. Franck, C. Lavigne, G. L. Lövei, A. Mansion-Vaquie, J. K. Pell, S. Petit, et al. 2017. A functional overview of conservation biological control. Crop Prot. 97: 145-158.

Begna, S. H., R. I. Hamilton, L. M. Dwyer, D. W. Stewart, D. Cloutier, L. Assemat, K. Foroutan-Pour, and D. L. Smith. 2001. Weed biomass production response to plant spacing and corn (*Zea mays*) hybrids differing in canopy architecture. Weed Technol. 15: 647-653.

Bereś, P. K., H. Kucharczyk, and D. Górski. 2017. Effects of insecticides used against the European corn borer on thrips abundance on maize. Plant Protection Science 53.

Bosco, L., and L. Tavella. 2010. Population dynamics and integrated pest management of *Thrips tabaci* on leek under field conditions in northwest Italy. Entomol. Exp. Appl. 135: 276-287.

Den Belder, E., J. Elderson, and P. F. G. Vereijken. 2000. Effects of undersown clover on host-plant selection by *Thrips tabaci* adults in leek. Entomol. Exp. Appl. 94: 173-182.

Doubi, B. T. S., K. I. Kouassi, K. L. Kouakou, K. K. Koffi, J.-P. Baudoin, and B. I. A. Zoro. 2016. Existing competitive indices in the intercropping system of *Manihot esculenta* Crantz and *Lagenaria siceraria* (Molina) Standley. J. Plant Interact. 11: 178-185.

El-Fakharany, S. K. M., M. A. Samy, S. A. Ahmed, and M. A. Khattab. 2012. Effect of intercropping of maize, bean, cabbage and toxicants on the population levels of some insect pests and associated predators in sugar beet plantations. J. Basic Appl. Zool. 65: 21-28.

García, E. 1988. Modificaciones al sistema de clasificación climática de Köppen. Instituto de Geografía, UNAM, México.

Gombač, P., and S. Trdan. 2014. The efficacy of intercropping with birdsfoot trefoil and summer savoury in reducing damage inflicted by onion thrips (*Thrips tabaci,* Thysanoptera, Thripidae) on four leek cultivars. J. Plant Dis. Protect. 121: 117-124.

Gorai, M., A. Moussa, and M. Neffati. 2016. Does mineral sulphur availability account for growth performance, bulb development and metabolically related traits in wild leek (*Allium ampeloprasum* L.; Alliaceae)? Flora 219: 8-17.

Huang, C., Q. Liu, F. Gou, X. Li, C. Zhang, W. van der Werf, and F. Zhang. 2017. Plant growth patterns in a tripartite strip relay intercrop are shaped by asymmetric aboveground competition. Field Crop Res. 201: 41-51.

Jalilian, J., A. Najafabadi, and M. R. Zardashti. 2017. Intercropping patterns and different farming systems affect the yield and yield components of safflower and bitter vetch. J. Plant Interact. 12: 92-99.

Jiao, N.-Y., C. Zhao, T.-Y. Ning, L.-T. Hou, G.-Z. Fu, Z.-J. Li, and M.-C. Chen. 2008. [Effects of maize-peanut intercropping on economic yield and light response of photosynthesis]. Ying Yong Sheng Tai Xue Bao = J. Appl. Ecol. 19: 981-985.

Knörzer, H., H. Grözinger, S. Graeff-Hönninger, K. Hartung, H.-P. Piepho, and W. Claupein. 2011. Integrating a simple shading algorithm into CERES-wheat and CERES-maize with particular regard to a changing microclimate within a relay-intercropping system. Field Crop Res. 121: 274-285.

Kucharczyk, H., and H. Legutowska. 2002. *Thrips tabaci* as a pest of leek cultivated in different conditions. Thrips and Tospoviruses 211.

Manandhar, R., and M. G. Wright. 2016. Effects of interplanting flowering plants on the biological control of corn earworm (Lepidoptera: Noctuidae) and thrips (Thysanoptera: Thripidae) in sweet corn. J. Econ. Entomol. 113.

Mushagalusa, G. N., J.-F. Ledent, and X. Draye. 2008. Shoot and root competition in potato/maize intercropping: effects on growth and yield. Environ. Exp. Bot. 64: 180-188.

Najda, A., L. Błaszczyk, K. Winiarczyk, J. Dyduch, and D. Tchórzewska. 2016. Comparative studies of nutritional and health-enhancing properties in the “garlic-like” plant *Allium ampeloprasum* var. *ampeloprasum* (GHG-L) and *A. sativum*. Sci. Hortic. 201: 247-255.

Pitan, O. O. R., and C. O. Filani. 2014. Effect of intercropping cucumber *Cucumis sativus* (Cucurbitaceae) at different times with maize *Zea mays* (Poaceae) on the density of cucumber insect pests. Int. J. Trop. Insect Sci. 34: 269-276.

Sankula, S., M. J. VanGessel, and R. R. Mulford. 2004. Corn leaf architecture as a tool for weed management in two corn production systems. Weed Sci. 52: 1026-1033.

Seran, T. H., and I. Brintha. 2010. Review on maize based intercropping. J. Agron. 9: 135-145.

Shiberu, T., and A. Mahammed. 2014. The importance and management option of onion thrips, *Thrips tabaci* (L.) (Thysanoptera: Thripidae) in Ethiopia: a review. Entomol. Appl. Sci. Letters 1: 1-8.

Theunissen, J., and G. Schelling. 1997. Damage threshold for *Thrips tabaci* (Thysanoptera: Thripidae) in monocropped and inter-cropped leek. Eur. J. Entomol. 94: 253-262.

Vandermeer, J. H. 1989. The Ecology of Intercropping. Cambridge University Press, Cambridge.

Williams, P. A., and A. M. Gordon. 1995. Microclimate and soil moisture effects of three intercrops on the rows of a newly-planted intercropped plantation. Agroforest Syst. 29: 285-302.

Wright, M. G., T. P. Kuhar, M. P. Hoffmann, and S. A. Chenus. 2002. Effect of inoculative releases of *Trichogramma ostriniae* on populations of *Ostrinia nubilalis* and damage to sweet corn and field corn. Biol. Control 149.

Yang, F., S. Huang, R. Gao, W. Liu, T. Yong, X. Wang, X. Wu, and W. Yang. 2014. Growth of soybean seedlings in relay strip intercropping systems in relation to light quantity and red: far-red ratio. Field Crop Res. 155: 245-253.

Yang, F., D. Liao, Y. Fan, R. Gao, X. Wu, T. Rahman, T. Yong, W. Liu, J. Liu, and J. Du. 2017. Effect of narrow-row planting patterns on crop competitive and economic advantage in maize–soybean relay strip intercropping system. Plant Prod Sci. 20: 1-11.

Zhang, G., Z. Yang, and S. Dong. 2011. Interspecific competitiveness affects the total biomass yield in an alfalfa and corn intercropping system. Field Crop Res. 124: 66-73.

Walking Capacity of *Lygus hesperus*[1] Fifth Instars in a Laboratory and Outdoors

Shimat V. Joseph[2,4]*and Jhalendra Rijal[3]

Abstract. The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), can cause serious losses to strawberry, vegetables, and field crops in the western US. Often, fifth instars are noticed walking at the perimeter of crop fields. However, little is known of their walking capacity and dispersal. A series of laboratory and field studies were used to record the walking capacity of fifth instars. Starved nymphs moved farther (~2×) than non-starved individuals in a laboratory. Fifth instars also traveled farther (as many as 12 m in 15 minutes) as surface temperature warmed. Wind velocity had no effect on nymphal movement.

Introduction

The western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), is a serious insect pest of several crops in California including strawberry (*Fragaria ananasa* Duch), lettuce (*Lactuca sativa* L.), and celery (*Apium graveolens* dulce (Mill.) Pers.) (Joseph et al. 2016, Zalom et al. 2018, SVJ unpublished data). In 2016-2017, the combined value of the crops was estimated to be >$5.8 billion in California (CDFA 2017). A female *L. hesperus* oviposits and feeds on plant and fruit structures of strawberry (Udayagiri and Welter 2000), petiole of celery (Joseph et al. 2016), and mid-rib of lettuce (SVJ unpublished data) that cause cat-faced strawberry fruit, elongated lesions on the petioles of celery, and brown lesions on the mid-rib of lettuce. These damages affect marketability or result in serious losses to growers (Bolda et al. 2008). Besides strawberry, celery, and lettuce, western tarnished plant bug is an important pest of several field and other vegetable crops such as alfalfa (*Medicago sativa* L.), cotton (*Gossypium* spp.), sweet potato [*Ipomoea batatas* (L.) Lam.], and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] in the western US. The insect has become successful in establishing in the new areas and causing significant economic damage to crops because of its wide host range, unique overwintering habit, and multiple generations per year (Hagler et al. 2016).

Dispersal behavior of western tarnished plant bug between managed and non-managed hosts is poorly understood, particularly for nymphs which can improve management options for western tarnished plant bug. An adult *L. hesperus* can walk as well as fly and is most likely to fly during dispersal. However, dispersal of nymphs of western tarnished plant bug is not well understood. In perimeter areas of strawberry and celery fields, *L. hesperus* nymphs were often found walking (SVJ unpublished data), which suggests they could disperse seeking suitable hosts for shelter, food, and water by walking in agricultural landscapes. During spring months on the Central Coast of California, *L. hesperus* nymphs were found on common weed hosts such as wild radish (*Raphanus raphanistrum* L.), common groundsel (*Senecio vulgaris* L.), and mustards (*Brassica* spp.) in non-managed areas (Bolda et al. 2008). The weeds provide food and water for the developing nymphs. As the season

progresses, weeds as they begin to senesce and die become unsuitable as food which can trigger dispersal where nymphs and adults disperse in search of suitable hosts including managed crops in agricultural landscapes elsewhere. Previous studies showed that food deprivation can alter normal locomotion and intensify host searching behavior of insects (Connolly 1966, Bell 1990, Browne and Withers 2002, Defagó et al. 2016). Movement of *L. hesperus* nymphs solely involves walking on the soil and plant surface. Because older *L. hesperus* nymphs walk and disperse and were not previously studied, walking capacity of fifth instars were studied in a laboratory and outdoors. This information will help refine management strategies for *L. hesperus*.

Materials and Methods

A sweep net was used to collect fifth-instar *L. hesperus* from mustard plants surrounding organic vegetable fields at Salinas and Chualar, CA, in 2015 and 2016. The nymphs were sorted and maintained for 24 hours on pods of green bean (*Phaseolus vulgaris* L.) in a mesh cage in the laboratory at University of California Cooperative Extension-Monterey County, Salinas, CA. Presence of five dots on the dorsal side and well-developed, two pairs of wing pads differentiated fifth from other instars of *L. hesperus* (Mueller et al. 2003, Zalom et al. 2018).

Horizontal walking of fifth instars of *L. hesperus* was examined in a laboratory. Insects were starved for 0, 2, 4, and 6 hours before the assay. For each starvation regime, 23-26 *L. hesperus* fifth instars were tested. The nymphs were individually introduced on each Petri dish (100 × 15 mm, Fisher Scientific, Hampton, NH). Two dishes (two nymphs) were placed on the base of a document visualizer (Visualizer re-350, Canon USA, Melville, NY) backlit by fluorescent lights. Videos of the arenas were recorded using an ethernet camera (acA1300-60gm, Basler, Exton, PA). The dark-colored *L. hesperus* contrasted with the light background enabled recording a good field vision of the arena. Experiments lasted 20 minutes, starting immediately after the insects were introduced, and videos were recorded. Movement parameters were analyzed using Noldus EthoVision XT software (Version 11.5, Noldus Information Technologies, Wageningen, Netherlands). The air temperature was recorded using four mini digital thermos-hygrometers (Avianweb, Mims, FL) at the four corners of the visualizer surface. All assays were done between 23.8 and 29ºC. The total distance covered (cm) and average speed (cm per second) of individual *L. hesperus* were calculated using EthoVision XT software.

Our method for quantifying outdoor walking movement by *L. hesperus* was modified from Lee et al. (2014). Only fifth instars were tested. The walking movement of individual *L. hesperus* was monitored by one to four human observers. The movement of nymphs was assessed by marking their locations every minute during 15 minutes of observation. The starting location was marked on the asphalt with colored chalk, and a nymph was placed at the spot to start the experiment. The observer moved at least 5 m away after each observation event to reduce observer effect. Twenty nymphs were observed. Total distance moved was calculated by summing the distances traveled between marked points. Net distance moved was measured as the distance between starting and final positions. Each experiment with an individual insect was a replication.

The assays were done between 0900 and 1600 hours on asphalt pavement at Salinas, CA. The pavement was free of cracks and swept clean of debris before the start of every observation period. The nymphs used were not starved. Surface

temperature of the pavement was recorded at three random locations by using a laser temperature gun thermometer (infrared 10:1, model 2267-20, Milwaukee Electric Tool Corporation, Brookfield, WI). Air temperature was recorded using two digital thermometers (model 00325, AcuRite, Lake Geneva, WI) on the pavement surface before the start of the assay. Wind speed was recorded at a height of ~1.2 m by using a handheld weather monitor (model WM-4, Ambient Weather, Chandler, AZ) from the cardinal directions at the release location of each nymph before and after the assay. All assays were done during cloud-free days. To relate surface asphalt temperature ("surface temperature") to soil surface temperature ("soil temperature"), soil temperature was measured at a production field at Salinas.

For a starvation assay with fifth instars in a laboratory, total distance covered and average speed data were square-root transformed to satisfy assumptions before subjected to ANOVA using the general linear model procedure (PROC GLM) (SAS Institute 2012) where starvation was the fixed effect. Means were separated using least significant difference (LSD) test ($\alpha = 0.05$) for laboratory assays. For the outdoor movement assay, regression analysis was done on movement data with surface temperature and wind velocity using the PROC REG procedure in SAS. Pearson's correlation analysis was used to determine the association between surface and air temperatures. Surface temperature probably was more influential than air temperature on *L. hesperus* behavior because nymphs were directly contacting the surface. The association between total and net distances moved by fifth instars was assessed using Pearson's correlation coefficient.

Results and Discussion

L. hesperus nymphs travelled greater distance ($F = 3.7$; df = 3,127; $P = 0.018$ and at faster pace ($F = 3.6$; df = 3,127; $P = 0.016$) after starved for 4 or 6 hours than did non-starved nymphs (Fig. 1A,B). Distance moved was not significantly different among nymphs starved for 2, 4, or 6 hours. Similarly, nymphs starved for 0 or 2 hours did not differ significantly.

L. hesperus nymphs traveled greater total and net distances with warmer surface temperatures (Fig. 2A,B). Wind velocity did not influence the total distance traveled (Fig. 3). Surface and air temperatures of individual assays were significantly correlated ($r = 0.515$, $P = 0.020$, N = 20). Total distance and net distance traveled by nymphs were significantly ($r = 0.881$, $P < 0.001$, N = 20) correlated.

Laboratory studies showed that starved fifth-instar nymphs of *L. hesperus* tended to move greater distances compared to non-starved individuals. Non-starved fifth instars moved about 2 m in 20 minutes, and the distance covered almost doubled after 4 hours of starvation in the laboratory. This was consistent with previous studies where insects deprived of food moved farther distances (Connolly 1966, Bell 1990, Browne and Withers 2002, Defagó et al. 2016). Increased locomotor behavior is associated with greater probability of finding suitable food.

The outdoor assay showed that movement of *L. hesperus* nymphs increased with warmer surface temperature where nymphs walked as far as 12 m in 15 minutes. Moreover, the total distance traveled by the nymphs positively correlated with net distance traveled which suggested that fifth instars were intent on moving away from the point of introduction rather than engaging in random walking around the point of introduction. Data suggested that older nymphs were capable of dispersal in search of suitable food which rarely is considered or incorporated as a factor before management decisions.

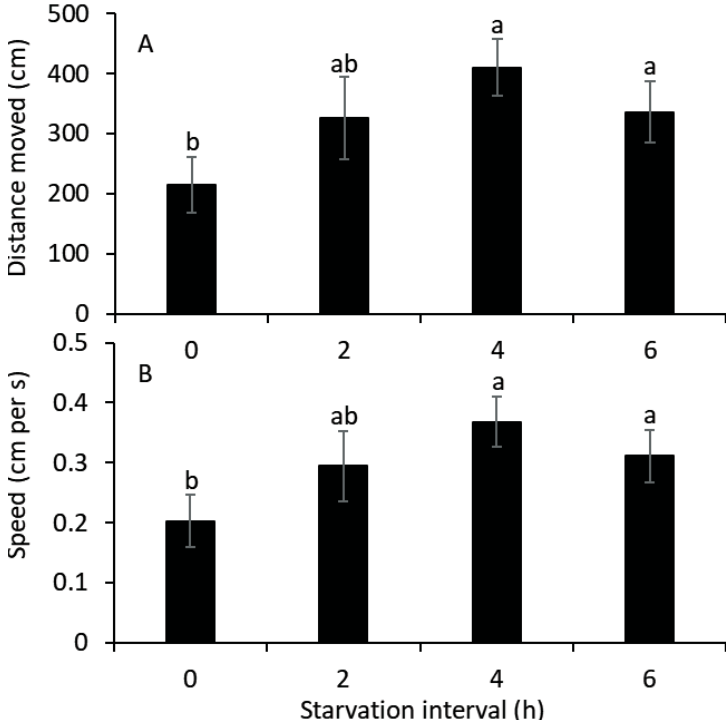

Fig. 1. Effects of starvation on distance moved (A) and speed of movement (B) by fifth instars of *L. hesperus* in a laboratory. Bars with same letters are not significantly different among the starvation status of *L. hesperus* (LSD test, $P > 0.05$).

Because *L. hesperus* is a pest that needs attention in several crops, results pertaining to nymphal behavior can be used to improve sampling and management strategies in several agroecosystems. For example, alfalfa is used as a trap crop to control *L. hesperus* in strawberry production especially by organic growers on the Central Coast of California (Swezey et al. 2007), and knowledge of walking capacity of older nymphs provides insight into potential movement capabilities within and between fields of alfalfa and strawberry. The information can be further incorporated to refine management decisions to improve timing of tractor-mounted vacuuming (bug vac) or insecticide application on a trap crop to control *L. hesperus*.

Acknowledgment

We appreciate technical assistance provided by A. Valdez and A. Alfaro with experiments. Agrochemical companies provided funding for the study.

References Cited

Bell, W. J. 1990. Searching behavior patterns in insects. Annu. Rev. Entomol. 35: 447-467.

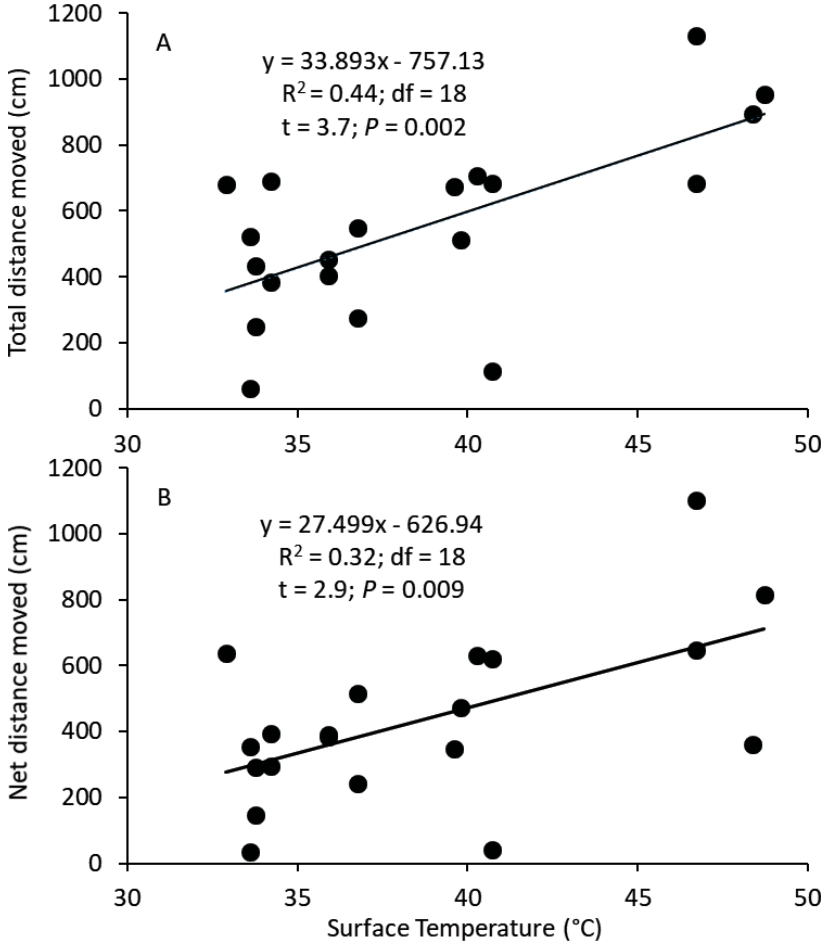

Fig. 2. Linear regression analysis showing relationship between total distance moved and surface temperature (A) and net distance moved and surface temperature (B) for fifth instars of *L. hesperus* in an outdoor setting.

Bolda, M. A., O. Daugovish, S. A. Fennimore, S. T. Koike, K. D. Larson, D. B. Marcum, and F. G. Zalom [technical coordinators]. 2008. Integrated Pest Management for Strawberries, 2nd ed. University of California, Division of Agriculture and Natural Resources Publication 3351.

Browne, L. B., and T. M. Withers. 2002. Time-dependent changes in the host-acceptance threshold of insects: implications for host specificity testing of candidate biological control agents. Biocontrol Sci. Technol. 12: 677-693.

California Department of Food and Agriculture (CDFA). 2017. Crop report https://www.cdfa.ca.gov/statistics/PDFs/2017-18AgReport.pdf Accessed 11 January 2019.

Connolly, K. J. 1966. Locomotor activity in *Drosophila* as a function of food deprivation. Nature 209: 224.

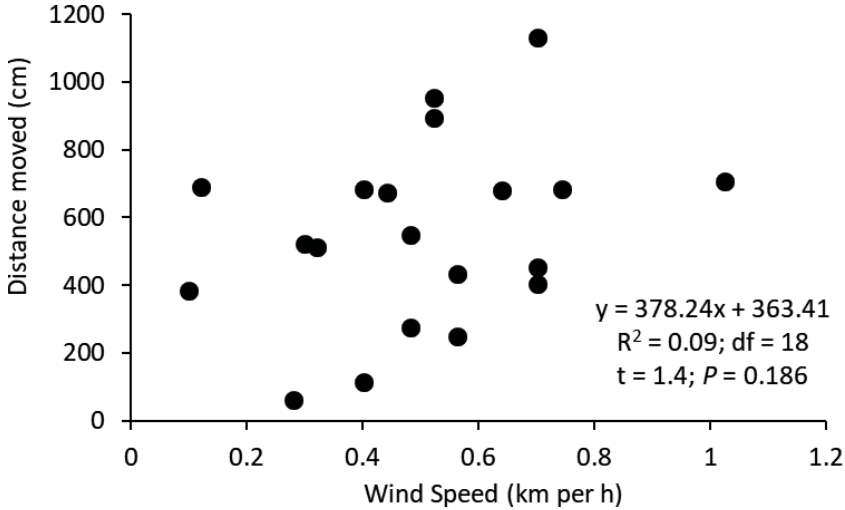

Fig. 3. Linear regression analysis showing relationship between total distance moved and wind speed for fifth instars of *L. hesperus* in an outdoor setting.

Defagó, M. T., M. Videla, and G. Valladares. 2016. To smell you better: prior food deprivation increases herbivore insect responsiveness to host plant odor cues. J. Insect. Behav. 29: 527-534.

Hagler, J. R., E. E. Tassone, T. A. Coffelt, and A. E. Lipka. 2016. Host plant preference of *Lygus hesperus* exposed to three desert-adapted industrial crops. Industrial Crops and Products 89: 363-367.

Joseph, S. V., R. Ahedo, and M. de la Fuente. 2016. Characterization of *Lygus hesperus* (Hemiptera: Miridae) feeding and oviposition injury on celery seedlings. Plant Health Prog. 17: 101-105.

Joseph, S. V., and M. Bolda. 2018. Evaluating the potential utility of an electrostatic sprayer and a tractor-mounted vacuum machine for *Lygus hesperus* (Hemiptera: Miridae) management in California's coastal strawberry. Crop Prot. 113: 104-111.

Lee, D-H., A. L. Nielsen, and T. C Leskey. 2014. Dispersal capacity and behavior of nymphal stages of *Halyomorpha halys* (Hemipetera: Pentatomidae) evaluated under laboratory and field conditions. J. Insect Behav. 27: 639-651.

Mueller, S. C., C. G. Summers, and P. B. Goodell. 2003. A field key to the most common *Lygus* species found in the agronomic crops of the Central Valley of California. ANR Publication 8104.

SAS Institute. 2012. SAS version 9.4. SAS Institute Inc., Cary, NC.

Swezey, S. L., D. J. Nieto, and J. A. Bryer. 2007. Control of western plant bug *Lygus hesperus* Knight (Hemiptera: Miridae) in California organic strawberries using alfalfa trap crops and tractor-mounted vacuums. Environ. Entomol. 36: 1457-1465.

Udayagiri, S., and C. Welter. 2000. Escape of *Lygus hesperus* (Heteroptera: Miridae) eggs from parasitism by *Anaphes iole* (Hymenoptera: Mymaridae) in strawberries: plant structure effects. Biol. Con. 17: 234-242.

Zalom, F. G., M. P. Bolda, S. K. Dara, and S. V. Joseph. 2018. Strawberry: Lygus bug. UC Pest Management Guidelines. UC ANR Publication 3468.

Physiological and Pathogenical Characterization of *Beauveria bassiana* and *Metarhizium anisopliae* Isolates for Management of Adult *Spodoptera frugiperda*[1]

Oscar Giovanni Gutiérrez-Cárdenas[2], Hipolito Cortez-Madrigal[2], Edi A. Malo[3], Jaime Gómez-Ruíz[3], and Rineaud Nord[2]

Abstract. To know the potential of entomopathogenic fungi for management of adults of *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) using the auto-dissemination technique, three isolates of *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin and six isolates of *Metarhizium anisopliae* (Metschnikoff) (Hypocreales: Clavicipitaceae) were characterized physiologically and pathogenically. At the time 50% of conidia germinated (GT_{50}), pathogenicity, effect of relative humidity, and horizontal transmission of the entomopathogen male-female in three ratios (1:5, 1:10, 1:15) were evaluated. In addition, a preliminary device for auto-dissemination of the entomopathogen was evaluated. All isolates were pathogenic (33.3-100%) toward adults of the insect. Pathogenicity and TG_{50} were very correlated (r = 0.8) and fluctuated from 14.1 to 20.3 hours. Because of intermediate pathogenicity (68.7%) and varying response to humidity (62-100%), isolation of *M. anisopliae* Ma-San Rafel-2 was used for auto-dissemination bioassays. Transmission of the entomopathogen was a function of the number of females exposed. After 11 days, a male transmitted the fungus to 15 healthy females. The males released in devices prepared with fungus + synthetic sex pheromone were attracted, infected, and killed 5 to 8 days later. Our results are fundamental for management of *S. frugiperda* through auto-dissemination of entomopathogenic fungi.

Resumen. Para conocer el potencial de los hongos entomopatógenos en el manejo de adultos de *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) mediante autodiseminación, se caracterizaron fisiológica y patogénicamente 3 aislamientos de *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin y seis de *Metarhizium anisopliae* (Metschnikoff) (Hypocreales: Clavicipitaceae). El tiempo en que germinó el 50% de los conidios (TG_{50}), patogenicidad, efecto de la humedad relativa y la transmisión horizontal del entomopatógeno macho-hembra en tres proporciones (1:5, 1:10 y 1:15) fueron evaluados. Adicionalmente, un dispositivo preliminar para la autodiseminación del hongo fue evaluado. Todos los aislamientos fueron patogénicos (33.3-100%) hacia adultos del insecto. Una estrecha correlación (r = 0.8) fue registrada entre patogenicidad *Vs* TG_{50}; éste fluctuó desde 14.1 a 20.3 horas. Por su intermedia patogenicidad (68.7%) y amplia

[1]Lepidoptera: Noctuidae

[2]Instituto Politécnico Nacional-CIIDIR, Unidad Michoacán. Justo Sierra # 28, Col. Centro, Jiquilpan, Michoacán, México. C.P. 59510. hcortezm@ipn.mx correspondence author.
[3]El colegio de la Frontera Sur (ECOSUR), Grupo de Ecología de Artrópodos y Manejo de Plagas. Km 2.5, Carretera Antiguo Aeropuerto, Apartado Postal 36, Tapachula, 30700, Chiapas, México.

respuesta a la humedad relativa (62-100%), el aislamiento Ma-San Rafel-2 de *M. anisopliae*, fue seleccionado para implementar bioensayos de autodiseminación. La transmisión del entomopatógeno estuvo en función del número de hembras expuestas. Después de 11 días, un macho fue capaz de transmitir el hongo a 15 hembras sanas. Los machos liberados en dispositivos cebado con el hongo + feromona sexual sintética, fueron atraídos, infectados y muertos entre 5 a 8 días después de la infección. Los resultados obtenidos son fundamentales para el manejo de *S. frugiperda* mediante autodiseminación de hongos entomopatógenos.

Introduction

Fall armyworm, *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), is native to the American continent and distributed from the southern USA to Argentina (Nagoshi and Meagher 2004). It also was recently reported in Africa (Goergen et al. 2016). It is one of the major agricultural pests in Latin-American countries (Burtet et al. 2017), with a large range of hosts that includes more than 180 species of plants, both wild and cultivated. Maize, *Zea mays* L., is the most economically important preferred host (Cruz et al. 2012, Farias et al. 2014).

In integrated pest management (IPM), use of semiochemicals (Witzgall et al. 2010, Guerrero et al. 2014), entomophagous insects, and entomopathogenic fungi are recommended management strategies. However, application of organosynthetic insecticides still is the main control strategy, notwithstanding disadvantages such as elimination of beneficial fauna (natural enemies and pollinators), environmental pollution, and development of resistant pests (Perez-Zubiri et al. 2016).

An important base for IPM is biological control in which entomopathogenic fungi are a main tool (Inglis et al. 2001, Meyling and Eilenberg 2007). For example, *Beauveria bassiana* (Balsamo-Crivelli) Vuillemin (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metschnikoff) (Hypocreales: Clavicipitaceae), are two of the most studied fungi (Meyling and Eilenberg 2007). Their mode of action (via cuticle) and easy *in vitro* reproduction encourage their popular use in pest management (Shah and Pell 2003, Mascarin and Jaronski 2016).

B. bassiana and *M. anisopliae* previously have been evaluated to control *S. frugiperda*; however, their use has been by spraying the larval stage (García et al. 2011, Romero-Arenas et al. 2014). This application technique has been the general paradigm in entomopathogen application, even though it is one of the least efficient techniques in biopesticide application (Bateman and Chapple 2001). Only 1% of the application impacts the target insect (Pimentel and Edwards 1982). An alternative technique is auto-dissemination that has proven advantageous in management of several pests of economic importance (Furlong et al. 1995, El-Sufty et al. 2011, Getahun et al. 2016). In Mexico, pioneer studies exist on pest management using the auto-dissemination technique of entomopathogenic fungi (García-Munguía et al. 2015, Suárez-Núñez et al. 2017). To our knowledge, this approach has not been assessed on *S. frugiperda*.

Seminal studies on the auto-dissemination technique used sex pheromone to attract pests (Furlong et al. 1995, Lopes et al. 2014, Kabaluk et al. 2015). Considering biological characteristics of *S. frugiperda* (King and Saunders 1984) and that traps baited with sex pheromone are used for monitoring *S. frugiperda* in maize and sorghum (*Sorghum bicolor* (L.) Moench) in Mexico (Malo et al. 2004),

use of auto-dissemination technique combined with sex pheromone could be good for managing the pest. The objective of this study was to characterize, physiologically and pathogenically, nine isolates of entomopathogenic fungi in combination with sex pheromone of fall armyworm to understand the potential of the auto-dissemination technique for management of adults of the pest.

Materials and Methods

Biological Material. Nine isolates of entomopathogenic fungi were collected in different regions of Mexico -- three *B. bassiana* and six *M. anisopliae* (Table 1). The fungi are part of the strain collection of entomopathogenic fungi of CIIDIR-IPN Unidad Michoacán, Jiquilpan, Michoacán, Mexico. The fungal cultures were preserved in Sabouraud dextrose agar + 0.1% yeast extract (ADS + YE BD-Bioxon-Mexico) at 25 ± 2°C and a photoperiod of 12:12 light:dark hours. A colony was established in the experimental conditions mentioned previously from *S. frugiperda* larvae collected in maize crops in the region. Each larva was put individually into a 3.6 x 3.3-cm plastic container with a lid with holes for aeration. A wheat germ-based semi-synthetic diet (16.5% maize flour, 4.4% brewer's yeast, 4.2% wheat germ, 0.6% ascorbic acid, 0.2% nipagin, 0.7% benzoic acid, and 1.5% agar) described by Greene et al. (1976) was used as food for the insects until they reached the pupal stage.

Brown paper bags (33 x 13 cm) were used for mating and oviposition by adults. Bags were checked daily, and once eggs were detected, the bags were placed for 2-3 minutes in a freezer to immobilize moths and collect the egg mass. Part of the adults were used for bioassays and the rest for rearing more insects.

Germination Time. Suspensions of $1x10^8$ conidia ml^{-1} were prepared from 20-day-old isolate cultures. Conidia of the fungus established in ADS + YE medium were collected in aseptic conditions, flooding the Petri dish with sterile distilled water and INEX-A® (0.05%; Cosmocel, Mexico), and scraping the surface with an inoculation loop. The conidia were shaken for 10 minutes in a magnetic stirrer (IKA®, Wilmington, DE) and counted in a Neubauer chamber. The concentration of conidia per milliliter was calculated by the formula of Lipa and Slizynski (1973) C = (Cc) (4 x 10^6) (Fd/80) in which C = number of conidia per milliliter; Cc = average number of conidia counted in the Neubauer chamber, and; Df = dilution factor.

Ten microliter of suspension were placed with ADS + YE at the center of a glass slide and spread with an inoculation loop. The glass slide with culture medium and fungal conidia was put on a glass tube triangle inside a Petri dish and incubated at 25 ± 2°C and photoperiod of 12:12 light:dark hours. Once germination started (approximately 10 hours), a microscope (400x; Zeiss, Germany) was used to examine 100 conidia of each isolate (N = 9) with three replications, at intervals of 1 hour. Reading stopped when ≥ 90% germination was reached. Germination was considered to have occurred when the length of a germ tube was equal to or more than half the length of the conidium (Jackson et al. 1985).

Pathogenicity Tests. Before inoculation of moths, circles of 0.9 cm of culture from 20-day-old cultures in ADS + YE were established to estimate conidia per milliliter per isolate. Samples were placed in 10-ml tubes of sterile distilled water, and two drops of sterile 0.05% INEX-A® surfactant were added. The tubes were shaken at 2,500 rpm in a vortex (Genie2® Scientific Industries, New York) for 10 minutes, and 10 µL were taken and placed in a Neubauer chamber. The

experiment had three replications (circles) for each isolate selected. Concentration of conidia was calculated from the formula of Lipa and Slizynski (1973).

Newly emerged moths were inoculated by contact with dry conidia from each 20-day-old isolate and cultured in ADS + YE media. Four couples (♂♀) were put with fungus in a dish for 5 minutes (inoculation time). The moths were transferred into a 1,000-ml plastic container with lid. A paper towel (40 x 40 cm) containing 7 ml of sterile distilled water was put in to the bottom of the container to provide humidity (100%) for development of fungus. A cotton ball was used to add honey solution (30%) as food. The experiment used a completely random design with three replications, with 16 individuals per replication. The number killed (expressed as percentage) was recorded daily, and corpses were put into a moist chamber for sporulation.

Effects of Relative Humidity. Four percentages of humidity were evaluated: 62, 75, 87 and 100. The bioassay was established in a clear 1,000-ml capacity plastic container with pierced walls for aeration. Inside, an 8-cm-tall stainless-steel wire base covered by organza fabric that supported the moths was installed. In the bottom of the container was added 50 ml of saturated solution of salts: NaBr (62%), NaCl (75%), and KCl (87%). Distilled water provided 100% relative humidity (Palem and Padmaja 2010). A cotton ball soaked in honey as food for the insect was put on the fabric of the wire stand.

Isolates evaluated were selected taking into account previous bioassays of pathogenicity at 3 days, from which were chosen three isolates: most pathogenic (Bb-Trips), a non-pathogenic one (Ma-Ornelas), and an intermediate one (Ma-San Rafael-2). Pure fungal cultures (20 days old) were used for a process similar to that for pathogenicity tests. Three replications were used for each amount of humidity evaluated, with 16 newly emerged individuals per replication and number of dead insects expressed in percentage. The bioassay lasted 10 days with experimental conditions mentioned previously.

Potential of Male *S. frugiperda* for Entomopathogen Auto-dissemination. Healthy females were put with one male inoculated with conidia of the best isolate (20 days old) inside wire cages (30 x 20 x 20 cm) covered by organza fabric. The isolate was selected based on pathogenicity and response to humidity bioassays. Male:female ratios analyzed were: 1:5, 1:10, and 1:15. Moths without fungus were used as a check. A cotton ball with honey solution (10%) was added as food. The experiment was replicated three times, incubated at 25 ± 2°C and a photoperiod of 12:12 light:dark hours. The moths were examined daily for 18 days. Death of the male was recorded, and corpses of moths were removed and put in a 100% humidity chamber until fungal sporulation.

Device for Attracting and Infecting Males. A piece of white fabric was put inside a white polyvinyl chloride (PVC) pipe 40 cm long and 7.6 cm internal diameter. Twenty day-old conidia (2.4 g) from the entomopathogen fungi selected from previous tests were spread on the fabric. Transparent, 4.5-liter containers were placed in the ends of the tube. Two holes were pierced into each container -- one in the lid to allow coupling to the PVC tube and another in the base to allow recovery of the moths presumably infected with fungus. Two balls of cotton soaked in honey (10%) as food for insects were put in one container. A rubber septum with 400 µg of sex pheromone of *S. frugiperda* was put in the other container (Cruz-Esteban et al. 2018).

Eight nonmated 4-day-old males of *S. frugiperda* were released into the container with the honey solution, and removed 12 hours later from the container with the sex pheromone. The experiment was done in triplicate, eight males per replication, with a check without fungi. Captured males were put into 30 x 20 x 20 cm cages and kept in the environmental conditions mentioned in the previous bioassay to record numbers that died. Corpses were put inside a humidity chamber for development of fungi.

Determination of Conidial Concentration in Moths. The moths inoculated with dry conidia were put into a freezer for 20 minutes to kill, then immediately put inside a sterile 20-ml tube with a screw-on lid to which 10 ml of sterile water and two drops of sterile 0.5% INEX-A® surfactant were added. The tube was agitated at 2,500 rpm in a vortex for 10 minutes. The suspension was filtered through 710-µm openings (Endecotts LTD, London) into another 20-ml tube. The suspension was agitated for 1 minute in a vortex at the rpm mentioned previously. An aliquot (10 µl) was used to count conidia in a Neubauer chamber (Reichert Scientific Instruments, Buffalo, NY) with the aid of a microscope (400x, Zeiss, Germany). Three replications were used.

Statistical Analyses. Data were analyzed with SAS (SAS Institute 2012). Before analysis of variance (ANOVA), the data were reviewed for homoscedasticity and normal distribution.

Results of germination time were analyzed by logistic regression analysis. Results of pathogenicity tests for the 3rd day, and the potential of males of *S. frugiperda* in auto-dissemination of the entomopathogen corresponding to Day 11, were analyzed by ANOVA. The time in which 50% (TL_{50}) of individuals died was estimated through Probit analysis.

Response to relative humidity on the 4th day was treated with a two-way ANOVA, using the entomopathogen as the first factor, relative humidity as the second factor, and portion of *S. frugiperda* killed as the variable of response. Mean separations for all cases were calculated with a Tukey test ($\alpha = 0.05$).

A mortality curve was used to determine the number of dead males of *S. frugiperda* in the device primed with sex pheromone. Twenty-four moths were used for the treatment and check. Results of determination of conidial concentration in moths and production of conidia per isolation were analyzed by descriptive statistics.

Results

Germination Time and Pathogenicity of Isolates. Results of the logistic regression analysis and the confidence intervals showed significant differences (x^2 <0.0001) among germination times (GT_{50}) of the isolates. Bb-Cat of *B. bassiana,* a tropical species, had the shortest germination time (GT_{50} = 14.1 hours), while the longest time (GT_{50} = 20.3 hours) was by Ma-Ornelas-3 of *M. anisopliae*, a species of subtropical origin (Table 1).

ANOVA applied to the number dead on the 3rd day indicated significant statistical differences (F = 33.8; df = 9, 20; p = 0.0001) among treatments. The most pathogenic isolate against adult *S. frugiperda* was Bb-Trips of *B. bassiana,* killing 100%, that entailed no difference in respect to Ma-Ornelas-3, Ma-Ornelas-2, Bb-M, Ma-Ornelas-4, and Ma-SR-Suelo isolates. Ma-San Rafael-2 showed intermediate pathogenicity (68.7%), Bb-Cat had low pathogenicity, but Ma-Ornelas was not pathogenic after 3 days on *S. frugiperda* (Fig. 1). After 7 days, all isolates

Table 1. Origin of Isolates and Time in which 50% of Conidia (GT_{50}) of the Fungi *Beauveria bassiana* and *Metarhizium anisopliae* Germinated

Isolate	Origin	GT_{50} (CI) in hours
Ma-Ornelas-3	VLRM	20.3 (20.1-20.5) a
Ma-San Rafael-2	SRTM	18.7 (18.6-18.8) b
Ma-Ornelas-4	VLRM	16.6 (16.5-16.8) c
Ma-Ornelas	VLRM	15.5 (15.4-15.6) d
Ma-SR-Suelo	SRTM	15.4 (15.3-15.5) d
Ma-Ornelas-2	VLRM	15.3 (15.1-15.5) d
Bb-M	Jiquilpan, Mich.	14.4 (14.3-14.6) e
Bb-Trips	VLRM	14.2 (14.2-14.3) f
Bb-Cat	Campeche	14.1 (14.0-14.2) f

CI: Confidence interval. Bb: *Beauveria bassiana*. Ma: *Metarhizium anisopliae.* VLRM = Valle de Los Reyes, Mich.; SRTM = San Rafael, Tingüindin, Mich.

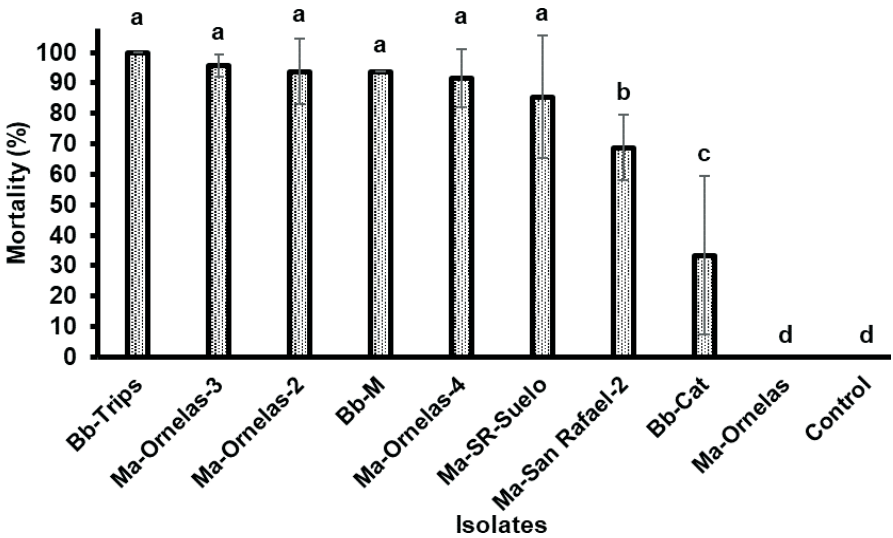

Fig. 1. Pathogenicity of different isolates of entomopathogenic fungi on *Spodoptera frugiperda* adults 3 days after inoculation.

killed 100% of *S. frugiperda* adults. Six days later, sporulation of fungi was evident on moths, although more mycelial growth was by *B. bassiana* isolates.

The relationship among germination, pathogenicity, and isolate origin was clear in some isolates; e.g., Bb-Trips, of subtropical origin, killed most, and also had one of the least GT_{50}. The number of conidia per milliliter estimated for the different isolates ranged from 20 x 10^6 to 36.2 x 10^6. It was possible to detect a close relationship between the number of conidia and pathogenicity (r = 0.8) (Table 2).

Effects of Relative Humidity. The fungal isolates killed 100% at all humidities after 6 days. However, time until death increased as relative humidity decreased. ANOVA at the 4th day showed significant differences among isolates (F = 201.8; df = 2, 32; p = 0.0001), among amounts of relative humidity (F = 58.3; df =

Table 2. Production of Conidia on Culture Medium and Numbers of Adults of *Spodoptera frugiperda* Killed by Different Isolates Evaluated

Isolate	Number of conidia ml^{-1}	Mortality (%)
Bb-Cat	20.3×10^6	33.3
Bb-M	24.7×10^6	93.7
Bb-Trips	23.1×10^6	100.0
Ma-Ornelas	36.2×10^6	0.0
Ma-San Rafael-2	20.0×10^6	68.7
Ma-Ornelas-3	23.7×10^6	95.8

3, 32; $p = 0.0001$), and in isolate by-relative humidity interaction ($F = 4.3$; df = 6, 32; $p = 0.0001$). The most pathogenic isolate at any humidity was Ma-San Rafael-2 that killed the most (91%) of any relative humidity, followed by Ma-Ornelas that killed more only at 100% humidity (Fig. 2A). The other isolates had statistically different pathogenicity with the most relative humidity (100%); however, they were inferior to pathogenicity by Ma-San Rafael-2 (Fig. 2B).

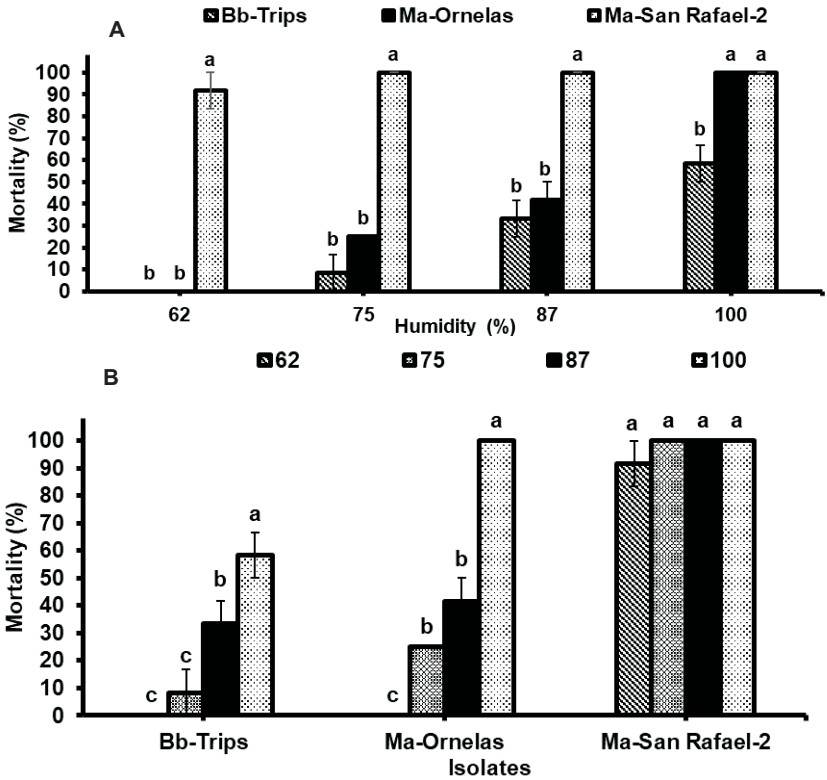

Fig. 2. Response of three isolates of entomopathogenic fungi to four amounts of relative humidity. A) isolate and B) humidity effects. Bars followed by the same letter within isolate or humidity were not statistically different (Tukey, $p \leq 0.05$).

Horizontal Transmission in Adult *S. frugiperda.* Isolate Ma-San Rafael-2 from *M. anisopliae* was tested. The three proportions of insects (infected males: healthy females) evaluated produced an infection rate and a final mortality of 100% between 15 and 19 days after inoculation; comparatively, no insects in the check died during the same amount of time. Based on the ANOVA of numbers dead on Day 11, there were no significant differences (F = 2.5, df = 2.6, $p \leq 0.05$) among treatments. The treatment that killed the most was the 1♂:10♀ ratio, with 78.8%. The treatment with the fewest dead was the 1♂:15♀ ratio, with 52.1% (Table 3).

Table 3. Horizontal Transmission of the Fungus *Metarhizium anisopliae* (Ma-San Rafael-2) from Infected Males to Healthy Females of *Spodoptera frugiperda* 11 Days after Inoculation

Ratio	Mortality + SD (%)
1♂:5♀	77.7** ± 25.4 a
1♂:10♀	78.7 ± 10.4 a
1♂:15♀	52.0 ± 7.2 a

**Means ± standard deviation followed by the same letter do not differ statistically (Tukey, $P < 0.05$).

Based on analysis of lethal time (LT_{50}), results of entomopathogen horizontal transmission (auto-dissemination) from inoculated males to healthy females showed significant differences (x^2 <0.0001) among treatments, which confirmed ANOVA trends. The least LT_{50} was recorded in the 1:5 treatment, with 8.5 days, which was similar to that in the 1:10 treatment, with 8.7 days. The 1:15 treatment had the greatest LT_{50} (11.7 days), significantly more than the other two ratios (Table 4). The calculated conidial concentration acquired by one moth (n = 3) varied with range between 6 to 32 x 10^6 per individual.

Table 4. Calculated Time until 50% (LT_{50}) of Female of *Spodoptera frugiperda* were killed by the Fungus *Metarhizium anisopliae* (Ma-San Rafael-2) Transmitted Horizontally by Males Infected with the Entomopathogen

Ratio	LT_{50} (CI) in days		Probability x^2
1♂:5♀	8.5** (7.9-9.1)	a	<0.0001
1♂:10♀	8.7 (8.4-8.9)	a	<0.0001
1♂:15♀	11.7 (11.4-12.0)	b	<0.0001

**LT_{50} (Confidence interval) followed by the same letter do not differ statistically ($x2$ <0.0001).

Attraction, Infection, and Death of *S. frugiperda* Males. The device primed with sex pheromone + conidia of Ma-San Rafael-2 of *M. anisopliae* attracted, infected, and killed male moths of *S. frugiperda*. The mortality curve showed that moths died 5 to 8 days after infection. In contrast, check moths died between 15 and 18 days after removing from the device (Fig. 3). Concentration of conidia acquired by males ranged from 1.5 to 7 x 10^6 conidia per moth.

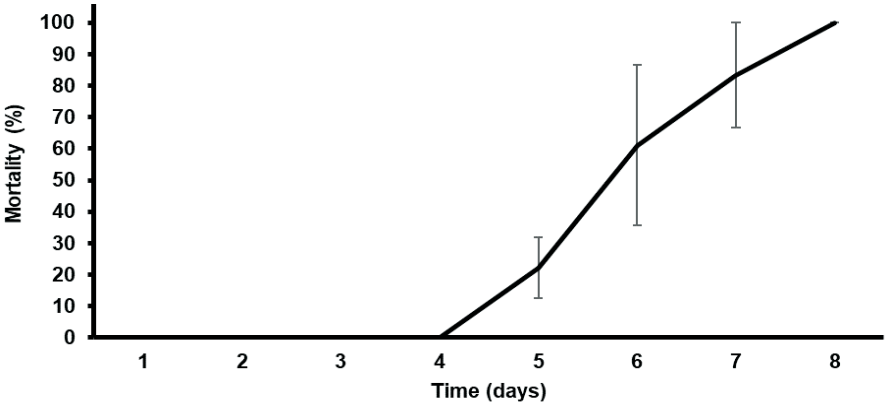

Fig. 3. *Spodoptera frugiperda* males dead in the device baited with sex pheromone and entomopathogenic fungi.

Discussion

Nine isolates of entomopathogenic fungi from different origins and hosts were characterized physiologically; and, for the first time, the pathogenicity of the fungi toward adult *S. frugiperda* was evaluated as a sustainable alternative in pest management. Application of entomopathogenic fungi has been under the application paradigm for organosynthetic insecticides (Bateman and Chapple 2001, Mora-Aguilera et al. 2017). However, microbial insecticides are living organisms that can be favored by surrounding environment (Inglis et al. 2001, Meyling and Eilenberg 2007). Velocity of conidia germination considered an important characteristic for isolation and selection of entomopathogenic fungi for application in biological control (Torres de La Cruz et al. 2013, Faria et al. 2015).

Results of this study about germination velocity can be associated with origin of an isolate. For example, *M. anisopliae* and *B. bassiana* isolates come from regions with contrasting climatic conditions -- cold regions, as the cases of *M. anisopliae* isolates from San Rafael-Tinguindín, Michoacán (1,700 m above sea level, 6.0-21.0°C), warm climates, such as Bb-Trips and Bb-M isolates for *B. bassiana* (1,000 and 1,500 m above sea level, respectively), or as is the case of Bb-Cat, from Campeche State, a tropical region at sea level. Entomopathogenic fungi have developed strategies adaptive to the environment, and one is rapid germination of conidial and enzyme production (Cortez-Madrigal et al. 2014). Consequently, they are more virulent than ones with low germination. Isolates with the shortest germination time have greater chance to infect insects, even when environmental conditions are adverse for fungi (Cortez-Madrigal et al. 2003, Torres de La Cruz et al. 2013).

The pathogenic characterization of isolates is necessary to understand details of the epidemic process in a population. Pathogenicity is part of a series of quantifiable biological processes, based on death of the target insect (Mora-Aguilera et al. 2017). Death evaluated in laboratory conditions allowed selection of the best isolates, as reported in other studies (Lacey et al. 2001, Cortez-Madrigal et al. 2014). Evaluation of isolates of *B. bassiana* and *M. anisopliae* against adult *S. frugiperda* showed most isolates pathogenic against the insect, with 33.3 and 100%

killed on the 3rd day. However, on the 7th day after inoculating the moths, all isolates (e.g., Ma-Ornelas) killed moths.

Among the three isolates, only Ma-San Rafael-2 responded with efficiency at amounts of humidity evaluated. It is important to know the energy potential of conidia to germinate and kill insects, even at low humidity (Doberski 1981), and to guarantee success of microbial control of pests. For example, adult coffee berry borer, *Hypothenemus hampei* Ferrari (Coleoptera: Curculionidae), was infected by *B. bassiana* at of 39 to 90% humidity (Arrubla et al. 2010).

Main natural dispersal strategies of entomopathogens occur through transmission provoked by insects themselves, which is denominated auto-dissemination (El-Sufty et al. 2011, Kabaluk et al. 2015, Getahun et al. 2016). This knowledge should be exploited for microbial control of pests. *S. frugiperda* males efficiently transmitted conidia in a horizontal way to healthy females (as many as 15) with a relatively short LT_{50} (8.5-11.7 days). However, rapid death of the male (72 hours) and transmission efficiency might explain rapidity with which the male transmitted the entomopathogen, or considering less space in cages, the females also might have participated in transmission of the entomopathogen.

The technique of auto-dissemination allows more specific handling of a pest, because deliberately infected insects can disseminate the entomopathogen among other members of the same species. In addition, the amount of inoculum is less than required for the spraying technique, which could decrease management costs (Furlong et al. 1995, Shah and Pell 2003). Studies on pest management through auto-dissemination have shown its benefit (El-Sufty et al. 2011, García-Munguía et al. 2015, Getahun et al. 2016). For example, Furlong et al. (1995) developed a device baited with sex pheromone and *Zoophthora radicans* (Brefeld) Batko (Zygomycetes: Entomophthorales) fungus to attract and infect male *Plutella xylostella* L. (Lepidoptera: Yponomeutidae), killing 76% of adults in 3.5 days.

In summary, based on physiological and pathogenic characteristics, *M. anisopliae* (Ma-San Rafael-2) was a good candidate for evaluation through the auto-dissemination and sex pheromone technique for management of *S. frugiperda*. However, information generated is at a basic level and requires validation through field studies that include the three main components of epidemiology: pathogen, host, and agroecosystem (environment), as well as design and evaluation of trap prototypes baited with insect sex pheromone and fungus. Information generated in different work will allow exploring alternative management of the insect such as massive trapping through insect attraction and infection devices that allow release in the field to cause epizootics, as was reported for management of *P. xylostella* (Furlong et al. 1995), *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) (Lopes et al. 2014), and *Agriotes obscurus* L. (Coleoptera: Elateridae) (Kabaluk et al. 2015).

Acknowledgment

We thank the Consejo Nacional de Ciencia y Tecnología (CONACYT) and the Programa de Becas de Estímulo de Formación de Investigadores-IPN (BEIFI), for financial support. We thank Dr. María Valentina Angoa Pérez, Dr. Monserrat Vázquez Sánchez, and Dr. Luis Fernando Ceja Torres from the Instituto Politécnico Nacional-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional CIIDIR-IPN Unidad Michoacán for support provided to do the work, and to Dr. Samuel Cruz-Esteban from the Colegio de la Frontera Sur (ECOSUR Unidad,

Tapachula, Chiapas), Dr. Julio S. Bernal of Texas A&M University, and Dr. Agustín Garzón Hidalgo of Crop Protection Unit, Technical University of Madrid for reviewing the manuscript.

References Cited

Arrubla, P. F., R. M. Cárdenas, and F. J. Posada. 2010. Efecto de la humedad relativa sobre la germinación de las esporas de *Beauveria bassiana* y la patogenicidad a la broca del café *Hypothenemus hampei*. Rev. U.D.C.A. Actual. Divulg. Cient. 13: 67-76.

Bateman, R., and A. Chapple. 2001. The spray application of mycopesticide formulations, pp. 289-309. *In* T. M. Butt, C. Jackson, and N. Magan [eds.], Fungi as Biocontrol Agents Progress, Problems and Potential. CABI Publishing, London, UK.

Borisade, O. A., A. Medina, and N. Magan. 2016. Interacting temperature and water activity modulate production of Destruxin A by *Metarhizium anisopliae* on *Galleria* larvae-modified agar based media *in vitro*. West Afr. J. Appl. Ecol. 24: 31-42.

Burtet, L. M., O. Bernardi, A. A. Melo, M. P. Pes, T. T. Strahl, and J. V. Guedes. 2017. Managing fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), with Bt maize and insecticides in southern Brazil. Pest Manage. Sci. 73: 2569-2577.

Cortez-Madrigal, H., R. Alatorre-Rosas, G. Mora-Aguilera, H. Bravo-Mojica, C. F. Ortiz-Garcia, and L. A. Aceves-Navarro. 2003. Characterization of multisporic and monosporic isolates of *Lecanicillium* (= *Verticillium*) *lecanii* for the management of *Toxoptera aurantii* in cocoa. BioControl 48: 321-334.

Cortez-Madrigal, H., J. M. Sánchez-Saavedra, G. Díaz-Godínez, and G. Mora-Aguilera. 2014. Enzymatic activity and pathogenicity of entomopathogenic fungi from central and southeastern Mexico to *Diaphorina citri* (Hemiptera: Psyllidae). Southwest. Entomol. 39: 491-502.

Cruz, I., M. D. L. C. Figueiredo, R. B. Da Silva, I. F. Da Silva, C. D. Paula, and J. E. Foster. 2012. Using sex pheromone traps in the decision-making process for pesticide application against fall armyworm (*Spodoptera frugiperda* [Smith] [Lepidoptera: Noctuidae]) larvae in maize. Int. J. Pest Manage. 58: 83-90.

Cruz-Esteban, S., J. C. Rojas, D. Sánchez-Guillén, L. Cruz-López, and E. A. Malo. 2018. Geographic variation in pheromone component ratio and antennal responses, but not in attraction, to sex pheromone among fall armyworm populations infesting corn in Mexico. J. Pest Sci. 3: 973-983.

Doberski, J. W. 1981. Comparative laboratory studies on three fungal pathogens of the elm bark beetle *Scolytus scolytus*: effect of temperature and humidity on infection by *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces farinosus*. J. Invertebr. Pathol. 37: 195-200.

El-Sufty, R., S. Al Bgham, S. Al Awash, A. Shahdad, and A. Al Bathra. 2011. A trap for auto-dissemination of the entomopathogenic fungus *Beauveria bassiana* by red palm weevil adults in date palm plantations. Egypt J. Biol. Pest Control 21: 271-276.

Faria, M., R. B. Lopes, D. A. Souza, and S. P. Wraight. 2015. Conidial vigor *vs* viability as predictors of virulence of entomopathogenic fungi. J. Invertebr. Pathol. 125: 68-72.

Farias, J. R., D. A. Andow, R. J. Horikoshi, R. J. Sorgatto, P. Fresia, A. C. dos Santos, and C. Omoto. 2014. Field-evolved resistance to *Cry1F* maize by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Brazil. Crop Prot. 64: 150-158.

Furlong, M. J., J. K. Pell, O. P. Choo, and S. A. Rahman. 1995. Field and laboratory evaluation of a sex pheromone trap for the auto-dissemination of the fungal entomopathogen *Zoophthora radicans* (Entomophthorales) by the diamondback moth, *Plutella xylostella* (Lepidoptera: Yponomeutidae). Bull. Entomol. Res. 85: 331-337.

García, C., M. B. González, and M. N. Bautista. 2011. Patogenicidad de aislamientos de hongos entomopatógenos contra *Spodoptera frugiperda* (Lepidoptera: Noctuidae) y *Epilachna varivestis* (Coleoptera: Coccinellidae). Rev. Colomb. Entomol. 37: 217-222.

García-Munguía, C. A., F. Reyes-Villanueva, M. A. Rodríguez-Pérez, H. Cortez-Madrigal, M. Acosta-Ramos, L. A. Ibarra-Juárez, M. A. Velázquez-Machuca, J. T. Silva-García, M. Rebollar-Plata, and A. M. García-Munguía. 2015. Auto-dissemination of *Metarhizium anisopliae* and *Beauveria bassiana* in *Musca domestica* L. results in less oviposition and short gonotrophic cycle. Southwest. Entomol. 40: 519-529.

Getahun, M. N., T. D. Biasazin, Y. Wolde-Hawariat, J. M. Bengtsson, Y. Hillbur, and E. Seyoum. 2016. *Metarhizium* spp. isolated from dead *Pachnoda interrupta* (Coleoptera: Scarabaeidae) as a potential entomopathogenic fungus for the pest insect: proof-of-concept for auto-dissemination. Int. J. Trop. Insect Sci. 36: 1-9.

Goergen, G., P. L. Kumar, S. B. Sankung, A. Togola, and M. Tamò. 2016. First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One. 11: doi: doi.org/10.1371/journal.pone.0165632.

Greene, G. L., N. C. Leppla, and W. A. Dickerson. 1976. Velvetbean caterpillar (Lepidoptera: Noctuidae) rearing procedure and artificial medium. J. Econ. Entomol. 69: 487-488.

Guerrero, A., E. A. Malo, J. Coll, and C. Quero. 2014. Semiochemical and natural product-based approaches to control *Spodoptera* spp. (Lepidoptera: Noctuidae). J. Pest. Sci. 87: 231-247.

Inglis, G. D., M. S. Goettel, T. M. Butt, and H. Strasser. 2001. Use of hyphomycetous fungi for managing insect pests, pp. 23-71. *In* T. M. Butt, C. Jackson, and N. Magan [eds.], Fungi as Biocontrol Agents Progress, Problems and Potential. CABI Publishing, London, UK.

Jackson, C. W., J. B. Heale, and R. A. Hall. 1985. Traits associated with virulence to the aphid *Macrosiphoniella sanborni* in eighteen isolates of *Verticillium lecanii*. Ann. Appl. Biol. 106: 39-48.

Kabaluk, J. T., J. P. Lafontaine, and J. H. Borden. 2015. An attract and kill tactic for click beetles based on *Metarhizium brunneum* and a new formulation of sex pheromone. J. Pest Sci. 88: 707-716.

King, A. B. S., and J. L. Saunders. 1984. Las plagas Invertebradas de Cultivos Anuales Alimenticios en América Central. Administración de Desarrollo Extranjero (ODA), London, UK.

Lacey, L. A., R. Frutos, H. K. Kaya, and P. Vail. 2001. Insect pathogens as biological control agents: do they have a future? Biol. Control 21: 230-248.

Lipa, J. J., and K. Slizynsky. 1973. Wskazówki metodyczne I. Terminologóa do wyznaczania snedniej dawki swiertelnej (LD_{50}). W Patologia Owadow I Tksykologia. Prace Navkowe Instytutu Ochrony Roslin. 15: 59-83.

Lopes, R. B., R. A. Laumann, D. Moore, M. W. Oliveira, and M. Faria. 2014. Combination of the fungus *Beauveria bassiana* and pheromone in an attract-and-kill strategy against the banana weevil, *Cosmopolites sordidus*. Entomol. Exp. Appl. 151: 75-85.

Malo, E. A., F. Bahena, M. A. Miranda, and J. Valle-Mora. 2004. Factors affecting the trapping of males of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) with pheromones in Mexico. Fla. Entomol. 87: 288-293.

Mascarin, G. M., and S. T. Jaronski. 2016. The production and uses of *Beauveria bassiana* as a microbial insecticide. World J. Microbiol. Biotechnol. 32: 1-26.

Meyling, N. V., and J. Eilenberg. 2007. Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: potential for conservation biological control. Biol. Control 43: 145-155.

Mora-Aguilera, G., H. Cortez-Madrigal, and G. Acevedo-Sánchez. 2017. Epidemiology of entomopathogens: Basis for rational use of microbial control of insects. Southwest. Entomol. 42: 153-169.

Nagoshi, R. N., and R. L. Meagher. 2004. Seasonal distribution of fall armyworm (Lepidoptera: Noctuidae) hosts strains in agricultural and turf grass habitats. Environ. Entomol. 33: 881-889.

Palem, P., and V. Padmaja. 2010. Impact of different relative humidities on *in vitro* growth and sporulation of entomopathogenic fungal isolates of *Beauveria bassiana* species. Int. J. Pharm. Biol. Sci. Arch. 1: 355-359.

Perez-Zubiri, J. R., E. Cerna-Chavez, L. A. Aguirre-Uribe, J. Landeros-Flores, M. K. Harris, and R. Rodriguez-Herrera. 2016. Population variability of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize (Poales: Poaceae) associated with the use of chemical insecticides. Fla. Entomol. 99: 329-331.

Pimentel, D., and C. A. Edwards. 1982. Pesticides and ecosystems. BioScience 32: 595-600.

Romero-Arenas, O., A. Rivera, A. Aragon, C. Parraguirre, E. Cabrera, and F. López. 2014. Mortality evaluation of armyworm (*Spodoptera frugiperda* JE Smith) by using *Metarhizium anisopliae in vitro*. J. Pure Appl. Microbiol. 8: 59-67.

SAS Institute. 2012. SAS/STAT Users Guide, Release 9.3. North Carolina.

Shah, P. A., and J. K. Pell. 2003. Entomopathogenic fungi as biological control agents. Appl. Microbiol. Biotechnol. 61: 413-423.

Suárez-Núñez, J., H. Cortez-Madrigal, and A. M. García-Munguía. 2017. Epidemiology of *Beauveria bassiana* in controlled populations of *Bactericera cockerelli*. Southwest. Entomol. 42: 1041-1056.

Torres de La Cruz, M., H. Cortez-Madrigal, G. Ortiz, F. Carlos, G. Cappello, and P. A. de la Cruz. 2013. Caracterización de aislamientos nativos de *Metarhizium anisopliae* y su patogenicidad hacia *Aeneolamia postica*, en Tabasco, México. Rev. Colomb. Entomol. 39: 40-46.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80-100.

Characterization of *Beauveria bassiana*[1] Isolates Associated with *Euwallacea* sp. nr. *fornicatus*[2] in *Populus* sp.

R. Montesinos-Matías[3], A. Gallou[3], A. M. Berlanga-Padilla[3],
M. G. Serna-Domínguez[3], B. Laureano-Ahuelicán[4], M. A. Ayala-Zermeño[3],
A. Ordáz-Hernández[5], J. A. López-Buenfil[6], and H. C. Arredondo-Bernal[3]*

Abstract. Introduction and spread of *Euwallacea* species in Mexico are a significant threat to production of avocado (*Persea americana* Mill.) and other potential hosts in urban and natural areas. Biological control with entomopathogenic fungi suggests an alternative to maintain pest abundance below the economic threshold. Nevertheless, isolation and characterization of strains capable of infecting *Euwallacea* spp. in local environmental conditions were previously required. Derived from the collection of natural enemies of Kuroshio shot hole borer, *Euwallacea* sp. nr. *fornicatus* (Eichhoff), mycosed adults were found in *Populus* sp. at Tijuana, Baja California, Mexico. Morphological (conidia and phialides were measured) and molecular (translation elongation factor and Bloc gene regions were sequenced) analyses resulted in the identification of two isolates of *Beauveria bassiana* (Bals.-Criv.) Vuill. Both strains produced greater growth between 25 and 27°C, and pathogenicity tests in Kuroshio shot hole borer ranged from 58.3 to 83.3%. It is the first report of *B. bassiana* in *Euwallace* sp. nr. *fornicatus* in Mexico.

Introduction

Ambrosia beetles are insects that have mycangia (Carrillo et al. 2014), which are specialized structures that assist in the transfer of symbiotic fungi (Freeman et al. 2016) from one host to another with the objective of growing the fungi for the beetle to feed on until the adult stage. Ambrosia beetles usually infest dead or stressed trees, but some exotic species attack healthy trees (Eskalen 2013, Freeman et al. 2016) and can cause death within weeks or months (Mendel et al. 2012). Shot hole borer-*Fusarium* dieback is a new pest-disease complex that affect several tree species in California and is vectored by two distinct, but related ambrosia beetles (*Euwallacea* sp. nr. *fornicatus* [Eichhoff]) called polyphagous shot hole borer and Kuroshio shot hole borer (Na et al. 2018). This pest-disease complex causes branch

---

[1]Hypocreales: Cordycipitaceae
[2]Coleoptera: Curculionidae: Scolytinae
[3]Centro Nacional de Referencia de Control Biológico. Km 1.5 Carretera Tecomán-Estación FFCC, Col. Tepeyac, C.P. 28110 Tecomán, Colima, México.
[4]Fitopatología, Postgrado en Fitosanidad, Entomología y Acarología. Colegio de Postgraduados, Km. 36.5 Carretera México-Texcoco, C.P. 56230 Montecillo, Texcoco, Estado de México, México
[5]Universidad de la Cañada. Carretera Teotitlán - San Antonio Nanahuatipán Km 1.7 s/n., Paraje Titlacuatitla, Teotitlán de Flores Magón, C.P. 68540 Oaxaca, México.
[6]Unidad Integral de Servicios, Diagnóstico y Constatación, Km 37.5. Carr. Federal México-Pachuca, C.P. 55740 Tecámac, Estado de México, México
*Corresponding authors: hugo.arredondo@senasica.gob.mx; montesinosroberto@yahoo.com.mx

dieback and kills trees of numerous wildland and landscape areas (Eskalen 2013, Freeman et al. 2016), as well as agricultural plants (Eskalen 2013), primarily avocado (*Persea americana* Mill.) in the southern United States. Kuroshio shot hole borer recently crossed the border with Mexico, in Baja California, affecting hosts in natural areas and parks (García-Avila et al. 2016).

The natural enemies of bark beetles influence the abundance and ecological dynamics in the place of origin. For example, Wegensteiner et al. (2015) reported that different species of entomopathogenic fungi are capable of infecting and killing members of the same species of bark beetles during each developmental stage, and described extensively fungi associated with different bark beetle species, although the authors did not report natural mycosis of *Euwallacea* spp.

Upon entry of Kuroshio shot hole borer into Baja California, Mexico (García-Avila et al. 2016), the Plant Health General Directorate-National Service for Agri-food Health, Safety and Quality (DGSV-SENASICA) designed a "Plan de acción para la vigilancia y aplicación de medidas de control contra complejos ambrosiales reglamentados en México: *Xyleborus glabratus* (Eichhoff)-*Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva y *Euwallacea* sp.-*Fusarium euwallaceae* S. Freeman, Z. Mendel, T. Aoki & O´Donnell" (http://sinavef.senasica.gob.mx/SIRVEF/ Manuales Operativos.aspx). The action plan presented an integrated management approach for ambrosia beetles and a series of strategies for monitoring and collecting entomopathogenic fungi to infect Kuroshio shot hole borer under local environmental conditions. The objectives of this research were to find and use morphology and molecular analysis to characterize isolates of entomopathogenic fungi associated with *Euwallacea* sp. nr. *fornicatus* at Tijuana, Baja California, Mexico.

Materials and Methods

With Kuroshio shot hole borer at Tijuana, Baja California, and aligned with the phytosanitary actions of confinement and eradication of the pest (http://sinavef.senasica.gob.mx/ALERTAS/inicio/pages/single.php?noticia=2849), exploration activities were done to collect entomopathogenic fungi associated with exotic ambrosia beetles. Samples were sent to the "Colección de Hongos Entomopatógenos" from the "Centro Nacional de Referencia de Control Biológico (CNRCB-CNRF-DGSV-SENASICA)" for analysis.

Conidia were removed from the surface of infected cadavers, isolated and purified on Sabouraud dextrose agar (Bioxon®, Becton Dickinson de Mexico S.A. de C.V. Cuautitlan Izcalli, Mexico) supplemented with yeast extract (1%). After 2 weeks, pure isolations were characterized by morphometric analysis, and identities were confirmed through molecular analysis. From microcultures, the length and width of conidia and phialides were measured (n = 30). Morphometric analysis was done with the aid of an optical microscope (Axio Scope A1, Carl Zeiss, Microscopy GmbH, Gottingen, Germany) using the 100-power objective with phase contrast. Development of entomopathogenic fungi at different incubation temperatures was evaluated in Sabouraud dextrose agar with yeast extract (Ayala-Zermeño et al. 2015).

Molecular identification was performed by phylogenetic analysis using sequences of the nuclear locus translation elongation factor-1α and the nuclear intergenic region Bloc (Rehner et al. 2011). Genomic DNA was extracted from 10 mg of lyophilized mycelium, disrupted with the TissueLyser II™ and one tungsten-carbide bead (3 mm diameter) (Qiagen, Valencia, CA) using a DNeasy Plant Mini Kit

(Qiagen). Amplification reactions were performed using the Phire Hot Start II DNA Polymerase and Veriti™ Thermal Cycler (Thermo Fisher Scientific, Waltham, MA). Purified amplicons were sequenced by the Macrogen Company (Seoul, South Korea). Sequences were edited and assembled using BioEdit 7.2.0 software (Hall 1999), and multiple alignments were done using MAFFT (Katoh et al. 2002). Phylogeny was by Bayesian inference using MrBayes 3.2.5 (Ronquist and Huelsenbeck 2003).

For the pathogenicity test (Koch's postulates), isolates were first grown on Sabouraud dextrose agar with yeast extract for 14 days to attain good sporulation, then adult females of Kuroshio shot hole borer (from a colony established in a laboratory of "Comité Estatal de Sanidad Vegetal de Baja California (CESAVBC)" at Tijuana, Mexico) were submerged for 30 seconds in conidial suspensions (1×10^7 ml^{-1}) prepared in 0.1% aqueous Triton x-100 for each isolate. One inoculated female of Kuroshio shot hole borer was placed in each Falcon tube with 15 ml of diet (Maner et al. 2013) previously sterilized. The experiment was replicated six times for each treatment and data on mortality were recorded at 10 days. Check insects were treated only with 0.1% Triton x-100 and the same conditions mentioned previously. The experiments were repeated three times. Dead insects were transferred to a 1% agar-water layer in Petri dishes to promote mycosis.

Results and Discussion

Mycosed (white muscardine) adult cadavers of *Euwallacea* sp. nr. *fornicatus* were found on poplar trees (*Populus* sp.) at Tijuana (32.53114° N, -117.01765° W), Baja California (Fig. 1a). The micosed insects were analyzed at the "Centro Nacional de Referencia de Control Biológico."

Morphology of the colony differed between the two cultures, and acronyms CHE-CNRCB 636 and CHE-CNRCB 637 were assigned for the two isolates. The average conidia and phialides dimensions were 2.51 ± 0.26 (2.13-3.06) × 2.05 ± 0.22 (1.57-2.59) µm and 2.93 ± 0.34 (2.36-3.93) × 2.23 ± 0.31 (1.5-2.8) µm for strain 636 (Fig. 2) and 3.02 ± 0.37 (2.41-3.81) × 2.34 ± 0.29 (1.9-2.88) µm and 3.86 ± 0.40 (3.1-4.55) × 3.42 ± 0.55 (2.77-4.24) µm for strain 637. The colonies of both isolates were dusty white, whereas the reverse had slightly orange-yellow tones. The colors are characteristic of *Beauveria bassiana* (Bals.-Criv) Vuill. Both strains produced greater growth between 25 and 27°C (Fig. 2), which are thermal conditions required in the local environment for development of *B. bassiana* on ambrosia beetles.

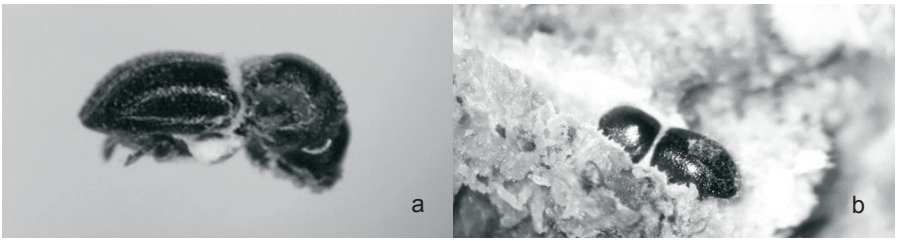

Fig. 1. Beetles collected in *Populus* sp. (a) and mycosed Kuroshio shot hole borer during pathogenicity tests (b).

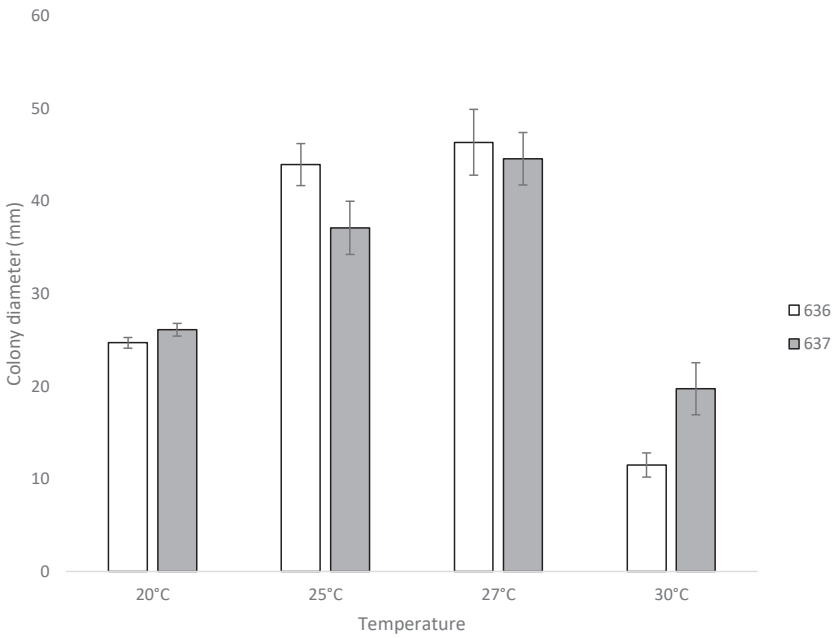

Fig. 2. Growth of *Beauveria bassiana* colonies after 14 days of incubation at different temperatures. Bars represent standard errors of the mean for temperature evaluated.

Bayesian inference confirmed the identity of the fungi as *B. bassiana* by grouping on the clade of the ex-type isolate ARSEF 1564 with a value of 100% of Bayesian inference posterior probabilities (Fig. 3) (GenBank accession numbers TEF: KY605378 and KY605379 and Bloc: KY605376 and KY605377 for isolates CHE-CNRCB 636 and 637, respectively).

The inoculum quality test for the bioassays showed 94.9 and 97.7% germination at 18 hours for isolates 636 and 637, respectively. The pathogenicity test showed mortality values of 83.3 and 58.3% for 636 and 637 isolates, respectively. Mycoses observed for the isolates were more than 66% (Fig. 1b). No insects were killed in the check.

Reports in the literature indicate the natural presence of entomopathogenic fungi at galleries of bark beetles; for example, Wegensteiner et al. (2015) reported that *B. bassiana* had been isolated from at least 11 scolytine species on different tree species. In India, Selvasundaram and Muraleedharan (2000) reported natural occurrence of *B. bassiana* on polyphagous shot hole borer in tea trees (*Camellia sinensis* L. O. Kuntze).

Beauveria bassiana was reported for the first time in association with Kuroshio shot hole borer, and both isolates showed pathogenicity to the ambrosia beetle. Discovery of pathogens adapted to weather conditions of the host promises to provide a viable alternative for development of technology for biocontrol of ambrosia beetles. Research should consider biological control as an option, since the use of pesticides in Mexico is restricted for national consumption and export of avocado, in addition to the large number of ecologically important hosts due to advance of the pest.

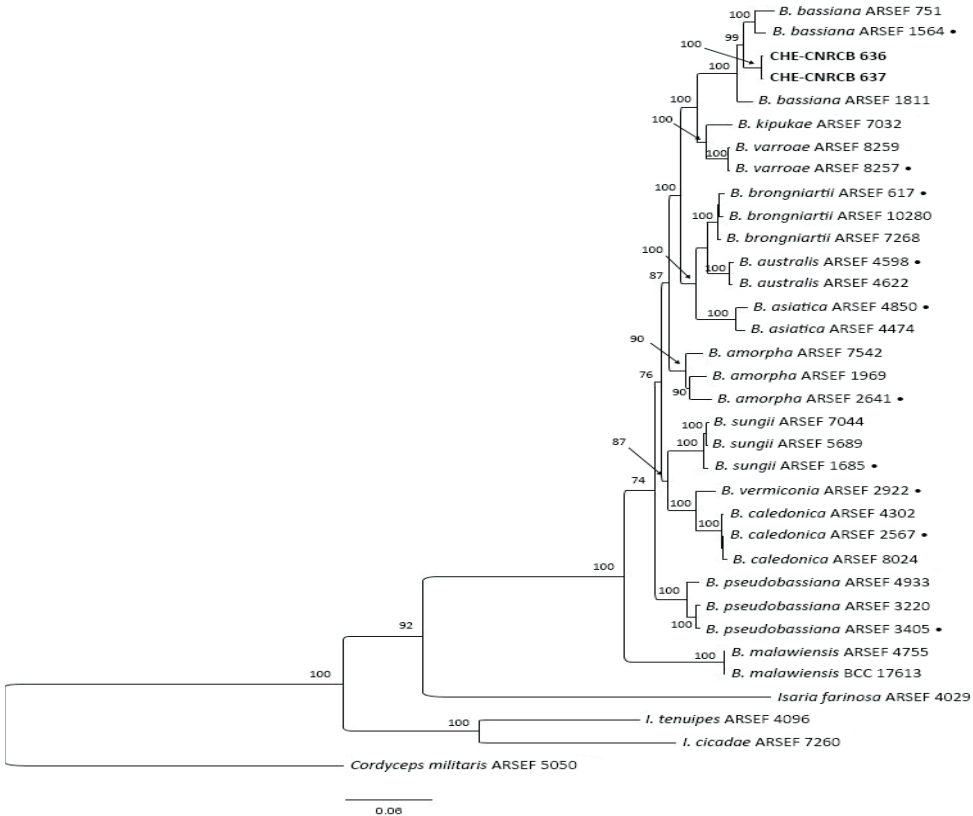

Fig. 3. Phylogram from Bayesian inference based on joint TEF and Bloc sequences. The analyses were run with 1,000,000 generations, sampling every 300 generations, under the general time-reversible evolution model with gamma distribution. Support of the branches is represented as a numerical value at each node. Ex-type isolates (Rehner et al. 2011) are marked with a (●).

Acknowledgment

Biol. Nallely Acevedo-Reyes (Departamento de Entomología y Acarología, Centro Nacional de Referencia Fitosanitaria, DGSV-SENASICA) for her support in the identification of ambrosia insects. Also, to Jaime González Cabrera and Beatriz Rodríguez Vélez for their comments to the final manuscript.

References Cited

Ayala-Zermeño, M. A, A. Gallou, A. M. Berlanga-Padilla, M. G. Serna-Domínguez, H. C. Arredondo-Bernal, and R. Montesinos-Matías. 2015. Characterisation of entomopathogenic fungi used in the biological control programme of *Diaphorina citri* in Mexico. Biocontrol Sci. Techn. 25: 1192-1207.

Carrillo, D., R. E. Duncan, J. N. Ploetz, A. F. Campbell, R. C. Ploetz, and J. E. Peña. 2014. Lateral transfer of a phytopathogenic symbiont among native and exotic ambrosia beetles. Plant Pathol. 63: 54-62.

Eskalen, A. 2013. Host range of Fusarium dieback and its ambrosia beetle (Coleoptera: Scolytinae) vector in Southern California. Plant Dis. 97: 938-951.

Freeman, S., M. Sharon, M. Dori-Bachash, M. Maymon, E. Belausov, Y. Maoz, and Z. Mendel. 2016. Symbiotic association of three fungal species throughout the life cycle of the ambrosia beetle *Euwallacea* nr. *fornicatus.* Symbiosis 68: 115-128.

García-Avila, C. J., J. T. Trujillo-Arriaga, J. A. López-Buenfil, R. González-Gómez, D. Carrillo, L. F. Cruz, and N. Acevedo-Reyes. 2016. First report of *Euwallacea* nr. *fornicatus* (Coleoptera: Curculionidae) in Mexico. Fla. Entomol. 99: 555-556. https://www.pestalerts.org/espanol/oprDetail.cfm? oprID=730).

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. (Oxf). 41: 95-98.

Katoh, K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 30: 3059-3066.

Maner, L., J. L Hanula, and S. K. Barman. 2013. Rearing redbay ambrosia beetle, *Xyleborus glabratus* (Coleoptera: Curculionidae: Scolytinae), onsemi-artificial media. Fla. Entomol. 96: 1042-1051.

Mendel, Z., A. Potasov, M. Sharon, A. Zveibil, S. B. Yehuda, K. O´Donnell, and S. Freeman. 2012. An Asian ambrosia beetle *Euwallacea fornicatus* and its novel symbiotic fungus *Fusarium* sp. pose a serious threat to the Israeli avocado industry. Phytoparasitica 40: 235-238.

Na, F., J. D. Carrillo, J. S. Mayorquin, et al. 2018. Two novel fungal symbionts *Fusarium kuroshium* sp. nov. and *Graphium kuroshium* sp. nov. of Kuroshio shot hole borer (*Euwallacea* sp. nr. *fornicatus*) Cause fusarium dieback on woody host species in California. Plant Disease 102: 1154-1164.

Rehner, S. A., A. M. Minnis, G. Sung, J. J. Luangsa-ard, L. Devotto, and R. A Humber. 2011. Phylogeny and systematics of the anamorphic, entomopathogenic genus *Beauveria*. Mycologia 103: 1055-1073.

Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes version 3.0: Bayesian phylogenetic inference under mixed models. Bioinformatics 19: 1572-1574.

Selvasundaram, R., and N. Muraleedharan. 2000. Occurrence of the entomogenous fungus *Beauveria bassiana* on the shot hole borer of tea. J. Plant. Crops 28: 229-230.

Wegensteiner, R., B. Wermelinger, and M. Herrmann. 2015. Natural enemies of bark beetles: predators, parasitoids, pathogens, and nematodes, pp. 247-304. *In* F. E. Vega and R. W. Hofstetter [eds.], Bark Beetles: Biology and Ecology of Native and Invasive Species. Elsevier, London.

Susceptibility of *Rhagoletis suavis*[1] Maggots to Entomopathogenic Fungi

Muhammad J. Nisar[2], Robert W. Behle[3], Christopher A. Dunlap[3], Erica J. Goett[3], Mubashar Iqbal[2], and Muhammad Dildar Gogi[2]

Abstract. Three entomopathogenic fungi, *Beauveria bassiana* (Bilsamo) Vuill. GHA, *Metarhizium brunneum* (Petch) F52, and *Isaria javanica* (Wise) strain 439 were evaluated for insecticidal activity against walnut husk maggot, *Rhagoletis suavis* (Loew), collected from husks of black walnut, *Juglans nigra* L., to identify the best pathogen for development of augmented biological control of the pest. The species of *Rhagoletis* from the field was confirmed to be *sauvis* by mitochondrial cytochrome c oxidase subunit I gene sequencing and by wing color patterns of adults. The larval stage of the walnut husk maggot was susceptible to each of the fungal pathogens. More field-collected maggots died from exposure to greater concentrations of fungal conidia. Probit analysis showed *M. brunneum* was 139 and 155 times more potent against maggots than were *B. bassiana* and *I. javanica*, respectively. Greater insecticidal activity of *Metarhizium* supports selection for further study as a biological control agent against *Rhagoletis*.

Introduction

Walnut husk maggot, *Rhagoletis suavis* (Loew) (Diptera: Tephritidae), is a pest of black walnut, *Juglans nigra* L., and English walnut, *J. regia* L. (Brooks 1921) and is distributed throughout the eastern third of the United States (Riedl 1993, Rull et al. 2013). Damage is caused by maggots feeding inside the husk surrounding the nut, making galleries, and causing spongy, rotten decomposition inside walnuts (Brooks 1921). This reduces walnut quality because the texture and color of the nuts change and the fruit shrivels and falls prematurely from the tree. The insect has one generation per year. Adults emerge and mate from July through October (Gibson and Kearby 1978). A female lays eggs by inserting her ovipositor inside the peel on the outer layer of developing walnuts. The eggs hatch in 5-7 days. When infested, fruit falls to the ground, and maggots move into soil to overwinter as pupae (Brooks 1921, Davis et al. 2015).

In the tephridid genus *Rhagoletis*, the *sauvis* group consists of six Neartic endemic species that have similar life cycles and feed on husks of walnuts in the United States and Mexico (Tadeo et al. 2013). The walnut husk fly, *Rhagoletis completa* Cresson, received attention in scientific literature because of range expansion to the western United States in 1929 (Boyce 1931) and western Europe in the late 1980s (Verheggen et al. 2017). Commercial production of English walnuts might require pest control measures to limit damage by *R. sauvis* and *R. completa*

[1]Diptera: Tephritidae

[2]Department of Entomology, University of Agriculture, Faisalabad Pakistan
[3]United States Department of Agriculture, Agriculture Research Service, National Center for Agricultural Utilization Research, Peoria, IL

(Van Steenwyk et al. 2010, Nix et al. 2014). Ninety percent of walnuts can be infested (Kasana and Aliazee 1996, Duso and Lago 2006). Life cycle similarities suggest that successful control measures for one species might be effective against other species in the group.

A variety of methods, including natural (Riedl 1993), cultural (Coats 2004), and chemical (Riedl and Hislop 1985, Van Steenwyk et al. 2010) are used to control flies in the *sauvis* group. Biological control techniques need study for use in an integrated approach against *Rhagoletis* spp. flies (Verheggen et al. 2017). Entomopathogenic fungi have been tested for insecticidal activity against related flies including European cherry fruit fly, *R. cerasi* L. (Daniel and Wyss 2009, 2010), and Western cherry fruit fly, *R. indifferens* Curran (Yee and Lacey 2005, Cossentine et al. 2010). There are no reports evaluating fungal pathogens to control the *suavis* group. In this research, maggots from field-collected walnuts were exposed to three entomopathogenic fungi to compare dosage response mortalities among the pathogens.

Experiments were conducted during 2017 at the National Center for Agricultural Utilization Research, Agricultural Research Service, USDA, Peoria, IL. Maggots were separated from walnuts collected at a single location (40.647, -89.424) in Tazewell County, Illinois. Infested walnuts were collected from a native stand of black walnut trees between 15 September and 5 November 2017.

The species of *Rhagoletis* maggots collected in the field was determined using PCR analysis on random insects surviving the check treatment for each fungal assay. Eighty-five pupae were placed individually into 1.5-ml microcentrifuge tubes and macerated with a pointed spatula in 50 µl of ATL (tissue lysis) buffer (Qiagen, Foster City, CA). The samples were shaken for 30 minutes at 60ºC and at 1,800 rpm for 30 minutes. The samples were centrifuged at 10,000 g for 10 minutes, the supernatant was decanted, and DNA was captured in 50 µl of AMpure XP magnetic beads (Beckman Coulter, Indianapolis, IN). The beads were washed twice with 70% ethanol, allowed to dry for 5 minutes, and resuspended in 30 µl of water. The cyctochrome oxidase I (COI) gene was amplified. A region of the 3' region of COI was amplified using primers "CI-J-2792 (George)" 5'- ATACCTCGACGTTATTCAGA-3' and "TK-N-3772" 5'- GAGACCATTACTTGCTTTCAGTCATCT-3' (Bogdanowicz et al. 1993). Resulting DNA sequences were visually edited and assembled using Sequencer® 5.4 software (Gene Codes Corporation, Ann Arbor, MI). Consensus sequences were compared with GenBank and Barcode of Life databases to identify the species.

Three fungi were evaluated for insecticidal activity against *Rhagoletis* maggots collected from black walnut husks. *Beauveria bassiana* (Bilsamo) Vuill. GHA and *Metarhizium brunneum* (Petch) F52, were selected as two commercial strains of entomopathogenic fungi. *Isaria javanica* (Wise) strain MBC 439 was selected for the study because it infects other Diptera (Jose Louis Ramirez, personal communication). Conidia were produced by growing cultures on potato dextrose agar in Petri dishes at 28ºC for as many as 30 days. Fungal spores were collected by scraping dry conidia from the dishes and diluting with water to create stock suspensions with concentrations of 1 to 50 × 10^8 conidia ml^{-1} determined by counting conidia using a hemocytometer (Bright-line, Housser Scientific, Horsham, PA). Viability was verified as > 90% germination of conidia on agar following procedures by Daniel and Wyss (2009). Application concentrations were based on numbers of conidia not corrected for spore viability.

Maggots were collected by dissecting infested walnuts, placed in a sieve, and washed with a gentle stream of distilled water. Washed maggots were placed in

plastic Petri dishes (100 × 15 mm) containing agar (2% w/w), wrapped with paraffin, and kept for 24 hours at room temperature (21ºC) to clear the intestines before use in insect exposure assays.

Three dosage-response experiments were done for each fungus on different days with different batches of maggots. For the first experiment, each fungal stock suspension was serially diluted (10×) to create four treatment concentrations of 3×10^7 to 3×10^4 conidia ml^{-1}. Each treatment (fungus × conidial concentration) was pipetted onto agar in Petri dishes (0.5 ml per dish, five dishes per treatment, and five nontreated check dishes), and conidia were uniformly dispersed over the agar surface with a spreader. Excess water was allowed to dry by leaving dishes open in a biological hood. Once dry, 10 healthy maggots were selected randomly and individually transferred to a treated dish. Dishes were wrapped with parafilm to retain moisture and kept at room temperature in the dark. After 7 days, number of dead larvae were counted. For the second and third experiments, conidial concentrations were adjusted to encompass 50% mortality indicated by the first experiment.

Mortality data were subjected to Abbott's correction because many insects of the check died. Probit analysis used POLOPlus software (LeOra Software LLC, CA) to determine LC_{50} values for each fungal treatment. Significant differences were identified based on the lethal dosage ratio for which the 95% confidence interval did not include the value of 1.0 (Robertson and Preisler 1992).

Results

DNA analysis confirmed the insects were *R. suavis* with 99% homology to GenBank sequence data. In total, 85 pupae were tested, and all COI sequence data were clonal. Because of a limited amount of COI sequence data available for the species in GenBank, our data were accessioned to support future studies (GenBank accession #MG916899). Insects that successfully pupated and subsequently emerged as adults also had the wing pattern of *R. suavis*.

The three fungal strains successfully infected field-collected maggots. Insect cadavers and subsequent mycosis expressed physical characteristics specific to each fungus. *Beauveria* resulted in pink cadavers and white sporulation often associated with the fungus. *Metarhizium* resulted in production of olive-green conidia after mycosis. *Isaria* had faster mycosis than did *Beauveria* or *Metarhizium*, and sporulation was slightly pink. The observations were typical for the pathogens.

The numbers of insects that died in the check varied among individual assays and as a result, treatment mortality data were corrected using Abbott's (1925) formula before probit analysis. Probit analysis of the full data set showed *Metarhizium* was 149× (ratio confidence limits 59.8 and 371.1) more potent than *Beauveria* against maggots. Activity of *Isaria* was similar to *Beauveria* (ratio = 1.03, confidence limits 0.50 and 2.12). The mortality data varied significantly from the probit model for *Beauveria* (X^2 = 98.6) and *Isaria* (X^2 = 64.5) fungi. Residual analysis indicated several data points were outliers (residual values >10 or <-10) that were omitted from the data set (Table 1). X^2 values were less, although the LC_{50} values remained similar to those from analysis of the full data set. *Metarhizium* was more effective than *Beauveria* (139×) or *Isaria* (155×).

Table 1. Probit Analysis Parameters of Dosage Response for *R. sauvis* Maggots Exposed to Entomopathogenic Fungi Using Reduced Set of Mortality Data Corrected for Check Mortality and Removing Outliers (residual value >10)

Fungus	n	LC_{50}, conidia cm^{-2}	-95% CL	+95% CL	Slope	X^2	df
B. bassiana	650	$2.00e^4$	$0.52e^4$	$30.10e^4$	0.580	44.9	8
M. brunneum	750	$1.44e^2$	$0.21e^2$	$4.56e^2$	0.384	26.2	10
I. javanica	650	$2.24e^4$	$1.09e^4$	$4.75e^4$	0.711	18.0	8

n = number of exposed maggots; X^2 = Chi-square value; df = degrees of freedom

Discussion

The research adds to understanding the potential for entomopathogenic fungi to provide biological control of fruit flies. Garrido-Jurado et al. (2011) reported more *Ceratitis capitata* (Weidemann) pupae were dead when exposed to *Metarhizium* rather than *Beauveria* conidia. By contrast, Cossentine et al. (2011) reported similar amounts of infection of *R. indifferens* pupae exposed to *Metarhizium*- or *Beauveria*-treated soil (Conssentine et al. 2010). This study compared three fungal agents against larvae and supports further development of *Metarhizium* as a component of an integrated pest management program.

In a field environment, *Metarhizium* could be applied to soil under infested trees, specifically targeting the pre-imaginal stage as maggots exit walnuts to pupate in the soil. Reducing the abundance of walnut husk maggots by treating soil under infested trees with entomopathogenic fungi has not been demonstrated. Yet, the potential for success seems good when considering recent reports of soil treatments for other pests (Cossentine et al. 2011). Yousef et al. (2017) reported 50 to 70% fewer adult olive fruit fly, *Bactrocera oleae* (Rossi), with two soil applications of conidia targeting pre-imaginals and emerging adults during a 4-year period. Behle et al. (2015) reported significantly fewer white grubs of Japanese beetle, *Popillia japonica* Newman, in turf grass where *M. brunneum* was applied as spray or granules. Most control was by treatments applied late in the fall -- September rather than August. The later season corresponds with the time the walnut husk maggot exits the walnut to pupate in the soil. Most maggots pupate within a few centimeters of the soil surface (Davis et al. 2015), which might increase the potential for contact with infective conidia from topically applied fungi.

Discovery that *Metarhizium* fungus has insecticidal activity against maggots supports further development of the fungus as a biological control agent. When considering this observation with recent improvement in fermentation production of *Metarhizium* (Behle and Jackson 2014), the combination warrants field evaluation for control of *Rhagoletis* flies that enter the soil to pupate. Benefits of successful development might include protecting beneficial insects in orchards by mitigating the need for application of broad-spectrum insecticides. Soil application of an entomopathogenic fungus probably will fit as a control suitable in organic production.

Disclaimer

Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Behle, R. W., and M. A. Jackson. 2014. Effect of fermentation media on the production, efficacy, and storage stability of *Metarhizium brunneum* microsclerotia formulated as a prototype granule. J. Econ. Entomol. 107: 582-590.

Behle, R. W., D. S. Richmond, M. A. Jackson, and C. A. Dunlap. 2015. Evaluation of *Metarhizium brunneum* F52 (Hypocreales: Clavicipitaceae) for control of Japanese beetle larvae in turfgrass. J. Econ. Entomol. 108: 1587-1595.

Bogdanowicz, S. M., W. E. Wallner, J. Bell, T. M. Odell, and R. G. Harrison. 1993. Asian gypsy moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. Ann. Entomol. Soc. Am. 86: 710-715.

Boyce, A. M. 1931. The diapause phenomenon, in insects, with special reference to *Rhagoletis completa* Cresson (Diptera: Trypetidae). J. Econ. Entomol. 24: 1018-1024.

Brooks, F. E. 1921. Walnut Husk-Maggot. U.S.D.A. Bulletin 992.

Coates, W. W. 2004. Walnut husk fly: varietal susceptibility and quality observations. Walnut Research Report, University of California, Berkeley, CA. http://info.ucanr.org/fnwalnuts/walnut_proc/2004/2004_179.pdf.

Cossentine, J., H. Thistlewood, M. Goettel, and S. Jaronski. 2010. Susceptibility of preimaginal western cherry fruit fly, *Rhagoletis indifferens* (Diptera: Tephritidae) to *Beauveria bassiana* (Balsamo) Vuillemin Clavicipitaceae (Hypocreales). J. Invertebr. Pathol. 104: 105-109.

Cossentine, J., S. Jaronski, H. Thistlewood, and W. Yee. 2011. Impact of *Metarhizium brunneum* (Hypocreales: Clavicipitaceae) on pre-imaginal *Rhagoletis indifferens* (Diptera: Tephritidae) within and on the surface of orchard soil. Biocont. Sci. Technol. 21: 501-1505.

Daniel, C., and E. Wyss. 2009. Susceptibility of different life stages of the European cherry fruit fly, *Rhagoletis cerasi*, to entomopathogenic fungi. J. Appl. Entomol. 133: 473-483.

Daniel, C., and E. Wyss. 2010. Field applications of *Beauveria bassiana* to control the European cherry fruit fly *Rhagoletis cerasi*. J. Appl. Entomol. 134: 675-681.

Davis, J. M., L. E. Coogan, and D. R. Papaj. 2015. Big maggots dig deeper: size-dependent larval dispersal in flies. Oecologia 179: 55-62.

Duso, C., and G. D. Lago. 2006. Life cycle, phenology and economic importance of the walnut husk fly *Rhagoletis completa* Cresson (Diptera: Tephritidae) in northern Italy. Ann. Soc. Entomol. France 42: 245-254.

Garrido-Jurado, I., J. Torrent, V. Barron, A. Corpas, and E. Quesada-Moraga. 2011. Soil properties affect the availability, movement, and virulence of entomopathogenic fungi conidia against puparia of *Ceratitis capitata* (Diptera: Tephritidae). Biol. Cont. 58: 277-285.

Gibson, K. E., and W. H. Kearby. 1978. Seasonal life history of the walnut husk fly and the husk maggot in Missouri. Environ. Entomol. 7: 81-87.

Kasana, A., and M. T. Aliazee. 1996. Seasonal phenology of the walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae). Can. Entomol. 128: 377-390.

Nix, K., C. Coots, P. Lambdin, J. Grant, D. Paulsen, G. Wiggins, and P. Merten. 2014. Concentrations of imidacloprid and olefin-imidacloprid metabolite in the walnut husk maggot (Diptera: Tephritidae). Fla. Entomol. 97: 1848-1851.

Riedl, H. 1993. Walnut husk fly. Orchard pest management: a resource book for the Pacific Northwest. Good Fruit.

Riedl, H., and R. Hislop. 1985. Visual attraction of the walnut husk fly (Diptera: Tephritidae) to color rectangles and spheres. Environ. Entomol. 14: 810-814.

Robertson, J. L., and J. K. Preisler. 1992. Pesticide Bioassays with Arthropods. CRC Press, Boca Raton, FL.

Rull, J., M. Aluja, E. Tadeo, L. Guillen, S. Egan, M. Glover, and J. L. Feder. 2013. Distribution, host plant affiliation, phenology, and phylogeny of walnut-infesting *Rhagoletis* flies (Diptera: Tephritidae) in Mexico. Biol. J. Linnean Soc. 110: 765-779.

Tadeo, E., M. Aluja, and J. Rull. 2013. Alternative mating tactics as potential prezygotic barriers to gene flow between two sister species of frugivorous fruit flies. J. Insect Behav. 126: 708-720.

Van Steenwyk, R. A., S. K. Zolbrod, R. M. Nomoto, J. K. Hasey, J. A. Grant, W. W. Coates, and R. W. Elkins. 2010. Walnut husk fly control with reduced risk insecticides. Acta Hort. 861: 375-382.

Verheggen, F., A. Verhaeghe, P. Giordanengo, X. Tassus, and A. Escobar-Gutierrez. 2017. Walnut husk fly, *Rhagoletis completa* (Diptera: Tephritidae), invades Europe: invasion potential and control strategies. Appl. Entomol. Zool. 52: 1-7.

Yee, W. L., and L. A. Lacey. 2005. Mortality of different life stages of *Rhagoletis indifferens* (Diptera: Tephritidae) exposed to the entomopathogenic fungus *Metarhizium anisopliae*. J. Entomol. Sci. 40: 167-177.

Yousef, M., I. Garrido-Jurado, M. Ruiz-Torres, and E. Quesada-Moraga. 2017. Reduction of adult olive fruit fly populations by targeting preimaginals in the soil with entomopathogenic fungus *Metarhizium brunneum*. J. Pest Sci. 90: 345-354.

Negative Impact of Selected Insecticides on Functional Response and Susceptibility of *Coccinella septempunctata*[1]

Amjad Sultan[2]*, Muhammad Farhanullah Khan[3], Muhammad Faheem Akbar[4], Qurban Ali Arain[5], Fayyaz Nawaz Soomro[6], and Shagufta Siddique[7]

Abstract. Insecticides negatively impact predators. The effect of insecticides on functional response of sevenspotted lady beetle, *Coccinella septempunctata* L., to melon aphid, *Aphis gossypii* Glover, was studied at controlled laboratory conditions of 26 ± 2°C, 65 ± 5% relative humidity, and photoperiod of 16:8 light:dark hours. In response to insecticides, all stages of *C. septempunctata* had Type II functional response, whereas Type III was observed for third, fourth, and adult stages in the check treatment. Least handling time, high attack rate, and greatest theoretical maximum predation were found with buprofezin in 1st instar (0.098±1.461h, 1.179±0.051 h^{-1}, 244.90), 2nd instar (0.04±0.278h, 0.404±0.008h^{-1}, 600.00), 3rd instar (0.032±0.313h, 1.182±0.060h^{-1}, 750.00), 4th instar (0.028±0.14h, 2.704±0.005h^{-1}, 857.14) and adult (0.031±0.159h, 1.137±0.159h^{-1}, 774.19) followed by pymetrozine. To evaluate the impact of insecticide on *C. septempunctata* at field conditions, selected insecticides were sprayed on *C. septempunctata* feeding on A. *gossypii* on chili, *Capsicum annuum* L. Fewest *C. septempunctata* were observed with buprofezin 55±3.56%, 65±8.97%, 85±6.56% and pymetrozine 32±4.45%, 71±10.12%, 84±7.54% at 24, 72, and 240 hours after spraying.

Introduction

Chili, *Capsicum annuum* L., in the family Solanaceae, originated in Latin America and is used throughout the world as a spice and an important condiment crop grown in Pakistan (Khan et al. 2006). It is the most important vegetable after potato, *Solanum tuberosum* L., and tomato, *Solanum esculentum* L. (Iqbal et al. 2011). Cultivation of chilli is capital-intensive because of production constraints, of which losses caused by pests are most important. About 25 insect pests that include aphids, cutworm, fruit borer, plant bug, thrips, whiteflies, and other minor insect pests infest chili (Hussain and Abid 2011). The melon aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is polyphagous and found worldwide in tropical, subtropical, and temperate regions. *A. gossypii* damages melon, cotton, *Gossypium hirsutum* L., and almost 100 other species of crops (Dong et al. 2018). *A. gossypii* also is responsible for transmitting cucumber mosaic virus (Perdikis et al. 2008).

[1]Coleoptera: Coccinellidae

[2]Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Sindh Pakistan
[3]Department of Zoology, University of Karachi, Sindh Pakistan; farhan.ullah.khan@hotmail.com
[4]faheemakbar@yahoo.com
[5]qurbanarain@gmail.com
[6]fayyaz_ali39@yahoo.com
[7]shagsiddique@gmail.com
*Corresponding author: e-mail: ams2410@gmail.com; Telephone: +923333088610

Predators play a vital role in most biological programs to mitigate pest abundance. Lady beetles (Coleoptera: Coccinellidae) are good biological control agents (Dixon et al. 1997). Sevenspotted lady beetle, *Coccinella septempunctata* L., is a generalist predator that preys on a variety of hosts including aphids, mealybugs, scales, and thrips (Arshad et al. 2017, Unal et al. 2017). *C. septempunctata* are good biological control agents in urban and agricultural landscapes throughout the world (Dixon 2000, Hodek and Honěk 2009, Hodek et al. 2012).

Lethal and sublethal effects of insecticide can kill a predator or reduce its efficiency. Sublethal effects of insecticide not only change development and reproductive attributes, but also predation rate and mobility of a predator (Řezáč et al. 2010, He et al. 2012).

Understanding predator-prey interaction is vital to finding functional response. This could be used to measure searching efficiency and repeatedly interrelated with effectiveness of biological control (Bayoumy 2011). Holling (1959a,b; 1965) proposed three categories of functional response. The sum of prey eaten by a predator as prey density increased might increase linearly (Type I), decelerate (Type II), or be sigmoidal (Type III). Functional response is observed mostly as the amount of prey eaten by a specific predator (Abrams and Ginzburg 2000, Sultan and Khan 2014). Functional response often has been presented not only as prey abundance, but other aspects that might affect predator abundance as well as combination with prey density-to-yield ratio (Arditi and Ginzburg 1989, Mills and Lacan 2004, Schenk et al. 2005), abundance of alternate prey (Tschanz et al. 2007), abundance of predator natural enemies (Křivan and Sirot 2004), searching substrate and abiotic factors like temperature (Jalali et al. 2010, Khan and Khan 2010), and insecticide (Poletti et al. 2007, Řezáč et al. 2010, He et al. 2012, Shen et al. 2017). With introduction of new insecticides, effect on predators needs to be identified for integrated pest management programs. The study was designed to observe effects of different newly introduced insecticides on functional response and death of sevenspotted lady beetle. Data from the study could help guide rational use of insecticide useful in improving IPM programs.

Materials and Methods

A camel-hair brush was used daily to collect from chili fresh first, second, third, fourth, and adult stages of sevenspotted lady beetle and third nymphal instar of *A. gossypii* that were put into Petri dishes. Insects were collected from the field at the Department of Agriculture and Agribusiness Management, University of Karachi Sindh Pakistan (latitude: 24º56'45" N, longitude: 67º07'16" E) during March 2017.

Filter paper was put into perforated plastic Petri dishes (100 x 15 mm), and recommended doses (Table 1) of insecticides and distilled water for the check were

Table 1. Insecticides and Recommended Doses on Bottle Labels

Common name	Trade name	Type	Source	Dose g a.i. ha^{-1}
Pymetrozine	Plenum 50% WG	Pyridine azomethine	Syngenta	300
Matrine	Legend 0.5% AS	Plant based	Kanzo AG	247
Nitenpyram+chlorfenapyr	Momentum 50% WDG	Neonicotinoid and pyrrole	Kanzo AG	7.41
Buprofezin	Applaud 25 WP	IGRs	Arysta	59.28

sprayed with the help of a hand sprayer until the filter paper was wet. The Petri dishes with filter paper were allowed to dry. Before the start of the experiment, *C. septempunctata* were starved for 12 hours. Ten, 20, 30, 40, 50, or 60 third-nymphal instars of *A. gossypii* were offered to each stage of *C. septempunctata* in the Petri dishes. The experiment lasted 1 day. Each day, freshly collected nymphs were provided to each stage of *C. septempunctata.* Nymphs of *A. gossypii* eaten by a single stage of C. *septempunctata* were counted, and nymphs not consumed were discarded at the end of each experiment. The experiment had 10 replications and was done at controlled laboratory conditions of 26 ± 2°C temperature, 65 ± 5% relative humidity, and photoperiod of 16:8 light:dark hours. The experiment was repeated from the beginning if any stage of *C. septempunctata* died.

The study was done in a randomized complete block design at the research field at the Department of Agriculture and Agribusiness Management, University of Karachi, Karachi, Sindh Pakistan during April 2017. Each block (5 x 3-m area) consisted of three replications and five treatments including the check. For *C. septempunctata*, 10 plants were randomly selected from each treatment and tagged. Insecticides (Table 1) were applied on plants containing *C. septempunctata* feeding on *A. gossypii*. The number of *C. septempunctata* was recorded after 24, 72, and 240 hours on each tagged plant.

The percentage of insects killed was calculated according to the modified Abbott formula (Henderson and Tilton 1955):

$$\% \text{ population reduction} = 100 * 1 - [(T_a \times C_b) / (T_b \times C_a)]$$

where T_a = number of insects after treatment, T_b = number of insects before treatment, C_a = number of insects in the check after treatment, C_b = number of insects in the check before treatment.

Types of functional response were estimated by applying logistic regression in Excel (Microsoft Office 365) in relation to prey eaten by a predator (Ne) versus the number of prey given to a test predator (No). At the end of analysis, if the linear coefficient value was less than zero (<0), the predator had Type II functional response. If the linear coefficient was greater than zero (>0) and quadratic coefficient was less than zero (<0), the insect had Type III functional response. The study was done without replacing prey. The parameters of attack rate (a) and handling time (T_h) were found by using a random predator equation.

Initially, in Type I functional response, the model was:

$$Ne = \alpha + \beta No \quad (1)$$

where Ne: number of prey eaten by a predator, No: number of prey offered to a predator, and α and β: intercept and slope

For functional response type II, the model was

$$N_e = N_o \{1 - \exp [a (T_h N_e - T)]\} \quad (2)$$

where Ne: Number of prey eaten by a predator, No: Number of prey offered to a predator, a: attack rate, and T: overall time available. Inserting a = (d - bNo) /(1 + cNo) in Equation 2, a Type III functional response was derived (Hassell 1978):

$$N_e = N_o \{1 - \exp [(d + bNo)(T_h N_e - T)/(1 + cNo)]\} \quad (3)$$

The predator had Type III functional response, and parameters c and d were non-significantly dissimilar from zero [i.e., 95% confidence interval (CI) included zero]. This reduced the model of Type III functional response (Hassanpour et al. 2009).

$$N_e = N_o \{1 - \exp [bNo(T_h N_e - T)/]\} \quad (4)$$

Parameters like attack rate (a) and handling time (T_h) for Type II functional response or attack coefficient (b) for Type III were estimated by applying nonlinear least square regression in Microsoft Office 365.

Data obtained were subjected to analysis of variance (ANOVA) by using SPSS statistical software version 19.0. Significant differences among treatment means were tested by a least significant difference (Tukey's) test using 5% probability.

Results

Fig. 1 shows the effect of insecticides on consumption of *A. gossypii* prey by *C. septempunctata.* Based on logistic regression (Table 2), the check had Type II functional response by the first two instars and Type III by the other stages of *C. septempunctata*. However, with insecticide, all stages of *C. septempunctata* had Type II functional response. Table 3 shows attack rate and handling time of different insecticides whereas Table 4 shows the greatest theoretical maximum predation. Least handling time (Th), greatest attack rate (a), and maximum predation were by lady beetles exposed to buprofezin, followed by pymetrozine, matrine, and nitenpyram + chlorfenapyr, respectively, indicating that buprofezin and pymetrozine

Table 2. Functional Response of *Coccinella septempunctata* with Different Insecticides Based on Logistic Regression Estimate using Ne/No

Stage	Treatment	Cubic	Quadratic	Linear	Model
1st instar	Check	-0.0003±0.0004	0.124±0.2354	-0.6421±0.47	Type II
	Pymetrozine	-0.0001±0.0001	0.0213±0.1656	-0.8774±0.27	Type II
	Nitenpyram+ chlorfenapyr	-9.00E-05±0.08	0.0157±0.2545	-0.811±0.1678	Type II
	Buprofezin	-9.0E-05±0.001	0.0266±0.2545	-0.811±0.1678	Type II
	Matrine	-0.0003±0.0002	0.031±0.02	-0.1398±0.599	Type II
2nd instar	Check	-0.0007±0.0009	0.0335±0.0017	-0.228±0.3334	Type II
	Pymetrozine	-0.0001±0.002	0.0187±0.12182	-0.683±0.1341	Type II
	Nitenpyram+ chlorfenapyr	-0.001±0.0024	0.0187±0.2161	-1.0748±0.323	Type II
	Buprofezin	-0.024±0.0024	0.0187±0.216	-1.0748±0.323	Type II
	Matrine	-0.004±0.0005	0.04±0.003	-0.5312±0.295	Type II
3rd instar	Check	0.0001±0.0001	-0.0416±0.016	1.443±0.6444	Type III
	Pymetrozine	-3.0E-05±0.001	0.0061±0.1237	-0.6679±0.321	Type II
	Nitenpyram+ chlorfenapyr	-7.00E-05±0.01	0.0113±0.1237	-0.575±0.122	Type II
	Buprofezin	-0.002±0.0121	0.0324±0.1237	-0.5748±0.127	Type II
	Matrine	-0.001±0.000	0.0310±0.022	-0.182±0.687	Type II
4th instar	Check	0.0003±0.00031	-0.0273±0.021	1.3146±0.574	Type III
	Pymetrozine	-8.0E-05±0.000	0.0145±0.0210	-0.2274±0.123	Type II
	Nitenpyram+ Chlorfenapyr	-0.0001±0.0113	0.0107±0.02111	-0.6707±0.234	Type II
	Buprofezin	-0.0001±0.0113	0.0216±0.0211	-0.6707±0.234	Type II
	Matrine	-0.0001±0.0001	0.011±0.01	-0.6679±0.457	Type II
Adult	Check	0.004±0.0001	-0.0311±0.0213	1.273±0.586	Type III
	Pymetrozine	-0.0001±0.0023	0.019±0.1123	-0.4738±0.63	Type II
	Nitenpyram+ chlorfenapyr	-0.0002±0.0213	0.0263±0.1123	-1.3517±0.425	Type II
	Buprofezin	-0.0023±0.0213	0.0642±0.1123	-1.3517±0.425	Type II
	Matrine	-0.0011±0.000	0.01±0.014	-0.670±0.460	Type II

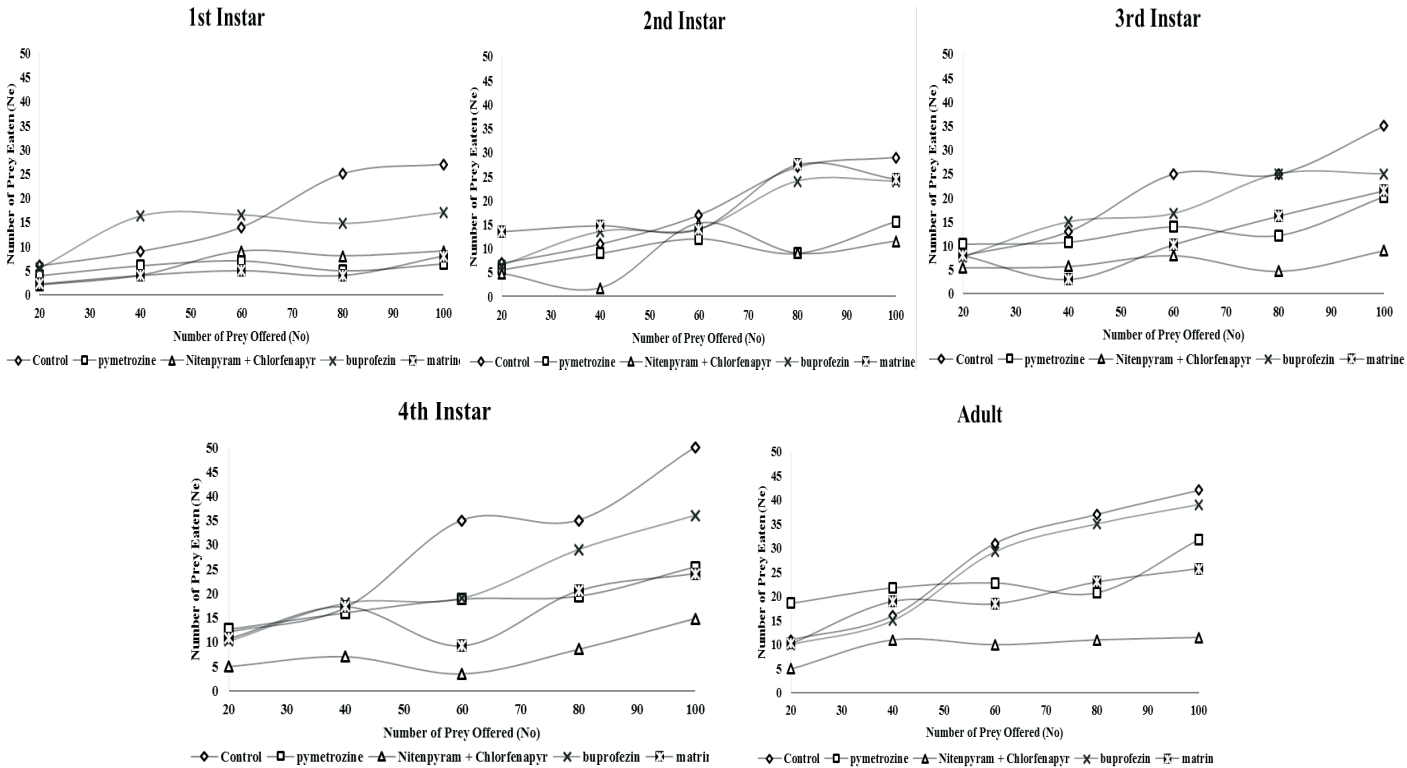

Fig. 1. Effect of insecticides on consumption of *A. gossypii* prey by *Coccinella septempunctata*.

Table 3. Parameters Attack Rate (a) and Handling Time (T_h) for Functional Response of *Coccinella septempunctata* with Various Insecticides

Treatment	Parameter	1st instar	2nd instar	3rd instar	4th instar	Adult
Check	a (h^{-1})	1.285±0.003a	1.111±0.014a	0.064±0.052e*	0.013±0.162e*	0.011±0.005d*
	T_h (h)	0.026±1.461E	0.019±0.278E	0.016±0.313E	0.014±0.159E	0.015±0.14E
Buprofezin	a (h^{-1})	1.179±0.051b	0.404±0.008b	1.182±0.060a	2.704±0.0058a	1.137±0.159a
	T_h (h)	0.098±1.461D	0.04±0.278D	0.032±0.313D	0.028±0.14D	0.031±0.159D
Pymetrozine	a (h^{-1})	0.751±0.023c	0.359±1.006c	1.115±0.171b	0.992±0.007b	0.607±0.238b
	T_h (h)	0.105±0.350C	0.053±0.634C	0.049±0.011C	0.037±0.14C	0.059±0.026C
Matrine	a (h^{-1})	0.324±0.007d	0.034±0.008c	0.709±0.060c	0.356±0.0058c	0.555±0.159b
	T_h (h)	0.324±1.461B	0.122±0.278B	0.14±0.313B	0.110±0.14B	0.122±0.159B
Nitenpyram + chlorfenpyr	a (h^{-1})	0.032±0.017d	0.024±0.023d	0.447±0.0121d	0.146±0.0032d	0.461±0.248c
	T_h (h)	1.733±1.461A	0.518±0.278A	0.430±0.313A	0.140±0.14A	0.419±0.159A

*attack coefficient (b)

Table 4. Greatest Theoretical Maximum Predation by *Coccinella septempunctata* with Various Insecticides

Treatment	First instar	Second instar	Third instar	Fourth instar	Adult
Check	923.08	1263.16	1500.00	1714.29	1600.0
Buprofezin	244.90	600.00	750.00	857.14	774.19
Pymetrozine	228.57	452.83	489.80	648.65	406.78
Matrine	74.07	196.72	171.43	218.18	196.72
Nitenpyram + chlorfenapyr	13.85	46.33	55.81	171.43	57.28

least affected predation by *C. septempunctata*, while greatest effect was by nitenpyram + chlorfenapyr and matrine.

Fig. 2 shows percentage reduction of *C. septempunctata* by different insecticides in the field. Most *C. septempunctata* were observed with Nitenpyram + chlorfenpyr 71.22±17.66% after 24 hours of spray with an increase of 85.13±%, 93.25±4.62% after 72 and 240 hours, followed by matrine 61.56±6.56%, 73.45±3.34%, 93.11±19.86% reduction. While buprofezin gave 55±3.56%, 65±8.97%, 85±6.56% and pymetrozine 32±4.45%, 71±10.12%, 84±7.54% at 24 hours, 72 hours , and 240 hours after spraying respectively.

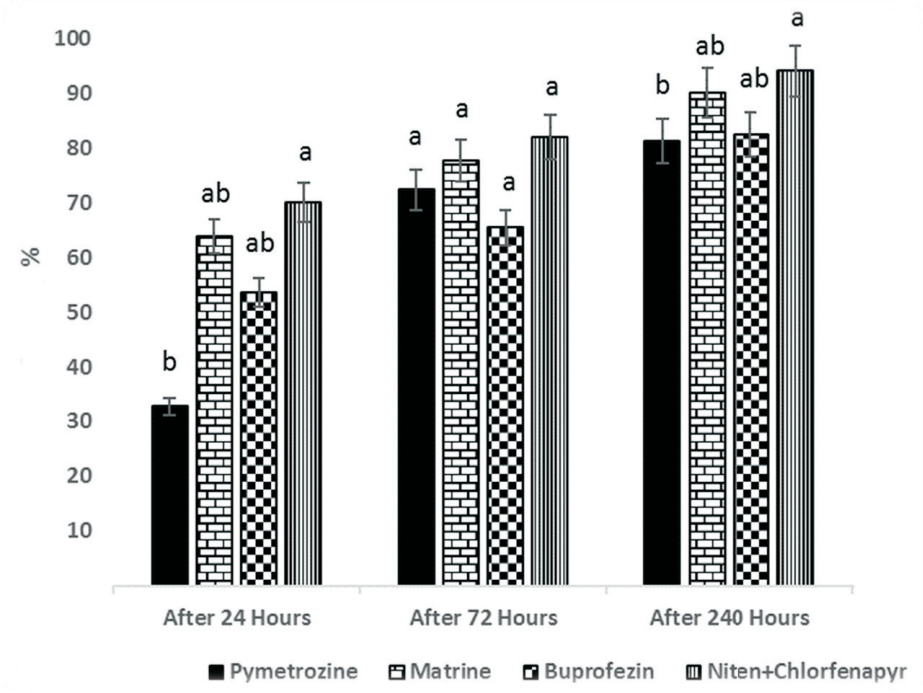

Fig. 2. Percentage reduction of *Coccinella septempunctata* with different insecticides at field conditions.

Discussion

C. septempunctata at all stages had Type II functional response in the presence of insecticides compared to Type III by the check. Shen et al. (2017) reported that pyridaben was most toxic to *Neoseiulus bicaudus* (Wainstein) (Acari: Phytoseiidae), whereas functional response remained the same in all treatments. He et al. (2012) reported that imidacloprid and its residues affected functional response of *Serangium japonicum* L. lady beetle on eggs of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) by prolonging handling time and reducing peak consumption. Poletti et al. (2007) found that neonicotinoid affected functional

response of *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae), but acetamiprid did not affect predation by *N. californicus*. Imidacloprid changed handling time and attack rate for both species, with reductions of 55 and 87% compared with peak consumption. Řezáč et al. (2010) studied the impact of selective insecticides on functional response of a spider *Philodromus cespitum* (Walckenaer) (Araneae: Philodromidae), with all insecticides except integro and mospilan affecting functional response.

In this study, buprofezin and pymetrozine least affected functional response and mortality of *C. septempunctata*. Buprofezin is a chitin-synthesis inhibitor (Cloyd 2012, Simon 2014) and does not affect natural enemies like arachnids, chrysopids, coccinellids, formicids, geocorids (Gogi et al. 2006), predatory bug (James 2004), *Chysopa rufilabris* (Burmeister) (Neuroptera: Chrysopidae) (Liu and Chen 2000), and foraging behavior of certain parasitoids including *Eretmocerus* sp. nr. *furuhashii* Rose & Zolnerowich (Hymenoptera: Aphelinidae) and *Encarsia luteola* Howard (Hymenoptera: Aphelinidae) (Gerling and Sinai 1994), and *Leptomastix dactylopii* Howard (Hymenoptera: Encyrtidae) (Cloyd and Dickinson 2006). Our results agreed with previous findings, although buprofezin was slightly harmful to natural enemies, according to the international organization of biological control (IOBC) category II (Ono et al. 2017, Barbosa et al. 2018). This might have been because of volatility (De Cock et al. 1990) or large doses of buprofezin (Liu and Chen 2000).

Pymetrozine selectively blocks feeding and like buprofezin does not affect natural enemies like ground beetles (Jansen et al. 2011b), predatory bugs, lacewings (Sechser et al. 2002), phytoseiid mites (Cuthbertson et al. 2012), aphidius wasp, carabid beetle, hoverfly, lady beetle, or rove beetle (Jansen et al. 2011a). In general, pymetrozine had no or minimal effect, as in reproduction of *N. californicus* (Castagnoli et al. 2005).

Nitenpyram + chlorfenapyr followed by matrine adversely affected functional response and killed many *C. septempunctata*. Results were inconsistent with those of Hwang et al. (2009) that matrine had moderately selective toxicity to natural enemies. Varenhorst and O'Neal (2012) studied effects of esfenvalerate, spirotetramat, imidacloprid, and a mixture of spirotetramat and imidacloprid and found abundance of *Harmonia axyridis* (Pallus) and *Orius insidiosus* (Say) reduced by all insecticide treatments compared with the check.

References Cited

Abrams, P. A., and L. R. Ginzburg. 2000. The nature of predation: prey dependent, ratio dependent or neither? Trends in Ecology & Evolution 15: 337-341.

Arditi, R., and L. R. Ginzburg. 1989. Coupling in predator-prey dynamics: ratio-dependence. J. Theoretical Biology 139: 311-326.

Arshad, M., H. A. A. Khan, F. Hafeez, R. Sherazi, and N. Iqbal. 2017. Predatory potential of *Coccinella septempunctata* L. against four aphid species. Pak. J. Zool. 49: 623.

Barbosa, P. R., M. D. Oliveira, E. M. Barros, J. Michaud, and J. B. Torres. 2018. Differential impacts of six insecticides on a mealybug and its coccinellid predator. Ecotoxicology and Environmental Safety 147: 963-971.

Bayoumy, M. H. 2011. Foraging behavior of the coccinellid *Nephus includens* (Coleoptera: Coccinellidae) in response to *Aphis gossypii* (Hemiptera: Aphididae) with particular emphasis on larval parasitism. Environ. Entomol. 40: 835-843.

Castagnoli, M., M. Liguori, S. Simoni, and C. Duso. 2005. Toxicity of some insecticides to *Tetranychus urticae, Neoseiulus californicus* and *Tydeus californicus*. BioControl 50: 611-622.

Cloyd, R. 2012. Indirect effects of pesticides on natural enemies. Pesticides-Advances in Chemical and Botanical Pesticides (in press).

Cloyd, R. A., and A. Dickinson. 2006. Effect of insecticides on mealybug destroyer (Coleoptera: Coccinellidae) and parasitoid *Leptomastix dactylopii* (Hymenoptera: Encyrtidae), natural enemies of citrus mealybug (Homoptera: Pseudococcidae). J. Econ. Entomol. 99: 1596-1604.

Cuthbertson, A. G. S., J. J. Mathers, P. Croft, N. Nattriss, L. F. Blackburn, W. Luo, P. Northing, T. Murai, R. J. Jacobson, and K. F. A. Walters. 2012. Prey consumption rates and compatibility with pesticides of four predatory mites from the family Phytoseiidae attacking *Thrips palmi* Karny (Thysanoptera: Thripidae). Pest Management Science 68: 1289-1295.

De Cock, A., I. Ishaaya, D. Degheele, and D. Veierov. 1990. Vapor toxicity and concentration-dependent persistence of buprofezin applied to cotton foliage for controlling the sweetpotato whitefly (Homoptera: Aleyrodidae). J. Econ. Entomol. 83 1254-1260.

Dixon, A. F., J.-L. Hemptinne, and P. Kindlmann. 1997. Effectiveness of ladybirds as biological control agents: patterns and processes. BioControl 42: 71-83.

Dixon, A. F. G. 2000. Insect Predator-prey Dynamics: Ladybird Beetles and Biological Control. Cambridge University Press.

Dong, Z., Y. Li, S. Liu, and Z. Zhang. 2018. Genetic diversity of the melon aphid *Aphis gossypii* (Hemiptera: Aphididae) in a diversified vegetable growing area. Applied Entomology and Zoology 53: 259-266.

Gerling, D., and P. Sinai. 1994. Buprofezin effects on two parasitoid species of whitefly (Homoptera: Aleyrodidae). J. Econ. Entom. 87: 842-846.

Gogi, M. D., R. M. Sarfraz, L. M. Dosdall, M. J. Arif, A. B. Keddie, and M. Ashfaq. 2006. Effectiveness of two insect growth regulators against *Bemisia tabac*i (Gennadius) (Homoptera: Aleyrodidae) and *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) and their impact on population densities of arthropod predators in cotton in Pakistan. Pest Management Science 62: 982-990.

Hassanpour, M., G. Nouri-Ganbalani, J. Mohaghegh, and A. Enkegaard. 2009. Functional response of different larval instars of the green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae), to the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). J. Food Agriculture & Environment 7: 424-428.

Hassell, M. P. 1978. The Dynamics of Arthropod Predator-prey Systems. Princeton University Press.

He, Y., J. Zhao, Y. Zheng, N. Desneux, and K. Wu. 2012. Lethal effect of imidacloprid on the coccinellid predator *Serangium japonicum* and sublethal effects on predator voracity and on functional response to the whitefly *Bemisia tabaci*. Ecotoxicology 21: 1291-1300.

Henderson, C. F., and E. W. Tilton. 1955. Tests with acaricides against the brown wheat mite. J. Econ. Entomol. 48: 157-161.

Hodek, I., and A. Honěk. 2009. Scale insects, mealybugs, whiteflies and psyllids (Hemiptera, Sternorrhyncha) as prey of ladybirds. Biol. Control 51: 232-243.

Hodek, I., H. F. van Emden, and A. Honek. 2012. Ecology and Behaviour of the Ladybird Beetles (Coccinellidae). John Wiley and Sons.

Holling, C. S. 1959a. The components of predation as revealed by a study of small-mammal predation of the European pine sawfly. Can. Entomol. 91: 293-320.
Holling, C. S. 1959b. Some characteristics of simple types of predation and parasitism. Can. Entomol. 91: 385-398.
Holling, C. S. 1965. The functional response of predators to prey density and its role in mimicry and population regulation. Mem. Entomol. Soc. Canada 97: 5-60.
Hussain, F., and M. Abid. 2011. Pest and diseases of chilli crop in Pakistan: a review. Int. J. Biol. Biotech 8: 325-332.
Hwang, I.-C., J. Kim, H.-M. Kim, D.-I. Kim, S.-G. Kim, S.-S. Kim, and C. Jang. 2009. Evaluation of toxicity of plant extract made by neem and matrine against main pests and natural enemies. Korean J. Applied Entomol. 48: 87-94.
Iqbal, S., M. Ashfaq, and H. Shah. 2011. Biological characterization of Pakistani isolates of cucumber mosaic cucumovirus (CMV). Pak. J. Botany 43: 3041-3047.
Jalali, M. A., L. Tirry, and P. De Clercq. 2010. Effect of temperature on the functional response of *Adalia bipunctata* to *Myzus persicae*. BioControl 55: 261-269.
James, D. G. 2004. Effect of buprofezin on survival of immature stages of *Harmonia axyridis*, *Stethorus punctum* picipes (Coleoptera: Coccinellidae), *Orius tristicolor* (Hemiptera: Anthocoridae), and *Geocoris* spp. (Hemiptera: Geocoridae). J. Econ. Entomol. 97: 900-904.
Jansen, J., T. Defrance, and A. Warnier. 2011a. Side effects of flonicamide and pymetrozine on five aphid natural enemy species. BioControl 56: 759-770.
Jansen, J. P., T. Defrance, and A. M. Warnier. 2011b. Side effects of flonicamide and pymetrozine on five aphid natural enemy species. BioControl 56: 759-770.
Khan, M. R., and M. R. Khan. 2010. The relationship between temperature and the functional response of *Coccinella septempunctata* (L.) (Coleoptera: Coccinellidae). Pak. J. Zool. 42: 461-466.
Khan, S. M., S. K. Raj, T. Bano, and V. K. Garg. 2006. Incidence and management of mosaic and leaf curl diseases in cultivars of chilli (*Capsicum annuum*). J. Food Agriculture Environment 4: 171.
Křivan, V., and E. Sirot. 2004. Do short-term behavioural responses of consumers in tri-trophic food chains persist at the population time-scale? Evolutionary Ecology Research 6: 1063-1081.
Liu, T.-X., and T.-Y. Chen. 2000. Effects of the chitin synthesis inhibitor buprofezin on survival and development of immatures of *Chrysoperla rufilabris* (Neuroptera: Chrysopidae). J. Econ. Entomol. 93: 234-239.
Mills, N., and I. Lacan. 2004. Ratio dependence in the functional response of insect parasitoids: evidence from *Trichogramma minutum* foraging for eggs in small host patches. Ecol. Entomol. 29: 208-216.
Ono, É. K., O. Z. Zanardi, K. F. Aguiar Santos, and P. T. Yamamoto. 2017. Susceptibility of *Ceraeochrysa cubana* larvae and adults to six insect growth-regulator insecticides. Chemosphere 168: 49-57.
Perdikis, D., E. Kapaxidi, and G. Papadoulis. 2008. Biological control of insect and mite pests in greenhouse solanaceous crops. Eur. J. Plant Sci. Biotechnology 2: 125-144.
Poletti, M., A. Maia, and C. Omoto. 2007. Toxicity of neonicotinoid insecticides to Neoseiulus californicus and Phytoseiulus macropilis (Acari: Phytoseiidae) and their impact on functional response to *Tetranychus urticae* (Acari: Tetranychidae). Biol. Control 40: 30-36.

Řezáč, M., S. Pekár, and J. Stará. 2010. The negative effect of some selective insecticides on the functional response of a potential biological control agent, the spider *Philodromus cespitum*. BioControl 55: 503-510.
Schenk, D., L,. F. Bersier, and S. Bacher. 2005. An experimental test of the nature of predation: neither prey-nor ratio-dependent. J. Anim. Ecol. 74: 86-91.
Sechser, B., B. Reber, and F. Bourgeois. 2002. Pymetrozine: selectivity spectrum to beneficial arthropods and fitness for integrated pest management. Anzeiger für Schädlingskunde 75: 72-77.
Shen, X.-Q., Y.-N. Zhang, T. Li, J.-Y.-Q. Jiang, and J.-P. Zhang. 2017. Toxicity of three acaricides to the predatory mite, *Neoseiulus bicaudus* (Acari: Phytoseiidae) and their impact on the functional response to *Tetranychus turkestani* (Acari: Tetranychidae). J. Econ. Entomol. 110: 2031-2038.
Simon, J. Y. 2014. The Toxicology and Biochemistry of Insecticides. CRC Press.
Sultan, A., and M. F. Khan. 2014. Functional response of *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) to sugarcane whitefly *Aleurolobus barodensis* (Maskell) in laboratory conditions. J. Insect Behav. 27: 454-461.
Tschanz, B., L.-F. Bersier, and S. Bacher. 2007. Functional responses: a question of alternative prey and predator density. Ecology 88: 1300-1308.
Unal, S., A. Er, E. Akkuzu, and L. Salek. 2017. Predation efficacy of the predator *Coccinella septempunctata* L. on the aphid species *Macrosiphum rosae* (L.) in Kastamonu Province, Turkey. Pak. J. Zool. 49: 327-327.
Varenhorst, A. J., and M. E. O'Neal. 2012. The response of natural enemies to selective insecticides applied to soybean. Environ. Entomol. 41: 1565-1574.

From the Forest to the Bottle: Entomochemicals from *Pterophylla beltrani* (Bolivar and Bolivar)[1] for the Beverage Industry

Luis Daniel García-García[2]*, Reyna Ivonne Torres-Acosta[3], Rodolfo Torres-de los Santos[3], Nohemí Niño-García[3], and Jorge Ariel Torres-Castillo[2]*

Abstract. Insects are consumed as food in many cultures of the world and are promoted through products that increase acceptance of the practice. Insects are a potential source of nutrients and bioactive compounds; however, lack of acceptance is an obstacle to widespread consumption. Extraction of *Pterophylla beltrani* (Bolivar and Bolivar) was evaluated in water, 50% ethanol, absolute ethanol, tequila, vodka, and rum. Total phenolic compounds (TPC) and free radical scavenging capacity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radicals were extracted, mostly with hydroalcoholic solutions and water rather than with absolute ethanol. The best extraction solvent for total phenolic compounds, DPPH, and ABTS was 50% ethanol with 37.21 milliequivalents of gallic acid per gram (mGAE/g), 412.40 milliequivalents of Trolox per gram (mTE/g), and 1,604.32 mTE/g. Of commercial beverages, tequila and vodka were better for extraction and activity of total phenolic compounds against the DPPH radical, while rum was better for extracting activity against the ABTS radical. Incorporation of total phenolic compounds and activity against the two radicals highlights the potential of entomochemicals of *P. beltrani* to be incorporated into alcoholic and other beverages. The insect has potential to be incorporated as a functional ingredient in the food industry.

Introduction

Entomophagy, in which insects are consumed directly or processed as food, is practiced by diverse cultures of the world (Gahukar 2011, Raubenheimer and Rothman 2013, Lensvelt and Steenbekkers 2014). This practice is an alternative to ensure the food supply for humans in the face of current and future problems (Gahukar 2011).

However, part of the success of entomophagy is acceptance of food derived from insects, because in industrialized countries insects are not accepted as food (Van Huis 2013, Tan et al. 2016). Therefore, innovation in preparation, presentation, and marketing for promotion of entomophagy plays a preponderant role for success (Caparros et al. 2013).

Insects are an important source of protein of animal origin, with contents similar to those of beef, pork, and chicken, as well as fats, carbohydrates, fiber, and

[1]Orthoptera: Tettigoniidae

[2]Universidad Autónoma de Tamaulipas, Instituto de Ecología Aplicada. Ave. División del Golfo 356, Col. Libertad, 87019. Ciudad Victoria, Tamaulipas, México. [3]Universidad Autónoma de Tamaulipas, Unidad Académica Multidisciplinaria Mante. Enrique Cárdenas González 1201 Pte., Col. Jardín. C. P. 89840. Ciudad Mante, Tamaulipas, México.
*Corresponding author: garcia.g.daniel@live.com.mx; jorgearieltorres@hotmail.com

minerals. Production of insects is more efficient, consumes less water, emits less greenhouse gas, and is considered a sustainable alternative (Van Huis 2013).

Most study of insect consumption focuses on nutrient content and not on bioactive compounds. Recently, contents of entomochemicals in *Pterophylla beltrani* (Bolivar and Bolivar) were reported and include phenolic compounds, alkaloids, amino acid derivatives, and terpenoids. These seem to be associated with capacity to collect free radicals in vitro and with antioxidant properties (Torres-Castillo et al. 2018).

Previously, *P. beltrani* was reported as a potential source of compounds of biotechnological interest and for the food industry, where antioxidants were highlighted as nutraceutical candidates with potential for food development (Torres-Castillo et al. 2015, Montiel-Aguilar et al. 2017). Due to this, we decided to explore its potential use for beverage supplementation. However, exploration of strategies for processing of *P. beltrani* and maintaining the stability of various bioactive components is necessary. In this work, extraction of phenolic compounds and free radical scavenging capacity of *P. beltrani* were evaluated to supplement aqueous solutions and alcoholic beverages.

Materials and Methods

Adult *P. beltrani* were collected in the foothills of the Sierra Madre Oriental, near Ciudad Victoria, Tamaulipas, Mexico (23.7702810°N, -099.2654220°W). Capture was by entomological nets. The insects were confined to plastic containers and transported to a laboratory for processing. Adults were kept at -20°C for 25 minutes and subsequently eviscerated to remove nondigested plant debris from the intestines. The material remaining was washed with running water and dried at 45°C for 72 hours. It was pulverized in a porcelain mortar and sieved through 0.7 mm (710 µm sieve 25, U.S.A. Standard test sieve W. S. Tyler) to obtain fine powder.

Initial extraction used water, 50% ethanol, and absolute ethanol and pulverized in a ratio of 1:5 (w/v) by shaking and resting for 10 minutes at 4°C, and centrifuging at 10,000 *g* for 8 minutes. Clarified supernatant obtained was stored at -20°C until used. Extraction was in 1:5 (w/v) ratio in commercial alcoholic beverages: tequila (El Jimador® white tequila with 35% alcohol content), vodka (Oso Negro® vodka with 38% alcohol content), and rum (Bacardi® rum with 40% alcohol content). Extraction was done in triplicate and allowed to rest for 24 hours. After resting, the extraction was poured and clarified by filtration through 0.25 µm to remove impurities and remaining debris. Samples were stored at -20°C until used.

Detection of total phenolic compounds was based on reaction with Folin-Ciocalteu reagent (Singleton et al. 1999). A standard calibration curve of gallic acid (Sigma-Aldrich, St. Louis, MO) (0-8 µg/ml gallic acid) was used to which water and 250 and 125 µl of 1 N Folin-Ciocalteu reagent (Sigma-Aldrich) were added. The mixture was incubated for 5 minutes, and 625 µl of 20% sodium carbonate (Na_2CO_3) (CTR, Monterrey, NL, Mexico) were added to each tube. The tubes were incubated in the dark for 1.5 hour at 28-30°C. Absorbance at 760 nm was recorded in a spectrophotometer. To a sample of 10 µl of each extract solution, 240 µl of water and 125 µl of Folin-Ciocalteu reagent were added, followed by 5 minutes of incubation and addition of 625 µl of 20% sodium carbonate. After incubation for 2 hours, absorbance was recorded as indicated for the standard curve. All quantifications were done in triplicate. The concentration was expressed as milliequivalents of gallic acid per milliliter (mGAE/ml) or per gram pulverized (mGAE/g).

Free radical scavenging activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, St. Louis, MO) according to Brand-Williams et al. (1995). A 0.002% solution of DPPH in methanol was prepared (CTR, Monterrey, NL, Mexico). The absorbance of the working solution was adjusted to 0.7 at 515 nm. The reactions were done by mixing 975 µl of adjusted DPPH solution and 25 µl of sample or standard. After incubation for 30 minutes at 28-30°C in the dark, absorbance variation was determined. The concentration was calculated with respect to a calibration curve prepared with a 4 mM stock solution of Trolox® ((±) -6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Sigma-Aldrich, St. Louis, MO)) and 80% methanol. A calibration curve was prepared with a range of 0 to 1,200 µM. The final units of the samples were expressed as mmol of Trolox equivalents per gram pulverized (mTE/g) or per milliliter (mTE/ml).

Determination of free radical scavenging activity against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) was based on that suggested by Re et al. (1999). A solution was prepared by mixing 20 ml of distilled water with 77.6 mg of ABTS reagent (Sigma-Aldrich, St. Louis, MO); subsequently 13.2 mg of potassium persulfate (Sigma-Aldrich) were added. The mixture was allowed to stand in the dark for 12 to 16 hours at 28-30°C. The resulting solution was diluted with absolute ethanol to obtain absorbance of 0.7 to 732 nm considered the working solution. Reactions of the extracts and standards were prepared by mixing 10 µl of sample or standard and 1 ml of ABTS working solution. The reaction was incubated in the dark for 5 minutes, and absorbance was measured at 732 nm. A calibration curve was prepared with Trolox in a range of 0 to 1,200 µM. The final units of the samples were expressed as mmol of Trolox equivalents per gram pulverized (mTE/g) or per milliliter (mTE/ml). The percentages of the contents of each parameter were calculated and compared by considering the greater value of each determination as 100% based on values presented in Table 1. All reactions were done in triplicate. The differences between the contents of antioxidant activity by solvent were determined by analysis of variance and means were compared by Tukey at $p \leq 0.05$ (SAS Institute 2011).

Results

Solvents (water, 50% ethanol, and absolute ethanol) differed in extraction capacity based on a gradient of polarities. The solvent that extracted the greatest concentrations of the three parameters was 50% ethanol; for total phenolic compounds, 25% more were extracted than with water and 72.9% more than with absolute ethanol. In the case of scavenging activity levels against DPPH, more than 27.4% was obtained with water and slightly less than 50% with absolute ethanol. Extraction of antioxidants against the ABTS radical showed that water enabled 21.1% and absolute ethanol enabled 80% less recovery than with 50% ethanol (Table 1).

Distilled alcoholic beverages were used because of their ethanol content that in all cases was less than 50%. Basal phases of 50% ethanol and commercial alcoholic beverages showed low and, in some cases, nondetectable total phenolic compounds and free radical-scavenging activities attributed to remnants of volatile or extractable compounds during elaboration of the products (Table 2). Vodka extracted most total phenolic compounds and activity against the DPPH radical. However, rum extracted most scavenging activity against ABTS. Differences could be explained by solubility of the compounds in different concentrations of ethanol, because rum had 40%, vodka 38%, and tequila 35%, in addition to several other compounds characteristic of each distilled alcoholic beverage (Table 3).

The beverage prototypes could be categorized as distilled spiced beverage with an amber hue and a sweet smell. The tests with working group personnel indicated the taste was essentially the same among all beverages, leaving a woody, slightly astringent sensation. No negative effect was observed by guests tasting the prototypes. The extractions were stored at 4°C for 1 week, and precipitates occasionally were observed even after clarification by filtration.

Table 1. Extraction by Different Solvents per Gram Pulverized
Tabla 1. Perfiles de Extracción con Diferentes Solventes por Gramo de Pulverizado

Solvent	TPC	DPPH	ABTS
Water	28.31b	299.60b	1,265.46b
50% ethanol	37.21a	412.40a	1,604.32a
Absolute ethanol	10.06c	217.03b	317.67c

Means with different letters in a column are significantly different, Tukey $p \leq 0.05$. Total phenolic compound expressed as mGAE/g; scavenging activity of free radicals DPPH and ABTS expressed as mTE/g.
Letras diferentes indican diferencias significares, Tukey $p \leq 0.05$. Los TPC expresados como mGAE/g, la capacidad colectora de radicales libres DPPH y ABTS expresada como mTE/g.

Table 2. Basal Concentrations of 50% Ethanol and Commercial Alcoholic Beverages
Tabla 2. Concentraciones Basales de Etanol al 50% y Bebidas Alcohólicas Comerciales

Solvent	TPC	DPPH	ABTS
50% ethanol	0.34	ND	ND
Tequila	ND	1.37	ND
Vodka	0.69	2.31	ND
Rum	0.11	ND	6.66

TPC expressed as mGAE/ml, scavenging activity of DPPH and ABTS free radicals expressed as mTE/ml, ND, not detectable.
Los TPC expresados como mGAE/ml, la capacidad colectora de radicales libres DPPH y ABTS expresada como mTE/ml.

Table 3. Concentrations of TPC and Activity against DPPH and ABTS in 50% Ethanol and Commercial Alcoholic Beverages
Tabla 3. Concentraciones de TPC y la Actividad contra DPPH y ABTS en el Etanol al 50% y Bebidas Alcohólicas Comerciales

Solvent	TPC	DPPH	ABTS
50% ethanol	49.00b	108.88a	39.60a
Tequila	55.10ab	139.52b	248.42b
Vodka	61.93a	179.26c	393.24c
Rum	34.07c	98.62a	433.68d

Means with different letters in a column are significantly different, Tukey $p \leq 0.05$. Total phenolic compound expressed as mGAE/ml; collecting activity of free radicals DPPH and ABTS expressed as mTE/ml.
Letras diferentes indican diferencias significares, Tukey $p \leq 0.05$. Los TPC expresados como mGAE/ml, la capacidad colectora de radicales libres DPPH y ABTS expresada como mTE/ml.

This type of preparation could be added with sweetener or supplemented with spices or aromatic herbs to develop a more competitive product. Like many drinks spiced with herbs, colors, flavors, and aromas are provided by metabolites extracted from the basal material, which in this case was from an insect. This is a first approach to encourage consumption and nutraceutical compounds of insects as an alternative to traditional entomophagy.

Discussion

The three solvents indicated most compounds were polar, because more total phenolic compound was extracted by water and 50% ethanol. Water and diluted alcohol solutions frequently are used in the food industry, and because of the behavior of phenolic and antioxidant compounds of *P. beltrani* makes them candidates for use. Entomochemicals of *P. beltrani* were diverse polar compounds extracted in hydroalcoholic phases, and little activity was observed for extractions in propanol (Torres-Castillo et al. 2018), which correlates with capacity for differential extraction observed in this work. The basal values in commercial drinks used might be associated directly with production processes, aging, or origin of vegetable material used in manufacture of the product, as indicated for tequila, vodka, and rum (Lin et al. 2005, Regalado et al. 2011, Magana et al. 2015).

Concentrations of phenolic compounds in beverages that include juices, teas, coffee, and alcoholic beverages such as wines, beers, and others are directly correlated with antioxidant properties and beneficial effects (Lugasi and Hóvári 2003), which reinforces use of alcoholic beverages enriched with phenolic compounds and antioxidant properties. Enrichment with phenolic compounds and antioxidants of alcoholic beverages by extraction from organic samples is useful for taking advantage of beneficial effects of phenolic compounds and their bioactive potential (Lin et al. 2005), which supports use of insects for consumption in enriched alcoholic beverages. Although, organoleptic characteristics must be evaluated through specific experimental designs and in different panels, attributes of color, smell, and taste are a starting point for evaluation of this kind of product.

Exploration of strategies to promote consumption of insects is necessary to increase acceptability of entomophagy, especially in countries where it is not commonly practiced (Van Huis 2013). Although there are many ways to introduce consumers to products from insects, innovation in presentations and especially incorporation of nutritional bioactive contents or entomochemical compounds are necessary to promote consumption and added value (Ayieko et al. 2010).

Entomochemicals of *P. beltrani* can be incorporated through rapid extraction to aqueous and alcoholic beverages as observed by extraction in water, 50% ethanol, and commercial drinks. The insect can be used in various areas of the food industry including sweets, desserts, ice cream, syrups, and jams that are water based, or in preparation of alcoholic beverages. Insects such as *P. beltrani* could be combined with management strategies for environmentally friendly use.

Acknowledgment

The authors are grateful to project UAT-PFI2017-ACP02 for partial financial support for detection of free radical-scavenging activity and total phenolic compounds.

References Cited

Ayieko, M., V. Oriaro, and I. A. Nyambuga. 2010. Processed products of termites and lake flies: improving entomophagy for food security within the Lake Victoria region. Afr. J. Food Agric. Nutr. Dev. 10.

Brand-Williams, W., M. E. Cuvelier, and C. Berset. 1995. Use of a free radical method to evaluate antioxidant activity. LWT 28: 25-30.

Caparros M., R., L. Sablon, M. Geuens, Y. Brostaux, T. Alabi, C. Blecker, D. Drugmand, E. Haubruge, and F. Francis. 2014. Edible insects acceptance by Belgian consumers: promising attitude for entomophagy development. J. Sens. Stud. 29: 14-20.

Gahukar, R. T. 2011. Entomophagy and human food security. Int. J. Trop. Insect Sci. 31: 129-144.

Lensvelt, E. J., and L. P. A. Steenbekkers. 2014. Exploring consumer acceptance of entomophagy: a survey and experiment in Australia and the Netherlands. Ecol Food Nutr. 53: 543-561.

Lin, Y. T., D. Vattem, R. G. Labbe, and K. Shetty. 2005. Enhancement of antioxidant activity and inhibition of Helicobacter pylori by phenolic phytochemical-enriched alcoholic beverages. Process Biochem. 40: 2059-2065.

Lugasi, A., and J. Hóvári. 2003. Antioxidant properties of commercial alcoholic and nonalcoholic beverages. Nahrung. 47: 79-86.

Magana, A. A., K. Wrobel, J. C. T. Elguera, A. R. C. Escobosa, and K. Wrobel. 2015. Determination of small phenolic compounds in tequila by liquid chromatography with ion trap mass spectrometry detection. Food Anal. Method. 8: 864-872.

Montiel-Aguilar, L., S. R. Sinagawa-García, and J. A. Torres-Castillo. 2017. *Pterophylla beltrany* (Bolivar and Bolivar): prospect for entomophagy. Southwest. Entomol. 42: 591-594.

Raubenheimer, D., and J. M. Rothman. 2013. Nutritional ecology of entomophagy in humans and other primates. Annu. Rev. Entomol. 58: 141-160.

Re, R., N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26: 1231-1237.

Regalado, E. L., S. Tolle, J. A. Pino, P. Winterhalter, R. Menendez, A. R. Morales, and J. L. Rodríguez. 2011. Isolation and identification of phenolic compounds from rum aged in oak barrels by high-speed countercurrent chromatography/ high-performance liquid chromatography-diode array detection-electrospray ionization mass spectrometry and screening for antioxidant activity. J. Chromatogr. A. 1218: 7358-7364.

SAS Institute. 2011. SAS User´s Guide: Statistics. Version 9.3. SAS Institute, Cary, NC.

Singleton, V. L., R. Orthofer, and R. M. Lamuela-Raventos. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299: 152-178.

Tan, H. S. G., E. van den Berg, and M. Stieger. 2016. The influence of product preparation, familiarity and individual traits on the consumer acceptance of insects as food. Food Qual. Prefer. 52: 222-231.

Torres-Castillo, J. A., S. R. Sinagawa-García, M. Lara-Villalón, G. C. G. Martínez-Ávila, A. Mora-Olivo, and F. A. Reyes-Soria. 2015. Evaluation of biochemical components from *Pterophylla beltrani* (Bolivar & Bolivar) (Orthoptera:

Tettigoniidae): a forest pest from northeastern Mexico. Southwest. Entomol. 40: 741-751.
Torres-Castillo, J. A., S. R. Sinagawa-García, R. I. Torres-Acosta, L. D. García-García, A. G. Ramos-Rodríguez, B. Villanueva-Bocanegra, B., and Y. del R. Moreno-Ramírez. 2018. Entomochemicals from *Pterophylla beltrani* Bolivar and Bolivar: antioxidants and other metabolites. Southwest. Entomol. 43: 369-382.
Van Huis, A. 2013. Potential of insects as food and feed in assuring food security. Annu. Rev. Entomol. 58: 563-583.

Chemicals in the Genital Chamber of Two Mexican Species of *Phyllophaga*[1]

A. A. Romero-López[2]*, R. Reyes-Chilpa[3], F. J. Pérez-Flores[3], G. A. Lugo-García[4], and J. I. Maldonado-Rodríguez[5]

Abstract. *Phyllophaga ravida* Blanchard and *Phyllophaga opaca* Moser (Coleoptera: Scarabaeoidea: Melolonthidae) are economically important in Mexico. An option for management of the insect pests is use of sex pheromones. To obtain the chemical profile of sex pheromones of both species the posterior section of the genital chamber of females was excised and macerated to release the contents. Eight compounds from *P. ravida* were identified by retention index and mass spectra: cyclohexane, 1,1'-(2-tridecyl-1,3-propanediyl) bis-; 10-methyl-eicosane; 5-butyl-hexadecane; 3-hexen-2-one; 3,3-diethyl-azetidine-2,4-dione; tetramethyl-oxirane; 3-methyl-2-pentanone; and 2-methoxy-2-methyl-butane. Seven compounds were identified for *P. opaca*: cholesta-4, 6-dien-3-ol; cholesterol; cholesta-3,5-dien; 2-methyl heptadecane; α-tocopherol succinate; 9,19-cyclolanost-24-en-3-ol, acetate; and lup-20 (29)-en-3-ol, acetate. Some identified chemicals, mainly 3,3-diethyl-azetidine-2,4-dione and 2-methyl heptadecane might be involved in sexual attraction of *P. ravida* and *P. opaca*, respectively. Several compounds probably are incorporated from plants into insects during feeding, and further transformed by metabolism.

Introduction

A sex pheromone is a multi-specific substance released by an organism to attract one or more individuals of the opposite sex for mating (Karlson and Butenandt 1959, Ward et al. 2002). Depending on the insect species, the male or female can produce the substance; both contribute to chemical communication related to mating (Jutsum and Gordon 1989). Sex pheromones are featured by their specificity, ability to cause long-distance responses, and persistence after release in the field (Foster and Harris 1997). Applied entomologists have been interested in identifying the substances to promote as alternatives for pest management, especially because of specificity (Thomson et al. 2000). In the case of coleopterans of the family Melolonthidae (Cherman and Morón 2014) whose larvae cause crop damage, agrochemicals have been used without consistent results. *Phyllophaga* pheromones have been identified by techniques such as gas chromatography-mass spectrometry, flame ionization detector-gas chromatography,

[1]Coleoptera: Melolonthidae

[2]Facultad de Ciencias Biológicas, Benemérita Universidad Autónoma de Puebla, México.
[3]Instituto de Química, Universidad Nacional Autónoma de México, México.
[4]Escuela Superior de Agricultura del Valle del Fuerte, Universidad Autónoma de Sinaloa, México.
[5]Universidad de la Amazonía, Florencia, Caquetá, Colombia.
*aaromelo@gmail.com, chilpa@unam.mx, jape@unam.mx, gabriel_lugo9010@hotmail.com, ironzi@hotmail.com

and electro-antennographic detection (Leal 1998, Zarbin et al. 2007). Sex pheromones of the insect species are composed mainly of blends of amino acids (Zhang et al. 1997, Leal et al. 2003, Nojima et al. 2003), sulfur derivatives (Robbins et al. 2003), and phenol (Zarbin et al. 2007). In Mexico, *Phyllophaga obsoleta* Blanchard was used in first attempts to identify sex pheromones of Melolonthidae, and some bioactive compounds were identified in the posterior section of the protractile genital chamber (Romero-López and Arzuffi 2010). The information stimulated our interest in related species such as *Phyllophaga ravida* Blanchard and *Phyllophaga opaca* Moser. *P. ravida* has a life cycle of 1 year and is distributed from the United States to Belize, Guatemala, and Mexico (Sanderson 1958, Moron et al. 1997). Larvae of the species feed on roots of diverse agricultural crops (Morón 2003), while adults feed on leaves of *Quercus* spp. (Fagaceae) (Romero-López et al. 2007). *P. opaca* has an annual life cycle and is restricted in distribution to the United States and Mexico (reported specifically for the states of Michoacán and Sinaloa) (Sanderson 1958, Morón 2003). The flight behavior is known. The diet is mainly Asteraceae and Fagaceae foliage (Aragón et al. 2009).

Data on chemical sexual communication was obtained for *P. obsoleta*, *P. ravida*, *P. opaca*, *P. macrocera* Bates, and *P. cristagalli* Arrow (Romero-López 2016). In general, before mating, females of the species show patterns of behavior that emphasize “sexual calling", approach to males, and finally mating. When a female exposes her genital chamber, chemicals that cause sexual attraction are hypothesized to be released. From *P. obsoleta*, butilhidroxianisole and other derivatives of hydrocarbons were identified (Romero-López and Arzuffi 2010). Therefore, the objective of this contribution was to extract and identify compounds in the genital chamber of *P. ravida* and *P. opaca* females as a first stage for identification of the sex pheromones.

Materials and Methods

P. ravida females were collected in zones with *Pinus* sp. (L., Pinaceae) (pine) and *Eucalyptus camaldulensis* (Dehnh., Myrtaceae) (eucaliptus) trees at Ciudad Universitaria (Benemérita Universidad Autónoma de Puebla, Puebla, México, 19° 00'05.06 "N 98° 12'02.97" O). *P. opaca* females were collected at Valle del Carrizo, Ahome, Sinaloa (26°18’19.2’’N, 108°56’33.8’’O, 36 m). Adults were captured in foliage of *Acacia farnesiana* (Fabaceae) where they were feeding and mating.

The same procedure for extracting chemicals was used for both species. The posterior section of the genital chamber of 10 females was excised, placed into a vial with 1 ml of hexane (C_6H_{14}, chromatographic grade Brand Burdick and Jackson), and macerated to release the contents. The solution was 10 female equivalents (10 FE). Afterward, 50 µl of the suspension were injected for analysis by gas chromatography-mass spectrometry. Twelve replications of each species (12 samples from 10 FE) were used.

Analysis of the samples by gas chromatography was done in a gas chromatograph (Agilent Technologies model 6890N) coupled with mass spectrometry (JEOL JMS GC mate II). The gas chromatograph was provided with a column (HP-5MS 30 m, 0.32 mm inner diameter, 0.25 µm film thickness). The injector ran in splitless mode at 225°C and the auxiliary to 280°C, with injection volume of 1 ml. The oven temperature program used an initial temperature of 50°C with an increase of 15°C per minute, to a final temperature of 250°C. The SCAN worked in mass spectrometry mode with a mass range of 35-550. Helium was used

as the carrier gas with a flow of 1 ml per minute and 34.33-minute flow time (start time of 1 minute and final 20 minutes) was used for each injected sample. The oven temperature program consisted of an initial temperature of 40°C per minute with an increase of 8°C per minute, to a final temperature of 310°C. The SCAN worked in mass spectrometry mode with a mass range of 10-550. NIST mass spectral search program version 2.0 was used.

Results

Eight compounds were extracted and identified in the posterior section of the genital chamber of *P. ravida* (Fig. 1): cyclohexane,1,1'-(2-tridecyl-1,3-propanediyl) bis-; 10-methyl- eicosane; 5-butyl- hexadecane; 3-hexen-2-one; 3,3-diethyl-azetidine-2,4-dione; tetramethyl-oxirane; 3-methyl-2-pentanone; and 2-methoxy-2-methyl-butane (Table 1, Fig. 2). For *P. opaca*, seven compounds were identified in the posterior section of the genital chamber (Fig. 3): cholesta-4,6-dien-3-ol; cholesterol; cholesta-3,5-dien; 2-methyl-heptadecane; α-tocopherol succinate; 9,19-cyclolanost-24-en-3-ol acetate; and lup-20-en-3-ol acetate (Table 2, Fig. 4).

Fig. 1. Genital chamber of *Phyllophaga ravida* female exposed after sexual calling.

Table 1. Chemicals Extracted with Hexane from Genital Chamber of *Phyllophaga ravida* Females and Identification by Gas Chromatography-Mass Spectrometry

Compound	CCF	MW	CAS	RT	AB
Cyclohexane, 1,1'-(2-tridecyl-1,3-propanediyl)bis- (1)	$C_{28}H_{54}$	390.7284	55255-74-8	45.15	+++
10-methyl- eicosane (2)	$C_{21}H_{44}$	296.5741	54833-23-7	43.85	+++
5-butyl- hexadecane (3)	$C_{20}H_{42}$	282.5475	6912-07-8	43.51	+++
3-hexen-2-one (4)	$C_6H_{10}O$	98.1430	4376-23-2	6.48	+++
3,3-diethyl-azetidine-2,4-dione (5)	$C_7H_{11}NO_2$	141.1677	42282-85-9	6.40	+++
3-methyl-2-pentanone (7)	$C_6H_{12}O$	100.1589	565-61-7	4.70	+
2-methoxy-2-methyl-butane (8)	$C_6H_{14}O$	102.1748	994-05-8	4.42	+
Tetramethyl-oxirane (6)	$C_6H_{12}O$	100.1589	5076-20-0	3.38	+

Identification from NIST

CCF = chemical condensed formula, MW = molecular weight, CAS = Chemical Abstract Services Number, RT = retention time (minutes), AB = abundance (+++ high, + low)

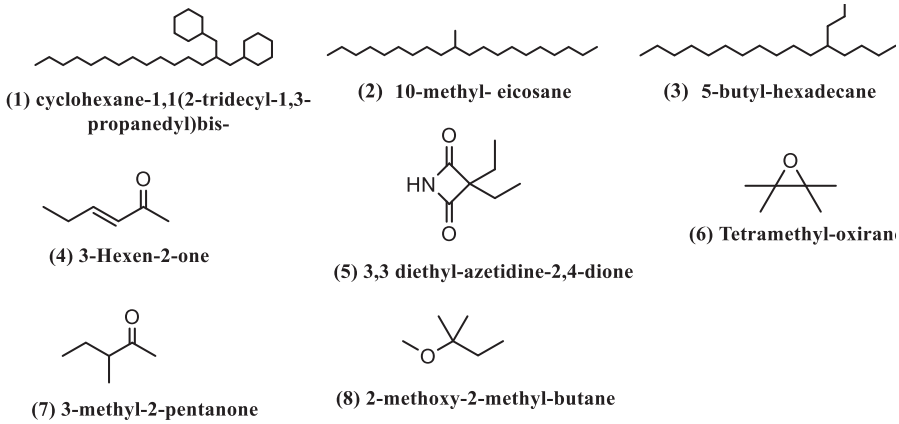

Fig. 2. Chemicals identified by gas chromatography-mass spectrometry in the genital chamber of *Phyllophaga ravida.*

Fig. 3. Separation of the genital chamber from a *Phyllophaga opaca* female by using dissecting forceps.

Table 2. Compounds Extracted with Hexane of the Protractile Genital Chamber of Female *Phyllophaga opaca* and Identification for Gas Chromatography-Mass Spectrometry

Compound	CCF	MW	CAS	RT	AB
Cholesta-4,6-dien-3-ol (9)	$C_{27}H_{44}O$	384.6400	14214-69-8	39.68	++
Cholesterol (10)	$C_{27}H_{46}O$	386.6535	57-88-5	38.24	+++
Cholesta-3,5-dien (11)	$C_{27}H_{44}$	368.6383	747-90-0	37.89	++
2-methyl heptadecane (12)	$C_{18}H_{38}$	254.4913	1560-89-0	29.96	+++
α-Tocopherol succinate (13)	$C_{33}H_{54}O_5$	530.7800	4345-03-3	29.67	+++
9,19-cyclolanost-24-en-3-ol acetate (14)	$C_{32}H_{52}O_2$	468.754	1259-10-5	27.05	+++
Lup-20 (29)-en-3-ol acetate (15)	$C_{32}H_{52}O_2$	468.7541	1617-68-1	26.03	++

Identification from NIST

CCF = chemical condensed formula, MW = molecular weight, CAS = Chemical Abstract Services Number, RT = retention time (minutes), AB = abundance (+++ high, ++ moderate)

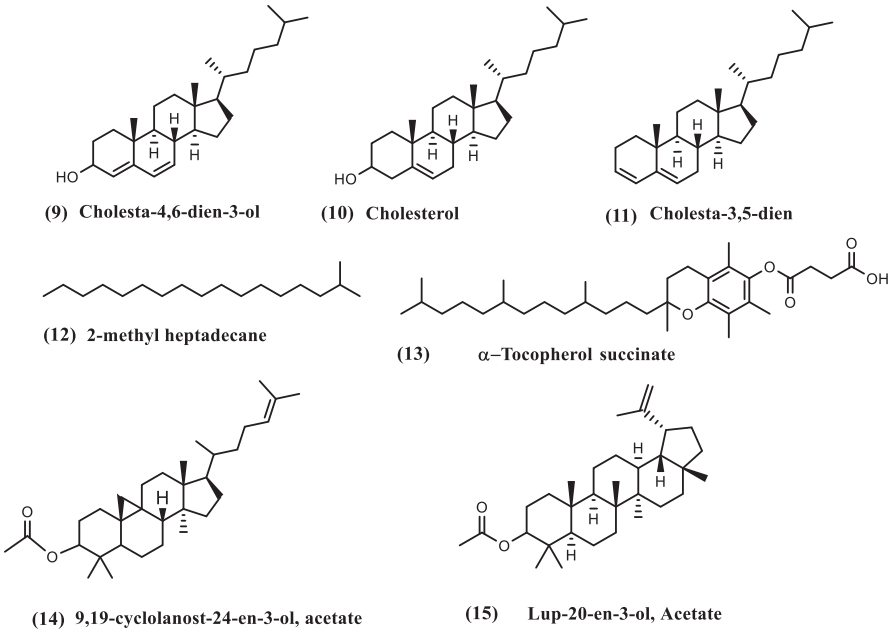

Fig. 4. Chemicals identified by gas chromatography-mass spectrometry in the genital chamber of *Phyllophaga opaca*.

Discussion

Chemicals from the genital chamber of *P. ravida* are derivatives of alkanes (cyclohexane,1,1'-(2-tridecyl-1,3-propanediyl) bis-, 10-methyl- eicosane and 5-butyl-hexadecane), amides (3,3-diethyl-azetidine-2,4-dione), ketones (3 hexen-2-one and 3-methyl-2-pentanone), and an epoxide (2-methoxy-2-methyl-butane). None of the compounds has been reported previously as a sex pheromone of any insect, with

the exception of the amide (azetidine dione derivative) that might be related to amino acids, which are the main components for sex pheromones of Melolonthidae (Leal 1998, Romero-López et al. 2005). Also, 3,3-diethyl-azetidine-2,4-dione might be a product of metabolism of the amino acid azetidine-2-carboxylic acid in some plants of the Liliaceae family (*Canavallia majalis*) and legumes (Fowden 1956). Azetidine-2-carboxylic acid is a metabolite that protects plants against pathogens and herbivores; it inhibits biosynthesis of proline. However, among strategies to avoid toxic effects of such defenses, many organisms use enzymes to degrade molecules and convert them to a source of nitrogen or other derivatives for their benefit. This might be the case for *P. ravida* that might incorporate azetidine-2-carboxylic acid from the diet and probably transform it as part of pheromones. However, other studies are needed to provide evidence of the role of 3,3-diethyl-azetidine-2,4-dione as a pheromone in the species.

For *P. opaca*, the seven compounds identified belong to several chemical classes: cholesterol and cholesterol derivatives (cholesta-4,6-dien-3-ol and cholesta-3,5-dien), ramified alkane (2-methyl-heptadecane), derivative of vitamin E α-(tocopherol succinate), and triterpenoids (9,19-cyclolanost-24-en-3-ol acetate and lup-20-en-3-ol, acetate). Cholesterol isolated from the phytophagous beetle *Popillia japonica* Newman, was proposed to be synthesized from sterols obtained from diet (Rogers and McLaughlin 1998). Other cholesterol derivatives, for example 5β-cholestane-3,24-dione, have been found in the *Malacosoma americanum* F. moth (Peterson and Fitzgerald 1991). The alcane 2-methyl heptadecane has been reported as the main component of sex pheromones of some *Holomelina* moths (Lepidoptera: Arctiidae) (Roelofs and Cardé 1971, Weatherston et al. 1974, Charlton and Roelofs 1991). Regarding sterols, which are common in plant species, none was documented as part of insect sex pheromones. The triterpene 9,19-cyclolanost-24-en-3-ol acetate was isolated in some plants such as *Xylosma flexuosa* (Flacourtiaceae) (Menezes de Oliveira et al. 2011).

Our results suggested that some compounds identified are in plants and incorporated into an insect during feeding. But this should be confirmed with further analysis. Appropriate bioassays still are needed to determine which compounds are found in sex pheromones of *P. ravida* and *P. opaca*. Identification of sex pheromones of both species can supplement information obtained for other species to learn more about bioecology and taxonomy of Melolonthidae. It also is expected to consolidate a multi-species approach to the "white grub complex" and possibly implement a pest management program based on the substances.

Acknowledgment

To Olinda Velázquez for technical support with collection of insects and to Carolina Contreras for English translation of the manuscript. This article is a contribution to research projects included in LGyAC “Ecología y Manejo de Recursos Bióticos”, Maestría en Ciencias Biológicas, BUAP.

References Cited

Aragón, A., G. A. Lugo-García, A. Reyes-Olivas, P. Casillas, J. R. Villegas-Cota, and M. A. Morón. 2009. Huéspedes vegetales de adultos de Coleoptera Scarabaeoidea en el Valle del Carrizo, Sinaloa, México. Southwest. Entomol. 35: 99-108.

Charlton, R. E., and L.R. Roelofs. 1991. Biosynthesis of volatile, methyl-branched hydrocarbon sex pheromone from leucine by arctiid moths *(Holomelina* spp.). Arch. Insect Biochem. Physiol. 18: 81-97.

Cherman, M. A., y M. A. Morón. 2014. Validación de la familia Melolonthidae Leach, 1819 (Coleoptera: Scarabaeoidea). Acta Zool. Mex. 30: 201-220.

Foster, S. P., and M. O. Harris. 1997. Behavioral manipulation methods for insect pest-management. Annu. Rev. Entomol. 42: 123-146.

Fowden, L. 1956. Azetidine-2-carboxylic acid: a new cyclic imino acid occurring in plants. Biochem. J. 64(2): 323-332.

Jutsum, A. R., and F. S. Gordon. 1989. Introduction. Pheromones: Importance to insects and role in pest management, pp. 1-13. *In* A. R. Jutsum and F. S. Gordon [eds.], Insect Pheromones in Plant Protection. Wiley, New York.

Karlson, P., and A. Butenandt. 1959. Pheromones (ectohormones) in insects. Annu. Rev. Entomol. 4: 39-58.

Leal, W. S. 1998. Chemical ecology of phytophagous scarab beetles. Annu. Rev. Entomol. 43: 39-61.

Leal, W. S., A. C. Oehlschlager, P. H. Zarbin, E. Hidalgo, P. J. Shannon, Y. Murata, L. González, R. Andrade, and M. Ono. 2003. Sex pheromone of the scarab beetle *Phyllophaga elenans* and some intriguing minor components. J. Chem. Ecol. 29: 15-25.

Menezes-de-Oliveira, D., M. I. Aguilar, B. King-Díaz, S. A. Vieira-Filho, L. Pains-Duarte, G. D. D. F. Silva, and B. Lotina-Hennsen. 2011. The triterpenes 3β-Lup-20 (29)-en-3-ol and 3β-Lup-20 (29)-en-3-yl acetate and the carbohydrate 1,2, 3, 4, 5, 6-hexa-O-acetyl-dulcitol as photosynthesis light reactions inhibitors. Molecules 16: 9939-9956.

Morón, M. A. 2003. Diversidad, distribución e importancia de las especies de *Phyllophaga* Harris en México (Coleoptera: Melolonthidae). Estudios sobre coleópteros del suelo en América. Publicación especial de la Benemérita Universidad Autónoma de Puebla, México.

Morón, M. A., B. C. Ratcliffe, y C. Deloya. 1997. Atlas de los escarabajos de México (Coleoptera: Lamellicornia). Vol. 1. Familia Melolonthidae (Subfamilias Rutelinae, Dynastinae, Cetoniinae, Trichiinae, Valginae y Melolonthinae). Sociedad Mexicana de Entomología, A.C. y CONABIO, México.

Nojima, S., P. S. Robbins, G. A. Salsbury, B. D. Morris, W. L. Roelofs, and M. G. Villani. 2003. L-leucine methyl ester: the female-produced sex pheromone of the scarab beetle *Phyllophaga lanceolata*. J. Chem. Ecol. 29: 2439-2446.

Peterson, S. C., and T. D. Fitzgerald. 1991. Chemoorientation of eastern tent caterpillars to trail pheromone, 5β-cholestane-3,24-dione. J. Chem. Ecol. 17: 1963-1972.

Robbins, P. S., R. L. Crocker, S. Nojima, B. D. Morris, W. L. Roelofs and M. G. Villani, 2003. Methyl 2-(methylthio) benzoate: the unique sulfur-containing sex pheromone of Phyllophaga crinita. *Naturwissenschaften*, 90(11), 517-520.

Roelofs, W. L., and R. T. Carde. 1971. Hydrocarbon sex pheromone in tiger moths (Arctiidae). Science 171: 684-686.

Rogers, I. L., and J. L. McLaughlin. 1998. Cholesterol from Japanese beetles, *Popillia japonica*. Nat. Prod. Lett. 11: 237-240.

Romero-López, A. A. 2016. Comunicación química de coleópteros Melolonthidae distribuidos en México: a una década de distancia. Dugesiana 23: 59-73.

Romero-López, A. A., y R. Arzuffi. 2010. Evidencias sobre la producción y liberación de compuestos bioactivos de un melolóntido mexicano, pp. 204-222. *In* L. A. Rodríguez del Bosque and M. A. Morón [eds.], Ecología y Control de Plagas Edafícolas, Instituto de Ecología A.C., Xalapa, México.

Romero-López, A. A., R. Arzuffi, y M. A. Morón. 2005. Feromonas y atrayentes sexuales de coleópteros Melolonthidae de importancia agrícola. Folia Entomol. Mex. 44: 233-245.

Romero-López, A. A., A. Aragón, y R. Arzuffi. 2007. Estudio comparativo del comportamiento sexual de cuatro especies de *Phyllophaga* (Coleoptera: Melolonthidae), pp. 275-281. *En* E. G. Estrada, A. Equihua, C. Luna, y J. L. Rosas-Acevedo [eds.], Entomología Mexicana 6. Sociedad Mexicana de Entomología, México.

Sanderson, M. W. 1958. Faunal affinities of Arizona *Phyllophaga*, with notes and descriptions of new species. J. Kans. Entomol. Soc. 31:158-173.

Thomson, D. R., L. J. Gut, and J. W. Jenkins. 2000. Pheromones for insect control. Strategies and successes, pp. 385-412. *In* F. R. Hall and J. J. Menn [eds.], Methods in Biotechnology. Biopesticides: Use and Delivery. Humana Press, Totowa, NJ.

Ward, A., C. Moore, V. Anitha, J. Wightman, and D. J. Rogers. 2002. Identification of the sex pheromone of *Holotrichia reynaudi*. J. Chem. Ecol. 28: 515-522.

Weatherston, J., L. M. Davidson, and D. Simonini. 1974. Attractants for several male forest Lepidoptera. Can. Entomol. 106: 781-782.

Zarbin, P. H., W. S. Leal, C. J. Ávila, and L. J. Oliveira. 2007. Identification of the sex pheromone of *Phyllophaga cuyabana* (Coleoptera: Melolonthidae). Tetrahedron Lett. 48: 1991-1992.

Zhang, A., P. S. Robbins, W. S. Leal, C. E. Linn Jr., M. G. Villani, and W. L. Roelofs. 1997. Essential amino acid methyl esters: major sex pheromone components of the cranberry white grub, *Phyllophaga anxia* (Coleoptera: Scarabaeidae). J. Chem. Ecol. 23: 231-245.

New State and County Records of Dung Beetles[1] of Oklahoma

Marcela S. Gigliotti[2], W. Wyatt Hoback[3], and Justin L. Talley[3]

Abstract. This work updates species and county distribution of Scarabaeinae dung beetles in Oklahoma. Historical collections and literature records, as well as data from a private regional collection, were compiled to document species in the state. The study adds eight new dung beetles for the state, bringing the total to 39 species. In addition, 78 new county records were reported. Two behavioral groups, tunnelers and rollers are present, but dwellers in the subfamily Scarabeinae have not been documented in Oklahoma. By updating the species list and known distribution, more research can be focused on dung beetles that provide numerous ecological services and benefit livestock producers.

Introduction

The family Scarabaeidae is a diverse group of Coleoptera with more than 7,000 described species in 12 subfamilies (Schoolmeesters 2015). The subfamily Scarabaeinae includes the true dung beetles that use vertebrate dung for food and rearing their larvae (Halffter and Matthews 1966). Dung beetles are detritivores that improve nutrient cycling, provide natural pest control of dung-feeding flies, reduce disease transmission, and improve secondary dispersal of seeds. Together dung beetles provide more than $380 million in ecosystem benefits to pasture and rangeland in North America (Losey and Vaughn 2006, Nichols et al. 2008, Whipple and Hoback 2012).

Dung beetles are divided into three behavioral groups based on how they use dung for rearing offspring. Tunnelers bury the resource in the soil, making tunnels near or below dung. The tunneling activities of species bring soil to the surface and fill the tunnel with loosened soil to protect the brood ball, improving soil aeration and moisture infiltration (Hanski and Cambefort 1991). Rollers take a portion of the fresh dung and create a ball on the surface, roll it a distance from the source of the dung, and bury it. Dwellers do not move the resource, but deposit eggs in the dung and rear the larvae in place (Halffter and Matthews 1966, Hanski and Cambefort 1991). Dung beetles are useful to the ecosystem where they occur naturally and have been introduced to many systems where they have improved pasture health especially when non-native herbivores such as cattle are present (Nichols et al. 2008).

Cattle producers and ecologists are interested in conserving and increasing the numbers of dung beetles. Widening the knowledge about dung beetles and cataloging their regional occurrence is the first necessary effort (Fiene et al. 2011). Scarabaeoidea that includes dung beetles has been studied in many states in North

[1]Coleoptera: Scarabaeinae
[2]Department of Zoology, University of Brasilia – Brasilia, Brazil.
[3]Department of Entomology and Plant Pathology, Oklahoma State University, Stillwater, OK.

America (Kriska and Young 2002, Riley and Wolfe 2003, Fiene et al. 2011, Whipple and Hoback 2012). However, Oklahoma with extensive beef production has little published information on the distribution and diversity of dung beetles. The only published data about the Scarabaeinae of Oklahoma is a taxonomic key and county distribution published in 1964 (Wallace and Drew).

Materials and Methods

To update dung beetle occurrence in Oklahoma, historical collections and literature records, personal records, and data from a private regional collection were compiled. All species reported here were confirmed by taxonomic keys (Wallace and Drew 1964). Specimens recorded were deposited in collections at Oklahoma State University at Stillwater, Sam Noble Oklahoma Museum of Natural History at Norman, and at the University of Oklahoma at Norman, curated by Kenneth Hobson.

Results

Eight new state records of dung species were recorded, bringing the total known to occur in Oklahoma to 39 species (Table 1). The species were organized according to county distributions (Figs. 1-14) and document 78 new county records.

Table 1. Checklist and Distributional Record of Species of Dung Beetles (Scarabaeidae: Scarabaeinae) in Oklahoma

Species	County
Ateuchus histeroides Weber, 1801	Alfalfa, Caddo*, Cherokee*, Cleveland, Comanche*, Craig, Jackson*, Jefferson*, Latimer*, Marshall*, McCurtain, Muskogee, Ottowa*, Payne, Pushmataha*, Stephens*, Tulsa*,
Ateuchus lecontei Harold, 1868	Cleveland, Comanche, Latimer, Wichita*
Canthon (Boreocanthon) lecontei Harold, 1868	Caddo*, Cleveland, Ellis, Logan*, Major*, McCurtain, Noble, Oklahoma*, Osage, Woodward*
Canthon (Glaphyrocanthon) viridis Harold, 1868	Caddo*, Comanche, Mayes*, McCurtain*, Oklahoma*, Payne*
Canthon (Melanocanthon) granulifer Schmidt, 1920	Latimer
Canthon (Melanocanthon) nigricornis Robison, 1941	Atoka, Cleveland, Comanche, Ellis*, Muskogee*, Payne*, Wichita*
Canthon chalcites Haldeman, 1843	Adair, Alfafa, Cleveland, Delaware, Ellis, Harper, Jay*, Latimer, McCurtain, Muskogee*, Nowata*, Payne*, Pushmataha,
Canthon imitator Brown, 1946	Cimarron*, Cleveland, Ellis*, Jackson*, Major*, Payne*, Roger Mills*, Washington*, Woodward*
Canthon pilurarius	Cleveland, Latimer, Murray, Wichita
Canthon practicola Leconte, 1859	Cimarron, Cleveland, Comanche, Grady, Noble, Payne, Wichita*
Canthon perplexus Leconte, 1847	McCurtain, Pontotoc
***Canthon simplex* LeConte, 1857**	Cleveland

Canthon laevis LeConte, 1859	Adair, Cherokee, Cimarron, Cleveland, Comanche, Custer, Ellis, Grant, Harmon, Latimer*, Logan, McClain, McCurtain, Murray, Noble, Oklahoma, Osage, Pawnee, Payne, Pontotoc, Woodward
Canthon vigilans Leconte, 1858	Alfafa*, Cleveland, Comanche, Delaware, Dewey, Ellis*, Jackson*, Latimer*, Marshall, Mayes*, McClain, McCurtain, Muskogee*, Oklahoma*, Payne*, Pontotoc, Roger Mills, Sequoyah*, Tulsa*, Woodward*
Canthon (Boreocanthon) ebenus Say, 1823	Alfafa, Beaver*, Beckham*, Caddo, Cherokee*, Cimarron, Cleveland, Ellis*, Grady, Major*, Marshall, McCurtain, Payne, Roger Mills*, Woods
***Copris fricator* (Fabricius, 1787)**	Cimarron, Kay, Mayes, McCurtain, Payne, Tulsa
Copris minutus (Drury, 1770)	Latimer*, Mayes, Payne, Sequoyah
Copris tullius	Canadian, Cleveland, Comanche, Dewey, Grady,, Kiowa, Latimer*, Marshall, McCurtain, Murray*, Oklahoma, Pawnee, Payne, Pontotoc, Roger Mills, Washington, Wichita*
Deltochilum gibbosum Fabricius, 1775	Atoka*, Cherokee*, Latimer*, McCurtain, Muskogee*, Ottowa, Pittsburg*, Pushmataha
Dichotomius carolinus (Linné, 1767)	Adair, Atoka, Big Cedar*, Bryan, Cherokee*, Cleveland, Comanche, Creek*, Grady*, Latimer*, LeFlore, Logan*, Marshall, Mayes*, McCurtain, Muskogee, Okfuskee, Oklahoma*, Pawnee, Payne, Seqouyah, Tulsa*, Wagoner*, Washington, Wichita*
***Digitonthophagus gazella*[1] (Fabricius, 1787)**	Bryan, Cherokee, Coal, Comanche, Creek, Jackson, Jefferson*, Latimer*, Leflore*, Logan*, Mayes*, McCurtain*, McIntosh*, Oklahoma*, Payne*, Pittsburg*, Pottawatomie*, Pushmataha*, Stephens*, Tillman*
***Euoniticellus intermedius*[1] (Reiche, 1849)**	Tillman*
Onthophagus (striatulus) striatulus	Cherokee*, Latimer, Muskogee*
Onthophagus cynomysis Brown, 1927	Cleveland, Grady, Noble, Payne, Woodward*
Onthophagus guatemalensis Bates, 1887	Cleveland, Grady, Latimer*
Onthophagus hecate (Panzer 1794*)*	Alfafa, Atoka*, Caddo*, Cherokee*, Cleveland, Comanche, Craig, Garfield*, Latimer, McCurtain, Muskogee*, Oklahoma*, Ottowa, Payne, Pontotoc*, Sequoyah, Texas, Wichita*
Onthophagus medorensis Brown, 1929	Cleveland, Ellis*, Grady, Latimer*, Muskogee*, Wichita, Woodward*
Onthophagus oklahomensis Brown, 1927	Cherokee*, Cleveland, Comanche, Ellis*, Grady, Latimer, Payne, Woodward*, Wichita*

Onthophagus orfheus (Panzer, 1794)	Bryan*, Latimer, Muskogee*, Payne*, Pottawatomie*
Onthophagus pennsylvanicus (Harold, 1871*)*	Alfafa, Beckham, Cimarron*, Cleveland*, Comanche, Dewey, Ellis*, Grady, Harmon, Leflore, McClain, McCurtain, Murray, Muskogee*, Payne, Wichita*, Woodward*
Onthophagus subaeneus (Beauvois, 1811)	Latimer*, Payne*
Onthophagus taurus[1] (Schreber, 1759)	Payne
Onthophagus tuberculifrons Harold, 1871	Ellis, Garvin, Latimer, Murray, Muskogee, Payne
Onthophagus velulinus	Cimarron, Muskogee
Phanaeus difformis Leconte, 1847	Cherokee, Cleveland, Ellis, Grady, Major, Woods
Phanaeus torrens LeConte, 1847	Latimer
***Phanaeus triangularis* (Say, 1823)**	Love
Phanaeus vindex MacLeay, 1819	Atoka, Blaine, Bryan, Caddo, Canadian, Cimarron, Cleveland, Comanche, Cotton, Creek, Custer, Dewey, Ellis, Grady, Jackson, Latimer, Leflore, Logan, Manhatten, Marshall, McClain, McCurtain, Murray, Muskogee, Pawnee, Payne, Pushmataha, Rogers, Stephens, Texas Tulsa, Oklahoma, Wichita, Woods, Woodward
Pseudocanthon perplexus (LeConte, 1847)	Beckham, Bryan, Kay, Latimer, Logan, Muskogee, Payne, Tulsa

Species in bold are new state records. *County record not reported in Wallace and Drew (1964). [1]Species introduced to North America.

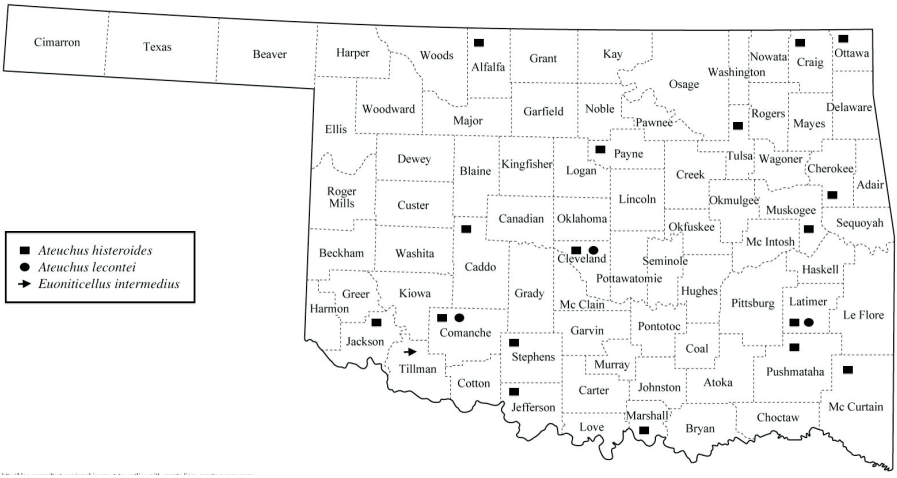

Fig. 1. Oklahoma county map with distribution of *Ateuchus histeroides* Weber, *Ateuchus lecontei* Harold, and *Euoniticellus intermedius* Reiche.

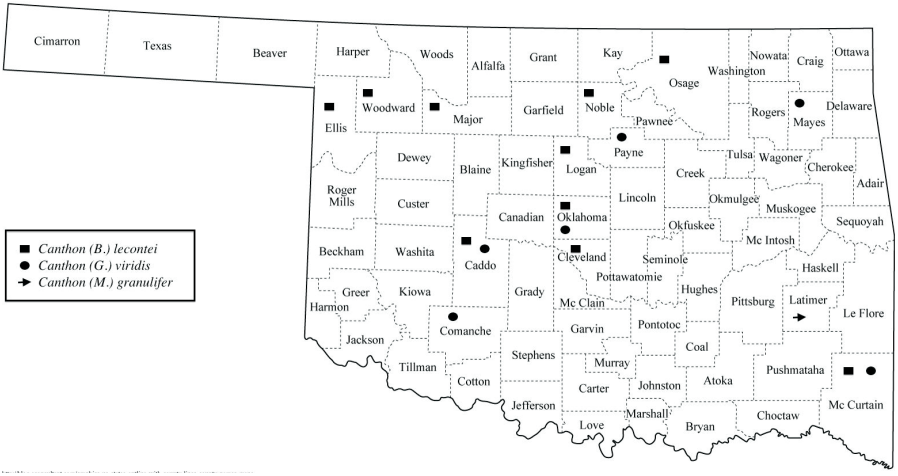

Fig. 2. Oklahoma county map with distribution of *Canthon (B.) lecontei* Harold, *Canthon (G.) viridis* Harold, and *Canthon (M.) granulifer* Schmidt.

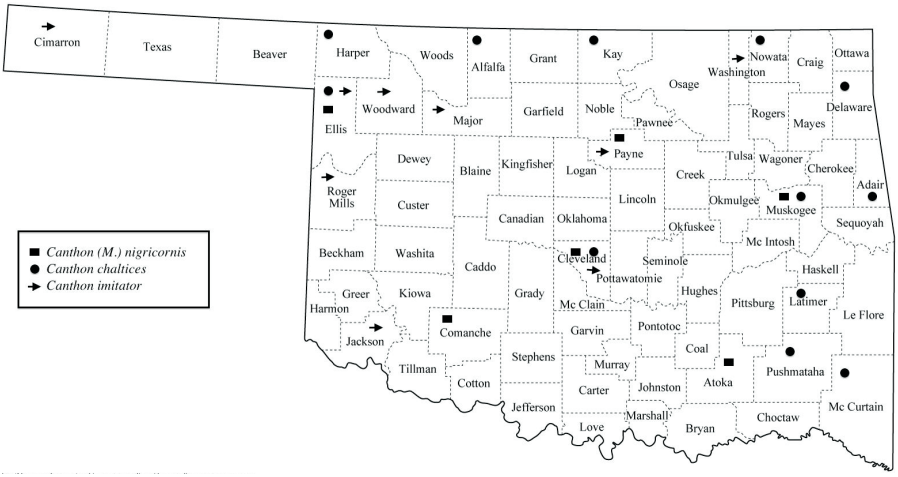

Fig. 3. Oklahoma county map with distribution of *Canthon (M.) nigricornis* Robison, *Canthon chalcites* Haldeman, and *Canthon imitator* Brown.

Fig. 4. Oklahoma county map with distribution of *Canthon pilurarius* (L.), *Canthon practicola* Leconte, and *Canthon simplex* LeConte.

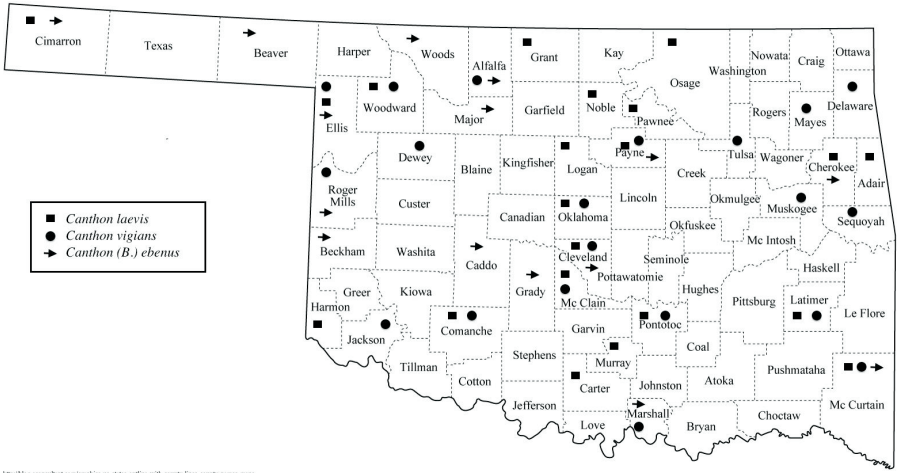

Fig. 5. Oklahoma county map with distribution of *Canthon laevis* LeConte, *Canthon vigilans* Leconte, and *Canthon (B.) ebenus* Say.

Fig. 6. Oklahoma county map with distribution of *Copris fricator* (F.), *Copris minutus* (Drury), and *Copris tullius* Olivier.

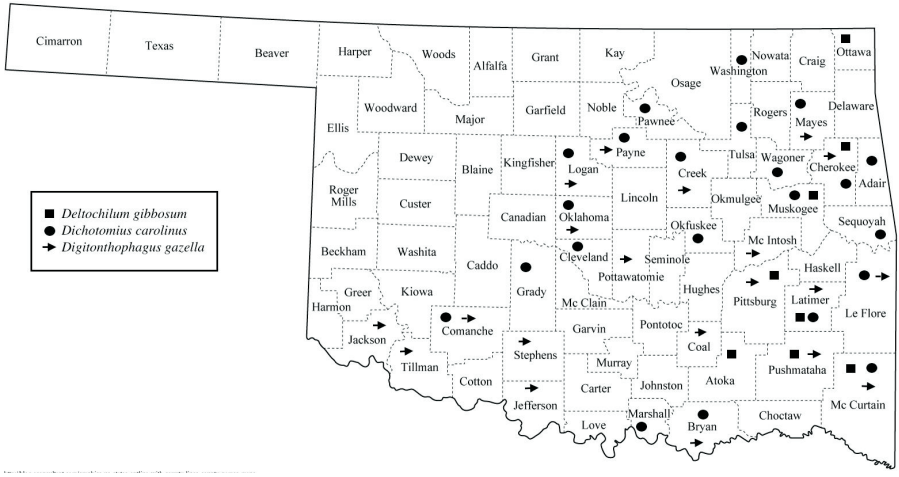

Fig. 7. Oklahoma county map with distribution of *Deltochilum gibbosum* Fabricius, *Dichotomius carolinus* (Linné), and *Digitonthophagus gazella* (F.).

Fig. 8. Oklahoma county map with distribution of *Onthophagus (S.) striatulus* Beauvois, *Onthophagus cynomysis* Brown, and *Onthophagus guatemalensis* Bates.

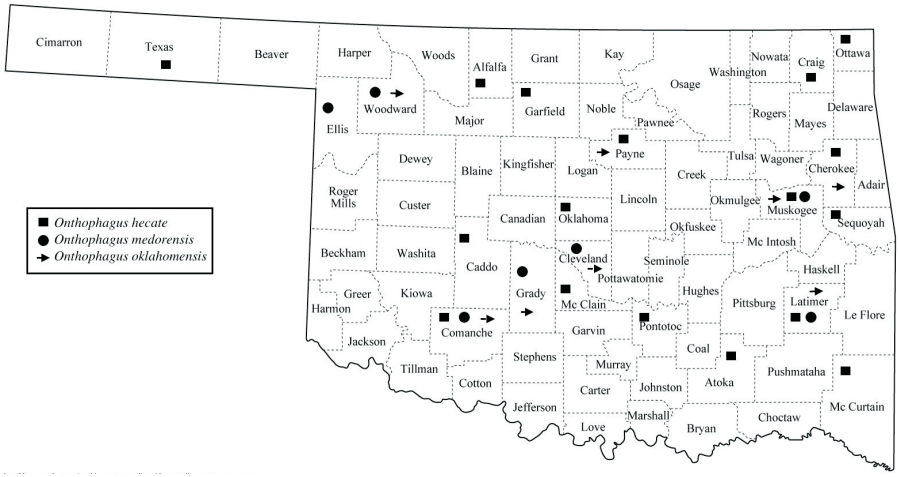

Fig. 9. Oklahoma county map with distribution of *Onthophagus hecate* (Panzer), *Onthophagus medorensis* Brown, and *Onthophagus oklahomensis* Brown.

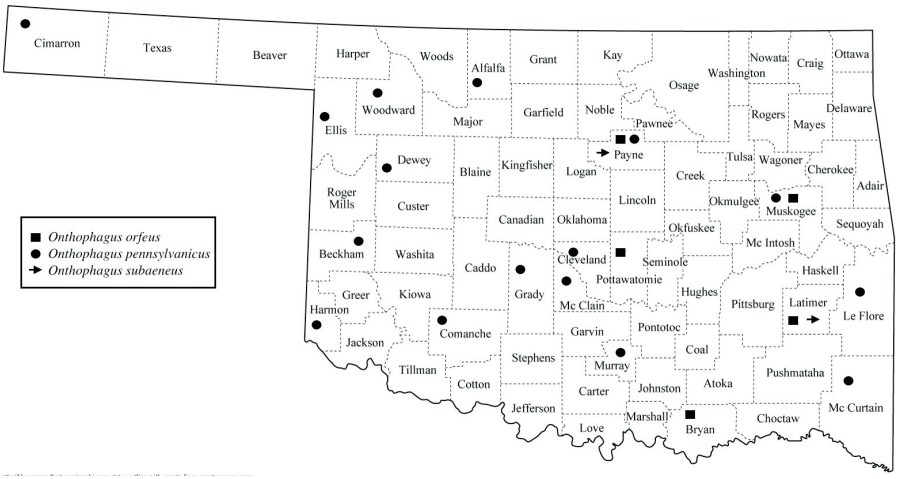

Fig. 10. Oklahoma county map with distribution of *Onthophagus orfheus* (Panzer), *Onthophagus pennsylvanicus* (Harold), and *Onthophagus subaeneus* (Beauvois).

http://blog.ecnsultant.com/graphics-us-states-outline-with-county-lines-county-names-maps

Fig. 11. Oklahoma county map with distribution of *Onthophagus tuberculifrons* Harold, *Onthophagus velulinus* Horn, and *Onthophagus taurus* (Schreber).

Fig. 12. Oklahoma county map with distribution of *Phanaeus difformis* Leconte, *Phanaeus torrens* LeConte, and *Phanaeus triangularis* (Say).

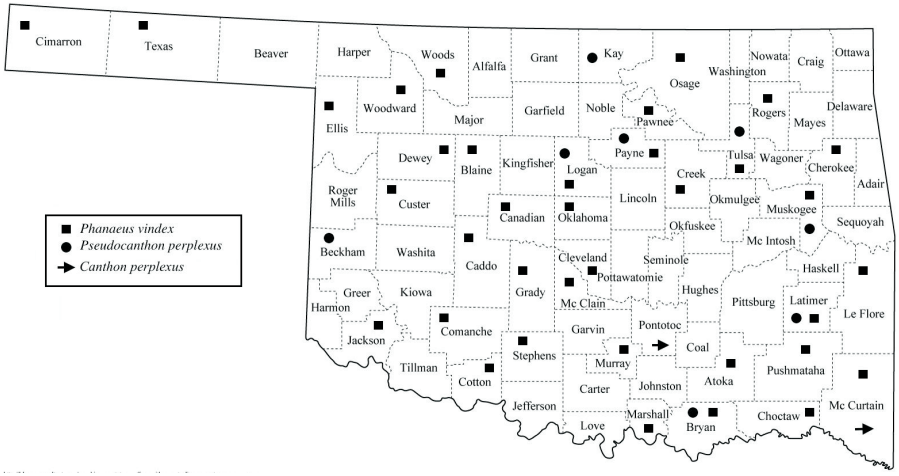

Fig. 13. Oklahoma county map with distribution of *Phanaeus vindex* MacLeay, *Pseudocanthon perplexus* (LeConte), and *Canthon perplexus* Leconte.

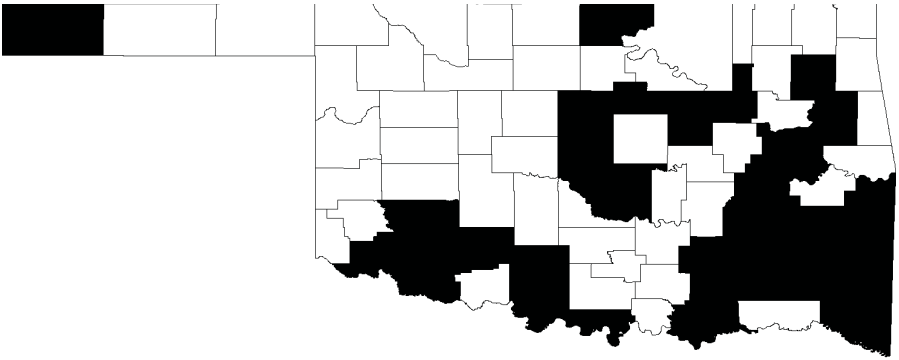

Fig. 14. Oklahoma counties with newly recorded state records of dung beetle species.

Discussion

Oklahoma, with more than 1.95 million head of cows, is usually one of the main beef-producing states (USDA-NASS 2017). However, diversity of agricultural commodities produced in Oklahoma lead to landscape fragmentation. Consequently, having accurate records of dung beetle species can lead to conservation programs that focus on ecosystem services dung beetles provide that increase the health of agroecosystems (Nichols et al. 2008). One benefit to agroecosystems is reduction of pestiferous flies associated with livestock production. Infestations of flies, especially horn flies, *Haematobia irritans* (L.), and face flies, *Musca autumnalis* De Geer, cause significant financial impact for livestock producers (Byford et al. 1992) and reduce livestock productivity (Haufe 1982). The impact to cattle usually is related to defensive activities when the animals react to the flies (Steelman et al. 1993). Studies by Hughes et al. (1978) and Ridsdill-Smith and Hayles (1990) found reduced abundance of flies by dung beetles in field settings. However, the reductions were not linked to individual dung beetle species (Feehan et al. 1985, Bishop et al. 2005), but to whole dung beetle assemblages (Horgan 2005, Manning et al. 2016).

Scarab dung beetles of Oklahoma are in two behavioral groups, with species of *Atheucus, Dichotomius*, *Digitonthophagus*, *Onthophagus,* and *Phanaeus* being tunnelers that move dung into the ground below the pat. Species of *Canthon*, *Copris*, *Deltochilum*, *Euoniticellus*, and *Pseudocanthon* are dung rollers that form a dung ball and move it to a new location. Neither this study nor Wallace and Drew (1964) found scarabaeid dwellers. Each dung beetle improves the soil by increasing percolation, introducing organic matter, and reducing non-point sources of organic pollution (Halffter and Mathews 1966, Hanski and Cambefort 1991, Scholtz and Chown 1995, Campos and Hernández 2013, Manning et al. 2016).

This study recorded eight new species in 30 Oklahoma counties (Fig. 14), including three new species not previously described in Oklahoma that were introduced to North America from Africa (Table 1). Of the eight new species described none of these species were previously detected by Wallace and Drew (1964). While most species were found in one or two counties in our study, *D. gazella*

was in 20 counties. In other studies, the species was an agresssive competitor that altered dung beetle communities and is considered invasive in some areas including Central and South America where it has spread and also been introduced intentionally (Noriega et al. 2010, Whipple et al. 2012).

The 10 ecoregions of Oklahoma range from plains to subtropical forests. The diversity of habitat supports an array of dung beetle species, and every county in the state has at least one record. Interestingly, there is a gap in new species being discovered in the northwestern section of Oklahoma (Fig. 14). This could be related to more crop-based agroecosystems, especially wheat (*Triticum aestivum* L.), that would result in less dung available for dung beetles to colonize. However, limited sampling occurred in many areas of the state, and additional species and many more county records probably will be discovered with continued sampling.

Acknowledgment

The authors thank the CAPES for a scholarship to Marcela Soares Gigliotti de Carvalho (Process: 88881.132834/2016-01). We also thank Dr. Kenneth Hobson from Oklahoma University – OU for hosting us at OU and providing access to his personal collection and Melissa Sadir from Sam Noble Museum, Norman, OK, for allowing us access to the entomology section of the museum. This work was approved for publication by the Director of the Oklahoma Agricultural Experiment Station, and supported, in part, under Projects OKL-03036 and OKL-02917 (Multi-State S-1076).

References Cited

Bishop, A. L., H. J. McKenzie, L. J. Spohr, and I. M. Barchia. 2005. Interactions between dung beetles (Coleoptera: Scarabaeidae) and the arbovirus vector *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae). Austral. J. Entomol. 44: 89-96.

Byford, R. L., M. E. Craig, and B. L. Crosby. 1992. A review of ectoparasites and their effect on cattle production. J. Ani. Sci. 70: 597-602.

Campos, R. C., and M. I. M. Hernandez. 2013. Dung beetle assemblages (Coleoptera, Scarabaeinae) in Atlantic forest fragments in southern Brazil. Revista Brasileira de Entomologia 57: 47-54.

Feehan, J., R. D. Hughes, M. A. Bryce, and S. Runko. 1985. Bush fly abundance and population events in relation to dung beetle catches on the south coast of New South Wales. J. Austral. Entomol. Soc. 24: 37-43.

Fiene, J. G., M. B. Connior, R. Androw, B. Baldwin, and T. Mckay. 2011. Surveys of Arkansas dung beetles (Coleoptera: Scarabaeidae and Geotrupidae): phenologies, mass occurrences, state and distributional records. Am. Mid. Nat. 165: 319-337.

Halffter, G., and E. G. Matthews. 1966. The natural history of dung beetles of the subfamily Scarabaeinae (Coleoptera: Scarabaeidae). Fol. Entomol. Mexi. 14: 1-312.

Hanski, I., and Y. Cambefort. 1991. Dung Beetle Ecology. Princeton University Press, New Jersey.

Haufe, W. O. 1982. Growth of range cattle protected from horn flies (*Haematobia irritans*) by ear tags impregnated with envalerate. Can. J. Ani. Sci. 62: 567-573.

Horgan, F. G. 2005. Effects of deforestation on diversity, biomass and function of dung beetles on the eastern slope of the Peruvian Andes. For. Ecol. Mgt. 216: 117-133.

Hughes, R., M. Tyndale-Biscoe, and J. Walker. 1978. Effects of introduced dung beetles (Coleoptera: Scarabaeidae) on the breeding and abundance of the Australian bushfly, *Musca vetustissima* Walker (Diptera: Muscidae). Bul. Entomol. Res. 68: 361-372.

Kriska, N. A., and D. K. Young. 2002. An annotated checklist of Wisconsin Scarabaeoidea (Coleoptera). Insecta Mundi 16: 31-48.

Losey, J. E., and M. Vaughan. 2006. The economic value of ecological services provided by insects. BioScience 56: 311-323.

Manning, P., E. M. Slade, S. A. Beynon, and O. T. Lewis. 2016. Functionally rich dung beetle assemblages are required to provide multiple ecosystem services. Agri. Eco. Envir. 218: 87-94.

Nichols, E., S. Spector, J. Louzada, T. Larsen, S. Amezquita, and M. E. Favila. 2008. Ecological functions and ecosystem services provided by Scarabaeinae dung beetles. Biol. Cons. 141: 1461-1474.

Noriega, J. A., F. G. Horgan, T. H. Larsen, and G. Valencia. 2010. Records of an invasive dung beetle species, *Digitonthophagus gazella* (Fabricius, 1787) (Coleoptera: Scarabaeidae), in Peru. Acta Zool. Mex. 26: 451-456.

Ridsdill-Smith, T., and L. Hayles. 1990. Stages of bush fly, *Musca vetustissima* (Diptera: Muscidae), killed by Scarabaeine dung beetles (Coleoptera: Scarabaeidae) in unfavourable cattle dung. Bul. Entomol. Res 80: 473-478.

Riley, E. G., and C. S. Wolfe. 2003. An annotated checklist of the Scarabaeoidea of Texas (Coleoptera). Southwest Entomol. 26: 1-37.

Scholtz, C. H., and S. L. Chown. 1995. The evolution of habitat use and diet in the Scarabaeoidea: a phylogenetic approach, pp. 355-374. *In* J. Pakaluk and S.A.S. Lipinski [eds.], Biology, Phylogeny, and Classification of Coleoptera. Museum Inst. Zool., Warsaw

Schoolmeesters, P. 2015. Scarabs: World Scarabaeidae Database (version Jan. 2015). *In* Y. Roskov et al. [eds.], Species 2000 & ITIS Catalogue of Life, 2015 Annual Checklist. www.catalogueoflife.org/annual-checklist/2015 [accessed 27 July 2017].

Steelman, C. D., E. E. Gbur, E. Tolley, and A. H. Brown. 1993. Individual variation within breeds of cattle in resistance to horn fly (Diptera: Muscidae). J. Med. Entomol. 30: 414-420.

USDA-NASS. 2017. Oklahoma Agriculture Statistics 2017. USDA. https://www.nass.usda.gov/Statistics_by_State/Oklahoma/index.php. Accessed 23 August 2017.

Wallace, F. L., and W. A. Drew. 1964. The Scarabaeinae of Oklahoma (Scarabainae, Coleoptera). Proc. Oklahoma Acad. Sci.

Whipple, S. D., and W. W. Hoback. 2012. A comparison of dung beetle (Coleoptera: Scarabaeidae) attraction to native and exotic mammal dung. Environ. Entomol. 41: 238-244.

Whipple, S. D., E. J. Lindroth, W. W. Hoback, M. C. Albrecht, and J. E. Foster. 2012. Genetic variability of *Digitonthophagus gazella* (F.) (Coleoptera: Scarabaeidae) from Vieques, Puerto Rico and South Africa. Coleop. Bul. 66: 45-50.

Sphecids of Mexico

Maximiliano Vanoye Eligio[1]* and Yazmín Guadalupe Martínez Téllez

Instituto Tecnológico Superior de Escárcega. Calle 85 s/n entre 10-B Col. Unidad Esfuerzo y Trabajo No. 1. Escárcega, Campeche CP 24350.

Abstract. The Sphecidae family is a group of predatory wasps that hunt other insects and spiders with which to supply their nests. The sphecids have certain characteristics that distinguish them from other groups of wasps and bees. The objective of this research was to gather current knowledge of predatory Sphecidae wasps for Mexico, which includes a general overview of Sphecidae worldwide, nationally, and by states. Available literature of the family and different databases were reviewed. In Mexico, 121 species and 14 genera have been reported. The genus *Ammophila* W. Kirby, 1798 contains the most species (43). Tamaulipas and Chihuahua are the states with the largest number of species in the family, which are part of the Nearctic region. The work contributes significant data of the number of species of Sphecidae in Mexico, updated to 121 from 91 species reported previously. However, a large number of species do not have locality data. Further studies are recommended of these insects that have an important role in tropical ecosystems as predators of other arthropods.

Introduction

Predatory solitary wasps are a group of insects that hunt other insects and spiders with which to supply their nests. This behavior is mostly by females of the families Ampulicidae, Crabronidae, Eumenidae, Pompilidae, Sphecidae, and Vespidae (Borror et al. 1989). Sphecids have characteristics that distinguish them from other wasps and bees; for example, they have a short pronotum lobed on the sides, simple hairs, a petiole formed from the sternum, and jugal lobe of the large posterior wing, including the anal vein. Wasps of the family are medium to large and colorful. Species of the family usually make their nests in different types of soil, mostly sandy, or soft stems and holes in trunks, with preference for areas lacking vegetation. For their pedotrophic diet, nests are supplied with species of the order araneae, nymphs of the order Orthoptera, and larvae of Lepidoptera, Hymenoptera, and other insects (Bohart and Menke 1976, Menke 1997). Prey supplied by solidary wasps to their nests can be subdivided into two categories; larvae during development when the nest is not completely closed, and complete supply of prey to the larva when the nest is completely closed (Evans and O'Neill 2007). The size of the young wasps depends mostly on the supply of prey offered by mother wasps. For the first case, progressive supply of prey to the larvae by species of the genus *Bembix* Fabricius, 1775 enables development of the offspring to be monitored by the mother. In the second case, wasps of the genus *Sceliphron* Klug, 1801 do not have direct contact

Tel. 982-596-0603. *maxvanoye@itsescarcega.edu.mx

with the offspring, and the number of prey varies according to size of the prey (Elgar and Jebb 2004).

Knowledge of the biology and distribution of the Sphecidae family has increased over the years. Sphecids are a cosmopolitan family with 783 species worldwide in five subfamilies (345 species of Ammophilinae, 20 Chloriontinae, 146 Sceliphrinae, 271 Sphecinae, and 1 Stangeellinae) (Hanson and Menke 2006, Pulawski 2018a). In North America, 102 species in 11 genera have been recorded. Ruiz et al. (2002) mentioned 87 species and 14 genera in Mexico, but in exhaustive review of the family, only 91 species and 14 genera were reported, of which 60% did not have distribution data in Mexico.

Over time, classification of Sphecidae has been proposed by different authors (Lomholdt 1982, Alexander 1992, Prentice 1998, Brothers 1999, Melo 1999), which showed that Sphecidae is paraphyletic in relation to bees (Hanson and Menke 2006). Currently, the classification of Sphecidae most accepted is by Prentice, Brothers, and Melo, in which Sphecidae, Ampulicidae, Apidae, Heterogynaidae, and Crabronidae are in the Superfamily Apoidea. The classification of Sphecidae used in the present work was by Pulawski (2007) who listed four subfamilies (Ammophilinae, Chloriontinae, Sceliphrinae, and Sphecinae) (Pulawski 2018a). The objective of this work was to update the catalog of Sphecidae species for Mexico.

Materials and Methods

Information on Sphecidae was reviewed in available data (Discoverylife 2018, GBIF 2018, Pulawski 2018b) and literature that made reference to species records in Mexico from books, articles in journal citation reports, Latindex, Scielo, scientific journal notes, as well as diversity studies on a local scale in Mexico. Only sphecid wasps in Mexico that were identified to species were selected.

Results

According to information generated from the Sphecidae family in Mexico, 121 species and 14 genera were recorded (Table 1, Annex 1). The genus *Ammophila*

Table 1. Number of Species by Genus of Sphecidae in Mexico

Genus	Number of species
Ammophila W. Kirby, 1798	43
Sphex Linnaeus, 1758.	21
Podalonia Fernald 1927	12
Prionyx Van Der Linden, 1827	8
Podium Fabricius, 1804	7
Isodontia (Saussure, 1867)	7
Sceliphron Klug, 1801	5
Chlorion Latrielle (1802)	4
Chalybion Dahlbom, 1843	4
Eremnophila Menke, 1964	3
Palmodes Kohl, 1890	3
Penepodium Menke, 1976	2
Dynatus Lepeletier, 1845	1
Trigonopsis Perty, I833	1
	121

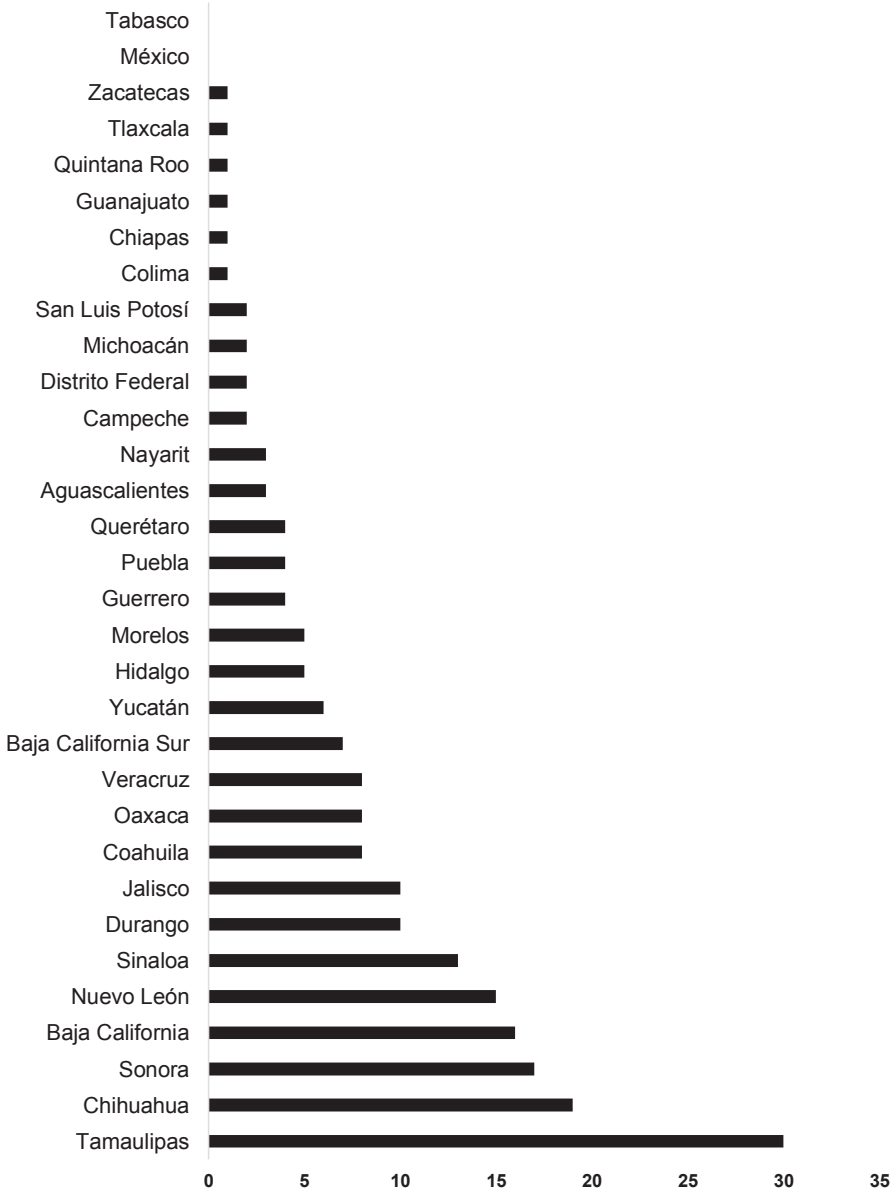

Fig. 1. Number of Sphecidae species recorded by state of the Mexican Republic.

W. Kirby, 1798 had the most species recorded for Mexico (43), but most genera (79%) had fewer than 10 species.

Distribution of Sphecidae species in Mexico also was identified. Tamaulipas is the state with the most species recorded (30), followed by Chihuahua and Sonora. The family had not been reported from two states (Tabasco and Mexico), while only one species was reported in other states (Colima, Chiapas, Guanajuato, Quintana Roo, Tlaxcala, and Zacatecas) (Annex 1). In general, 45% of the species registered in the country have been reported for the Nearctic region of which Mexico is a part, 4% in the Neotropical region, 19% in both regions, and 31% only mentioned in the country (Fig. 1).

Discussion

The Sphecidae family has been studied from different approaches, from taxonomy (Bohart and Menke 1976, Menke and Fernández 1996), ecology (Coville 1981, Genaro 1994, Horta et al. 2007, Vanoye-Eligio et al. 2015), and faunistics (Ruiz et al. 2002, Gayubo and Izquierdo 2006, Vanoye-Eligio et al. 2014), among others. According to studies of the family in Mexico, 91 species and 14 genera were reported by Ruiz et al. (2002) and Horta et al. (2007), while González-Hernández and Carpenter (2011) reported 64 species, and the Discoverylife database (2018) and GBIF (2018) reported 40 to 78 species. In this study, 121 species and 14 genera were reported, which according to the most complete review (Ruiz et al. 2002) increased the number of Sphecidae species (30) in Mexico by 33%.

More Sphecidae species in Mexico have been reported in states that are part of the Nearctic region, such as Tamaulipas and Chihuahua, while Sinaloa, Oaxaca, and Veracruz are states that excel in registry of species of the family in the neotropical region. Seventy-six percent of the species of Sphecidae reported for the Nearctic region are in Mexico, with 18% of the 159 species from the Neotropical region reported in Mexico (Table 2).

Table 2. Number of Sphecidae Species Worldwide, Regionally, Nationally, and In the Present Study

				Study		
World	Neartic	Neotropical	Mexico	Neartic	Neotropical	Mexico
660	102	159	91	78	28	121

Bohart and Menke 1976; Finnamore and Michener 1993; Gauld and La Salle 1993; Wesbauer 1995; Fernández 2000, 2002; Ruiz et al. 2002; González 2006; Horta et al. 2007; Morrone and Márquez 2008; Pulawski 2011, 2018c)

References Cited

Alexander, B. A. 1992. A cladistic analysis of the subfamily Philanthinae (Hymenoptera: Sphecidae). Systematic Entomology, 17(2), 91-108.

Amarante, S. T. P. 2002. A synonymic catalog of the Neotropical Crabronidae and Sphecidae (Hymenoptera: Apoidea). Arquivos de Zoologia (São Paulo) 37: 1-139.

Ayala, L. P., and J. E. Rocha. 2015. Insectos potencialmente comestibles del estado de Aguascalientes, México. Investigación y Ciencia 23: 19-25.

Bohart, R. M., and A. S. Menke. 1976. Sphecid Wasp of the World. A Generic Revision. University of California Press.

Borror, D. J., C. A. Triplehorn and N. F. Johnson. 1989. An introduction to the study of insects (No. Ed. 6). Saunders college publishing.

Brothers, D. J. 1999. Phylogeny and evolution of wasps, ants and bees (Hymenoptera, Chrysidoidea, Vespoidea and Apoidea). Zoologica Scripta, 28(1-2), 233-250.

Cancino, E. R., D. R. Kasparyan, J. M. C. Blanco, S. N. Myartseva, V. A. Trjapitzin, S. G. H. Aguilar, and J. G. Jiménez. 2015. Himenópteros de la Reserva "El Cielo", Tamaulipas, México. Dugesiana 17.

Coville, R. E. 1981. Biological observations on three *Trypoxylon* wasps in the subgenus *Trypargilum* from Costa Rica: T. *nitidum schultessi, T. saussurei and T. lactitarse* (Hymenoptera: Sphecidae). Pan-Pacific Entomol. 57: 332-340.

DiscoveryLife. 2018. https://www.discoverlife.org/.

Elgar, M. A., and M. Jebb. 2004. Nest provisioning in the mud-dauber wasp *Sceliphron laetum* (F. Smith): body mass and taxa specific prey selection. Behaviour 136(2): 147-159

Evans, H. E., and K. M. O'Neill. 2007. The Sand Wasps: Natural History and Behavior. Harvard University Press.

Fernández, C. 2002. Sinopsis de las Familias y Subfamilias de Hymenoptera en La región Neotropical. Red Iberoamericana de Biogeografía y Entomología Sistemática PrIBES 2: 239-243.

Fernánez, F. 2000. Sistemática y Filogenia de los Himenópteros de la Región Neotropical: Estado del Conocimiento y Perspectivas. *En* F. Martínes-Piera, J. J. Morrone, y A. Melic [eds.], Hacia un Proyecto Cyted para el Inventario y Estimación de la Diversidad Entomológica en Iberoamérica: PrIBES, Monografías Tercer Milenio 1: 211-231.

Finnamore, A. T., and C. Michener. 1993. Serie Spheciformes. *In* H. Goulet and J. T. Huber [eds.], Hymenoptera of the World: An Identification Guide to Families, Research Branch Agriculture Canada Publication 1894/E. Ottawa.

Gauld, I. D., and J. La Salle [eds.]. 1993. Hymenoptera and Biodiversity. CAB International.

Gayubo, S. F., and M. I. Izquierdo. 2006. Presencia de la especie invasora *Sceliphron curvatum* (F. Smith 1870) en la peninsula Iberica (Hymenoptera: Apoidea: Sphecidae).

GBIF (Global Biodiversity Information Facility). 2018. Listado de especies en México. https://www.gbif.org/es/occurrence/search?country=MX&taxon_key=4352&advanced=1. Consultado 29 Noviembre 2018.

Genaro, J. A. 1994. Inquilinos de *Sceliphron assimile*, con énfasis en *Podium fulvipes* (Hymenoptera: Vespidae, Sphecidae, Megachilidae). Caribbean J. Sci. 30: 268-270.

González, H. A. 2006. Generación del catálogo de autoridad taxonómica para Hymenoptera: aculeata no apiformes de la República Mexicana. Universidad Autónoma de Nuevo León Facultad de Ciencias Biológicas. Informe final SNIB-CONABIO proyecto DE019. México, DF.

González-Hernández, A., and J. M. Carpenter. 2011. Avispas con aguijón (Insecta: Hymenoptera: Aculeata).

Hanson, P. E., and A. S. Menke. 2006. Capitulo 17. Las avispas apoideas: Ampulicidae, Sphecidae, Crabronidae. Hymenoptera de la Región Neotropical//Memoirs of the American Entomological Institute, 77, 694-733.

Horta, V. J. V., O. N. P. Domínguez, L. L. Barrientos, and S. A Correa. 2007. Sphecidae y Crabronidae (Hymenoptera) de algunos municipios del centro y sur de Tamaulipas, México. Acta Zoológica Mexicana 23: 35-48.

Jiménez-Jiménez, M. L., and C. Palacios-Cardiel. 2008. Historia natural de la anidación de *Ammophila aberti* Haldeman (Hymenoptera: Sphecidae) en Baja California Sur, México. Acta Zoológica Mexicana 24: 227-230.

Lomholdt, O. 1982. On the origin of the bees (Hymenoptera: Apidae, Sphecidae). Insect Systematics & Evolution, 13(2), 185-190.

Melo, G. A. 1999. Phylogenetic relationships and classification of the major lineages of Apoidea (Hymenoptera), with emphasis on the crabronid wasps.

Menke, A. S., and F. Fernández. 1996. Claves ilustradas para las subfamilias, tribus y géneros de esfécidos neotropicales (Apoidea: Sphecidae). Inst. Nacional de Biodiversidad.

Menke, A. S. 1997. Family-group names in Sphecidae (Hymenoptera: Apoidea). Journal of Hymenoptera Research, 6(2), 243-255.

Morrone, J. J., and J. Márquez. 2008. Biodiversity of taxonóm terrestrial arthropods (Arachnida and Hexapoda): a biogeographical puzzle. Acta Zoológica Mexicana 24: 15-41.

Prentice, M. A. 1998. The Comparative Morphology and Phylogeny of Apoid Wasps (Hymenoptera: Apoidea). – Unpublished Dissertation Thesis. University of California, Berkeley.

Pulawski, W. J. 2007. Catalog of Sphecidae. Available on: http://www.calacademy.org/research/entomology/Entomology_Resources/Hymenoptera/sphecidae/Genera_and_s pecies_PDF/introduction.htm.

Pulawski, W. J. 2011. Sphecidae. http://research.calacademy.org/sites/research.calacademy.org/files/Departments/ent/sphecidae/Family_group_names_and_classification.pdf. Última actualización: Julio 2018.

Pulawski, W. J. 2018a. Family group names and classification. http://researcharchive.calacademy.org/research/entomology/entomology_resources/hymenoptera/sphecidae/family_group_names_and_classification.pdf.

Pulawski, W. J. 2018b. Bibliography of Sphecidae sensu lato (= Apoidea excluding Apidae sensu lato) (= Heterogynaidae, Ampulicidae, Sphecidae, and Crabronidae). http://researcharchive.calacademy.org/research/entomology/entomology_resources/hymenoptera/sphecidae/bibliography_a-j.pdf.

Pulawski, W. J. 2018c. Catalog of Sphecidae. http://www.calacademy.org/research/entomology/Entomology_Resources/Hymenoptera/sphecidae/Genera_and_species_PDF/introduction.htm.

Rau, P. 1942. The nesting habits of certain sphecid wasps of México, with notes on their parasites. Ann. Entomol. Soc. Am. 36: 647-653.

Ruiz, C. E., B. J. M. Coronado, S. E. Varela, and V. J. V. Horta. 2002. Sphecoidea, pp. 657-670. *En* B. J. Llorente and J. J. Morrone [eds.], Biodiversidad, Taxonomía y Biogeografía de Artrópodos de México. Vol. 3. Facultad de Ciencias de la U.N.A.M. México.

Ruíz, C. E., B. J. M. Coronado, and V. J. V. Horta. 2005. Conocimiento actual de las avispas depredadoras (Hymenoptera) en Tamaulipas, México, pp. 164-170. *In* L. Barrientes Lozano, A. Correa Sandoval, and J. García Jiménez. Biodiversidad Tamaulipeca. Vol 1. Instituto Tecnológico de Cd. Victoria, Ciudad Victoria, Tamaulipas, México.

Saldívar, L. D., H. J. C. Gutiérrez, and J. E. Rocha. 2001. Biodiversidad de himenópteros del estado de Aguascalientes, México. Investigación y Ciencia: de la Universidad Autónoma de Aguascalientes 24: 20-27.

Starr, C. K., A. Falcón-Brindis, and M. L. Jiménez. 2018. Brood success of the mud-daubing wasp Sceliphron jamaicense (Hymenoptera: Sphecidae) in a desert environment. Rev. Mex. Biodiversidad 89.

Vanoye-Eligio, M., V. M. Ramírez, R. Ayala, J. Navarro, and H. Delfin-González. 2014. Predatory Wasps (Hymenoptera) of the Yucatan Peninsula. Southwestern Entomologist, 39(3), 635-647.

Vanoye-Eligio, M., V. Meléndez-Ramírez, R. Ayala, J. Navarro-Alberto, and H. Delfín-González. 2015. Avispas depredadoras de áreas naturales protegidas del estado de Yucatán, México. Rev. Mex. Biodiversidad 86: 989-997.

Wesbauer, M. S. 1995. Pompilidae, pp. 522-539. Chapter 14. *En* P. E. Hanson and I. D. Gauld [eds.], The Hymenoptera of Costa Rica. Oxford Science.

Annex 1. List of Sphecidae Species in Mexico

Subfamily	Species	States where reported	References
AMMOPHILINAE	*Ammophila aberti* Haldeman, 1852	AGS, BC, BCS, COAH, CHIHDGO, NL, SIN, SON	Ruiz et al. 2002, Jiménez-Jiménez y Palacios-Cardiel 2008, Discoverylife 2018
	Ammophila aellos Menke, 1966	GRO, JAL, MOR	Ruiz et al. 2002, Discoverylife 2018
	Ammophila aucella Menke, 1966	GRO, JAL, MOR	Ruiz et al. 2002, Discoverylife 2018
	Ammophila azteca azteca Cameron, 1888	BC, NL	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Ammophila azteca clemente Menke 1967	BC	Ruiz et al. 2002
	Ammophila bella Menke, 1966	GRO, PUE, SLP, SIN	Ruiz et al. 2002, Discoverylife 2018
	Ammophila bellula Menke, 1964	México	Ruiz et al. 2002, Discoverylife 2018
	Ammophila boharti Menke, 1964	BC	Discoverylife 2018
	Ammophila breviceps Smith, 1856	TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018, GBIF 2018
	Ammophila centralis Cameron, 1888	TAM	Ruiz et al. 2002, Horta et al. 2007, Cancino et al. 2015, Discoverylife 2018
	Ammophila cleopatra Menke, 1964	COAH, DGO, NL, SIN, SON, TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018, GBIF 2018
	Ammophila cora Cameron, 1888	México	Discoverylife 2018
	Ammophila dejecta Cameron, 1888	SON	Ruiz et al. 2002, Discoverylife 2018
	Ammophila evansi Menke, 1964	México	Ruiz et al. 2002

Ammophila femurrubra Fox, 1894	BCS	Discoverylife 2018
Ammophila fernaldi (Murray, 1938)	México	Ruiz et al. 2002
Ammophila ferruginosa Cresson, 1865	CHIH, SON	Discoverylife 2018
Ammophila formicoides Menke, 1964	DGO, SON	Ruiz et al. 2002, Discoverylife 2018
Ammophila gaumeri Cameron, 1888	TAM, YUC	Ruiz et al. 2002, Cancino et al. 2015, Vanoye et al. 2015, Discoverylife 2018, GBIF 2018
Ammophila gracilis Lepeletier, 1845	México	GBIF 2018
Ammophila hermosa Menke, 1966	CHIH	Ruiz et al. 2002, Discoverylife 2018
Ammophila imitator Menke, 1966	SON	Ruiz et al. 2002
Ammophila juncea Cresson, 1865	CHIH, SON	Ruiz et al. 2002, Discoverylife 2018
Ammophila karenae Menke, 1964	BC	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
Ammophila macra Cresson, 1865	BC	Discoverylife 2018
Ammophila mcclayi Menke, 1964	BC	Discoverylife 2018
Ammophila mescalero Menke, 1966	AGS, CHIH, JAL, OAX, PUE, QRO, ZAC	Ruiz et al. 2002, Discoverylife 2018
Ammophila murrayi Menke, 1964	BC	Discoverylife 2018
Ammophila nancy Menke, 2007	BC, SON	Discoverylife 2018
Ammophila nicclayi Menke	B	Ruiz et al. 2002
Ammophila nigricans Dahlbom, 1843	México	GBIF 2018
Ammophila novita (Fernald, 1934)	México	Ruiz et al. 2002, Discoverylife 2018
Ammophila peckhami (Fernald, 1934)	México	Ruiz et al. 2002, Discoverylife 2018
Ammophila picipes Cameron, 1888	CHIH, TAM, YUC	Ruiz et al. 2002, Horta et al. 2007, Vanoye et al. 2015, Discoverylife 2018
Ammophila pictipennis Walsh, 1869	DF, GTO, QRO	Ruiz et al. 2002, Discoverylife 2018
Ammophila placida Smith, 1856	CHIH	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
Ammophila procera Dahlbom, 1843	AGS, CHIH, DGO, JAL, NL, TAM	Ruiz et al. 2002, Horta et al. 2007, Ayala y Rocha 2015, Discoverylife 2018, GBIF 2018

	Ammophila pruinosa Cresson, 1865	México	Ruiz et al. 2002, GBIF 2018
	Ammophila regina Menke, 1964	México	Ruiz et al. 2002
	Ammophila strenua Cresson, 1865	México	Ruiz et al. 2002, Discoverylife 2018
	Ammophila varipes Cresson, 1865	SON	Ruiz et al. 2002, Discoverylife 2018
	Ammophila wrightii (Cresson, 1868)	BC, BCS, SIN, SON	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Ammophila zanthoptera Cameron, 1888	México	Ruiz et al. 2002, Discoverylife 2018
	Eremnophila aureonotata (Cameron, 1888)	JAL, SIN, TAM, VER, YUC	Ruiz et al. 2002, Horta et al. 2007, Vanoye et al. 2015, Discoverylife 2018, GBIF 2018
	Eremnophila melanaria (Dahlbom, 1843)	TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
	Eremnophila opulenta (Guérin-Méneville, 1838)	México	Ruiz et al. 2002, Discoverylife 2018
	Podalonia argentifrons (Cresson, 1865)	México	Discoverylife 2018, GBIF 2018
	Podalonia argentipilis (Provancher, 1887)	SON	Discoverylife 2018
	Podalonia atriceps (Smith, 1856)	DF	Ruiz et al. 2002, Discoverylife 2018
	Podalonia communis atriceps (F. Smith, 1856)	México	Ruiz et al. 2002
	Podalonia communis communis (Cresson, 1865)	AGS	Saldívar et al. 2001, Ruiz et al. 2002
	Podalonia communis intermedia Murray, 1940	México	Ruiz et al. 2002, GBIF 2018
	Podalonia mexicana (Saussure, 1867)	México	Ruiz et al. 2002, Discoverylife 2018
	Podalonia mickeli Murray, 1940	SIN	Discoverylife 2018
	Podalonia montana (Cameron, 1888)	DGO	Ruiz et al. 2002, Discoverylife 2018
	Podalonia pubescens Murray, 1940	CHIH	Ruiz et al. 2002, Discoverylife 2018
	Podalonia robusta (Cresson, 1865)	México	Ruiz et al. 2002, Discoverylife 2018
	Podalonia sonorensis (Cameron, 1888)	SON	Ruiz et al. 2002, Discoverylife 2018
CHLORIONTINAE	*Chlorion aerarium* Patton, 1879	CHIH, NL, PUE, SIN, SON, TAM	Ruiz et al. 2002, Cancino et al. 2015, Discoverylife 2018, GBIF 2018
	Chlorion boharti Menke, 1961	BC, BCS, TAM	Ruiz et al. 2002, Discoverylife 2018
	Chlorion cyaneum Dahlbom, 1843	CHIH, DGO, TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018

	Chlorion harrisi Fernald (Rymal and Folkerts 1982)	TAM	Rau 1942
SCELIPHRINAE	*Chalybion californicum* (Saussure, 1867)	TAM	Ruiz et al. 2002, Cancino et al. 2015, GBIF 2018
	Chalybion zimmermani aztecum (Saussure, 1867)	COL, HGO, JAL, MOR, NAY, TAM, YUC	Ruiz et al. 2002,Horta et al. 2007, Vanoye et al. 2015
	Chalybion zimmermani peninsularum Bohart y Menke, 1963	BC	Ruiz et al. 2002
	Chalybion zimmermani zimmermani Dahlbom, 1843	HGO, TAM, VER	Rau 1942, Horta et al. 2007
	Dynatus crassipes (Cameron, 1913)	MOR, VER	Ruiz et al. 2002, Discoverylife 2018
	Penepodium albovillosum (Cameron, 1888)	México	Amarante 2002
	Penepodium foeniforme (Perty, 1833)	México	Ruiz et al. 2002, Discoverylife 2018
	Podium chalybaeum Kohl, 1902	México	Ruiz et al. 2002, Discoverylife 2018
	Podium denticulatum Smith, 1856	México	Ruiz et al. 2002, Discoverylife 2018
	Podium friesei Kohl, 1902	OAX	Ruiz et al. 2002, Discoverylife 2018
	Podium iridescens Kohl, 1902	OAX, TAM	Rau 1942, Ruiz et al. 2002, Discoverylife 2018
	Podium krombeini Bohart and Menke, 1963	MOR, OAX, PUE	Ruiz et al. 2002, Discoverylife 2018
	Podium luctuosum Smith, 1856	YUC	Vanoye et al. 2015, Discoverylife 2018
	Podium rufipes Fabricius, 1805	TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
	Sceliphron annulatum lucae (Saussure, 1867)	BC	Ruiz et al. 2002
	Sceliphron assimile (Dahlbom, 1843)	COAH, GRO, HGO, JAL, MICH, NAY, NL, OAX, SLP, SIN, TAM, VER, YUC	Rau 1942, Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018, GBIF 2018
	Sceliphron caementarium (Drury, 1773)	COAH, CHIH, HGO, JAL, MICH, NL, OAX, QRO, SLP, TAM, TLAX	Ruiz et al. 2002, Horta et al. 2007, Cancino et al. 2015, Discoverylife 2018, GBIF 2018
	Sceliphron fistularium (Dahlbom, 1843)	CAM, CHIAP, OAX, SIN, VER	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Sceliphron jamaicense (Fabricius, 1775)	BCS	Discoverylife 2018, Starr et al. 2018
	Trigonopsis cameronii (Kohl, 1902)	México	Ruiz et al. 2002, Discoverylife 2018

	Isodontia apicalis F. Smith, 1856	TAM	Horta et al. 2007
	Isodontia azteca (Saussure, 1867)	VER	Ruiz et al. 2002, Discoverylife 2018
	Isodontia cyanipennis Fabricius, 1793	México	GBIF 2018
	Isodontia elegans F. Smith, 1856	COAH, CHIH, JAL, SIN, TAM	Horta et al. 2007, GBIF 2018
	Isodontia fuscipennis (Fabricius, 1804)	TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
	Isodontia mexicana (Saussure, 1867)	TAM, VER	Ruiz et al. 2002, Horta et al. 2007, Cancino et al. 2015, Vanoye et al. 2015, Discoverylife 2018
	Isodontia philadelphica (Lepeletier, 1845)	México	Ruiz et al. 2002, Discoverylife 2018
	Palmodes dimidiatus (DeGeer, 1773)	COAH, CHIH, TAM	Ruiz et al. 2002, Horta et al. 2007
	Palmodes lissus Bohart y Menke	México	Ruiz et al. 2002, GBIF 2018
	Palmodes praestans (Kohl, 1890)	COAH	Ruiz et al. 2002, Discoverylife 2018
	Prionyx atratus (Lepeletier, 1845)	DGO	Ruiz et al. 2002, Discoverylife 2018
	Prionyx biforeolanis	México	GBI 2018
	Prionyx canadensis Provancher, 1887	TAM	Horta et al. 2007
SPHECINAE	*Prionyx fervens* (Linnaeus, 1758)	BCS, CHIH, NL	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Prionyx foxi Bohart and Menke, 1963	BC, COAH	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Prionyx parkeri Bohart y Menke, 1963	CHIH, NL, SON, TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
	Prionyx subatratus Bohart, 1958	CHIH	Ruiz et al. 2002, GBIF 2018
	Prionyx thomae (Fabricius, 1775)	BC, BCS, SON, TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
	Sphex ashmeadi (Fernald, 1906)	COAH, NL, TAM	Ruiz et al. 2002, Ruiz et al. 2005, Discoverylife 2018
	Sphex caliginosus Erichson, 1848	SON, VER	Discoverylife 2018, GBIF 2018
	Sphex dorsalis Lepeletier, 1845	JAL, NL, SIN	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Sphex flavitarsis (Fernald, 1906)	DGO	Discoverylife 2018
	Sphex flavovestitus flavovestitus F. Smith, 1856	DGO	Amarante 2002, Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
	Sphex flavovestitus saussurei (Fernald, 1906)	México	Amarante 2002

Sphex guatemalensis Cameron, 1888	México	Ruiz et al. 2002, Discoverylife 2018
Sphex habenus Say, 1832	NL, OAX, SIN, TAM	Ruiz et al. 2002, Cancino et al. 2015, Discoverylife 2018, GBIF 2018
Sphex ichneomoneus (Linnaeus, 1758)	NL, QROO, SIN, TAM	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018, GBIF 2018
Sphex lucae Saussure, 1867	CHOIH, DGO, HGO, MOR, NL	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018
Sphex maximiliani Kohl, 1890	México	Ruiz et al. 2002, Discoverylife 2018
Sphex melanopus Dahlbom, 1843	México	GBIF 2018
Sphex nigropilosus Rohwer, 1912	México	GBIF 2018
Sphex nitidiventris Spinola, 1851	México	Ruiz et al. 2002, Discoverylife 2018
Sphex opacus Dahlbom, 1845	México	Ruiz et al. 2002, Discoverylife 2018, GBIF 2018
Sphex peckhami Fernald, 1934	México	GBIF 2018
Sphex pensylvanicus Linnaeus, 1763	NL	Ruiz et al. 2002, Discoverylife 2018
Sphex servillei Lepeletier, 1845	México	Ruiz et al. 2002, Discoverylife 2018
Sphex tepanecus Saussure, 1867	CHIH, QRO	Ruiz et al. 2002, Horta et al. 2007, Discoverylife 2018, GBIF 2018
Sphex tinctipennis Cameron, 1888	México	Ruiz et al. 2002, Discoverylife 2018
Sphex willistoni Fernald, 1934	México	GBIF 2018

Digonogastra kimballi Kirkland[1] as a Parasitoid of *Diatraea lineolata* (Walker)[2]: New Record at Tabasco, México

Azalia Correa-Méndez[3], Rodolfo Osorio-Osorio[3]*, José Isaac Figueroa-de-la-Rosa[4], Luis Ulises Hernández-Hernández[3], Efraín de la Cruz-Lázaro[3], and Cesar Márquez-Quiroz[3]

Abstract. The Neotropical cornstalk borer, *Diatraea lineolata* (Walker) 1856 (Lepidoptera: Crambidae), is a pest of economic importance for cultivation of maize, *Zea mays* L., in Mexico. *Digonogastra kimballi* Kirkland 1989 (Hymenoptera: Braconidae) was identified as a gregarious ectoparasitoid of fifth-instar larvae of *D. lineolata*. Parasitism was low, 0.39 to 0.98% on cornstalk borers during three maize planting seasons. This is the first record of *D. kimballi* parasitizing *D. lineolata* larvae in maize at Macuspana, Tabasco, which broadens the distribution of the species in Mexico.

The Neotropical cornstalk borer, *D. lineolata* (Walker), is a major pest of maize, *Zea mays* L. (Dyar and Heinrich 1927, Peairs and Saunders 1980, Rodríguez-del-Bosque et al. 1988b, Osorio-Osorio et al. 2015). It also has been found in sugarcane, *Saccharum officinarum* L. (Bleszynski 1969), sorghum, *Sorghum bicolor* L. (Agnew et al. 1988), Johnsongrass, *Sorghum halepense* L., and other species of Poaceae (Rodríguez-del-Bosque et al. 1988a). Its distribution in the Neotropical region of the world ranges from Venezuela to Colombia, Ecuador, Central America, Caribbean Islands, Mexico, and southern Texas, USA (Dyar and Heindrich 1927, Bleszynski 1969). In Mexico, *D. lineolata* causes significant damage to maize crops at Jalisco (Pérez-Domínguez and Ireta-Moreno 2017), Tabasco (Osorio-Osorio et al. 2015), Tamaulipas (Rodríguez-del-Bosque et al. 1988b), and Veracruz (Aguirre 2007). In the State of Tabasco, 62.4% of stalks of a crop of 'Mejen' maize were bored, thereby warranting control (Osorio-Osorio et al. 2015). Chemical control of stalk borers of the genus *Diatraea* is problematic because of the cryptic feeding habits of the insect, use of alternate host plants throughout the year (De Freitas et al. 2007), and overlapping of generations of mature and immature insects during outbreaks of the pest.

Parasitoid wasps of the family Braconidae are the second-most abundant family of the order Hymenoptera (Quicke 2015). In Mexico, 704 species of Hymenoptera have been recorded, of which 19 species are found at Tabasco

[1]Hymenoptera: Braconidae.
[2]Lepidoptera: Crambidae.
[3]Universidad Juárez Autónoma de Tabasco. División Académica de Ciencias Agropecuarias. Km. 25 Villahermosa-Teapa road, Centro, Tabasco, México. correa.azalia@gmail.com; hluish@yahoo.com.mx; efrain.delacruz@ujat.mx; cesar_quiroz23@hotmail.com
[4]Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo, Km. 9.5 Morelia-Zinapécuaro road, Tarímbaro, Michoacán, México. figueroaji@yahoo.com.mx
*Corresponding author: rodolfo.osorioo@gmail.com

(Coronado-Blanco and Zaldívar-Riverón 2014). Species of *Digonogastra* Viereck, 1912 are solitary or gregarious idiobiont ectoparasitoids of concealed larvae of Coleoptera (Cerambycidae) and Lepidoptera (Crambidae, Psychidae, and Pyralidae) (Achterberg and Polaszek 1996). Most species of *Digonogastra* formerly were included in *Iphiaulax* Foerster or *Ipobracon* Dalla Torre (Wharton et al. 1997). Quicke (1988) restricted the definition of *Iphiaulax* to Old World species, resulting in transfer of New World species to *Digonogastra*, a name previously treated as a synonym of *Iphiaulax*. *D. kimballi* is a gregarious ectoparasitoid of *D. considerata* Heinrich, *D. grandiosella* Dyar, *D. lineolata*, *D. magnifactella* Dyar, and *D. saccharalis* (Fabricius) (Achterberg and Polaszek 1996)

During development of a study to evaluate damage by stalk borers in maize during the summer-autumn cropping season of 2014 (Osorio-Osorio et al. 2015), a species of ectoparasitoid attacking *D. lineolata* larvae was detected. Interest followed in identifying the parasitoid and evaluating natural parasitism of the pest in a Mejen maize crop at the Chinalito ejido municipality of Macuspana, Tabasco, Mexico (17º 45´12´´ N, 92º15´47´´ W, 11 m above sea level). Under rainfed agricultural conditions in the study area, Mejen maize was planted in a 1-ha area in autumn-winter 2016, spring-summer 2017, and summer-autumn 2018. In each planting season, parasitism by *D. kimballi* was studied by sampling on *D. lineolata* during peak abundance (Osorio-Osorio et al. 2015), which coincided with physiological maturity of the maize plants. Three sample periods were 85 to 93 days after maize was planted (21 January 2016, 26 August 2017, and 1 November 2018). On each sampling date, 500 maize plants were randomly selected and cut at the base. The stalks were opened longitudinally to extract larvae and pupae of *D. lineolata*, which were later placed individually into plastic containers. The larvae were maintained with diet proposed by Badilla-Fernández et al. (1994) to continue development until emergence of borer adults or parasitoids. The containers were kept at 28 ± 5ºC, 70 ± 5% relative humidity, and photoperiod of 12:12 light:dark hours in a breeding chamber. The identity of larvae of the Neotropical cornstalk borer was confirmed by studying the genitalia of adult males (Dyar and Heinrich 1927, Bleszynski 1969). The parasitoid was identified by keys by Wharton et al. (1997) and Wharton and Smith (1989).

D. kimballi was identified as a gregarious ectoparasitoid of fifth-instar larvae of *D. lineolata* (Fig. 1). According to Wharton and Smith (1989), adults of the species are distinguished from other species of *Digonogastra* by a combination of relatively smooth metasomal sculpture, length of the ovipositor (2.4-5.0 mm), black head, red mesonotum, dark propodeum with pale median streak, and completely red female metasoma. The sculpture of the face varies from almost smooth to punctated. The clypeal depression is bordered dorsally by a distinctive carina, and the dorsal margin of the clypeus clearly rises above the level of the face.

During any sampling period, parasitism of *D. lineolata* by *D. kimballi* was low. Of a total of 719 specimens of *D. lineolata*, only four parasitized larvae (all fifth ínstars) were found. One larva was collected on 21 January 2016 (autumn-winter 2016), another on 26 August 2017 (spring-summer 2017), and two more on 1 November 2018 (summer-autumn 2018). The percentages of parasitism by sampling date were 0.98, 0.39, and 0.54%, respectively. On average, 6.5 wasps emerged per larva, and the sex ratio (female:male) of *D. kimballi* was 1:1.2.

In Mexico, the parasitoid was recorded at Morelos (Jojutla, Miacatlán, Palo Bolero, San Miguel, Tetela, and Xochitepec), Guanajuato (Pénjamo), and Veracruz (Kirkland 1982, Wharton et al. 1989). At Veracruz, it was associated with *D.*

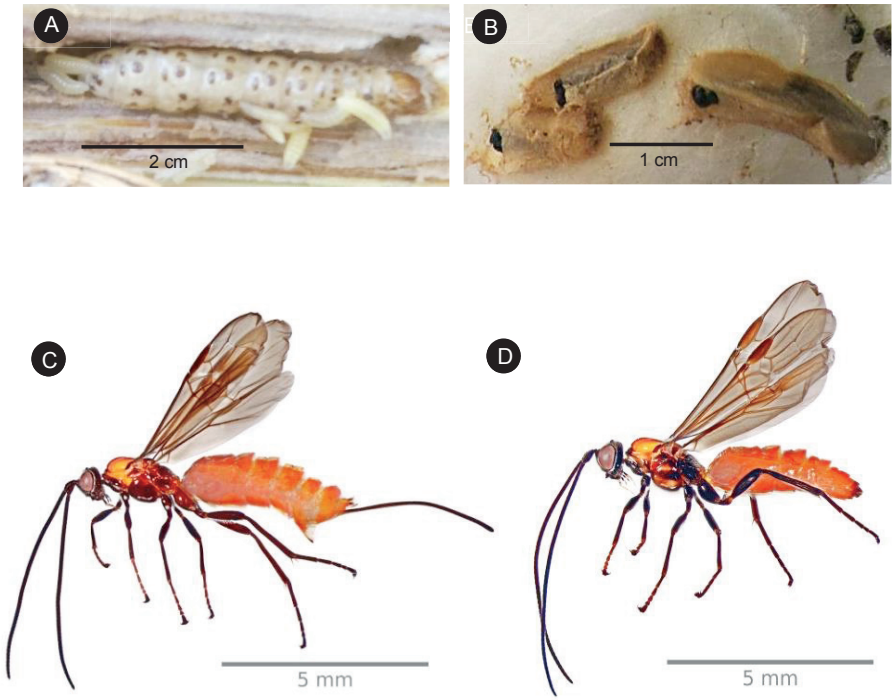

Fig. 1. *Digonogastra kimballi* larvae feeding on a *D. lineolata* larva (A), cocoons from emerged wasps (B), and female (C) and male (D) parasitoids.

(Poza Rica) associated with *D. grandiosella*, *D. lineolata*, and *D. sacharalis magnifactella*, at Jalisco (Autlán) and Nayarit (Tepic) with *Diatraea* sp., and at Sinaloa (Culiacán) with *D. considerata*).

This is the first record of *D. kimballi* parasitizing *D. lineolata* larvae in a maize crop at Macuspana, Tabasco, which broadens the distribution of the species in Mexico. Kirkland (1982) indicated that *D. kimballi* rarely exceeded 5% parasitism of stalk borer populations at sites he visited in Mexico (Jojutla, Miacatlan, Palo Bolero, San Miguel, Tetela, and Xochitepec [Morelos], Poza Rica [Veracruz], and Penjamo [Guanajuato]), which coincides with the current study. In a maize crop at Tamaulipas, *Digonogastra* sp. parasitized 0.9 and 0.6% of the *D. lineolata* and *D. saccharalis* stalk borers, respectively (Rodríguez-del Bosque et al. 1990). In contrast, Overholt and Smith (1990) mentioned that parasitism by *D. kimballi* on *D. grandiosella* was 11.3%. Under laboratory conditions, *D. kimballi* parasitized 60% of diapausing *D. lineolata* larvae (Rodríguez-del Bosque and Smith 1989).

In conclusion, few detailed studies recorded *D. kimballi* associated with stalk borers in Mexico. Natural presence of the parasitoid in seven states of Mexico, together with large percentages of parasitism recorded in the laboratory indicated the species of parasitoid is a promising agent for biological control of the Neotropical cornstalk borer.

Acknowledgment

The authors thank Jorge Valdez for the digitalization of the parasitoid, Bernardino Hernández Cardoza for his support in the sampling of the corn stalk borer and Mexico's National Science and Technology Council (CONACYT) for the master's degree scholarship granted to the first author.

References Cited

Achterberg, C. van, and A. Polaszek. 1996. The parasites of cereal stem borers (Lepidoptera: Cossidae, Crambidae, Noctuidae, Pyralidae) in Africa, belonging to the family Braconidae (Hymenoptera: Ichneumonoidea). Zool. Verh. 304.

Agnew, C. W., L. A. Rodríguez-del-Bosque, and J. W. Smith, Jr. 1988. Misidentifications of Mexican stalkborers in the subfamily Crambinae (Lepidoptera: Pyralidae). Folia Entomológica Mexicana 75: 63-75.

Aguirre, H. A. 2007. Pérdida del Rendimiento de Maíz por Daño del Barrenador Neotropical *Diatraea lineolata* (Walker) y *Fusarium* spp., en el Trópico. Tesis de Licenciatura, Saltillo, Coahuila, México.

Badilla, F. F., S. A. I. Solis, y S. D. Alfaro. 1994. Manual de Producción del Parasitoide *Cotesia flavipes* para el Control Biológico de los Taladradores de la Caña de Azúcar *Diatraea* spp. en Costa Rica. Dirección de Investigación y Extensión de la Caña de Azúcar (DIECA), Ed. Conmemorativa del 10 Aniversario del Programa de Entomología.

Bleszynski, S. 1969. The taxonomy of the Crambinae moth borers of sugar cane, pp. 11-41. *In* J. R. Williams, J. R. Metcalfe, R. W. Montgomery, and R. Mathes [eds.], Pest of Sugar Cane. Elsevier Publishing, Amsterdam.

Coronado-Blanco, J. M., y A. Zaldívar-Riverón. 2014. Biodiversidad de Braconidae (Hymenoptera: Ichneumonoidea) en México. Revista Mexicana de Biodiversidad, Supl. 85: 372-378.

De Freitas, M. Do. R. T., L.A. Da Silva, E. D. L, Mendonca, A. P.P., Da Fonseca, A. De L., Mendonca, S. De Santos, R. R. Do Nascimento, and A. E. G Santana. 2007. The biology of *Diatraea flavipennella* (Lepidoptera: Crambidae) reared under laboratory conditions. Fla. Entomol. 90: 309-313.

Dyar, H. C., and C. Heinrich. 1927. The American moths of the genus *Diatraea* and allies. Proceedings of the United States National Museum 71: 1-48.

Kirkland, R. L. 1982. Biology of *Iphiaulax kimballi* (Hym.: Braconidae), a parasite of *Diatraea grandiosella* (Lep.: Pyralidae). Entomophaga 27: 129-134.

Osorio-Osorio, R., V. Hernández-García, K. M. Harris, L. U. Hernández-Hernández, E., Cruz-Lázaro, C. Márquez-Quiroz, D. Mota-Sánchez, and U. L. A. Aguirre-Uribe. 2015. Species of stalk borers (Lepidoptera: Crambidae) and damage to maize in Southeastern México. Southwest. Entomol. 40: 831-835.

Overholt, W. A., and J. W. Smith. 1990. Comparative evaluation of three exotic insect parasites (Hymenoptera: Braconidae) against the southwestern corn borer (Lepidoptera: Pyralidae) in corn. Environ. Entomol. 19: 1156-1164.

Peairs, F., y J. Saunders. 1980. *Diatraea lineolata* y *D. saccharalis*: una revisión en relación con el maíz. Agronomía Costarricense 41: 123-135.

Pérez-Domínguez, J. F., y J. Ireta-Moreno. 2017. Daños causados por barrenadores del tallo y pudrición de tallo en maíz de la región Ciénega de Chapala, Jalisco. Entomología Agrícola 5: 428-434.

Quicke, D. L. J. 1988. *Digonogastra*: the correct name for Nearctic *Iphiaulax* of authors (Hymenoptera, Braconidae). P. Entomol. Soc. Wash. 90: 196-200.

Quicke, D. L. J. 2015. The Braconid and Ichneunonid Parasitoid Wasps: Biology, Sistematics, Evolution and Ecology. John Wiley and Sons, Oxford, UK.

Rodríguez-del-Bosque, L. A., and J. W. Smith. 1989. Parasitization of *Diatraea lineolata* pupae and diapausing larvae by several exotic parasites. Fla. Entomol. 72: 703-705.

Rodríguez-del-Bosque, L. A., J. W. Smith, Jr., and H. W. Browning. 1988a. Bibliography of the Neotropical cornstalk borer *Diatraea lineolata* (Lepidoptera: Pyralidae). Fla. Entomol. 71: 176-186.

Rodríguez-del-Bosque, L. A., J. W. Smith, Jr., and H. W. Browning. 1988b. Damage by stalkborers (Lepidoptera: Pyralidae) to corn in Northeastern Mexico. J. Econ. Entomol. 81: 1775-1780.

Rodríguez-del-Bosque, L. A., H. W. Browning, and J. W. Smith, Jr. 1990. Seasonal parasitism of cornstalk borers (Lepidoptera: Pyralidae) by indigenous and introduced parasites in Northeastern México. Environ. Entomol. 19: 393-402.

Wharton, R. A., and J. W. Smith, Jr. 1989. Two new species of *Digonogastra* Viereck (Hymenoptera: Braconidae) parasitic on Neotropical pyralid borers (Lepidoptera) in maize, sorghum and sugarcane. Bull. Entomol. Res. 79: 401-410.

Wharton, R. A., P. M. Marsh, and M. Sharkey. 1997. Manual of the New World Genera of the Family Braconidae (Hymenoptera). Special publication of The International Society of Hymenopterologist 1. Washington, DC.

Magnolia schiedeana[1] as a New Host Plant Record of Four Lepidoptera Species in a Tropical Montane Cloud Forest

Luis Alberto Lara-Pérez[2,3]*, Claudia Suárez-Ramírez[3], Iván Oros-Ortega[2], Antonio Andrade-Torres[3], Iván Córdova-Lara[4], and Luis Sáenz-Carbonell[4]

Abstract. Four Lepidoptera species that feed on leaves of *M. schiedeana* were recorded for the first time in tropical montane cloud forest at Veracruz, Mexico. Adult and immature stages of the species are illustrated, including months and occurrence and behavioural traits of larvae of *Janospyge jonas*.

Magnolia schiedeana Schlecht. (Magnoliaceae) is a rare and endemic Mexican species restricted to scattered populations in tropical montane cloud forest (Vázquez-García 1994, Cicuzza et al. 2007). The species is threatened because of deforestation, expansion of agriculture, and human population growth, low fruit production, and low natural establishment of seedlings (Dieringer and Espinosa 1994). As a result, *M. schiedeana* is on the Mexican list of protected species in NOM-059-ECOL (SEMARNAT 2010). Unfortunately, little information is available on Mexican arthropods associated with magnolias (Robinson et al. 2010). The only known insects associated with *M. schiedeana* are *Myrmecocephalus* (=*Stenagria*) sp. (Staphylinidae) and *Cyclocephala jalapensis* Casey (Scarabaeidae) that are specialist pollinators on the species and also use flowers and pollen as food (Dieringer and Delgado 1994, Dieringer and Espinosa 1994). As far as we know, there are no records of herbivory on the foliage of the species, probably because the *Magnolia* genus contains secondary metabolites such as magnolol and biphenyl ether that are toxic to several insect herbivores (Kelm and Nair 2000).

During fieldwork on September 2010, we observed larvae of two species feeding on *M. schiedeana* leaves in a fragment of tropical montane cloud forest in the Conservation Area at La Martinica, Banderilla, Veracruz, Mexico, at 1,580 m altitude (19°35' 12.11''/96°56'59.20'' W). During the next 7 months, we found larvae of two additional species. All larvae were reared to adults for identification. All except one species failed to reach the adult stage. The adults were identified as: *Papilio garamas abderus* (Hopffer 1856) (Papilionidae) (Fig. 1A), *Paradirphia hoegei* (Druce 1886) (Saturniidae: Hemileucinae) (Fig. 1B), and *Jonaspyge jonas* (Felder and Felder 1859) (Hesperiidae) (Fig. 1C). Because of the absence of one adult, the species of one larva could not be identified (Fig. 1D). It resembled the family Apatelodidae but

[1]Magnoliales: Magnoliaceae

[2]Instituto Tecnológico de la Zona Maya, Tecnológico Nacional de México. Carretera Chetumal-Escárcega km 21.5 Ejido Juan Sarabia, C.P. 97200 Quintana Roo, México.
[3]Instituto de Biotecnología y Ecología Aplicada, Universidad Veracruzana, Av. de las Culturas Veracruzanas No. 101, Campus para la Cultura, las Artes y el Deporte, col. Emiliano Zapata, C.P. 91090, Xalapa, Veracruz, México.
[4]Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, CICY. Calle 43 No. 130 Col. Chuburná de Hidalgo, C. P. 97200 Mérida.
*Corresponding author: ingluislara@gmail.com

Fig. 1. Immature stages and adults of Lepidoptera species that feed on *Magnolia schiedeana*. Column (A) *Papilio garamas abderus* third instar (bird dropping mimic), last instar, and adult. Column (B) *Paradirphia hoegei* last instar and adult. Column (C) *Jonaspyge jonas* first instar shelter, late instar, and adult. (D) Larva of Lepidoptera species not identified.

needs confirmation. The species was recorded only in September on seven trees with abundance ranging from five to 22 larvae on one tree of *M. schiedeana*. To the best of our knowledge, this is the first record of the four Lepidoptera species using *M. schiedeana* as a host plant, and this is the only documented case of herbivory on *M. schiedeana.*

Larvae of *P. garamas abderus* were recorded in September, October, January, February, and May 2011. The family Papilionidae has 570 species worldwide, with 48 species in Mexico (van Nieukerken et al. 2011, Llorente-Bousquets et al. 2014) and 39 species recorded from the state of Veracruz (Luis et al. 2011). The species *P. garamas abderus* is restricted to Mexico (Warren et al. 2013), and information is scarce on host plants of the species. Glasberg (2007) mentioned only that the species fed on a variety of host plants, but there is no additional information on plant species. Robinson et al. (2010) indicated host plants for 10 species of the family Papilionidae including eight species of the genus *Papilio* and two of *Protesilaus* that feed on *Magnolia* spp. Interestingly, interaction of the species *P. glaucus australis* with *Magnolia virginiana* resulted in an ecologically monophagous population adapted to their toxic compounds (Scriber 1986).

The species *P. hoegei* was observed only in April. Saturniidae with 3,454 described species is distributed worldwide (Kitching et al. 2018). In Mexico are 212 species with 112 taxa in the subfamily Hemileucinae (Beutelspacher-Baigts and Balcazar-Lara 1994). The subfamily has some of the most polyphagous species with as many as 84 species used as host plants by *Automeris zugana* Druce, 1886 (Janzen 2003). *Paradirphia hoegei* is distributed in southeastern Mexico and Guatemala (Beutelspacher-Baigts and Balcazar-Lara 1994, Wolfe 2009). The host plants recorded for the species included: *Platanus lindeniana* (Platanaceae), *Salix chilensis* (Salicaceae), *Prunus* sp. (Rosaceae), *Robinia pseudoacacia* (Fabaceae), and *Pyrus malus* (Rosaceae) (Stone 1991). The first author also raised several cohorts of the species on *Ficus* sp. (Moraceae), *Inga* sp. (Fabaceae), *Persea americana*, *P. schiedeana* (Lauraceae), and *Psidium guajava* (Myrtaceae). The immature stage is used as food by local people at Puebla State, Mexico (Ramos-Elorduy et al. 2011).

Larvae of *J. jonas* were found in October, February, and May. The family Hesperiidae has 4,113 species (van Nieukerken et al. 2011), of which only two (*Quadrus francesius* and *Dyscophellus* sp.) have been reported to feed on the Magnoliaceae family (Janzen and Hallwachs 2009, Robinson et al. 2010). *J. jonas* is distributed in eastern and southwestern Mexico, Guatemala, and Nicaragua (Warren et al. 2013). As far as we know, the ecology and biology of the species are unknown. This is the first record of the immature stage of *J. jonas* and its host plant. The last instar larva is reddish brown with strongly contrasting yellow rings and sparse long white hair on the body and on the black head (Fig 1C). Larvae of *J. jonas* are easy to identify on trees, because larvae of the first instar construct conspicuous circular shelters and silk shelters in a folded leaf by the last instar. The family Hesperiidae contains the greatest diversity of larval shelters by Lepidoptera (Greeney and Jones 2003). Understanding evolutionary patterns of shelter architecture is not well understood (Greeney 2009). The behavior of *J. jonas* larvae is similar to those of South American species *Pyrrhopyge papius* Hopffer, 1874 and *Elbella luteizona* (Mabille, 1877) (Greeney et al. 2010, Lepesqueur et al. 2017).

This is the first record of foliage herbivory on endemic *Magnolia* of Mexico and a new host plant record of *P. garamas abderus*, *P. hoegei*, and *J. jonas.* Further investigations of plant-insect interactions on a larger geographical scale of Mexican

magnolias are needed to understand ecological interactions for conservation of both Magnoliacae and arthropod species.

References Cited

Beutelspacher-Baigts, C. R., and M. Balcázar-Lara. 1994. Catálogo de la familia Saturniidae de México. Trop. Lepid. 5: Suppl. 1: 28.

Cicuzza, D., A. Newton, and S. Oldfield. 2007. The red list of Magnoliaceae. Fauna and Flora International, Cambridge.

Dieringer, G., and J. E. Espinosa. 1994. Reproductive ecology of *Magnolia schiedeana* (Magnoliaceae), a threatened cloud forest tree species in Veracruz, Mexico. Bull. Torrey Bot. Club 121: 154-159.

Dieringer, G., and L. Delgado. 1994. Notes on the biology of *Cyclocephala jalapensis* (Coleoptera: Scarabaeideae): an endemic of eastern Mexico. Southwest. Entomol. 19: 309-311.

Glassberg, J. 2007. A Swift Guide to the Butterflies of Mexico & Central America. Sunstreak, Morristown, NJ.

Greeney, H. F. 2009. A revised classification scheme for larval hesperiid shelters, with comments on shelter diversity in the Pyrginae. J. Res. Lepid. 41: 53-59.

Greeney, H. F., and M. T. Jones. 2003. Shelter building in the Hesperiidae: a classification scheme for larval shelters. J. Res. Lepid. 37: 27-36.

Greeney, H. F., T. R. Walla, J. Jahner, and R. Berger. 2010. Shelter building behavior of *Pyrrhopyge papius* (Lepidoptera: Hesperiidae) and the use of the Mayfield method for estimating survivorship of shelter-building Lepidopteran larvae. Zoologia 27: 867-872.

Janzen, D. H. 2003. How polyphagous are Costa Rican dry forest saturniid caterpillars? pp. 369-379. *In* Y. Basset, V. Novotny, S. E. Miller, and R. L. Kitching [eds.], Arthropods of Tropical Forests. Spatio-temporal Dynamics and Resource Use in the Canopy. Cambridge University Press, Cambridge, UK.

Janzen, D. H., and W. Hallwachs. 2009. Dynamic database for an inventory of the macrocaterpillar fauna, and its food plantsand parasitoids, of Area de Conservacion Guanacaste (ACG), northwestern Costa Rica. http://janzen.sas.upenn.edu. Accessed on 21 February 2019.

Kelm, M. A., and M. G. Nair. 2000. A brief summary of biologically active compounds from Magnolia spp. Stud. Nat. Prod. Chem. 24: 845-873.

Kitching, I. J., R. Rougerie, A. Zwick, C. A. Hamilton, R. A. St Laurent, S. Naumann, L. Ballesteros-Mejía, and A. Y. Kawahara. 2018. A global checklist of the Bombycoidea (Insecta: Lepidoptera). Biodivers. Data J. 6: e22236-13.

Lepesqueur, C., M. Neis, N. A. Silva, T. Pereira, H. P. A. Rodrigues, T. Trindade, and I. R. Diniz. 2017. *Elbella luteizona* (Mabille, 1877) (Lepidoptera, Hesperiidae: Pyrginae) in Brazilian Cerrado: larval morphology, diet, and shelter architecture. Rev. Bras. Entomol. 61: 282-289.

Llorente-Bousquets, J., I. Vargas-Fernández, A. Luis-Martínez, M. Trujano-Ortega, B. C. Hernández-Mejía, and A. D. Warren. 2014. Biodiversidad de Lepidoptera en México. Rev. Mex. Biodivers. 85: 353-371.

Luis, A. M., J. Llorente-Bousquets, I. F. Vargas, and F. Hernández-Baz. 2011. Mariposas diurnas Papilionoidea y Hesperioidea (Insecta: Lepidoptera), pp. 339-354. *In* A. A. Cruz, F. G. Lorea, V. O. Hernández, and J. E. M. Morales [eds.], La Biodiversidad de Veracruz. Estudio de Estado. Vol. II. Consejo Nacional para el Conocimiento y Uso de la Biodiversidad/Gobierno del Estado

de Veracruz/Universidad Veracruzana/ Instituto de Ecología, Xalapa, Veracruz.
Nieukerken, E., J. van, L. Kaila, I. J. Kitching, N. P. Kristensen, C. Lees, D. J. Minet, et al. 2011. Order Lepidoptera Linnaeus, 1758, pp. 212-221. *In* Z. Q. Zhang [ed.], Animal Biodiversity: an Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa.
Ramos-Elorduy, J., J. M. Moreno, A. I. Vázquez, I. Landero, H. Oliva-Rivera, and V. H. Camacho. 2011. Edible Lepidoptera in Mexico: geographic distribution, ethnicity, economic and nutritional importance for rural people. J. Ethnobiol. Ethnomed. 7: 1-22.
Robinson, G. S., P. R. Ackery, I. J. Kitching, G. W. Beccaloni, and L. M. Hernández. 2010. HOSTS – a database of the world's lepidopteran host plants. Retrieved 5 January 2019. http://www.nhm.ac.uk/researchcuration/projects/hostplants/index.html.
Scriber, J. M. 1986. Origins of the regional feeding abilities in the tiger swallowtail butterfly: ecological monophagy and the *Papilio glaucus australis* subspecies in Florida. Oecol. 71: 94-103.
SEMARNAT. 2010. Norma Oficial Mexicana NOM-059-ECOL-2010. Diario Oficial de la Federación, México.
Stone, S. F. 1991. Food plants of world Saturniidae. Memoirs J. Lepid. Soc. 4.
Vázquez-García, J. A. 1994. *Magnolia* (Magnoliaceae) in Mexico and Central America: a sinopsis. Brittonia 46: 1-23.
Warren, A. D., K. J. Davis, E. M. Stangeland, P. J. Pelham, and N. V. Grishin. 2013. Illustrated Lists of American Butterflies. http://www.butterfliesofamerica.com/
Wolfe, K. 2009. The Kirby Wolfe Saturniidae Collection. http://www.silkmoths.bizland.com/kirbywolfe.htm. Accessed on 6 January 2019.

First Report of *Trigona fuscipennis* and *T. nigerrima*[1] Chewing Tissues of *Jatropha curcas* L.[2] in Southern Chiapas, Mexico

Primer Reporte de *Trigona fuscipennis* y *T. nigerrima*[1] Mordiendo Tejidos de *Jatropha curcas* L.[2] en el Sur de Chiapas, México

Guillermo López-Guillén[3]*, Eduardo R. Chamé-Vázquez[4], Jaime Gómez Ruiz[4], and Juan F. Barrera[4]

Abstract. The social stingless bees of the genus *Trigona* (Hymenoptera: Apidae: Meliponini) are important floral visitors and pollinators. However, some *Trigona* species can cause damage when they chew the tissues of plants of agricultural importance to obtain resins and fibers, they use to build their nests. *Trigona fuscipennis* Friese and *T. nigerrima* (Cresson) are reported chewing different vegetative and reproductive parts of physic nut, *Jatropha curcas* L. (Euphorbiaceae), in southern Chiapas, Mexico.

Resumen. Las abejas sociales sin aguijón del género *Trigona* (Hymenoptera: Apidae: Meliponini), son importantes visitantes y polinizadores florales. Sin embargo, en ocasiones algunas especies de *Trigona* causan lesiones cuando muerden los tejidos de plantas de interés agrícola para obtener resinas y fibras que utilizan para construir sus nidos. En este trabajo se reporta a *Trigona fuscipennis* Friese and *T. nigerrima* (Cresson) mordiendo distintas partes vegetativas y reproductivas del piñón *Jatropha curcas* L. (Euphorbiaceae) en el sur de Chiapas, México.

The Meliponini tribe (Hymenoptera: Apidae) is a group of social stingless bees in tropical and subtropical areas of the world (Roubik 1989). In America are 391 species, and approximately 75% of them are geographically restricted to the Neotropical region (Michener 2000, Camargo and Pedro 2008). Stingless bees usually form permanent colonies of many individuals. The bees are important floral visitors and pollinators in tropical ecosystems (Kerr et al. 2001). Widely distributed on the American continent, *Trigona* is the genus of the Meliponini tribe with the most species (Michener 2000). In the Neotropical region, six subgenera of *Trigona* with at least 52 species are recognized; among them, more than half of the species described are *Trigona* (*Trigona* s. str.) (Fernández 2001).

Although stingless bees are important floral visitors and pollinators (Heard 1999, Kerr et al. 2001, Slaa et al. 2006), some species of *Trigona* are pests because

[1]Hymenoptera: Apidae
[2]Malpighiales: Euphorbiaceae
[3]Campo Experimental Rosario Izapa, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Tuxtla Chico, Chiapas, C. P. 30780, México.
[4]Grupo Académico Ecología de Artrópodos y Manejo de Plagas, Departamento de Agricultura, Sociedad y Ambiente, El Colegio de la Frontera Sur, Tapachula, Chiapas, CP 30700, México.
*Corresponding author e-mail: lopez.guillermo@inifap.gob.mx

they damage crops (Silva et al. 1997, Peruquetti et al. 2010, Nunes dos Santos et al. 2012). These species, use their mandibles to cut to soft and woody vegetative and floral tissue or fruit of agricultural crops to obtain resin and fiber for construction of their nests (Sacramento et al. 2007, Telesca Da Silveira et al. 2010). For example, some *Trigona* species cause considerable loss when they cut flowers of citrus (*Citrus* spp.) and blueberry (*Vaccinium ashei* J.M. Reade) crops (Wille 1965, Telesca Da Silveira et al. 2010), and leaves of *Macadamia integrifolia* Maiden & Betche and *M. tetraphylla* L.A.S. Johnson (Wille 1965). Recently, we observed damage by *Trigona* spp. in physic nut, *Jatropha curcas* L. (Euphorbiaceae), at southern Chiapas, Mexico. The objectives of this work were to identify *Trigona* species involved and describe damage to *J. curcas*.

Physic nut is a tree endemic to tropical America; its center of origin is in southeastern Mexico (Heller 1996, Pecina-Quintero et al. 2014). This multipurpose plant is an important bioenergy crop because of the quantity and quality of the oil in the seeds (Heller 1996, Li et al. 2017). Since the end of the 2001-2010 decade, plantations of *J. curcas* were established in Mexico (Martinez et al. 2010) to obtain oil for biodiesel production in refineries (Rodríguez-Acosta et al. 2010). Problems caused by phytophagous insects have been reported in the monoculture *J. curcas* plantations (e.g., Gómez-Ruiz et al. 2015, López-Guillén et al. 2018).

Trigona have been observed every year on *J. curcas* plants at the germplasm bank of Campo Experimental Rosario Izapa of the Instituto Nacional de Investigaciones, Forestales, Agrícolas y Pecuarias (INIFAP) at Tuxtla Chico, Chiapas, Mexico (14° 58'35" N; 92° 9'18" W; 314 m). Some stingless bee specimens were collected and mounted on entomological pins. They were identified using the taxonomic keys by Ayala (1999). The species were *Trigona fuscipennis* Friese and *Trigona nigerrima* (Cresson). Identified specimens were deposited in the ECO-TAP-E insect collection at El Colegio de la Frontera Sur at Tapachula, Chiapas (registration number SEMARNAT CHIS-INV-133-09-02).

Species of *Trigona* are characterized by a longitudinal elevation on the inner surface of the posterior tibia, which is covered with keirotrichia. The inner surface of the posterior basitarsus also has a more or less circular silky basal area and mandibles with four or five teeth. *T. fuscipennis* is distinguished by black mandibles with reddish brown distal margin and clypeus with setae shorter than the diameter of the scapus. *T. nigerrima* is larger than *T. fuscipenis*, and the setae in its clypeus are as long as the diameter of the scapus (Fig. 1a).

T. fuscipennis and *T. nigerrima* damaged *J. curcas* plants by chewing the bark of stems, branches, leaf petiole, leaf veins, fruit peduncles, and fruit pericarp (Fig. 1). No inflorescence damage was observed. The lesions on the plants secreted latex and healed after several days. Healed lesions were 1-5 mm wide, 2-19 mm long, and 1-2 mm deep. The fruit dropped when the peduncle was severely damaged. When fruit with a damaged peduncle did not fall, it was as many as 10 mm in diameter smaller than fruit without a damaged peduncle. The two species of *Trigona* alone or as many as five bees chewed the same plants throughout the productive cycle of *J. curcas*.

This is the first report of *T. fuscipennis* and *T. nigerrima* chewing tissue of *J. curcas* in Mexico and the world, respectively. Rasmussen et al. (2009) previously reported that *T. fuscipennis*, *T. amalthea* (Olivier), and *T. truculenta* Almeida in Peru caused damage like that in plantations of *J. curcas*. According to the literature, because species of the genus *Trigona* build their nests exposed to weather conditions, they require fiber and resin for construction and maintenance of the colony

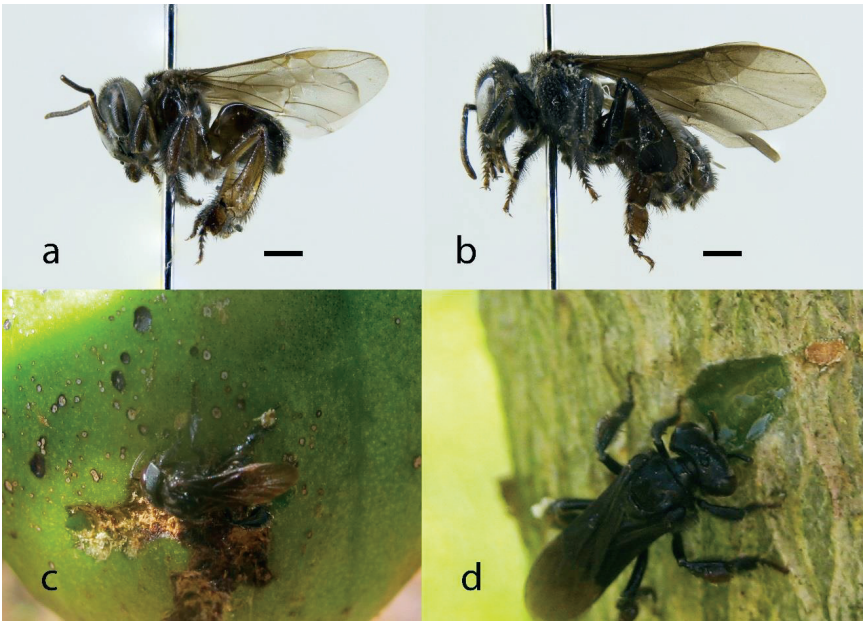

Fig. 1. Stingless bees *T. fuscipennis* (a) and *T. nigerrima* (b). *Trigona* sp. chewing on fruit (c) and branch of *J. curcas* (d).
Fig 1. Abeja sin aguijón *T. fuscipennis* (q) y *T. nigerrima* (b). *Trigona* sp. mordiendo fruto (c) y rama de *J. curcas* (d).

(Rasmussen and Camargo 2008, Rasmussen et al. 2009). Because stingless bees are important floral visitors and pollinators, nests of *T. fuscipennis* and *T. nigerrima* should be relocated to avoid serious damage to commercial plantations of *J. curcas*.

Acknowledgment

We thank the Instituto Nacional de Investigaciones, Forestales, Agrícolas y Pecuarias (INIFAP) for funding SIGI project number 1021634081.

References Cited

Ayala, R. 1999. Revisión de las abejas sin aguijón de México (Hymenoptera: Apidae: Meliponini). Fol. Entomol. Mex. 106: 1-123.

Camargo, J. M. F., and S. R. M. Pedro. 2008. Meliponini Lepeletier, 1836, pp. 272-578. *In* J. S. Moure, D. Urban, and G. A. R. Melo [eds.], Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical region. www.moure.cria.org.br/catalogue.

Fernández, C. F. 2001. Checklist of genera and subgenera of *Aculeate* Hymenoptera of the Neotropical region (Hymenoptera: Vespomorpha). Biota Colomb. 2: 87-130.

Gómez-Ruiz, J., G. López-Guillén, J. F. Barrera, A. M. Solis, and A. Zamarripa-Colmenero. 2015. First record of *Ectomyelois muriscis* (Lepidoptera: Pyralidae) on physic nut (*Jatropha curcas*), a biofuel plant. Biomass Bioenerg. 75: 150-154.

Heard, T. A. 1999. The role of stingless bees in crop pollination. Annu. Rev. Entomol. 44: 183-206.

Heller, J. 1996. Physic nut (*Jatropha curcas* L.): promoting the conservation and use of underutilized and neglected crops. Rome: Institute of Plant Genetics and Crop Plant Research. https://www.bioversityinternational.org/fileadmin/_migrated/uploads/tx_news/Physic_nut__Jatropha_curcas_L._161.pdf.

Kerr, W. E., G. A. Carvalho, A. Coletto da Silva, and M. G. P. Assis. 2001. Aspectos pouco mencionados da biodiversidade amazônica. Parcerias Estratégicas 12: 1-22.

Li, H., S. Tsuchimoto, K. Harada, M. Yamasaki, H. Sakai, N. Wada, A. Alipour, T. Sasai, A. Tsunekawa, H. Tsujimoto, T. Ando, H. Tomemori, S. Sato, H. Hirakawa, V. P. Quintero, A. Zamarripa, P. Santos, A. Hegazy, A. M. Ali, and K. Fuku. 2017. Genetic tracing of *Jatropha curcas* L. from its Mesoamerican origin to the world. Front. Plant Sci. 8: 1539.

López-Guillén, G., F. Hernández-Baz, J. Gómez Ruiz, and Juan F. Barrera. 2018. *Jatropha curcas* L., a new host plant of *Lycomorpha pholus* (Drury) in Southeastern Chiapas, Mexico. Southwest. Entomol. 43: 1051-1054.

Martinez, H. J., A. L. Martinez Ayala, H. Makkar, G. Francis, and K. Becker. 2010. Agroclimatic conditions, chemical and nutritional characterization of different provenances of *Jatropha curcas* L. from Mexico. Eur. J. Sci. Res. 39: 396-407.

Michener, C. D. 2000. Bees of the World. Johns Hopkins University Press.

Nunes dos Santos, A. J., M. Forti Broglio, N. da Silva Dias-Pini, L. Alves de Souza, and T. J. Araújo Barbosa. 2012. Stingless bees damage broccoli inflorescences when collecting fibers for nest building. Sci. Agric. 69: 281-283.

Pecina-Quintero, V., J. L. Anaya-López, A. Zamarripa-Colmenero, C. A. Núñez-Colín, N. Montes-García, J. L. Solís-Bonilla, and M. F. Jiménez-Becerril. 2014. Genetic structure of *Jatropha curcas* L. in Mexico and probable centre of origin. Biomass Bioenerg. 60: 147-155.

Peruquetti, R.C., L. S. M. Da Costa, V. S. Da Silva, and P. M. Drumond. 2010. Frugivory by a stingless bee (Hymenoptera: Apidae). Neotrop. Entomol. 39: 1051-1052.

Rasmussen, C., and J. M. F. Camargo. 2008. A molecular phylogeny and the evolution of nest architecture and behavior in *Trigona* s.s. (Hymenoptera: Apidae: Meliponini). Apidologie 39: 102-118.

Rasmussen, C., P. Orihuela-Pasquel, and V. H. Sánchez-Bocanegra. 2009. *Trigona* Jurine, 1807 bees (Hymenoptera: Apidae) as pests of physic nut (Euphorbiaceae: *Jatropha curcas*) in Peru. Entomotropica 24: 31-34.

Rodríguez-Acosta, M., J. Sandoval-Ramírez, and R. Zeferino-Díaz. 2010. Extraction and characterization of oils from three Mexican *Jatropha* species. J. Mex. Chem. Soc. 54: 88-91.

Roubik, D. W. 1989. Ecology and natural history of tropical bees. Cambridge University Press, New York.

Sacramento, C. K., E. Coelho Jr., J. E. Urano De Carvalho, C. H. Müller, and W. M. Oliveira Do Nascimento. 2007. Cultivo do mangostão no Brasil. Rev. Bras. Frutic., Jaboticabal-SP 29: 195-203.

Silva, M. M., C. H. Buckner, M. Picanço, and C. D. Cruz. 1997. Influência de *Trigona spinipes* Fabr. (Hymenoptera: Apidae) na polinização do maracujazeiro-amarelo. An. Soc. Entomol. Bras. 26: 217-221.

Slaa, E. J., L. A. Sánchez Chaves, K. S. Malagodi-Braga, and F. E. Hofstede. 2006. Stingless bees in applied pollination: practice and perspectives. Apidologie 37: 293-315.

Telesca Da Silveira, T. M., M. C. Bassols Raseira, D. Edson Nava, and M. Couto. 2010. Influência do dano da abelha-irapuá em flores de mirtileiro sobre a frutificação efetiva e as frutas produzidas. Rev. Bras. Frutic., Jaboticabal-SP 32: 303-307.

Wille, A. 1965. Las abejas atarrá de la región mesoamericana del género y subgénero *Trigona* (Apidae-Meliponini). Rev. Biol. Trop. 13: 271-291.

Comparison of Microscope Slide-Mounting Media for Preparation of Fungus Gnats (Diptera: Mycetophilidae)

Stephen W. Taber

Biology Department, Saginaw Valley State University, 7400 Bay Road,
University Center, MI 48710

Abstract. Nine microscope slide-mounting media were compared to determine which types were best, worst, and of intermediate value for the preparation of fungus gnats for microscope examination. The best were Euparal, Carolina Canada balsam, and fingernail polish. The worst were Eukitt and PVA. Those of intermediate value were Benz synthetic Canada balsam, Rublev Canada balsam, and Kleermount. One slide was made using Hoyer's slide-mounting medium.

Introduction

While making microscope slides of fungus gnat specimens during a search for new species, and while examining slides previously prepared by others, I wondered which type of mounting medium was best. For example, some specimens had shriveled parts after preparation. The most susceptible parts are antennae and legs. Age of preparation also affects the quality of slide-mounted material. Some media are easier and/or faster to use than others. Some media are safer than others. I am unaware of any previously published articles that compare a spectrum of slide-mounting media for the preparation of animals. A recent book that evaluates media for insect slides is valuable (Chick 2016) but understandably does not make the kind of detailed comparisons offered here.

Materials and Methods

The nine mounting media compared herein are: Canada balsam (Carolina Biological Supply, Burlington, NC), Rublev Canada balsam (Natural Pigments, Willits, CA), synthetic Canada balsam (Benz Microscopic Optics Center, Ann Arbor, MI), Kleermount (Carolina Biological Supply), Eukitt (Electron Microscopy Sciences, Hatfield, PA), Euparal (BioQuip Products, Rancho Dominguez, CA), polyvinyl alcohol (PVA) (BioQuip), and fingernail polish (Maybelline longwearing clear, Maybelline, New York, NY). Near the conclusion of comparisons, Euparal was discontinued by BioQuip but remained available from Agar Scientific (Stansted, England). A few slides were made using Euparal from that vendor. One slide using Hoyer's Slide Mounting Medium (Hempstead Halide, League City, TX) was prepared 1 year after the others. Advice and procedures for mounting insects on microscope slides are available elsewhere (Oldroyd 1958, Brown 1997, Triplehorn and Johnson 2005, Gibb and Oseto 2006, Chick 2016).

Qualities assessed were clarity; hardness; presence or absence of bubbles; the effect of the preparation on antennae, legs, and other body parts; ease and safety of preparation; and crazing (production of a fine network of cracks on a surface) and cracking on a larger scale.

Most slides were made in April 2016. At the time of comparisons, the preparations were 2.5 years old. Most flies were species of *Boletina* Staeger, but some were *Docosia* Winnertz, a few were other mycetophilid genera, and some were chironomids. Most specimens were males because males are used in the search for new species. Some females were prepared to see if any difference between sexes regarding abdominal contents such as egg material would influence the quality of slides. No problems were encountered with female results. Some slides were made using insects of other orders.

All specimens were frozen after collection from 6-m Malaise traps (BioQuip) in the field. Upon thawing, the specimens were macerated in KOH solution (two pellets per 25 ml H_2O) overnight to clear dark exoskeletons and spread male terminalia. They were soaked in H_2O (at least a half hour but this should be increased to overnight to reduce the excessive clearing noticed in some mounted specimens). The treatment following maceration in water depended on the mounting medium used. Round cover slips were used because the presence of corners might cause irregularities to form under the slip. All slides were stored horizontally in cardboard slide folders. A total of 204 slides was prepared. Photographs shown here were taken with a stereoscopic compound microscope (Olympus SZ40 Zoom, Tokyo, Japan).

Media Procedures. Carolina balsam [24 slides], Rublev Canada balsam [10 slides], Benz synthetic Canada balsam [11 slides], Kleermount [12 slides], and Eukitt [52 slides]: After the KOH and water treatments, specimens were immersed in increasing concentrations of isopropyl alcohol. Starting with 50% and through approximately 80% (both for 30 minutes or so) and terminating with 100% (overnight). A soak in xylene, usually overnight, followed the isopropyl treatments. Mounting medium and a cover slip completed the preparation. Variations were tried – less time in water, more or less time in isopropyl, more or less time in xylene. No differences were noticed, but it is best to use longer immersion times. Leaving medium on the specimen overnight before adding one or two additional drops just before placing the coverslip helped complete the seal and smooth the preparation.

Euparal [38 slides]: As for the balsam treatments except xylene was not used. After 100% isopropyl, the specimen was covered with Euparal.

PVA [46 slides]: As for the Euparal treatments except sometimes the alcohol step was omitted and PVA was added immediately after a second immersion in water (final rinse). Similar results were obtained both ways. PVA slides were ringed with Japan gold size varnish (Winsor and Newton, London, England) to minimize evaporation of water from under the cover slip.

Fingernail polish [10 slides]: Treatment like that for PVA except alcohol dehydrations were always used after immersion in water. After immersion in 100% isopropyl and addition of fingernail polish, a cover slip was added with no further processing.

Hoyer's Slide Mounting Medium [1 slide]: After soaking overnight in KOH and 1 hour of immersion in water, the specimen was stored for 3.5 months in 80% ethanol (but not for this purpose). After application of medium and a square cover slip, the slide was warmed for 2 days at 40°C in an incubator. The cover slip was

ringed with Japan gold size varnish. Because Hoyer's medium was purchased late in the study, a single slide was prepared.

Depending upon the medium used, immersion in water can be followed by alcohol and xylene (the balsams, Kleermount, Eukitt), or by alcohol without xylene (Euparal, PVA, fingernail polish), or directly into the medium from water (PVA).

Results

Canada balsam (Carolina) (Fig. 1): specimen morphology fine, medium color slightly yellowed but nearly clear, medium rather firm but light touch of teasing needle on medium just outside the cover slip left a small indentation.

Fig. 1. Fungus gnat mounted with Carolina Canada balsam.

Canada balsam (Rublev) (Fig. 2): color like Carolina balsam but a little clearer, medium not as firm – similar pressure with teasing needle evoked the appearance of liquid water moving under ice when one steps upon newly frozen ice. Some specimens had shrunken, flattened antennomeres, terminal tarsomeres and even some of the larger, more basal leg segments; occasionally the abdomen was flattened. Male terminalia were fine.

Benz synthetic balsam (Fig. 3): firmness like the other balsams, sometimes parts cleared beyond the level seen when they were mounted, color slightly yellowed, sometimes slight cloudiness. Two slides had a large air bubble. Morphology of specimens fine.

Fig. 2. Fungus gnat mounted with Rublev balsam.

Fig. 3. Fungus gnat mounted with Benz synthetic balsam.

Kleermount (Fig. 4): results as for Carolina balsam but terminalia especially were slightly bleached (cleared). Small wasp looked good.

Eukitt (Fig. 5): medium clear, very firm (product selling point), air bubbles more common than in other media except fingernail polish (inside the flies and in the surrounding medium), specimen morphology sometimes shrunken and/or shriveled, the medium with a unique problem – many specimens had a whitish, frosty appearance from unknown cause.

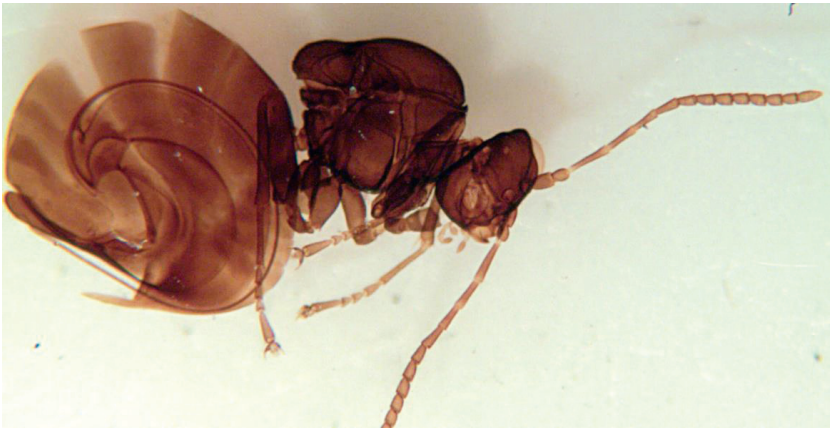

Fig. 4. Wasp mounted with Kleermount.

Fig. 5. Fungus gnat mounted with Eukitt.

Euparal (Fig. 6): Medium clear, fairly firm but slightly softer than balsam, antennae and legs good, often paler than when mounted so clearing continued after preparation, some specimens had shrunken, flattened antennae and leg segments and air bubbles inside the segments (rarely inside flagellomeres), one small coleopteran had excellent antennae, bubbles not present in medium, a bibionid fly was mounted directly from water into Euparal without clearing – leg segments were excellent, juvenile phasmid with long, thin legs and antennae showed how susceptible such structures can be to shrinking and flattening,

Fig. 6. Fungus gnat mounted with Euparal.

PVA (Fig. 7): medium not as firm as the others, mouthparts, antennae and terminal leg segments (even basal ones), abdomen, terminalia sometimes flattened/ shrunken, medium clear, lace bug looked good all around, one specimen had three air bubbles at edge of cover slip, tendency for parts of specimens to move beyond the cover slip.

Fingernail polish (Fig. 8): Very clear medium, harder than the others with the possible exception of Eukitt, antennae, legs, terminalia fine, the biggest difference was the presence of huge and sometimes numerous air bubbles (occasionally wider than the body of the animal) at the periphery of the medium (pressing against cover slip with part of the bubble protruding beyond the slip), bubbles often crowd against body parts but the bubbles seldom interfered with the observation of details (on one specimen much of the antennae were occluded by overlying bubbles and inside another specimen air entered and affected the appearance of the thorax). Four slides with four bubbles, one with five, one with six, and two with 11.

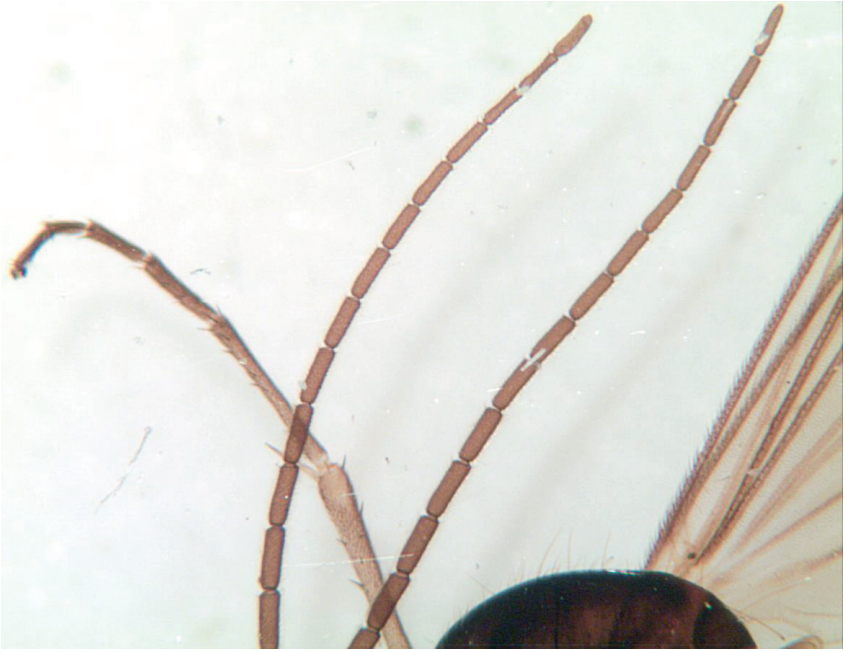

Fig. 7. Fungus gnat mounted with PVA.

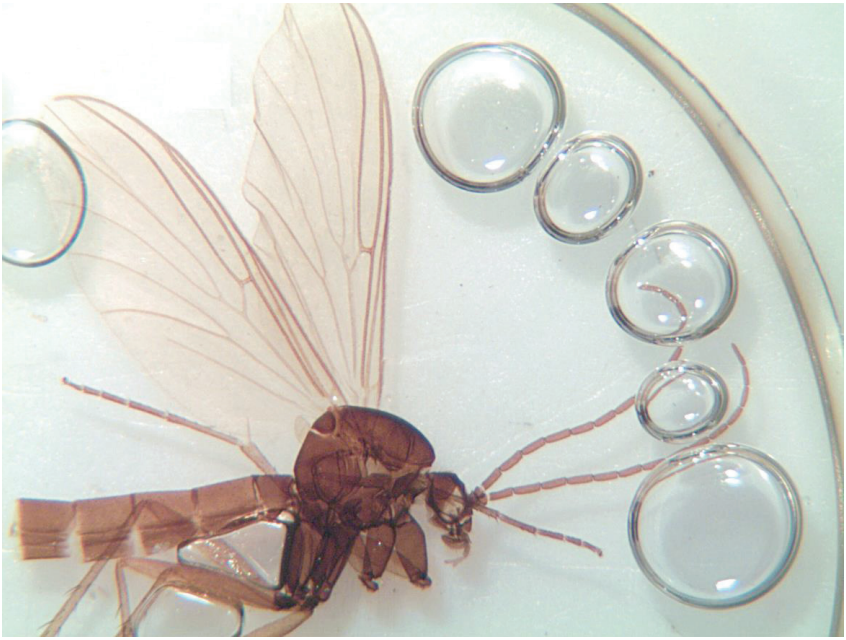

Fig. 8. Fungus gnat mounted with fingernail polish.

Hoyer's medium (Fig. 9): The medium was clear, there were no bubbles, the male terminalia looked satisfactory. Hoyer's is watery so attempts to check its firmness at the edge of the cover slip tested only the ringing substance (Japan gold size) instead. The specimen was poorly chosen for comparison because it lacked antennae and legs. It was flatter than specimens in the other media. It is best to reserve judgement on Hoyer's medium until more and better material is tested.

Two slides of each medium (except Hoyer's) were transferred from room temperature (24°C) to 35°C in an incubator for 4 months starting 5 months after preparations were made before returning to room temperature. Appendages looked as good or better than on slides kept at the cooler temperature for the entire 2.5 years. Media were mostly firmer after treatment at the warmer temperature and neither Eukitt slide showed the white, frosty damage common among slides kept at room temperature. Warmer temperature did not remove the bubbles from fingernail polish slides. The medium probably already was hard before the slides were placed in the incubator.

Crazing or cracking was not a problem for any medium. Bubbles disappeared by the time of comparison except in Eukitt where bubbles were common, in fingernail polish where bubbles were the rule, and in rare cases among the other media.

Fig. 9. Fungus gnat mounted with Hoyer's medium.

The best slides were made with Euparal, Carolina Canada balsam, and fingernail polish. Inferior results were obtained with Eukitt and PVA. Intermediate quality results were obtained with Benz synthetic Canada balsam, Rublev Canada balsam, and Kleermount. Carolina Canada balsam and the other balsams are known for slightly yellow or amber tint but when illuminated for observation with a microscope, the tint was hardly noticed. Specimens mounted in Kleermount and Benz synthetic balsam were more bleached or cleared than material of the other

balsam types. Advantages of Euparal include clarity and avoidance of xylene during preparation, but Euparal is difficult to find. Advantages of fingernail polish include clarity, ease of preparation, and low cost.

Discussion

Results could be improved by retaining material in water and alcohol for longer periods of time. In the study, rinsing in water and dehydration with alcohol often lasted only hours or even minutes. Perhaps overnight in each condition is ideal. Clearing overnight with KOH always produced satisfactory results. To minimize additional, unwanted clearing, glacial acetic acid can be added (Chick 2016). Glacial acetic acid was never used in the study. Other clearing agents such as clove oil might be as good or better for clearing. Two specimens stored for several months in Eukitt at warmer temperature were better than the many specimens stored at room temperature for the entire 2.5 years. But, two is a small sample size. Effect of temperature on all nine media needs further evaluation.

Acknowledgment

The research was supported by a Ruth and Ted Braun Fellowship and by the Earl L. Warrick Award for Excellence in Research at Saginaw Valley State University.

References Cited

Brown, P. A. 1997. A review of techniques used in the preparation, curation and conservation of microscope slides at the Natural History Museum, London. The Biology Curator 10 Supplement 1-4.

Chick, A. 2016. Insect Microscopy. The Crowood Press, Ramsbury, England.

Gibb, T. J., and C. Y. Oseto. 2006. Arthropod Collection and Identification. Academic Press, New York, NY.

Oldroyd, H. 1958. Collecting, Preserving and Studying Insects. Macmillan Company, New York, NY.

Triplehorn, C. A., and N. F. Johnson. 2005. Borror and DeLong's Introduction to the Study of Insects, 7th ed. Thomson Brooks/Cole, Belmont, CA.

Méndez-Herrera Technique: New Clearing Technique Proposed for Immature Stages and Internal Structures of Adult Insects

N. Acevedo-Reyes, D. H. Zetina, E. Blanco-Rodríguez*, J. A. López-Buenfil, and R. Martínez-Rosas

Centro Nacional de Referencia Fitosanitaria (CNRF), Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria (SENASICA), Dirección General de Sanidad Vegetal (DGSV), Km 37.5 de la Carretera Federal México-Pachuca, Tecámac, Estado de México, C. P. 55740, México

Abstract. Insects must be preserved before identification. The objective of this work was to propose a fast and effective clearing technique for immature stages, mostly larvae and internal adult structures, for permanent mounting in lamellae, thus reducing time. The technique was modified from the conventional dehydration process. The technique reduced the times for clearing larvae and internal structures of insects without need for dehydration of alcohols that can require 3 or more hours of time.

Resumen. Los insectos deben pasar por un tratamiento de preservación para su identificación. El objetivo de este trabajo es proponer una técnica rápida y efectiva en etapas inmaduras, principalmente larvas y estructuras internas de adultos y el montaje permanente en laminillas, reduciendo así el tiempo. La técnica se modifica del proceso de deshidratación convencional. Esta técnica permite reducir los tiempos de limpieza de larvas y estructuras internas de insectos, sin necesidad de pasar por el proceso de la deshidratación de alcoholes que pueden tardar hasta tres horas o más tiempo.

Introduction

Insects, with more than 1 million species identified in the world (Zhang 2015), are an important part of biological diversity (Morrone et al. 1999). Some species are of economic, ecological, medical-veterinary, and taxonomic importance (Wilson 1992). Márquez-Luna (2005) mentioned that insects must be preserved to be studied for identification. Preservation of insects implies permanent, semi-permanent, or temporal preparations, the first being most common. Gaviño et al. (1977) and Aguilar-Morales et al. (1996) indicated that a small puncture with a pin or very fine dissecting needle in the ventral region of the abdomen of the organism was required for permanent preparation. The insect is put into a test tube with 10% potassium hydroxide for clearing and heated little by little. When the exoskeleton is cleared, it can be stained with dye such as gentian violet for 5 minutes, if necessary.

*Corresponding author: Colegio de Postgraduados, Campus Montecillo. Km 36.5, Carretera México-Texcoco, C.P. 56230, Texcoco, Estado de México; e-mail: eblancor23@hotmail.com

After that, it is dehydrated with 30, 50, 60, and 70% ethyl alcohol and absolute alcohol. The time the organism must remain in each kind alcohol is 1 minute, with excess drained between each change. Other groups of insects do not need to be stained, although all are based on established and well-known steps in classical literature. Palma (1978) suggested 10 steps for clearing and mounting insects (Mallophaga and Diplura), but a long time is required for dehydration.

Wirth and Marston (1968) used potassium hydroxide and alcohol for dehydration with a traditional method with Canada balsam for mounting insects in lamellae, although it sometimes has been unsatisfactory because of shrinkage of delicate structures such as antennae and palps. Carter et al. (1920) and Lutz (1922) called attention to times of dehydration and clearing, but proposed rinsing in carbolic acid (phenol) for mounting specimens of the families Ceratopogonidae and Chironomidae, and adapted for Cecidomyiidae. Carter et al. (1920) and Lutz (1922) argued that the advantage of the technique was the elasticity of chitin because it was easy to dissect, hairs and scales did not come off easily, and wings did not need to be treated separately but could be kept indefinitely in storage. Ronderos et al. (2000) provided modifications or proposed new techniques for insects.

The objective of this work was to propose a fast and effective clearing technique for immature stages, mostly larvae, and internal adult structures, for permanent mounting in lamellae, thus reducing time (Table 1).

Table 1. Differences between Technique Méndez-Herrera and other Techniques

Parameter	Technique Méndez-Herrera	Other techniques
Times for dehydration	Short	Long
Shrinkage of structures	No present	Present
Clarification	KOH 40% (5-7 minutes)	-Phenol -Lactophenol (20 minutes) -10% KOH (3-5 minutes) - Glacial acetic acid (2-3 minutes)
Distilled water	3-4 washes (3-4 minutes)	5 or more washes (5-10 minutes)
Ethyl alcohol (96%)	All taxa and internal adult structures (2 minutes)	30, 50, 70, 80, 95, 96, 100 Aphids (1-2 minutes) Mealybugs (3-4 minutes)
Clove oil	All taxa and internal adult structures (3-5 minutes)	Aphids (10-20 minutes) Mealybugs (20 minutes)
Canada balsam	Present	Present
Hoyer liquid	Absent	Dermestidae

The technique was modified to reduce time from the conventional dehydration process. The modification was attributed to Mr. Méndez-Herrera (retired employee of Centro Nacional de Referencia Fitosanitaria, Department of Entomology and Acarology) who developed tests and adjustments for reduced time for dehydration. For this reason, it was named Technique Méndez-Herrera. This technique can be used for mounting specimens in 70% ethanol:

1. Put the specimen in a Petri dish and make a small puncture or dissection (Table 2).

Table 2. Taxonomic Group and Incision Point for Clearing

Taxon	Incision point or punction	References
Aphididae	Dorsal (4 to 6 punctions)	Blackman & Eastop (2000)
Thripidae	-	Borror et al. (1992)
Pseudococcidae	Dorsal (1 incision or 4 to 6 punctions)	Williams and Granara (1992). Modified.
Coleoptera	Cut last 3 abdominal segments	Borror et al. (1992)
Dermestidae (larvae)	Dorsal. Last segment incision, remove excess hairs.	Rodríguez (1994)
Dermestidae (larvae)	Dorsal (4 to 6 punctions)	Blanco-Rodríguez, personal communication (2018)
Lepidoptera	Cut last 3 abdominal segments	Borror et al. (1992)
Diptera (larvae)	1 incision on last segments	Lutz (1922), Borror et al. (1992)

2. Put the sample into a test tube with approximately 2 ml of 40% potassium hydroxide (KOH) [For 100 ml preparation, weigh 46.5 g flakes of potassium hydroxide, then add to a volumetric flask, and add 100 ml of distilled water. Stir gently with circular movement until the potassium hydroxide is dissolved].
3. Place the sample or adult structure in a test tube in a water bath. When the water boils, leave the tube in the water bath and stir every 2 minutes to accelerate KOH entering the body of a specimen. The time will depend on the size and morphological structures of the insect.
4. Drain the KOH from the tube and rinse repeatedly with plenty of distilled water (two to four washes), shaking gently to remove organic material from the specimen.
5. Place the sample in 96% ethyl alcohol for 2 to 4 minutes, and repeat the process at least twice.
6. Transfer to clove oil for 1 to 2 minutes before mounting in Canada balsam.

The conventional clearing technique uses several reagents with special functions so the specimen or structure of interest is visible. Potassium hydroxide, being a base, has an alkaline pH of 14.11 according to figures by a Corning pinnacle brand potentiometer. Other reagents counteract the alkalinity of potassium hydroxide, because 96% ethanol (5.22 pH), clove oil (3.06 pH), and Canada balsam (5.0 pH) have acidic pH.

The technique allows reduction in time for clearing larvae and internal structures of insects, without need for dehydration of alcohols that requires 3 or more hours. Structures or specimens must be washed in a thorough and detailed way, because if not done properly the specimen will dye bright yellow when moved from 96% ethanol into clove oil. The color indicates inadequate washing of the specimen that should be returned to 96% alcohol as soon as the color is detected.

References Cited

Aguilar-Morales, M., B. Coutiño-Bello, and P. Salinas-Rosales. 1996. Manual General de Técnicas Histológicas y Citoquímicas. Facultad de Ciencias, UNAM, México.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the World's Crops. An Identification and Information Guide. Chichester, UK.

Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1992. An Introduction to the Study of Insects. 6th ed. Saunders College Publishing, Orlando.
Carter, H. F., A. Ingram, and J. W. S. Macfie. 1920. Observations on the ceratopogonine midges of the Gold Coast, with descriptions of new species. Part I. Ann. Trop. Med. Parasitol. 14: 187-210.
Gaviño G., C. Juárez, and H. H. Figueroa. 1977. Técnicas Selectas de Laboratorio y de Campo. Limusa, México, DF.
Lutz, A. 1922. Contribution aux methods d'obseryations microscopiques de biologiques. Ann. Biol. Lacustre 11: 90-102.
Márquez-Luna, J. 2005. Técnicas de colecta y preservación de in*sectos*. Boletín Sociedad Entomológica Aragonesa 37: 385-408.
Morrone, J. J., D. Espinosa, A. D. Fortino, and P. Posadas. 1999. El arca de la biodiversidad. Universidad Nacional Autónoma de México, México, DF.
Palma, R. L. 1978. Slide-mounting of lice: a detailed description of the Canada balsam technique. New Zealand Entomologist 6: 432-436.
Rodriguez, N. S. 1994. Manual de Diagnóstico e identificación del Gorgojo Khapra *Trogoderma granarium* Everts. Serie Sanidad Vegetal. México.
Ronderos, M. M., G. R. Spinelli, and P. Sarmiento. 2000. Preparation and mounting of biting midges of the genus *Culicoides* Latreille (Diptera: Ceratopogonidae) to be observed with a scanning electron microscope. Trans. Am. Entomol. Soc. 126: 125-132.
Wilson, E. O. 1992. The Diversity of Life. W. W. Norton & Company, New York.
Williams, D. J., and M. C. Granara de Willink. 1992. Mealybugs of Central and South America. CAB International, Wallingford, UK.
Wirth, W. W., and N. Marston. 1968. A method for mounting small insects on microscope slides in Canada balsam. Ann. Entomol. Soc. Am. 61: 783-784.
Zhang, Z-Q. 2015. Phylum Arthropoda, pp. 17-27. *In* Z.-Q. Zhang [ed.], Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness. Zootaxa 3703: 1-82.

Una Comparación de Técnicas de Rehidratación Abdominal y Coriónica en Especies de *Emesis* Fabricius[1] y *Lasaia* H. Bates[1]

A Comparison of Techniques for Abdominal and Chorionic Rehydration on Species of *Emesis* Fabricius[1] and *Lasaia* H. Bates[1]

Jorge Llorente-Bousquets[2]*, Sandra Nieves-Uribe[2], Marysol Trujano-Ortega[2], y Arturo Arellano-Covarrubias[2]

Resumen. El estudio de la biodiversidad es imprescindible ante la dinámica de la modificación ambiental. Para ello es necesario emplear diferentes técnicas que permitan la obtención de resultados óptimos de la manera más eficiente y estandarizada. En Lepidoptera, el estudio de la estructura del exocorion ofrece un sistema de información útil en la discriminación taxonómica a diferentes niveles jerárquicos. En este estudio se describen y valoran tres técnicas de rehidratación abdominal a partir de muestras alojadas en colecciones científicas y se compara con el material recién recolectado en el campo, con el fin de examinar su efectividad y costos. En el campo a menudo es difícil o imposible obtener hembras para estos estudios, por lo que el uso del material de museos, con el empleo de estas técnicas, podría ser un gran avance en el conocimiento de este sistema de caracteres morfológicos en mariposas, ya que se podrían estudiar hembras de especies raras, crípticas y/o con muestreo deficiente. Además, también permitiría el registro de la variación temporal y geográfica de tales caracteres. A pesar que lo más recomendable es usar material fresco, de acuerdo con los resultados hallados en el presente estudio, las técnicas de rehidratación podrían permitir la recuperación del corion en un grado aceptable para su análisis y comparaciones, aunque se necesita más tiempo para limpiar la muestra. La técnica de rehidratación de proteinasa K obtuvo los huevos más conservados, incluso con material que se recolectó hace más de treinta años.

Abstract. Research on biodiversity is crucial in the current dynamics of environmental modification. To optimize results is necessary to apply multiple techniques and standardize them, to increase efficiency. The analysis of the exochorionic structure of Lepidoptera provides a useful information system for taxonomic discrimination at different hierarchical levels. In this study, three abdominal rehydration techniques are described and tested utilizing samples deposited in scientific collections. The effectiveness and costs of the techniques was tested by comparing the results with the morphology of preserved exochorion with

[1]Lepidoptera: Riodinidae

[2]Museo de Zoología (Entomología), Departamento de Biología Evolutiva, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, 04510, CDMX, México; llorentebousquets@gmail.com, s.nieves.uribe@outlook.com, marysol_trujano@yahoo.com.mx, arellano.covarrubias@gmail.com
*Autor de correspondencia

freshly obtained samples. Using these techniques over museum specimens could be an advance in the study of this system of morphological characters in female butterflies since it would increase the knowledge about rare, cryptic, and/or little-sampled species. Besides, it would increase the amount of data on the geographical and temporal variation of the chorionic characters. Although the results show that fresh material is the best option of rehydration techniques allow recovering the exochorion in acceptable conditions for analysis and comparisons, but it needs more time to clean these samples. The best rehydration technique was the use of proteinase K, since it obtained the most conserved eggs, even with specimens collected 30 years ago.

Introducción

En años recientes, los estudios sobre huevos de mariposas y en particular de su capa más externa —el exocorion— se realizaron con el empleo de dos técnicas de visualización: el empleo de microscopio electrónico de barrido (MEB) y la tinción con cloruro de metiltionina (azul de metileno) con observaciones en microscopio estereoscópico (Nieves-Uribe et al. 2016a). Estos trabajos se efectuaron en Noctuidae (Dolinskaya y Geryak 2010; Dolinskaya 2010, 2011; Dolinskaya y Ponomarenko 2013), Hesperiidae (Hernández-Roldán et al. 2012), Pieridae (Eitschberger y Ströhle 1990; Hernández-Mejía et al. 2013, 2014a,b, 2015; Nieves-Uribe et al. 2016a,b, 2018a,b; Llorente-Bousquets et al. 2018), Nymphalidae (Heliconiini: Dell'Erba et al. 2005; Biblidinae: Nieves-Uribe et al. 2015, 2016d, 2017a,b, 2019; Satyrinae: Thomson 1992, García-Barros y Martín 1995), Lycaenidae (Downey y Allyn 1981, Munguira et al. 2015), y Riodinidae (Downey y Allyn 1980). Los huevos en la mayoría de estos estudios, así como otros donde se abarcan principalmente ciclos de vida, se obtienen mediante la observación y recolección de oviposiciones de huevos fecundados en el campo o en el laboratorio (*v. gr.* Downey y Allyn 1980, Freitas y Brown 2004, Hernández-Roldán et al. 2012, Munguira et al. 2015); o bien al presionar los abdómenes desde en medio hacia su extremo distal, para conseguir al menos un huevo fecundado (Freitas y Brown 2004). Sin embargo, hay trabajos donde la obtención del corion maduro se efectúa mediante la disección de abdómenes que fueron almacenados en etanol al 70% desde el momento de la recolecta de los imagos en el campo (Llorente-Bousquets y Castro-Gerardino 2007, 2008; Hernández-Mejía et al. 2013, 2014a,b, 2015; Nieves-Uribe et al. 2015, 2016a,b,c,d, 2017a,b, 2018a,b, 2019; Llorente-Bousquets et al. 2018).

La composición del huevo en insectos se caracteriza por su gran complejidad estructural (Rezende et al. 2016). Ésta consiste en una serie de capas y subcapas que proveen protección al embrión en desarrollo; éstas son la membrana vitelina, la capa serosa y las capas propiamente coriónicas: el endocorion —con ciertas capacidades de intercambio gaseoso— y una capa superficial externa y 'ornamentada' llamada exocorion (Rezende et al. 2016). Ambas capas poseen más de 100 polipéptidos diferentes (hasta 186 en *Antheraea;* Telfer 2009), y se han secuenciado 413 proteínas coriónicas en mariposas diurnas y nocturnas (Giannopoulos et al. 2013). Los polipéptidos del corion se agrupan en tres categorías o familias, que a su vez se dividen en varios grupos. La mayoría de sus aminoácidos se encuentran en una región central evolutivamente conservada, lo que permite inferir una estructura secundaria tripartita en los aminoácidos coriónicos (Telfer 2009); posiblemente primaria y antiparalela de modo principal (Srivastava et al. 2011). No obstante, la formación del exocorion y cómo se agrupan las proteínas que

lo componen sigue sin comprenderse en su totalidad. La forma en la que las proteínas se agrupan y forman la diversidad de microestructuras diferenciadas u 'ornamentaciones', así como la ontogenia exocoriónica en la oogénesis, sigue sin ser satisfactoriamente comprendida (Srivastava et al. 2011). Los autores se encuentran estudiando el desarrollo o génesis de tales microestructuras en Pieridae y Nymphalidae.

El exocorion presenta algunos carbohidratos inmersos en las proteínas que lo componen, que es una de sus diferencias estructurales principales respecto al endocorion (Richards y Davies 1977, Hinton 1981, Trougakos y Margaritis 2008). Aunque es una estructura de protección, el corion carece de quitina; el exocorion se endurece por los enlaces disulfuro entre las fibras de proteínas ricas en cisteína (Telfer 2009). En éste hay regiones especializadas, los polos anterior y posterior, así como aperturas micropilares que permiten la entrada de espermatozoides, hidrópilos para la captación de agua y aerópilos para la respiración. En algunas especies se desarrollan diferentes tipos de superficies y estructuras de fijación (*v. gr.* ganchos, dentículos, espinas) (Skudlik et al. 2005, Kumar et al. 2007). El exocorion se genera también por las células foliculares ováricas envueltas por el epitelio de la ovariola (Telfer 2009, Srivastava et al. 2011), por lo que puede considerarse la última expresión morfológica directa del fenotipo materno. Por eso varios autores consideran que las características del corion son informativas en estudios taxonómicos (Duarte et al. 2005; Llorente-Bousquets y Castro-Gerardino 2007, 2008; Duarte y Robbins 2009; Hernández-Mejía et al. 2013, 2014a,b, 2015; Kaminski y Carvalho-Filho 2013; Nieves-Uribe et al. 2015, 2016a,b,c,d, 2017a,b, 2018a,b, 2019; Srivastava y Kumar 2016).

Hasta ahora la recuperación del corion maduro por disección abdominal presenta ciertas ventajas respecto a otras técnicas. Entre ellas están el registro de caracteres que con la oviposición se pueden perder —tales como la forma y patrones en la base del corion; así también la observación de rasgos típicos de cada taxón y aquellos que son anómalos y solo aparecen en una ovariola de las ocho que constituyen los órganos de producción de huevos (Nieves-Uribe et al. 2015). En los casos donde se estudian huevos a partir de oviposiciones, si se colocan de forma individual, a menudo no hay un escrutinio de huevos distintos para comparaciones; mientras que en las oviposiciones colectivas (*v. gr. Eucheira socialis,* género *Chlosyne,* subtribu Acreina; JLB obs. pers.) solo se fotografían sin detalle o carecen de una evaluación minuciosa de los caracteres exocoriónicos, especialmente a escalas de mayor resolución.

El examen del exocorion obtenido después de una disección brinda mayor certeza en la identificación adecuada de los ejemplares de donde provienen; esto es de especial importancia con especies crípticas o muy similares, que ovipositan en las mismas plantas de alimentación larval. Sin embargo, la gran dificultad en conseguir hembras de ciertos taxones en el campo —sea por encontrarse en hábitats de difícil acceso, los hábitos peculiares de algunas especies, o la rareza de algunas hembras durante las recolectas— limitan la recopilación de caracteres coriónicos en especies de gran importancia o significación filogenética. Esto implica impedimentos para poner a prueba hipótesis en varios grupos específicos. Ejemplo de ello es la ausencia del género *Patia* dentro del trabajo de Llorente-Bousquets et al. (2018), después de muchos años de esfuerzo en trabajo de campo en América del Sur. Este género es el único que falta por estudiar de toda la subfamilia Dismorphiinae (Pieridae). Otros casos han ocurrido en los estudios de Nieves-Uribe et al. (2015, 2016d) para la subfamilia Biblidinae, así como en los estudios de Pierinae y

Coliadinae de Hernández-Mejía et al. (2013, 2014a,b, 2015) y Nieves-Uribe et al. (2016a,b,c, 2018a,b).

Otro modo de compilar información coriónica sin tener ejemplares frescos y/o huevos ovipuestos, es mediante ilustraciones que aparecen en la literatura o en la red electrónica. En libros o monografías publicados principalmente en Europa (Döring 1955, Henriksen y Kreutzer 1982), Asia (Yu-Feng 2002, 2006; Igarashi y Harada 2015) y África (Dickson y Kroon 1978), se pueden encontrar fotografías y dibujos del corion en gran variedad de taxones del Viejo Continente. Sin embargo, estas ilustraciones presentan limitaciones visuales y conceptuales que no permiten un reconocimiento y caracterización al nivel que se obtiene mediante disecciones. Además, a menudo se carece de certeza en la procedencia (localidad o taxón) de los huevos ilustrados, precisión en las determinaciones taxonómicas, o bien los datos de las descripciones son bastante generalizados y omiten la variación.

Ante la obtención diversa de las muestras, su modo de preservación y preparación, la falta de uniformidad en las estructuras informativas que hay que registrar, así como los errores e incertidumbres asociados en cada uno de los procesos, el desarrollo de una técnica de rehidratación estandarizada para ejemplares secos podría permitir la recuperación del corion de una manera equivalente a la reportada por Llorente-Bousquets y Castro-Gerardino (2007, 2008) y trabajos sucesivos. Se podrían aprovechar las colecciones para este propósito y así se podría conocer la 'ornamentación' o arquitectura exocoriónica de gran diversidad de taxones que son muy raros para recolectar en campo; o bien aquellos que por logística es difícil de acceder por sus hábitats. Además, posibilita la disposición de una muestra mayor que revele la variación temporal, fisiográfica y/o climática. En el presente trabajo se presentan las pruebas técnicas realizadas y los resultados con tres diferentes líquidos rehidratantes, en especies de dos géneros de Riodinidae, cuyo corion se encuentra reportado en la literatura con estudios en MEB y por lo tanto es posible hacer comparaciones.

Materiales y Métodos

Obtención de los Ejemplares. Se eligieron hembras de especies de los géneros *Emesis* y *Lasaia* (Lepidoptera: Riodinidae), depositados en la colección de Lepidoptera del Museo de Zoología 'Alfonso L. Herrera' de la Facultad de Ciencias (MZFC) de la Universidad Nacional Autónoma de México (UNAM). Las especies de *Emesis* seleccionadas corresponden con las publicadas por Downey y Allyn (1980), salvo *E. zela* Butler que no se encuentra en dicho trabajo. Respecto a *Lasaia,* la elección es de una subespecie diferente a la publicada (*L. sula sula* Staudinger) y también se anexa para comparación a *L. agesilas callaina* Clench. Los rótulos de los ejemplares de *Emesis* tienen fechas de recolecta de hasta más de 35 años, entre 1984 y 1986, mientras que para *Lasaia* sus ejemplares se recolectaron entre 2007 y 2013.

Los abdómenes se retiraron con ayuda de unas pinzas planas para sujetar el tórax del imago; así como tijeras de disección con el fin de seccionar el abdomen a partir del metatórax. En el caso de las hembras recién recolectadas, el abdomen desprendido se conservó en un microtubo de 1.5 ml con etanol al 70%, mientras que para las hembras de museo el abdomen se almacenó en seco. En ambos casos se asignaron números de identificación, tanto en el espécimen como en el microtubo con el abdomen, para su incorporación a la colección de abdómenes (ABD) del MZFC de la UNAM.

Datos Curatoriales de los Ejemplares. *Emesis zela:* México: Guerrero: Omiltemi, Camino a Potrerillo (17°33'45" N, 99°41'30" W), 2300 msnm, 21-X-1985, leg. J. Llorente y A. Luis (ABD-2054; MZFC LEP 02510).

E. mandana furor: México: Oaxaca: El Puente, 3 Km al E de Choapam (17°22'38" N, 95°55'20" W), 620 msnm, 05-X-2015, leg. A. Luis y cols. (ABD-1706); Michoacán: Presa Hidroeléctrica Cupatitzio Gabriel Zamora (19°16'07" N, 102°04'39"W), 920-1460 msnm, 10-IX-1994, leg. L. L. González (ABD-2052; MZFC LEP 400793).

E. tenedia: México: Oaxaca: El Puente, 3 Km al E de Choapam (17°22'38" N, 95°55'20" W), 620 msnm, 27-VIII-2015, leg. J. Llorente y col. (ABD-1506); Guerrero: La Cascada, Km. 8, Taxco-Ixcateopan de Cuauhtémoc (18°33'12.42"N, 99°37'08.25"W), 1900 msnm, 14-XII-1986, leg. J. Llorente (ABD-2067; MZFC LEP 59357); Tetipac, Arroyo Las Damas (18°39'29"N, 99°37'53.7"W), 1850 msnm, 17-XII-1986, leg. A. Luis (ABD-2068; MZFC LEP 02061); Atoyac de Álvarez, Nueva Delhi (17°25'20.05" N, 100°11'37.87"W), 1400 msnm, 02-XII-1984, leg. J. Llorente y A. Luis (ABD-2070; MZFC LEP 02083).

Lasaia sula sula: México: Oaxaca: Pluma Hidalgo, Rancho Hagia Sofía (15°22'42"N, 96°22'05"W), 410 msnm, 30-V-2013, leg. J. Llorente y A. Luis (ABD-487, ABD-2071; MZFC LEP 378818, ABD-2072; MZFC LEP 378837).

Lasaia agesilas callaina: México: Oaxaca: Pluma Hidalgo, Rancho Hagia Sofía (15°22'42"N, 96°22'05"W), 410 msnm, 25-VI-2013, leg. A. Luis (ABD-500); Candelaria Loxicha, El Azulillo (15°53'25"N, 96°29'27"W), 380 msnm, 24-IX-2007, leg. J. Llorente (ABD-2073; MZFC LEP 242892).

Pruebas de Rehidratación y Calibración de las Variables. Las pruebas de rehidratación y calibración solo se efectuaron en el género *Emesis*, mientras que para *Lasaia* se empleó la técnica preliminar de rehidratación con hidróxido de potasa (KOH) (Llorente-Bousquets 1984). Las tres técnicas examinadas se realizaron con agua, KOH o proteinasa K como líquidos hidratantes; únicamente para las dos primeras el calentamiento fue indirecto por convección térmica con agua destilada (baño María) en un sistema abierto. Este consistió en un recipiente plástico con un volumen de 200 ml de agua destilada, ya sea a 75 u 85°C. Los abdómenes recibieron el volumen del líquido correspondiente al tubo que los contiene y se colocaron en el 'baño María' por diferentes intervalos de tiempo.

Luego, cada abdomen se lavó con agua destilada para retirar excesos; en los casos donde se empleó KOH, los abdómenes pasaron por un tratamiento con ácido acético glacial al 7.5% durante 30 segundos, para detener la reacción de la base empleada, seguido de un segundo lavado con agua destilada. Finalmente, cada abdomen se colocó en un vial de 1.5 ml con agua destilada a temperatura ambiente (23°C en promedio) para su disección inmediata. Detalles de cada técnica con sus resultados se dan en la sección de Resultados y Discusión.

Disección del Abdomen y Aislamiento de los Huevos. Los abdómenes se diseccionaron bajo microscopios estereoscópicos Leica S6D y Olympus SXZ9. La primera parte de la disección de los abdómenes fue en cajas de Petri de vidrio con agua destilada; esto se requiere para facilitar la limpieza de las estructuras a partir de restos degradados y otros tejidos abdominales. Después se retiró la grasa, pero en los casos donde los abdómenes se encontraban muy endebles luego de la rehidratación, se empleó inmersión en etanol al 70% para endurecerlos. Con el fin de reconocer las estructuras y tener una visualización mejor de los genitales y huevos, el etanol se tiñó con una gota de azul de metileno a partir de una solución madre de 0.05 g/ml. Se aislaron los huevos maduros de cada ejemplar, mientras

que la *bursa copulatrix* y otras estructuras asociadas se conservaron en un vial de 1.5 ml con etanol al 70%. Todos los ejemplares rehidratados se incorporaron a la colección ABD del MZFC.

Con el fin de comparar el estado del corion después de la rehidratación, se realizó la disección de abdómenes almacenados en etanol al 70% desde el momento de recolecta del ejemplar, tal como se describe en Hernández-Mejía et al. (2013, 2014a,b, 2015). Estos abdómenes son de los mismos taxones que fueron rehidratados, para evitar sesgos en los resultados y sus comparaciones.

Montaje y Observación en Microscopio Electrónico de Barrido (MEB). Para su observación bajo el MEB se seleccionaron tres de los huevos obtenidos de cada especie y condición técnica (24 en total). Adicionalmente, a los huevos obtenidos mediante rehidratación se les aplicó una limpieza en agua con jabón biológico (marca EXTRAN) previo al montaje; esto consistió en dejar las muestras durante 72 horas, con un recambio diario del líquido; la limpieza se efectuó con el empleo de pinzas de punta fina y lavados con agua destilada.

Ya limpios, los huevos se colocaron de manera individual en una caja de Petri de vidrio y cada diez minutos se cambiaron a otra, cuya concentración de alcohol fuese mayor; los cambios de concentración de alcohol empezaron al 70%, con subsecuentes aumentos a 80%, 90%, y alcohol absoluto. Posteriormente, se fijaron con una solución de glutaraldehído al 2.5% y alcohol absoluto en proporción 1:1 durante 10 minutos, y 15 más en solo glutaraldehído al 2.5%. Transcurrido este tiempo, se lavaron con agua destilada y deshidrataron en alcoholes graduales (10, 30, 50, 70, 80, y 96%) por diez minutos en cada concentración, para terminar en alcohol absoluto.

Todas las muestras se transfirieron a bolsas de papel arroz con etanol al 100% y sus datos respectivos, luego se secaron a punto crítico con CO_2 en un equipo Emitech K 850. El montaje de cada una se realizó en un porta muestras de aluminio con cinta adhesiva conductora, para recubrirse con una capa de oro de 20 mÅ de grosor durante dos minutos en una evaporadora de metales Quorum Q15OR ES.

Las observaciones y tomas fotográficas se efectuaron en el MEB Hitachi SU 3500 a un voltaje de 10 Kv. Los aumentos utilizados van desde x95 a 2.70k. Con propósitos de comparación, se tomaron fotografías en las mismas vistas y aumentos similares que las presentadas por Downey y Allyn (1980).

Resultados y Discusión

El resultado principal es la estandarización de las diferentes técnicas de rehidratación empleadas para la recuperación de coriones en buen estado, a partir de material de colecciones científicas con diferentes fechas. A continuación, se describe cada una de las tres técnicas de rehidratación de abdómenes propuestas.

Técnica 1: a) con agua, b) con potasa, c) con proteínasa K. En ellas se detallan las concentraciones de cada sustancia rehidratante, los tiempos de exposición y la temperatura. También se describe el producto y estado de conservación que se obtiene en la estructura del huevo (Tabla 1). Los resultados de las pruebas de rehidratación, así como las variables en cada caso, se refieren en la Tabla 1. Solo se describirá de manera más amplia la técnica preliminar.

1a) Técnica de rehidratación con agua destilada. Se agrega a cada vial de los abdómenes seccionados el volumen correspondiente en ml de agua destilada a temperatura ambiente ($\bar{x}$ = 23°C). Después éstos se colocan en un baño María con 200 ml de agua destilada a 85°C durante 20 minutos. Al concluir este lapso, el

Tabla 1. Variables y Resultados de la Rehidratación Abdominal y Coriónica
Table 1. Results and Variables of the Abdominal and Chorionical Rehydration

Concentración KOH	Temperatura empleada	Tiempo de calentamiento	Resultado
0% (agua destilada)	85°C	20 minutos	Abdomen rehidratado, pero con muchas adherencias; para realizar la disección se necesitó dejar en EtOH 70% por 16 horas.
0% (agua destilada)	85°C	180 segundos	Abdomen seco y duro; no se logró su rehidratación.
10%	85°C	180 segundos	Solo se recuperó la *bursa copulatrix.*
10%	75°C	180 segundos	Tejido degradado; solo se recuperó la *bursa copulatrix.*
7.5%	75°C	180 segundos	Se conserva el endocorion con daño severo. No hay rastro del exocorion.
7.5%	75°C	90 segundos	Endo y exocorion rodeados por grasa/otras estructuras se conservan en buen estado; caso contrario solo se conserva el endocorion.
20 mg/ml (proteinasa K)	45°C	24 horas	Exocorion en muy buen estado, pero con muchas adherencias

abdomen se retira del agua caliente y se pone en otro con agua a temperatura ambiente previo a su disección.

1b) Técnica de rehidratación con proteinasa K. El área de trabajo se limpia con etanol al 70% para evitar la contaminación. En un tubo se coloca 600 µl de buffer de lisis y 25 µl de proteinasa k (20 mg/ml), para posterior adición del abdomen con ayuda de pinzas de puntas finas. Se agita suavemente cada tubo durante 20 segundos y se colocan en una incubadora a 45°C por 24 horas. Es conveniente agitar la muestra suavemente, una vez transcurridas 12 horas. Transcurridas las 24 horas, se separa el abdomen de la solución de lisis y se coloca en un tubo nuevo con etanol al 70%. El tubo con la solución de lisis contiene el ADN suspendido, el cual puede ser empleado en siguientes etapas de extracción de material genético (Hillis et al. 1996, Knölke et al. 2004, Falcón y Valera 2007).

1c) Técnica preliminar de rehidratación con KOH. Después de seccionar el abdomen de cada ejemplar, se deposita en un vial con 0.6 ml de KOH al 7.5% a ~23°C. Inmediatamente, se coloca en un baño María con 200 ml de agua destilada a 75°C durante 90 segundos. Luego, el abdomen se retira del KOH y enjuaga con agua destilada, se sumerge en 10 ml de ácido acético glacial al 7.5% durante 30 segundos, y se lava con agua destilada. Entonces, el abdomen se coloca en un vial con agua destilada a temperatura ambiente previo a su disección. Para imagos con más de 20 años de antigüedad, los abdómenes se dejaron cinco minutos en etanol al 70% antes de exponer a baño María. En estos casos, si los huevos maduros se encuentran rodeados de grasa abdominal u otras estructuras (tejido conectivo,

genitales, conducto intestinal), el exocorion se conserva en buen estado. De encontrarse muy cercano a la cutícula del abdomen o no encontrarse rodeado de algún otro tejido/estructura, únicamente se conserva el endocorion; quizá debido a que la reacción del KOH es más acentuada en aquellos huevos que no presentan otras adherencias que degradar antes de llegar a su capa más externa (Ver Tabla 1).

Resultados de Cada Técnica y Comparación de Resultados. Se logró la recuperación del exocorion de las especies de *Emesis* y *Lasaia* mediante las tres técnicas de rehidratación antes mencionadas. Cada caso presentó variaciones y calidades distintas entre sí, así como con la técnica de disección abdominal de material fresco (Llorente-Bousquets y Castro-Gerardino 2007, 2008). La recuperación coriónica con proteinasa K permitió obtener el exocorion en un buen estado (Lám. 1 A-C), pero su limpieza requiere más trabajo, debido a la gran cantidad de membranas y otros tejidos conectivos y epiteliales adheridos al corion (Lám. 1 A). Esto es especialmente notorio bajo la observación a través de un microscopio electrónico, donde algunas partes quedan ocultas (Lám. 1 A, B) o las 'ornamentaciones' carecen de nitidez, al encontrarse cubiertas por restos de tejido (Lám. 1 C). Aunque la técnica brinda buenos resultados en general, el costo de sus materiales es mayor respecto a las demás, al tratarse de materiales empleados para la extracción de material genético.

La técnica de rehidratación con agua destilada es conveniente por la facilidad y accesibilidad de sus materiales. Sin embargo, los resultados de limpieza son de menor calidad a la presentada por proteinasa K o rehidratación por KOH (Lám. 1 D-F). En huevos como los de *Emesis*, donde se encuentran proyecciones exocoriónicas, se dificulta aún más debido al riesgo de romper o dañar tales estructuras mediante una limpieza mecánica (Lám. 1 D, F). Con esta técnica se pueden producir deformidades coriónicas (Lám. 1 E); en este caso no se logró recuperar un corion sin alteraciones en la forma respecto a los ovipuestos (Downey y Allyn 1980), o aquellos recuperados mediante disección de abdómenes frescos conservados en etanol desde su momento de recolecta (Lám. 2 D-F).

La técnica con KOH al 7.5% permitió una recuperación de huevos con menor cantidad de deformidades respecto a la hidratación con agua destilada; e incluso se conservaron estructuras delgadas sin colapsamientos como las observadas en el género *Lasaia* (Lám. 3 A-C). Esto es notorio en la estructura tipo 'olán o faralá' en las zonas ecuatorial y basal del corion, proyectándose por encima de la superficie exocoriónica; en las fotografías de Downey y Allyn (1980) de *Lasaia sula peninsularis,* en algunas partes se observa colapsada y pegada al corion (Downey y Allyn 1980, Fig. 1).

El mayor inconveniente en las tres técnicas de rehidratación se presenta en la limpieza coriónica, con gran cantidad de adherencias aun después de pasar por el jabón biológico, tanto para *Lasaia* (Lám. 3-B) como para *Emesis* (Lám. 2 A-C). Esto no ocurre en aquellos huevos montados a partir de disecciones de abdómenes conservados en fresco para *Emesis* (Lám. 2 D-F) y *Lasaia* (Lám. 3 D-F). Se puede considerar hasta ahora que esta última técnica presenta los mejores resultados para la recuperación de caracteres coriónicos.

Al afinar alguna de las técnicas presentes en este trabajo, o alguna otra implementada para las muestras antes mencionadas, se permitirá ampliar los estudios en ciertos géneros importantes para Pieridae o Biblidinae. Entre estas técnicas también se encuentra la recuperación del corion en huevos expuestos a temperaturas bajo cero por nitrógeno líquido (SNU y JLB, obs. pers. en *Neophasia*

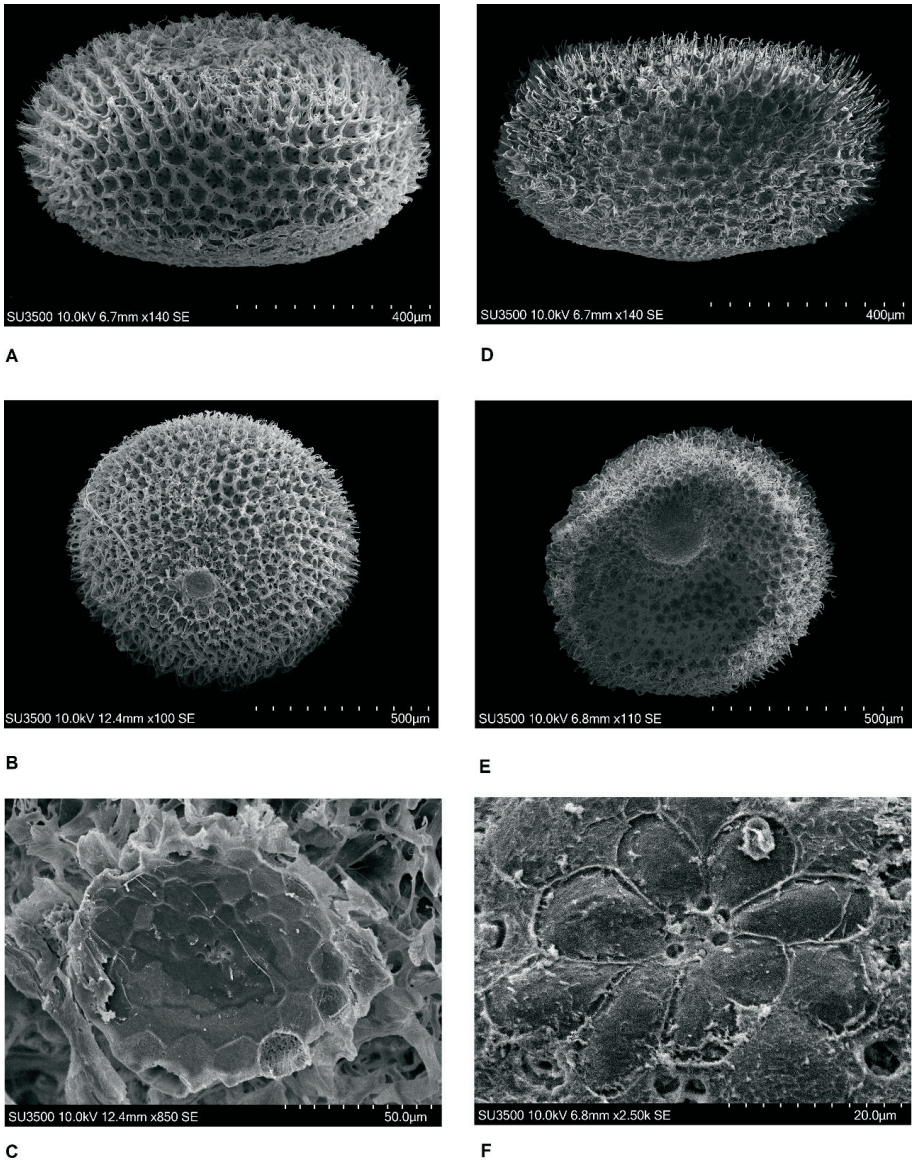

Lámina 1. Rehidratación coriónica exitosa en *Emesis.* A-C. Con proteinasa K en *E. zela;* D-F. Con agua destilada en *E. mandana furor*. En ambos casos se presentan fotografías en vista lateral (A, D), apical (B, E) y de la región micropilar (C, F).
Plate 1. Success chorionic rehydration in *Emesis*. A-C. With K proteinase in *E. zela;* D-F. With distilled water in *E. mandana furor.* Lateral view (A, D), apical view (B, E), micropylar region (C, F).

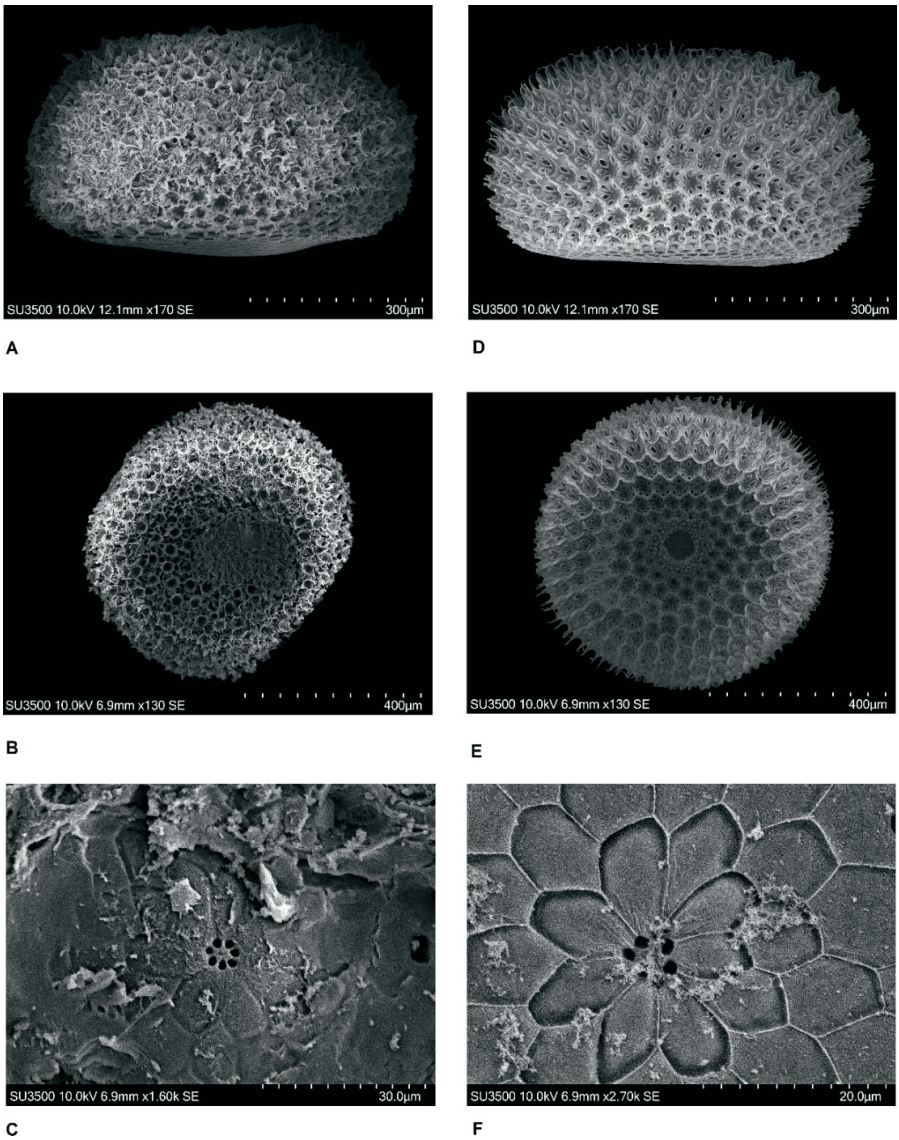

Lámina 2. Corion de *Emesis tenedia.* A-C. Rehidratación coriónica exitosa con KOH al 7.5%*;* D-F. Corion recuperado a partir de disección abdominal (material preservado con alcohol al momento de su captura). Fotos en vista lateral (A, D), apical (B, E), y de la región micropilar (C, F).

Plate 3. Chorion of *Emesis tenedia*. A-C. Successful chorionic rehydration with 7.5% KOH*;* D-F. Recuperated chorion from abdominal dissection (preserved material with alcohol at the time of capture). Photos lateral (A, D) and apical view, also the micropylar region (C, F).

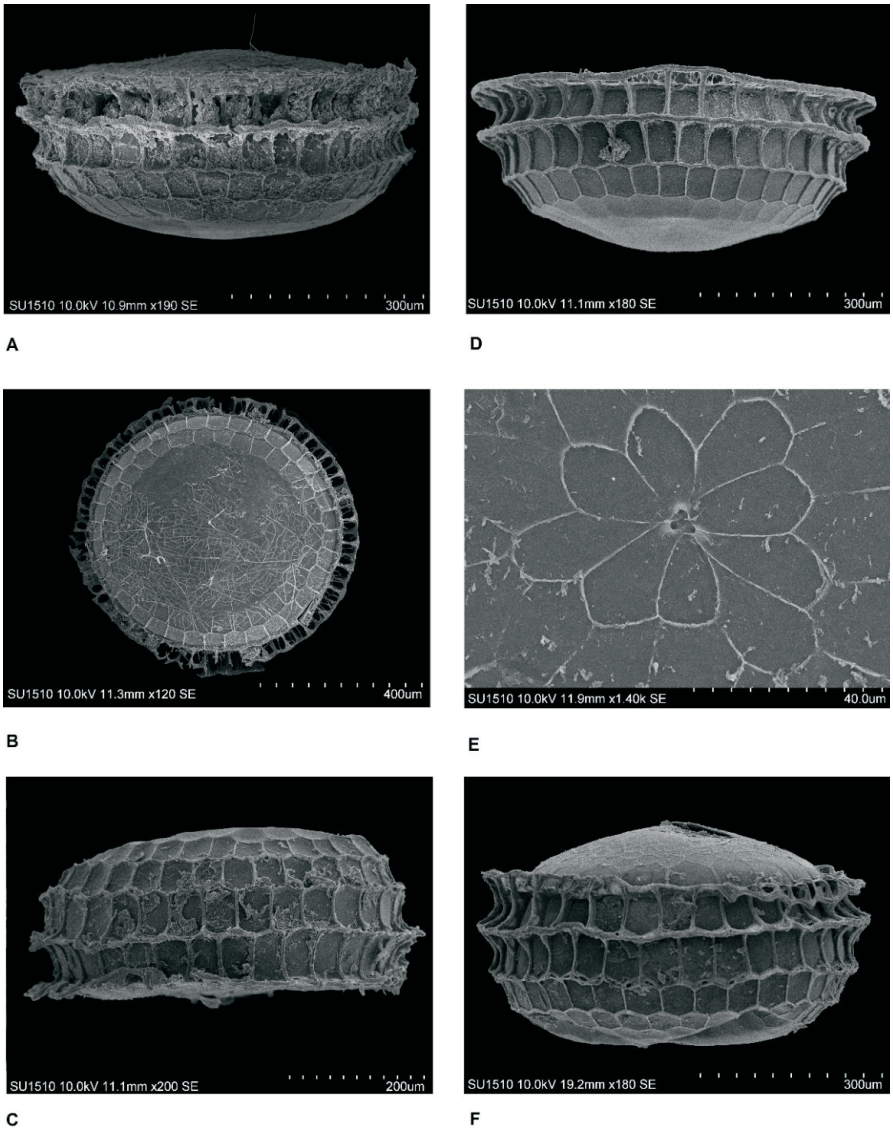

Lámina 3. Corion de *Lasaia* rehidratado con KOH al 7.5% (izq.) y a partir de disecciones de abdómenes conservados en fresco (der.). Nótese que los coriones rehidratados conservan la forma general del corion, a pesar de tener más adherencias. *L. sula sula* en vista lateral (A, D), apical (B), y micropilar (E); *L. agesilas callaina* en vista lateral (C, F).

Plate 3. Rehydrated or moisturized chorion of *Lasaia* with 7.5% KOH (left) and from dissection of abdomens conserved from fresh (right). The rehydrated conserved the chorionic general form even with all the connective tissue on it. *L. sula sula* on lateral (A, D), apical (B), and mycropilar view (E); *L. agesilas callaina* on lateral view (C, F).

Behr, Pieridae). Estudios coriónicos en *Patia* Klots, *Pseudopontia* Plötz, *Leptosia* Hübner o *Elodina* C. Felder & R. Felder podrían apoyar o refutar hipótesis filogenéticas sobre su posición en los clados de Dismorphiinae y Pierinae (*v. gr.* Braby et al. 2006, Wahlberg et al. 2014). El conocimiento del corion en *Batesia* Felder & Felder y *Panacea* Godman & Salvin (Nymphalidae), a partir de ejemplares cuya identificación sea certera, permitirá aclarar si las descripciones en la literatura corresponden o no a estos géneros; problema que aún no se resuelve por morfología exocoriónica respecto a si este clado hermano se separaría de Ageroniina al ser de un tipo de corion distinto al registrado para *Hamadryas* Hübner y *Ectima* Doubleday (Nieves-Uribe et al. 2015, 2016d, 2017a, 2019). No obstante, un análisis reciente (Dias et al. 2019) indica que *Batesia* y *Panacea* son géneros hermanos, donde posiblemente Ageroniini se separe en dos subtribus.

El empleo de dispositivos fotográficos nuevos ha superado las técnicas empleadas por Peterson (1960, 1970) en el estudio de huevos en campo; aún estos ofrecen resultados escasos de comparación con fines taxonómicos, pues se requiere la aplicación de técnicas histológicas y microscópicas para develar los rasgos exocoriónicos de detalle (*v. gr.* Van der Poorten y Van der Poorten 2014). Por otra parte, en los huevos fecundados operan procesos, uno de los más importantes es el cambio de color durante la incubación (Peterson 1968). A la fecha ignoramos si hay cambios exocoriónicos con el endurecimiento después de la oviposición.

Agradecimiento

El financiamiento del trabajo de gabinete, a lo largo de esta investigación, fue otorgado por DGAPA-UNAM-PAPIIT (IN202415 y 212418) y CONACyT 284966. Al grupo de trabajo del Museo de Zoología 'Alfonso L. Herrera', en especial a Armando Luis por su ayuda durante las recolectas de campo de los ejemplares mexicanos. A las M. en C. Berenit Mendoza Garfias e Isabel Vargas Fernández por su ayuda y colaboración en los montajes y tomas fotográficas de las muestras para el MEB. La Facultad de Ciencias, UNAM, en su Departamento de Biología Evolutiva, nos ha apoyado durante todos estos años con transporte, instalaciones y tiempo académico para estas investigaciones entomológicas.

Referencias Citadas

Braby, M. F., R. Vila, and N. E. Pierce. 2006. Molecular phylogeny and systematics of the Pieridae (Lepidoptera: Papilionoidea): higher classification and biogeography. Zool. J. Linn. Soc. 147: 239-275.

Dell'Erba R., L. A. Kaminski, and G. R. P. Moreira. 2005. O estágio de ovo dos Heliconiini (Lepidoptera, Nymphalidae) do Rio Grande do Sul, Brasil. Iheringia (Sér. Zool.) 95: 29-46.

Dias, F. M. S., R. R. Siewert, A. V. L. Freitas, G. Lamas, L. M. Magaldi, O. H. H. Mielke, and M. M. Casagrande. 2019. An integrative approach elucidates the systematics of *Sea* Hayward and *Cybdelis* Boisduval (Lepidoptera: Nymphalidae: Biblidinae). Sys. Entomol. 44: 226-250.

Dickson, C. G. C., and D. K. Kroon [eds.]. 1978. Pennington's Butterflies of Southern Africa. Ad. Donker, 1ª ed. Johannesburgo, Sudáfrica.

Dolinskaya, I. V. 2010. The chorionic sculpture in eggs of some Hadeninae (Lepidoptera, Noctuidae) from Ukraine. Ukr. Entom. 1: 3-15.

Dolinskaya, I. V. 2011. The chorionic sculpture of the eggs of some Xyleninae (Lepidoptera, Noctuidae). Vestnik Zool. 45: 41-56.
Dolinskaya, I. V., and Y. N. Geryak. 2010. The chorionic sculpture of the eggs of some Noctuinae (Lepidoptera, Noctuidae) from Ukraine. Vestnik Zool. 44: 421-432.
Dolinskaya, I. V., and M. G. Ponomarenko. 2013. The chorionic sculpture in eggs of some Noctuidae (Lepidoptera). Vestnik Zool. 47: 33-41.
Döring, E. 1955. Zur Morphologie der Schmetterslingseier. Akademie Verlag, Berlín.
Downey, J. C., and A. C. Allyn. 1980. Eggs of Riodinidae. Bull. Allyn Mus. 34: 133-145.
Downey, J. C., and A. C. Allyn. 1981. Chorionic sculpturing in eggs of Lycaenidae. Part I. Bull. Allyn Mus. 61: 1-29.
Duarte, M., and R. K. Robbins. 2009. Immature stages of *Calycopis bellera* (Hewitson) and *C. janeirica* (Felder) (Lepidodptera, Lycaenidae, Techlinae, Eumaeini): taxonomic significance and new evidence for detritivory. Zootaxa 2325: 39-61.
Duarte, M., R. K. Robbins, and O. H. H. Mielke. 2005. Immature stage of *Calycopis caulonia* (Hewitson 1877) (Lepidoptera, Lycaenidae, Theclinae, Eumaeini), with notes on rearing detritivorous hairstreaks on artificial diet. Zootaxa 1063: 1-31.
Eitschberger, U., und M. Strohle. 1990. Zehnte Ergänzung zu “Systematische Unterschungen am *Pieris-napi-bryoniae* Komplex (s.1.)”. Aufzucht und Beschreibung der Praimaginalstadien von *Pieris bryoniae adalwinda* Fruhstorfer, 1909 und der Phaenotyp der Imagines (Lepidoptera, Pieridae). Atalanta 21: 239-252.
Falcón, L. I., y A. Valera. 2007. Extracción de ácidos nucleídos, pp. 499-516. *En* L. E. Eguiarte, V. Souza, y X. Aguirre [eds.], Ecología Molecular. INE, SEMARNAT, CONABIO, UNAM. México, DF.
Freitas, A. V. L., and K. S. Brown Jr. 2004. Phylogeny of the Nymphalidae (Lepidoptera). Syst. Biol. 53: 363-383.
García-Barros, E., and J. Martín. 1995. The eggs of the European satyrine butterflies (Nymphalidae): external morphology and its use in systematics. Zool. J. Linn. Soc. 115: 73-115.
Giannopoulos, N. G., I. Michalopoulos, N. C. Papandreou, A. Malatras, V. A. Iconomidou, and S. J. Hamodrakas. 2013. LepChorionDB, a database of Lepidopterian chorion proteins and a set of tools useful for the identification of chorion proteins in Lepidopteran proteomes. Ins. Biochem. Mol. Biol. 43: 189-196.
Henriksen, H. J., and I. B. Kreutzer [eds.]. 1982. The Butterflies of Scandinavia in Nature. Lubrecht and Cramer.
Hernández-Mejía, B. C., A. Flores-Gallardo, y J. Llorente-Bousquets. 2013. Comparación morfológica del corion de especies de los géneros *Pieriballia*, *Itaballia* y *Perrhybris* (Lepidoptera: Pieridae: Pierinae), y sus implicaciones filogenéticas. Southwest. Entomol. 38: 275-292.
Hernández-Mejía, B. C., A. Flores-Gallardo, y J. Llorente-Bousquets. 2014a. Morfología del corion en especies de los géneros *Ascia* y *Ganyra* y su comparación con otros géneros próximos de Pierinae (Lepidoptera: Pieridae). Southwest. Entomol. 39: 119-134.

Hernández-Mejía, C., A. Flores-Gallardo, y J. Llorente-Bousquets. 2014b. Morfología del corion en la subfamilia Coliadinae (Lepidoptera: Pieridae). Southwest. Entomol. 39: 853-886.
Hernández-Mejía, C., A. Flores-Gallardo, y J. Llorente-Bousquets. 2015. Morfología del corion en *Leptophobia* (Lepidoptera: Pieridae) y su importancia taxonómica. Southwest. Entomol. 40: 351-368.
Hernández-Roldán, J. L., M. L. Munguira, W. Wagner, and R. Vila. 2012. Comparative analysis and taxonomic use of the morphology of immature stages and natural history traits in European species of *Pyrgus* Hübner (Lepidoptera: Hesperiidae, Pyrginae). Zootaxa 3470: 1-71.
Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. Sinauer Associates, Sunderland, Massachusetts.
Hinton, H. E. 1981. Biology of Insect Eggs. 3 volumes. Pergamon, Oxford.
Igarashi, S., and M. Harada. 2015. Sequel to "The life histories of Asian butterflies Vols. 1 and 2. Tokai University Press.
Kaminski, L. A., and F. S. Carvalho-Filho. 2013. Life history of *Aricoris propitia* (Lepidoptera: Riodinidae) - a myrmecophilous butterfly obligately associated with fire ants. Psyche 2013: 1-10.
Knölke, S., S. Erlacher, A. Hausmann, M. A. Miller, and A. H. Segerer. 2004. A procedure for combined genitalia dissection and DNA extraction in Lepidoptera. Ins. Syst. Evol. 35: 401-409.
Kumar, V., B. K. Kariappa, A. M. Babu, and S. B. Dandin. 2007. Surface ultrastructure of the egg chorion of Eri silkworm *Samia ricini* (Donovan) (Lepidoptera: Saturniidae). J. Entomol. 4: 68-81.
Llorente-Bousquets, J. 1984. Sinopsis sistemática y biogeográfica de los Dismorphiinae de México con especial referencia al género *Enantia* Hübner (Lepidoptera: Pieridae*).* Fol. Entomol. Mex. 58: 1-207.
Llorente-Bousquets, J., y J. Castro-Gerardino. 2007. Estudios en sistemática de Dismorphiini (Lepidoptera: Pieridae) I. Morfología de huevos y su importancia taxonómica. Rev. Acad. Colomb. Ci. Exact. Fís. Nat. 31: 145-164.
Llorente-Bousquets, J., y J. Castro-Gerardino. 2008. Morfología del corion en Dismorphiini (Lepidoptera: Pieridae), pp. 71-90. *En* J. Llorente-Bousquets y A. Lanteri [eds.], Contribuciones Taxonómicas en Órdenes de Insectos Hiperdiversos. Las Prensas de Ciencias, UNAM, México DF.
Llorente-Bousquets, J., S. Nieves-Uribe, A. Flores-Gallardo, B. C. Hernández-Mejía, and J. Castro-Gerardino. 2018. Chorionic sculpture in the Dismorphiinae subfamily (Lepidoptera: Pieridae). Zootaxa 4429: 201-246.
Munguira, M. L., J. Martín, E. García-Barros, G. Shahbazian, and J. P. Cancela. 2015. Morphology and morphometry of Lycaenid eggs (Lepidoptera: Lycaenidae). Zootaxa 3937: 201-247.
Nieves-Uribe, S., A. Flores-Gallardo, B. C. Hernández-Mejía, y J. Llorente-Bousquets. 2015. Exploración morfológica del corion en Biblidinae (Lepidoptera: Nymphalidae): aspectos filogenéticos y clasificatorios. Southwest. Entomol. 40: 589-648.
Nieves-Uribe, S., J. Castro-Gerardino, A. Flores-Gallardo, y J. Llorente-Bousquets. 2016a. Corion en los géneros *Anteos* y *Rhabdodryas* (Pieridae: Coliadinae): su significado e implicaciones. Southwest. Entomol. 41: 485-504.
Nieves-Uribe, S., J. Castro-Gerardino, A. Flores-Gallardo, y J. Llorente-Bousquets. 2016b. Microrretícula coriónica en los géneros *Nathalis* Boisduval y

Kricogonia Reakirt (Lepidoptera: Pieridae): implicaciones taxonómicas y tendencias evolutivas. Southwest. Entomol. 41: 715-733.
Nieves-Uribe, S., J. Castro-Gerardino, A. Flores-Gallardo, y J. Llorente-Bousquets. 2016c. Estudio del corion de tres especies del género *Colias* Fabricius, 1807 y *Zerene cesonia cesonia* (Stoll, 1790). Southwest. Entomol. 41: 1121-1141.
Nieves-Uribe, S., A. Flores-Gallardo, y J. Llorente-Bousquets. 2016d. Morfología coriónica de once especies de Biblidinae (Lepidoptera: Nymphalidae) de México y Colombia: un examen de predicciones. Southwest. Entomol. 41: 505-532.
Nieves-Uribe, S., A. Flores-Gallardo, y J. Llorente-Bousquets. 2017a. Corion de *Ectima thecla thecla* (Fabricius, 1796) y su comparación con el género *Hamadryas*. Southwest. Entomol. 42: 851-864.
Nieves-Uribe, S., A. Flores-Gallardo, y J. Llorente-Bousquets. 2017b. Corion de *Orophila cardases campaspe* (Hewitson, 1869) y caracterización coriónica de la subtribu Callicorina. Southwest. Entomol. 42: 865-878.
Nieves-Uribe, S. A. Flores-Gallardo, y J. Llorente-Bousquets. 2018a. Corion de *Leucidia brephos* (Hübner, [1809]), comparaciones con otros géneros de Coliadinae y tendencias reticulares. Southwest. Entomol. 43: 239-255.
Nieves-Uribe, S., A. Flores-Gallardo, y J. Llorente-Bousquets. 2018b. Estructura exocoriónica en tres especies de *Colias* Fabricius, 1807. Southwest. Entomol. 43: 485-503.
Nieves-Uribe, S., A. Flores-Gallardo, J. Llorente-Bousquets, A. Luis-Martínez, and C. Pozo. 2019. Use of Exochorion characters for systematics of the genus *Hamadryas* in relation to *Ectima* (Nymphalidae: Biblidinae: Ageroniini). Zootaxa. En prensa.
Rezende, G. L., H. C. M. Vargas, B. Moussian, and E. Cohen. 2016. Composite eggshell matrices: chorionic layers and sub-chorionic cuticular envelopes, pp. 325-366. *In* E. Cohen and B. Moussian [eds.], Extracellular Composite Matrices in Arthropods. Springer International Publishing, Cham.
Richards, W., and R. G. Davies. 1977. Imms' General Textbook of Entomology. Vol. I. Structure, Physiology and Development. Chapman and Hall, London.
Skudlik, J., I. Poprawa, and M. M. Rost. 2005. The egg capsule of *Spodoptera exiqua* Hübner, 1808 (Insecta, Lepidoptera, Noctuidae): morphology and ultrastructure. Zoolog. Pol. 50: 25-31.
Srivastava, A. K., and K. Kumar. 2016. Ultrastructure of egg chorion of castor butterfly *Ariadne merione* (Crammer) (Lepidoptera: Nymphalidae). Zool. Anz. 263: 1-5.
Srivastava, A. K., V. A. Iconomidou, G. D. Chryssikos, V. Gionis, K. Kumar, and S. J. Hamodrakas. 2011. Secondary structure of chorion proteins of the Lepidoptera *Pericallia ricini* and *Ariadne merione* by ATR FT-IR and micro-Raman spectroscopy. Int. J. Biol. Macromol. 49: 317-322.
Telfer, W. H. 2009. Egg formation in Lepidoptera. J. Ins. Sci. 9, Art. 50: 1-21.
Thomson, G. 1992. Egg surface morphology of Manioline butterflies (Lepidoptera, Nymphalidae, Satyrinae). Atalanta 23: 195-214.
Trougakos, I. P., and L. H. Margaritis. 2008. Novel morphological and physiological aspects of insect eggs, pp. 3-36. *In* M. Hilker and T. Meiners [eds.], Chemoecology of Insect Eggs and Egg Deposition. Blackwell Publishing Company, Berlin.
Van der Poorten, G., and N. Van der Poorten. 2014. New and revised descriptions of the immature stages of some butterflies in Sri Lanka and their larval food plants

(Lepidoptera: Pieridae). Part 2: Subfamily Pierinae (in oart). Tijdsch. Vor. Entomol. 157: 1-25.
Wahlberg, N., J. Rota, M. F. Braby, N. E. Pierce, and C. W. Wheat. 2014. Revised systematics and higher classification of pierid butterflies (Lepidoptera: Pieridae) based on molecular data. Zool. Scr. 43: 641-650.
Yu-Feng, H. 2006. Butterflies of Taiwan. Vol. III. National Phoenix Valley Bird Park.
Yu-Feng, X. 2002. Butterflies of Taiwan. Vol. II. National Phoenix Valley Bird Park.

Diversidad de Hormigas en Ecorregiones de Durango, México

Ant Diversity in Different Ecological Regions of Durango, Mexico

Miguel Angel Soto-Cárdenas[1], Miguel Vásquez-Bolaños[2], Cipriano Gutiérrez-García[3], Miguel Mauricio Correa-Ramírez[4], René Torres-Ricario[5], Martha Celina González-Güereca[6], y Isaías Chairez-Hernández[7]*

Resumen. Dentro de los ecosistemas terrestres las hormigas son organismos muy diversos, exitosos, y abundantes. Alrededor del mundo se han descrito 15,945 especies pertenecientes a 290 géneros. Las ecorregiones influyen de manera directa en la diversidad y composición de la fauna de hormigas, ya que proporcionan sitios de nidificación, recursos alimentarios y microclimas. El objetivo de este trabajo fue conocer la diversidad de hormigas en las diferentes ecorregiones presentes en el estado de Durango, México. Se colectaron 2,340 hormigas de 40 especies, 24 géneros y cinco subfamilias, con diferencias en la composición de especies entre las ecorregiones: desiertos de América de Norte, elevaciones semiáridas meridionales, selvas cálido secas, y sierras templadas.

Abstract. In terrestrial ecosystems, ants are diverse, successful, and abundant organisms. Around the world, 15,945 species belonging to 290 genera have been described. Ecoregions directly influence the diversity and composition of ant fauna, since they provide nesting sites, food resources, and microclimates. This work aimed to learn the diversity of ants in the different ecoregions of Durango State, Mexico. We collected 2,340 individuals belonging to 40 species in 24 genera and five subfamilies. Differences in species composition were observed between the ecoregions of North American deserts, meridional semi-arid elevations, warm dry forest, and temperate sierra.

Introducción

Dentro de los ecosistemas terrestres las hormigas son organismos muy diversos, exitosos y abundantes. Se utilizan para evaluar la diversidad y el grado de disturbio de los ecosistemas (Majer 1983, Hölldobler y Wilson 1990, Fernández et al.

[1,4,5,6,7]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Durango, Sigma 119 Fracc. 20 de Noviembre II, 34220 Durango, Durango, México.
[2]Entomología, Centro de Estudios en Zoología, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km. 15.5 Carr. Nogales, Las Agujas, Zapopan, Jalisco. CP. 45110, México. Apdo. Postal 134.
[3]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Sinaloa, Bulevar Juan de Dios Bátiz Paredes #250, Colonia San Joachin. Guasave, Sinaloa.
*Autor de correspondencia ichairez@hotmail.com

1996, Agosti et al. 2000, Fernández 2003, Vásquez-Bolaños y Quiroz-Rocha 2013). Las hormigas, junto con abejas, avispas y otros insectos del orden Hymenoptera, poseen la organización social más compleja (Vásquez-Bolaños y Quiroz-Rocha 2013). Alrededor del mundo se han descrito 15,945 especies pertenecientes a 290 géneros, en 20 subfamilias, de las cuales 16 tienen representantes vivos, mientras que cuatro se conocen solo a partir de fósiles (Bolton et al. 2006, AntWeb 2017). Se presentan dentro de una amplia diversidad de ecosistemas terrestres (bosques templados, bosques tropicales, zonas áridas, urbanas, y cultivos) desde el nivel del mar hasta los 4000 msnm, concentrándose en su mayoría entre los 800 y 1,400 msnm (Hölldobler y Wilson 1990, Jaffé et al. 1993, Suárez et al. 2010, Ward 2010). Además, constituyen cerca del 15% de la biomasa animal alrededor del mundo (Agosti et al. 2000).

Las ecorregiones influyen de manera directa en la diversidad y composición de la fauna de hormigas, ya que proporcionan sitios de nidificación, recursos alimentarios. y microclimas (Wisdom y Whitford 1981, Leal 2003). Diversos estudios han descrito la diversidad y ensamblaje de las comunidades de hormigas en diferentes ecosistemas. Mackay y Mackay (2002) reportan un amplio estudio acerca de la mirmecofauna en el estado de Nuevo México, EEUU, en el cual reportaron 239 especies representando el 37% del total de las presentes para Estados Unidos de América, varias de estas especies se presentan en el estado de Durango, México, mientras que Ríos-Casanova et al. (2004), analizaron la composición de hormigas en una zona árida del Valle de Tehuacán en Puebla, México, encontrando 28 especies pertenecientes a cinco subfamilias, de las cuales Myrmicinae fue la que presentó el mayor número de especies. Del Toro et al. (2009), realizaron un estudio acerca del género *Liometoupum,* donde destaca solo una especie para Durango y González-Castillo et al. (2014), analizaron la composición y estructura de poblaciones de hormigas en una zona árida del estado de Durango, encontrando 15 especies pertenecientes a 15 géneros. A pesar de esto, son pocos los estudios que consideran más allá de la ecorregion desiertos de América del Norte en Durango, es por ello que el objetivo de este trabajo fue conocer la diversidad de especies de hormigas dentro de las ecorregiones del estado y observar la variación entre la composición de especies entre las ecorregiones. Ya que el estado posee una amplia diversidad de ambientes y aunado a un gradiente altitudinal, presenta una variedad de sitios de nidificación para las hormigas (Fernández 2003).

Materiales y Métodos

Área de Estudio. El presente trabajo se realizó en el estado de Durango, México, tomando en cuenta las cuatro ecorregiones presentes en el estado, que han sido el resultado de diversos factores físicos y biológicos: desiertos de América del Norte (región árida y semiárida), elevaciones semiáridas meridionales (región de los valles), sierras templadas (región de la sierra), y selvas cálido secas (región de las quebradas) (González-Elizondo et al. 2007). Se muestreó en 56 localidades distribuidas en 19 municipios (Fig. 1).

Colecta de Ejemplares. Se realizaron colectas desde agosto de 2016 hasta septiembre de 2017, en cada localidad en cuatro transectos de 100 m. Se colectaron los ejemplares que se encontraban forrajeando y en nidos que se observaron a simple vista. Además, se buscó bajo piedras, hojarasca, árboles, y arbustos hasta una altura de 1.75 m. Los ejemplares se preservaron en alcohol (96%) y fueron identificados hasta género y especie utilizando las claves: Del Toro et al. 2009,

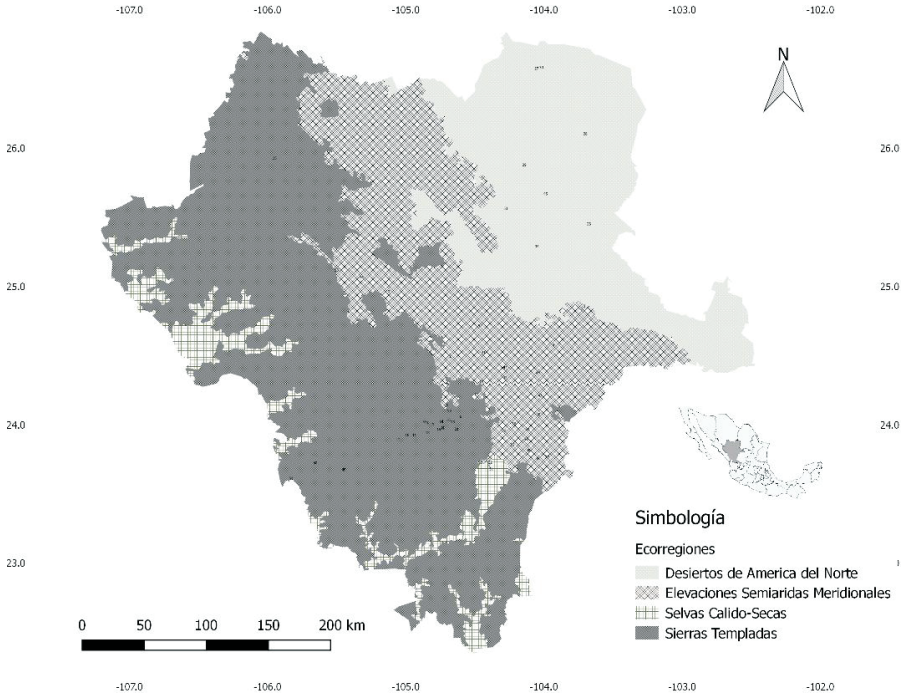

Fig. 1. Sitios muestreados y ecorregiones de Durango, México.
Fig. 1. Sampled sites and ecoregions of Durango, Mexico.

Demarco y Cognato 2015, Mackay y Mackay 1989, 2002, 2017. Los especímenes se depositaron en la colección entomológica del CIIDIR-Durango, México y en la del centro de Estudios en Zoología de la Universidad de Guadalajara, México.

Análisis de Diversidad. Para analizar la riqueza de hormigas se realizó una fluctuación altitudinal de especies. Para cada ecorregión se calcularon los índices de diversidad de Shannon (H´) y con ello estimar los patrones de ambientes perturbados (Majer 1983) y mediante los números de diversidad de Hill, especies abundantes (N_1) y muy abundantes (N_2) (Ludwing y Reynolds 1988). Adicionalmente, se obtuvieron los índices de Simpson (λ) para observar si dos individuos provenientes de una muestra al azar pertenezcan a la misma especie y Margalef (D_{mg}) para observar las zonas de baja y alta diversidad (Moreno 2001). Se realizó un ANOVA para observar las diferencias entre las ecorregiones. También, se llevó a cabo un análisis de similitud entre los sitios y entre las especies mediante la presencia/ausencia de estas, utilizando el algoritmo de Ward y distancia euclidiana con el paquete estadístico PAST versión 3.16 (Hammer et al. 2001).

Resultados y Discusión

Se colectaron 2,340 individuos los cuales pertenecen a 40 especies dentro de 24 géneros y cinco subfamilias (Dolichoderinae, Dorylinae, Formicinae, Myrmicinae,

y Ponerinae), donde Myrmicinae fue la más diversa con 21 especies, seguida de Formicinae con 12, Dolichoderinae con cinco, y Dorylinae y Ponerinae con una. La Fig. 2 muestra que Myrmicinae tiene la mayor abundancia relativa (60%) mientras que Ponerinae tiene la menor abundancia relativa (<1%).

La subfamilia Myrmicinae y Formicinae son las más ricas y abundantes respecto al número de especies que pertenecen a estas, alrededor del mundo y dentro de los diferentes ecosistemas (Ward 2010), en este trabajo se encontraron 21 especies de la subfamilia Myrmicinae y 12 especies de la subfamilia Formicinae, coincidiendo con los patrones mundiales reportados por este autor, debido a que, muestran un grado de evolución avanzado en su comportamiento y con ello la adaptación a diferentes ecosistemas. Por otro lado, estudios realizados en zonas áridas de México mostraron que la subfamilia Myrmicinae presenta una mayor riqueza en estas zonas (Rojas-Fernández 2001, Ríos-Casanova et al. 2004) y observaron que la subfamilia Ponerinae mostró proporciones bajas en otras zonas áridas del norte de México donde representa menos del 3% (Ríos-Casanova et al. 2004). Los resultados en este estudio coinciden en la proporción de especies de esta subfamilia presentes en el área de estudio, que, representó menos del 1% (Fig. 2). En regiones templadas de la Sierra Madre Occidental en Durango se han realizado pocos trabajos en esta zona destacando el de Del Toro et al. (2009) donde reporta una especie *Loimetopum apiculatum,* la cual se presenta desde los 1500 m hasta los 2500 m, a partir de esta altura es remplazada por *Liometopum luctuosum*, sin embargo, la información acerca de esta ecorregión es escasa (Rojas 2001).

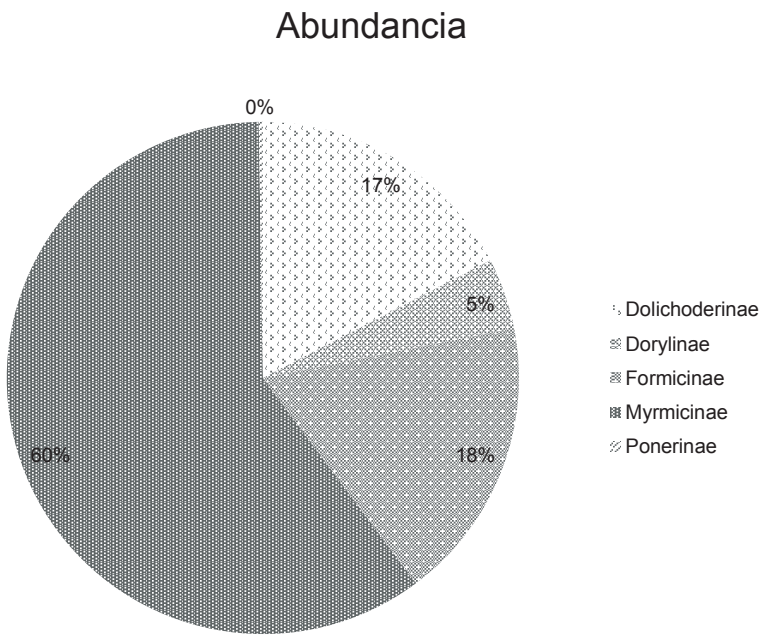

Fig. 2. Abundancia relativa de las subfamilias de hormigas en las ecorregiones de Durango, México.
Fig. 2. Relative abundance of ant subfamilies in ecoregions of Durango, Mexico.

En la Fig. 3 se observa la fluctuación altitudinal de especies en cada uno de los sitios muestreados dentro de las ecorregiones, en este estudio la mayor concentración de especies se presentó entre los 1800-2400 msnm, Bustos y Ulloa-Chacón (1996) señalan que los cambios en la composición de especies dentro de gradientes altitudinales se atribuyen a los diferentes hábitos de alimentación, anidación, y mircoclimáticos de las hormigas, lo que puede explicar que algunas especies en una de las ecorregiones se presenten en otra que cumpla con requeriemientos para el desarrollo de las mismas.

El cálculo de los números de especies abundantes (N_1) y número de especies más abundantes (N_2) para cada una de las ecorregiones fue: desiertos de América de Norte, N_1 = 4.1661 y N_2 = 1.5146; elevaciones semiáridas meridionales, N_1 = 9.1705 y N_2 = 1.1973; selvas cálido secas, N_1 = 3.8612 y N_2 = 1.3672; y sierras templadas, N_1 = 22.6917 y N_2 = 1.0555. Hölldobler y Wilson (1990) reportan que la mayor concentración de especies de hormigas está entre los 800 a 1,400 msnm. En este trabajo se encontró la mayor concentración de especies entre los 1,600 a 2,600 msnm (Fig. 3), abarcando principalmente las ecorregiones de elevaciones semiáridas meridionales y sierras templadas, las cuales se encuentran de los 1,600 msnm hasta los 3,340 msnm en su punto más alto, siendo parte de la región Neártica, donde los requerimientos de cada especie son pieza clave para el establecimiento de las mismas, así como la heterogeneidad ambiental (Fernández 2003, González-Elizondo et al. 2007). Fernández (2003) y Ward (2010) reportan que la mayoría de las especies de hormigas se encuentran en la región Neotropical, que incluye la ecorregión de las selvas cálido secas presente en el estado de Durango, sin embargo, la mayor parte de este pertenece a la región Neártica, por lo que, no se pudo comprobar lo establecido por los autores mencionados.

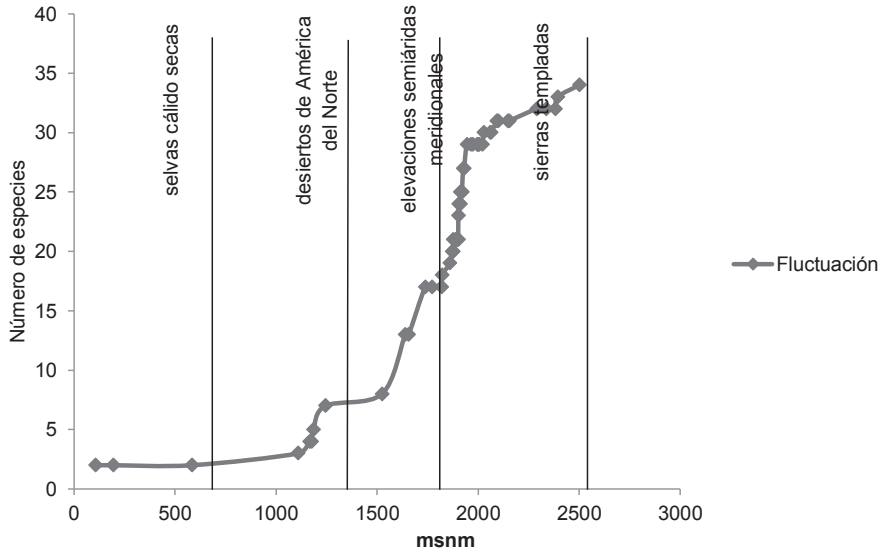

Fig. 3. Fluctuación altitudinal de especies de hormigas en las ecorregiones de Durango, México.
Fig. 3. Altitudinal fluctuation of ant species in ecoregions of Durango, Mexico.

El índice de diversidad de Shannon (H´) expresó los siguientes valores: desiertos de América de Norte, H´ = 1.42, elevaciones semiáridas meridionales, H´ = 2.21, selvas cálido secas, H´ = 1.35, y sierras templadas, H´ = 3.12. De acuerdo con González-Castillo et al. (2014) valores H´ <1.5 indican patrones de ambientes perturbados, en este estudio se encontraron valores menores a este en las ecorregiones desiertos de América del Norte y selvas cálido secas, por lo que, existen estos patrones en éstas. Rojas y Fragoso (2000) reportaron valores promedios de H´ = 3.4 en la región desiertos de América del Norte en una Reserva de la Biósfera, en este trabajo se encontró H´ = 1.42 para esta misma región, ya que, se realizó en sitios sin estatus de conservación y algunos de ellos perturbados por actividades agropecuarias. González-Castillo et al. (2014) encontraron H´ = 1.3 en elevaciones semiáridas meridionales en tres sitios de muestreo, este estudio abarcó 26 sitios de muestreo en esta misma región, obteniendo H´ = 2.21, la diferencia radica en el número de sitios de muestreo. Los valores del índice de Simpson para las especies de hormigas fueron de: desiertos de América de Norte de $\lambda = 0.54$, elevaciones semiáridas meridionales, $\lambda = 0.72$, selvas cálido secas, $\lambda = 0.73$, y para las sierras templadas, $\lambda = 0.94$. González-Castillo et al. (2014) reportaron valores de $\lambda = 0.38$ en elevaciones semiáridas meridionales en tres sitios de muestreo, mientras que en este trabajo el número de sitios fue de 26 y $\lambda = 0.72$, la diferencia es debido al número de sitios de muestreo. El índice de Simpson (λ) mostró que la probabilidad de tomar dos individuos provenientes de una muestra al azar pertenezcan a la misma especie es mayor en la ecorregión de la sierra templada, seguida por selvas cálido secas y elevaciones semiáridas meridionales, donde la heterogeneidad del hábitat representa un factor de gran importancia para este patrón (Bestelmeyer y Schooley 1999). El índice de Margalef (D_{mg}) mostro los siguientes valores: desiertos de América de Norte de $D_{mg} = 1.35$, elevaciones semiáridas meridionales, $D_{mg} = 3.07$, selvas cálido secas, $D_{mg} = 0.97$, y para las Sierras templadas, $D_{mg} = 4.35$, donde las ecorregiones desiertos de américa del Norte y selvas cálido secas presentan valores <2, lo que indica que son zonas de baja diversidad. En cambio, las ecorregiones elevaciones semiáridas meridionales y sierras templadas mostraron diversidad media con valores $2 \geq$ y ≤ 5, valores $D_{mg} < 2$ se consideran zonas de baja diversidad y valores >5 se consideran zonas de alta diversidad (Moreno 2001).

No se encontraron diferencias significativas entre las ecorregiones selvas cálido secas y desiertos de América del Norte para los tres índices medidos Shannon H´, Simpson (λ) y Margalef D_{mg}. (Cuadro 1), lo que indica que la riqueza y dominancia de especies dentro de estas no es diferente. Estrada y Fernández (1999) realizaron una comparación entre diferentes ecosistemas en Colombia (Potrero, Pastizal, y Bosque) encontrando diferencias entre los sitios de muestreo para la equitatibilidad (H´) $p < 0.0457$. En este estudio también se encontraron diferencias entre las distintas ecorregiones, este debido a la heterogeneidad botánica y microclimatica de los sitios muestreados, debido a que aportan requerimientos de hábitat y nutricionales para las diferentes especies que ahí habitan.

La Fig. 4, muestra la similitud entre los sitios de las ecorregiones de Durango, basado en la composición de especies. En el grupo $a_{1.1}$ se presentaron *Pogonomyrmex rugosus* y *Liometopum apiculatum.* El grupo $a_{1.2}$ se forma debido a la presencia de especies presentes en la ecorregión de las elevaciones semiáridas meridionales, mientras que el grupo a_2 es formado debido a la presencia de *Novomessor cockerelli*, la cual es una especie afín a ecosistemas áridos y semiáridos, se encontró en elevaciones semiáridas meridionales y semidesierto principalmente lo cual coincide con lo reportado en Antwiki (2019). En el grupo b, en

el subgrupo $b_{1.1}$ se formó por la presencia de la especie *Pogonomyrmex barbatus* en desiertos de América del Norte, elevaciones semiáridas meridionales y principalmente en sierras templadas, encontrando similitud en lo reportado por Mackay y Mackay (2002). El subgrupo $b_{1.2}$ fue formado por la diversidad presente en este, donde se concentran una gran parte de las especies en desiertos de América del Norte, selvas cálido secas y principalmente en elevaciones semiáridas meridionales. El grupo $b_{2.1}$ fue formado por especies que se presentan en zonas templadas como la ecorregión de sierras templadas y solamente en este grupo se encontró la especie *Lasius alienus* dentro de todos los sitios de muestreo. El subgrupo $b_{2.2}$ se generó debido a que en este se concentran especies que están asociadas a ecosistemas altamente degradados.

La Fig. 5, muestra el ensamblaje de las especies, donde se generaron grupos influenciados por el ambiente en el que se encontraron. El grupo a se formó principalmente por especies de la subfamilia Myrmicinae y asociadas a regiones áridas y semiáridas, mientras que el grupo b se formó por especies de varias subfamilias, y se formó un subgrupo b_1 en donde estas especies se presentaron en tres de las cuatro ecorregiones presentes en el estado de Durango México. Se formó otro subgrupo b_2, el que se compone de dos subgrupos internos, $b_{2.1}$ y $b_{2.2}$. El primero se presentó principalmente en la ecorregión de elevaciones semiáridas meridionales donde la mayoría de los géneros coinciden con los reportados por González-Castillo et al. (2014), mientras que, el segundo en la ecorregión de sierras templadas, donde, se observó que son especies con afinidad a ambientes fríos y templados. Es necesario tener en cuenta la estacionalidad ya que en este estudio se desconoció si esta afectó la abundancia de hormigas en el estado de Durango, México. La información generada puede ser de utilidad para futuras investigaciones.

Cuadro 1. Diferencias entre las Ecorregiones Mediante los Índices de Diversidad Medidos

Table 1. Differences between Ecoregions through Measured Diversity Indices

	DAN	ESM	SCS	ST
		Shannon H´		
DAN	1	0.001*	0.692	0.001*
ESM	0.001*	1	0.049*	0.001*
SCS	0.692	0.049*	1	0.001*
ST	0.001*	0.001*	0.001*	1
		Simpson (λ)		
	DAN	ESM	SCS	ST
DAN	1	0.001*	0.138	0.001*
ESM	0.001*	1	0.214	0.001*
SCS	0.138	0.214	1	0.001*
ST	0.001*	0.001*	0.001*	1
		Margalef D_{mg}		
	DAN	ESM	SCS	ST
DAN	1	0.001*	0.635	0.001*
ESM	0.001*	1	0.003*	0.001*
SCS	0.635	0.003*	1	0.001*
ST	0.001*	0.001*	0.001*	1

*Significativo $p < 0.05$; desiertos de América de Norte (DAN), elevaciones semiáridas meridionales (ESM) selvas cálido secas (SCS) y sierras templadas (ST).

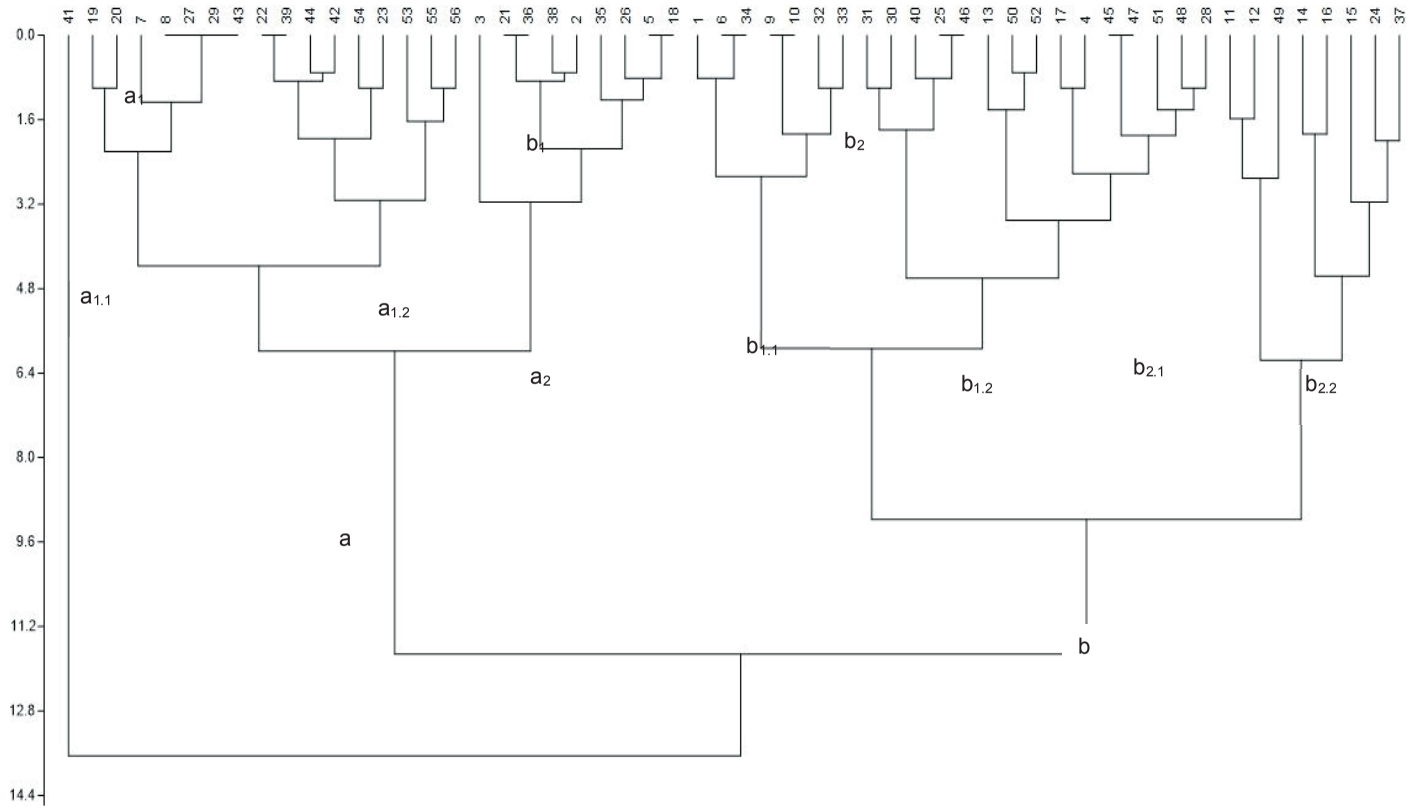

Fig. 4. Similitud entre los sitios basado en la composición de especies de hormigas en las ecorregiones de Durango, México.
Fig. 4. Similarity between sites based on composition of ant species in ecoregions of Durango, Mexico.

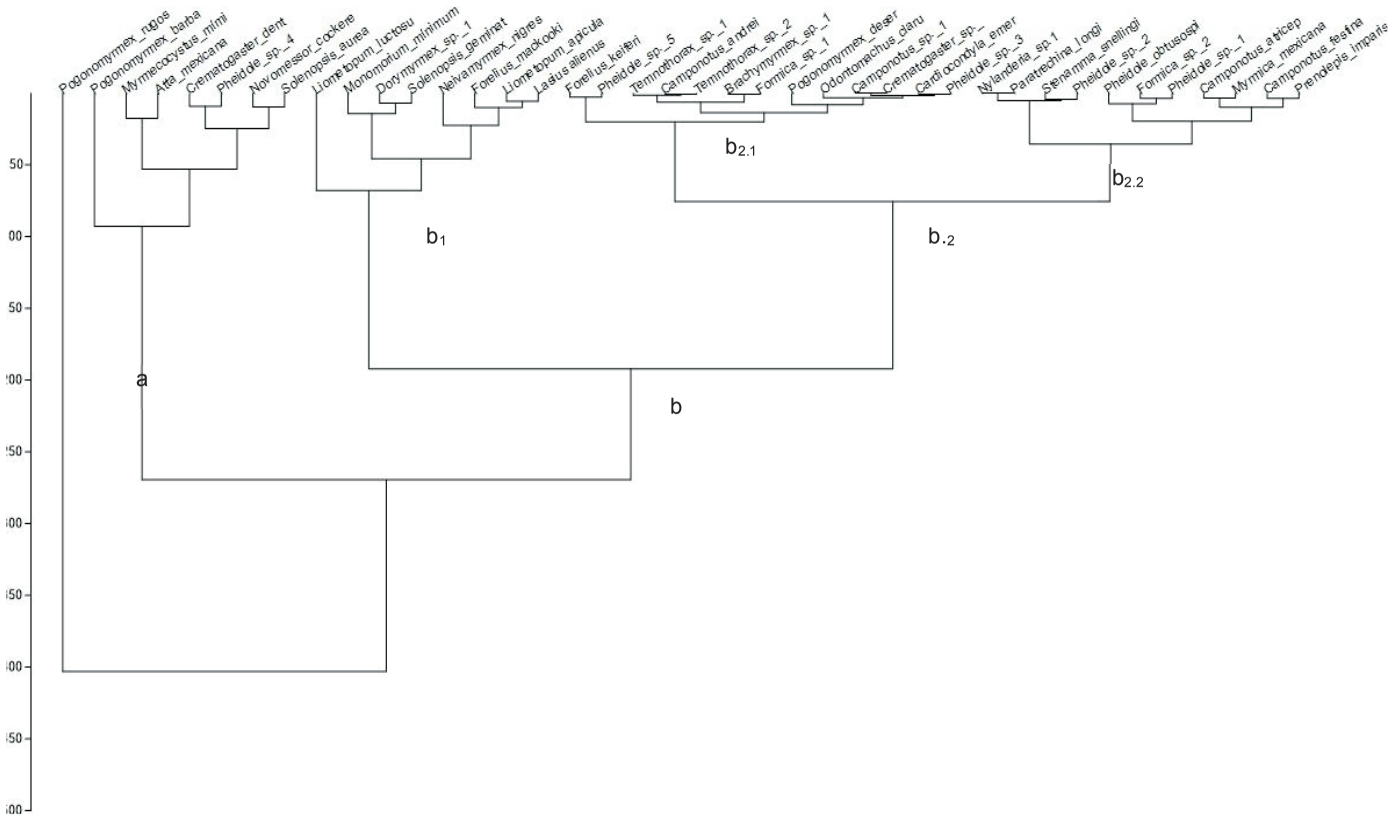

Fig. 5. Agrupamiento de especies de hormigas en los sitios muestreados en las ecorregiones de Durango, México.
Fig. 5. Clustering of ant species at sampled sites in ecoregions of Durango, Mexico.

Agradecimiento

Al Instituto Politécnico Nacional, al proyecto SIP: 20181619 y SIP: 20170564, parte del proyecto multidisciplinario 1808, por las facilidades prestadas para realizar el presente trabajo, así como al CONACyT por la beca de posgrado otorgada.

Referencias Citadas

Agosti, D. M., J. D. Alonso, L. E. Schultz, and R. Ted. 2000. Ants, pp. 304. Standard Methods for Measuring and Monitoring Biodiversity. Smithsonian Institution Press, Washington, DC.

AntWeb. 2017. AntWeb. https://www.antweb.org/ Accesado 28 Octubre 2017.

Antwiki. 2019. Antwiki. https:// http://www.antwiki.org/wiki/Novomessor_cockerelli/ Accesado 20 Enero 2019.

Armbrecht, I., y P. Ulloa-Chacon. 1999. Rareza y diversidad de hormigas en fragmentos de bosque seco colombianos y sus matrices. Biotropic. 31: 646-653. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1744-7429.1999.tb00413.x

Bestelmeyer, B. T., and R. L. Schooley. 1999. The ants of the southern Sonoran desert: community structure and the role of trees. Biodivers. Conserv. 8: 643-657.

Bolton, B., G. Alpert, P. S. Ward, and P. Naskrecki. 2006. Bolton's Catalogue of Ants of the World: 1758-2005. Harvard University Press, Massachusetts. CD-ROM.

Bustos, J., y P. Ulloa-Chacón. 1996. Mirmecofauna perturbación en un bosque de niebla neotropical (Reserva Natural Hato Viejo, Valle del Cauca, Colombia). Rev. Biol. Trop. 44/45: 259-266. https://revistas.ucr.ac.cr/index.php/rbt/ article/ view/22042/22220

Del Toro, I., J. A. Pacheco, and W. P. Mackay. 2009. Revision of the ant genus *Liometopum* (Hymenoptera: Formicidae). Sociobiol. 53: 299-369.

Demarco, B., and A. Cognato. 2015. Phylogenetic analysis of *Aphaenogaster* supports the resurrection of *Novomessor* (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 108: 201-210.

Estrada M., C., y C. F. Fernández. 1999. Diversidad de hormigas (Hymenoptera: Formicidae) en un gradiente sucesional del bosque nublado (Nariño, Colombia). Rev. Biol. Trop. 47: 189-201. http://www.scielo.sa.cr/scielo.php?pid=S0034-7441999000100019&script=sci_arttext

Fernández, F. 2003. Introducción a las Hormigas de la Región Neotropical, pp. 424. Bogotá, Colombia: Instituto de Investigación de Recursos. https://core.ac.uk/download/pdf/30440979.pdf

Fernández, F., E. E. Palacio, W. Mackay, y E. Mackay. 1996. Introducción al estudio de las hormigas, pp. 351-416. *En* M. G. Andrade, G. Amat-García, y G. Fernández [eds.], Insectos de Colombia: Estudios Escogidos Santafé de Bogotá, Colombia: Academia Colombiana de Ciencias Exactas, Físicas y Naturales.

González-Castillo, M. P., G. Hinojosa-Ontiveros, and D. Ramírez-Noya. 2014. Contribution to the current knowledge of ant communities (Hymenoptera: Formicidae) in a xerophitic scrubland of southeast Durango, Durango. Am. J. Appl. Sci. 11: 740-747.

González-Elizondo, M. D., M. González-Elizondo, y M. A. Márquez-Linares. 2007. Vegetación y ecorregiones de Durango. Instituto Politécnico Nacional, México, DF.

Hammer, Ø., D. Harper, and P. D. Rayan. 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electronica 4: 9.

Hölldobler, B., and E. O. Wilson. 1990. The Ants. Harvard University Press.

Jaffé, K., J. Lattke, y E. Pérez. 1993. El Mundo de las Hormigas. Equinoccio Ediciones, Universidad Simón Bolívar, Carácas, Venezuela.

Leal, I. R. 2003. Diversidade de formigas em diferentes unidades de Paisagem da Caatinga, pp. 435-461. *En* I. R. Leal, M. Tabarelli, y J. C. Silva [eds.], Ecologia e Conservação da Caatinga. Recifw, PE, Brasil: Editora Universitária UFPE.

Ludwing, J. A., and J. F. Reynolds. 1988. Statistical Ecology: a Primer on Methods and Computing. Wiley, New York.

Mackay, W. P., y E. Mackay. 1989. Clave de los géneros de hormigas en México (Hymenoptera: Formicidae). *En* Memorias del II Simposio Nacional de Insectos Sociales. Oaxtepec, Morelos. https://www.researchgate.net/profile/William_Mackay5/publication/255645332_CLAVE_DE_LOS_GENEROS_DE_HORMIGAS_EN_MEXICO_HYMENOPTERA_FORMICIDAE/links/53d673260cf2a7fbb2eaa573.pdf

Mackay, W., and E. Mackay. 2002. The Ants of New Mexico (Hymenoptera: Formicidae). Edwin Mellen Press.

Mackay, W., and E. Mackay. 2017. UTEP Biodiversity collections. http://www.utep.edu/leb/ants/Camponotus.htm Accessed 13 May 2017.

Majer, J. D. 1983. Ants: bio-indicators of minesite rehabilitation, land-use, and land conservation. Environ. Manag. 7: 375-383.

Majer, J. D., and G. Beeston. 1996. The biodiversity integrity index: an illustration using ants in Western Australia. Conserv. Biol. 10: 65-73.

Moreno, C. E. 2001. Métodos para Medir la Biodiversidad. Zaragoza: M&T–Manuales y Tesis SEA, Vol.1.

Ríos-Casanova, L., A. Valiente-Banuet, y V. Rico-Gray. 2004. Las hormigas del Valle de Tehuacán (Hymenoptera: Formicidae): una comparación con otras zonas áridas de México. Acta Zool. Mex. 20: 37-54. http://www.scielo.org.mx/pdf/azm/v20n1/v20n1a4.pdf

Rojas Fernández, P. 2001. Las hormigas del suelo en México: diversidad, distribución e importancia (Hymenoptera: Formicidae). Acta Zool. Mex. (nueva serie) (Especial 1): 189-238. http://www.redalyc.org/pdf/575/57500010.pdf

Rojas, P., and C. Fragoso. 2000. Composition, diversity, and distribution of a Chihuahuan Desert ant community (Mapimí, México). J. Arid Environ. 44: 213-227. https://www.sciencedirect.com/science/article/abs/pii/S0140196399905839

Suárez, A. V., T. P. McGlynn, and N. D. Tsutsui. 2010. Biogeographic and taxonomic patterns of introduced snts, pp. 233-244. *En* L. Lach, C. L. Parr, and K. L. Abbott [eds.], Ant Ecology. Oxford University Press, New York.

Vásquez-Bolaños, M., y G. A. Quiroz-Rocha. 2013. Introducción, pp. 7-22. *En* M. Vásquez-Bolaños, G. Castaño-Meneses, A. Cisneros-Caballero, G. A. Quiroz-Rocha, y J. L. Navarrete-Heredia [eds.], Formicidae de México. Órganica Editores, Guadalajara, Jalisco, México.

Ward, P. S. 2010. Taxonomy, Phylogenetics, and Evolution, pp. 3-17. *En* L. Lach, C. L. Parr, and K. L. Abbott [eds.], Ant Ecology. Oxford University Press, New York.

Wisdom, W. A., and W. G. Whitford. 1981. Effects of vegetation change on ant communities of arid rangelands. Environ. Entomol. 10: 893-897.

Nuevos Registros de Especies de Hormigas[1] para el Estado de Sinaloa, México

New Records of Ant[1] Species for Sinaloa, Mexico

Madai Rosas-Mejía[2]*, Miguel Vásquez-Bolaños[3], Griselda Gaona-García[2], y Venancio Vanoye-Eligio[2]

Resumen. Para obtener información sobre la presencia de especies de hormigas en Sinaloa, México; se realizaron recolectas directas en suelo y vegetación. Los especímenes fueron depositados en la colección entomológica del Instituto de Ecología Aplicada de la Universidad Autónoma de Tamaulipas (Ciudad Victoria, Tamaulipas, México). Se reporta por primera vez la presencia de 3 especies nativas: *Brachymyrmex depilis* Emery, *Monomorium cyaneum* Wheeler, y *Pseudomyrmex seminole* Ward, y una especie introducida: *Hypoponera opaciceps* Mayr. El número de géneros y especies conocidas para Sinaloa incrementó a 23 géneros y 52 especies, esta información sugiere que el inventario de especies aún es incompleto para este grupo, siendo esta entidad de las menos exploradas.

Abstract. To obtain information on the presence of ant species in Sinaloa, Mexico, direct collections were made in soil and vegetation. The specimens were deposited in the entomological collection of Instituto de Ecología Aplicada of Universidad Autónoma de Tamaulipas (Ciudad Victoria, Tamaulipas, Mexico). The presence of three native species is reported for the first time: *Brachymyrmex depilis* Emery, *Monomorium cyaneum* Wheeler, and *Pseudomyrmex seminole* Ward, and an introduced species: *Hypoponera opaciceps* Mayr. The number of genera and species known for Sinaloa increased to 23 genera and 52 species. This information suggests that the inventory of species is still incomplete for this group, considering the state is one of the least explored.

El grupo de insectos más exitoso en ecosistemas terrestres son las hormigas (Agosti y Johnson 2005). A nivel mundial se conocen 16,029 especies y subespecies de hormigas pertenecientes a 474 géneros en 20 subfamilias (Bolton 2018). En México se menciona el registro de 973 (Ríos-Casanova 2014) y 923 (Vásquez-Bolaños 2015) especies. Sin embargo ambos autores concluyen que es necesario incrementar la investigación en diversas regiones aún sin explorar.

---

[1]Hymenoptera: Formicidae

[2]Universidad Autónoma de Tamaulipas, Instituto de Ecología Aplicada, División del Golfo 356, Colonia Libertad, C.P. 87019, Cd. Victoria, Tamaulipas, México.
[3]Entomología, Centro de Estudios en Zoología, Departamento de Botánica y Zoología, Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km. 15.5 Carr. Nogales, Las Agujas, Zapopan, Jalisco. C.P. 45110, México. Apdo. Postal 134.
*E-mail address of corresponding autor: marosas@docentes.uat.edu.mx

El estado de Sinaloa con 22 géneros y 48 especies se encuentra dentro de las 10 entidades menos estudiadas en México (Vásquez-Bolaños 2015). En esta nota se reporta por primera vez la presencia de 3 especies nativas: *Brachymyrmex depilis* Emery, *Monomorium cyaneum* Wheeler, y *Pseudomyrmex seminole* Ward, y una especie introducida: *Hypoponera opaciceps* Mayr.

Se coleccionaron hormigas por medio de captura directa sobre el suelo y vegetación entre junio y septiembre del 2014, y entre febrero y abril del 2015 en los municipios de Ahome y Guasave: Tajito (25°39'27.4"N, 108°38'04.8"W), Juan José Ríos (25°45'23.7"N, 108°49'13.0"W), y Villa de Ahome (25°55'10.6"N, 109° 10'27.2"W). La distribución actual y la clasificación vigente de las especies se basó en Ríos-Casanova (2014), Vásquez-Bolaños (2015), Guénard et al. (2017), y AntWeb (2018). Los especímenes recolectados fueron depositados en la colección entomológica del Instituto de Ecología Aplicada de la Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, México.

Las especies con nuevos registros se presentan a continuación con información de recolecta y distribución.

Subfamilia Formicinae

Tribu Myrmelachistini

Brachymyrmex depilis Emery 1893. MÉXICO. Sinaloa, Ahome, Villa de Ahome. 25°55'10.6"N, 109°10'27.2"W, 10 m. 6.III.2015. Vegetación secundaria sobre *Ficus* sp. (6 obreras) M. Rosas-Mejía. Se distribuye desde Costa Rica hasta Canadá (Guénard 2017). Primer registro para Sinaloa. Se conocía de Baja California, Baja California Sur, Durango, Guerrero, Puebla, Sonora, Tamaulipas, y Veracruz (Vásquez-Bolaños 2015). Esta especie es oportunista de dieta generalista, habita en pastizales (Cook 2003, MacGown y Brown 2006).

Subfamilia Myrmicinae

Tribu Solenopsidini

Monomorium cyaneum Wheeler 1914. MÉXICO. Sinaloa, Ahome, Villa de Ahome. 25°55'10.6"N, 109°10'27.2"W 10 m. 8.IV.2015. Vegetación secundaria sobre *Leucaena leucocephala* (5 obreras). M. Rosas-Mejía. Se distribuye en México y Estados Unidos (Guénard et al. 2017). Primer registro para Sinaloa. Se conocía de Baja California Sur, Guanajuato, Hidalgo, Jalisco, Nayarit, Nuevo León, Puebla, Sonora, Tamaulipas, Veracruz, Estado de México, y Querétaro (Vásquez-Bolaños 2015). Esta especie se encuentra en diversos hábitats, desde zonas áridas hasta bosques húmedos de pinos (Mackay y Mackay 2002), se alimenta de néctar extrafloral y eliosoma de semillas (Oliveira et al. 1999, Salazar-Rojas 2012).

Subfamilia Ponerinae

Tribu Ponerini

Hypoponera opaciceps (Mayr 1887). MÉXICO. Sinaloa, Ahome, Juan José Ríos. 25°45'23.7"N, 108°49'13.0"W 12 m. 5.VI.2014. Cultivo de maíz, debajo de una roca (6 obreras). M. Rosas-Mejía. Su rango nativo de distribución se extiende desde Argentina hasta Guatemala, en América se distribuye como especie exótica desde el sur de México hasta Washington, Estados Unidos (Guénard 2017). Primer registro para Sinaloa. Se conocía para Baja California, Baja California Sur, Morelos, Quintana Roo, Tabasco, Tamaulipas, y Yucatán (Vásquez-Bolaños 2015). Habita en áreas húmedas, es una especie depredadora que se ha encontrado en el suelo de plantas en macetas, de esta manera puede ser transportada por el comercio (Fellers y Fellers 1982, MacGown y Hill 2009).

Subfamilia Pseudomyrmecinae

Tribu Pseudomyrmecini

Pseudomyrmex seminole Ward 1985. MÉXICO. Sinaloa, Guasave, El Tajito. 25°39'27.4"N, 108°38'04.8"W 15 m. 8.VII.2014. Vegetación secundaria, sobre *Acacia* sp. (2 obreras). M. Rosas-Mejía. Se distribuye desde Belice y Cuba hasta la costa del Golfo de México en los Estados Unidos (Guénard 2017). Primer registro para Sinaloa. Se conocía de Nuevo León, Tabasco, Tamaulipas, y Nuevo León (Vásquez-Bolaños 2015). Esta especie es arborícola, habita en tallos de las familias Poaceae y Fabaceae, presenta una dieta generalista, son depredadoras y visitantes extraflorales (Ward 1985, Wheeler y Stocks 2009).

Con los nuevos registros presentados se incrementa a 23 géneros y 52 especies de Formicidae para Sinaloa.

Agradecimiento

A Benoit Guénard por el acceso a la información de la base de datos: Global Ant Biodiversity Informatics (GABI).

Referencias Citadas

Agosti, D., and N. F. Johnson. 2005. Antbase. World Wide Web electronic publication. antbase.org, version (05/2005). Viewed 15 July 2018.

AntWeb. 2018. All AntWeb. https://www.antweb.org/. Viewed 10 June 2018.

Bolton, B. 2018. AntWeb. Bolton World Catalog. https://www.antweb.org/project.do? name=worldants. Viewed 21 May 2018.

Cook, J. L. 2003. Conservation of biodiversity in an area impacted by the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Biodivers. Conserv. 12: 187-195.

Fellers, J. H., and G. M. Fellers. 1982. Status and distribution of ants in the crater district of Haleakala National Park. Pac. Sci. 36: 427-437.

Guénard, B., M. D. Weiser, K. Gomez, N. Narula, and E. P. Economo. 2017. The Global Ant Biodiversity Informatics (GABI) database: synthesizing data on ant species geographic distribution. Myrmecol. News. 24: 83-89.

MacGown, J. A., and R. L. Brown. 2006. Observations on the high diversity of native ant species coexisting with imported fire ants at a microspatial scale in Mississippi. Southeast. Nat. 5: 573-586.

MacGown, J. A., and J. G. Hill. 2009. *Tapinoma melanocephalum* (Hymenoptera: Formicidae), a new exotic ant in Mississippi. J. Miss. Acad. Sci. 54: 172-174.

Mackay, W. P., and E. Mackay. 2002. The Ants of New Mexico (Hymenoptera: Formicidae). Edwin Mellen Press, Lewiston, NY.

Oliveira, P. S., V. Rico-Gray, C. Diaz-Castelazo, and C. Castillo-Guevara. 1999. Interaction between ants, extrafloral nectaries and insect herbivores in Neotropical coastal sand dunes: herbivore deterrence by visiting ants increases fruit set in *Opuntia stricta* (Cactaceae). Funct. Ecol. 13: 623-631.

Ríos-Casanova, L. 2014. Biodiversidad de hormigas en México. Rev. Mex. Biodivers. 85: 392-398.

Salazar-Rojas, B., V. Rico-Gray, A. Canto, and M. Cuautle. 2012. Seed fate in the myrmecochorous Neotropical plant *Turnera ulmifolia* L., from plant to germination. Acta Oecol. 40: 1-10.

Vásquez-Bolaños, M. 2015. Taxonomía de Formicidae (Hymenoptera) para México. MES 10: 1-53.

Ward, P. S. 1985. The Nearctic species of the genus *Pseudomyrmex* (Hymenoptera: Formicidae). Quaest. Entomol. 21: 209-246.

Wheeler Jr., A. G., and I. C. Stocks. 2009. *Wolcottia* (= *Isohydnocera*) *aegra* (Coleoptera: Cleridae): association with grasses (Poaceae), native *Spartina* spp. and the introduced *Eragrostis curvula*, and resemblance to co-occurring pseudomyrmecine ants. Fla. Entomol. 92: 648-652.

SCIENTIFIC NOTE

Influence of Cations on the Intestinal Proteolytic Activity of *Anticarsia gemmatalis* (Hübner)[1] Larvae

Nohemí Niño-García, Rodolfo Torres-delosSantos, Miguel Ángel Delgado García, Ernesto Rangel-Torres, Eduardo Padrón-Torres, and Reyna Ivonne Torres-Acosta*

Universidad Autónoma de Tamaulipas: Unidad Académica Multidisciplinaria Mante. Blvd. Enrique Cárdenas González No. 1201 Pte. Col. Jardín C.P. 89840. Cd. Mante, Tamaulipas, México

The digestive process of insects is key to developing mechanisms that regulate crop pests; various mechanisms have been sought to affect their activity, mainly, proteases and amylase inhibitors (Rodrigues and Machado 2011). Control strategies based on enzyme inhibition can be developed with knowledge of insect digestive physiology, particularly, enzyme diversity and digestive dynamics. Velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), is a voracious and abundant defoliator of soybean, *Glycine max* (L.) Merr., in tropical Mexico (Ávila and Rodríguez 2004). The most destructive larval instars are fourth through sixth. Mendonca et al. (2012) reported that larvae have trypsin-like serine proteinases and cysteine-proteinases in their digestive systems; these are possible control targets through their inhibition. However, reduction in the activity of digestive enzymes can be affected by other agents such as divalent cations including copper or zinc that can reduce hydrolytic capacity. Cations such as calcium, copper, manganese, and zinc have been used as foliar sprays to control insects (Fried et al. 2018). This indicates the potential of understanding the digestive process to develop control strategies against *A. gemmatalis*. The effect of cations on total intestinal proteolytic activity was evaluated from fourth-instar larvae of *A. gemmatalis* from soybean crops at Mante, Tamaulipas, Mexico, in September 2018. Enzymes were extracted according to Aguirrezabala-Campano et al. (2013).

Proteolytic activity was detected by using BApNA (Sigma-Aldrich, St. Louis, MO), a prototype substrate for trypsin-like enzymes and useful for detecting cysteine-proteinase (Mendonça et al. 2012, Torres-Castillo et al. 2016). Proteolytic activity was measured by 0.1 increases in absorbance at 410 nm after incubation for 20 minutes at 37°C. Serine proteinase activity was confirmed with 10 mM phenyl methanesulfonyl fluoride solution, and cysteine proteinase was confirmed with 10 mM trans-epoxysuccinyl-L-leucylamido(4-guanidino) butane (E-64) solution (Sigma-Aldrich, St. Louis, MO). Reactions were prepared with a 10 mM EDTA solution (Sigma-Aldrich) to determine their sensitivity to a chelating agent.

The effect of cations was determined by developing the proteolytic activity reaction using aliquots of 50, 100, and 200 µl of different cation solutions and adjusting final volumes as needed. Each cation was added using 100 mM solutions

[1]Lepidoptera: Noctuidae

*Corresponding author: ivonnetorresacosta@yahoo.com.mx

of $CaCl_2$, $MgCl_2$, $MnCl_2$, $ZnSO_4$, $CoCl_2$, $CuSO_4$, $NiCl_2$, and $AlCl_2$ in distilled water. All reactions were done in quadruplicate and analyzed by ANOVA and Tukey ($p \leq 0.05$).

Total proteolytic activity in the samples ranged between 2,600 and 4,500 proteolytic activity units per gram of fresh tissue. The predominant proteolytic activity was associated with trypsin and cysteine-poteinase activity, according to inhibition registered before the respective inhibitors. No inhibition against EDTA was observed, indicating independent activity associated with naturally occurring divalent cations (Fig. 1A). Total proteolytic activity in the presence of different cations showed

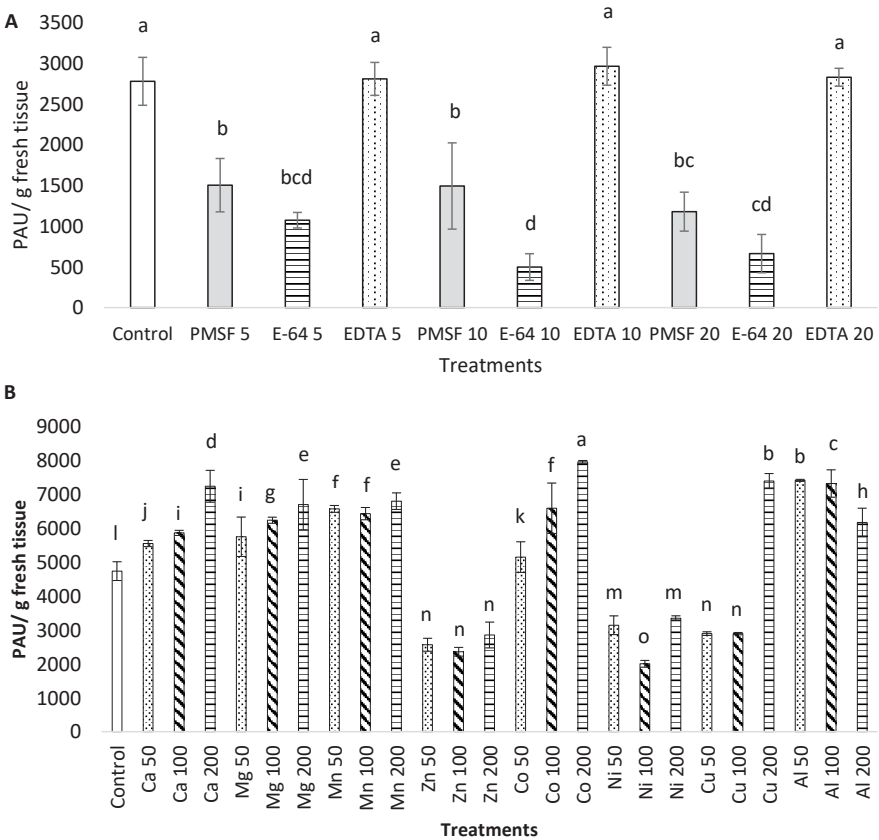

Fig. 1. Proteolytic activity from larvae of *Anticarsia gemmatalis*. A. Total proteolytic activity in the presence of specific inhibitors the respective volume. B. Total proteolytic activity in the presence of divalent cations, the chemical symbol of each element is shown, followed by the volume (50, 100, and 200 microliters) added to the reaction. Different letters indicate statistical differences ($p \leq 0.05$).

Fig. 1. Actividad proteolítica de larvas de *Anticarsia gemmatalis*. A. Actividad proteolítica total en presencia de inhibidores específicos con los volúmenes respectivos. B. Actividad proteolítica total en presencia de cationes divalentes, el símbolo químico de cada elemento se indica, seguido de la alícuota añadida a la reacción. Letras diferentes indican diferencias estadísticas ($p \leq 0.05$).

differential effect, because salts that increased activity were Ca, Mg, Mn, Co, and Al, while salts that decreased activity were Zn, Cu, and Ni with 50% less than the check (Fig. 1B). Increase in proteolytic activity is complements and potentiates the catalytic site of proteinases such as trypsin with calcium, although some cations reduce activity, either by altering charges on the molecule or competing with naturally occurring divalent cations associated with the active site. Results support differential sensitivity of proteinases from *A. gemmatalis* in the presence of the different cations, some of which have been indicated as commercial agents used to control pests (Fried et al. 2018). Although the mechanism of action is not yet known, cations are an interesting option with potential to affect the digestive process in insect pests.

Acknowledgment

The authors are thankful for financial support from PRODEP project UAT-PTC-241.

References Cited

Aguirrezabala-Cámpano, M. T., R. I. Torres-Acosta, A. Blanco-Labra, M. E. Mediola-Olaya, S. R. Sinagawa-García, A. Gutiérrez-Díez, and J. A. Torres-Castillo. 2013. Trypsin inhibitors in xoconostle seeds (*Opuntia joconostle* Weber.). J Plant Biochem. Biotechnol. 22: 261-268.

Ávila Valdez, J., and L. A. Rodríguez del Bosque. 2004. Control biológico del gusano terciopelo con el nucleopoliedrovirus de *Anticarsia gemmatalis* en soya. Folleto Técnico 17, Campo Experimental Sur de Tamaulipas, CIRNE-INIFAP. Altamira, Tam., México.

Fried, A., E. Schell, G. Steinecke, and K. Köppler. 2018. Control of *Drosophila suzukii* with DS-lime in black berries, pp. 196-199. *In* Proceedings of the 18th International Conference on Organic Fruit-Growing, 19-21 February 2018, Hohenheim, Germany. FÖKO.

Mendonça, E. G., M. G. A. Oliveira, L. E. Visôtto, and R. N. C. Guedes. 2012. Midgut cysteine-proteinase activity in the velvetbean caterpillar (*Anticarsia gemmatalis* (Hübner)). J. Pest Sci. 85: 117-123.

Rodrigues Macedo, M. L., and M. G. Machado Freire. 2011. Insect digestive enzymes as a target for pest control. Invertebrate Surviv. J. 8: 190-198.

Torres-Castillo, J. A., S. R. Sinagawa-García, G. Ruiz-De-La-Cruz, A. K. Gámez-Huerta, M. C. Juárez-Aragón, M. Lara-Villalón, and A. Mora-Olivo. 2016. Digestive proteinases and antioxidant capacity from *Pterophylla beltrani* Bolivar & Bolivar fed two natural diets. Southwest. Entomol. 41: 613-624.

MINUTES OF THE 2019 ANNUAL MEETING OF THE EXECUTIVE COMMITTEE OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS

The Executive Committee met Monday, 15 April 2019 before the annual meeting of the ESA Southwestern Branch at Tulsa, OK. President Manuel Campos presided. Others present were Bonnie Pendleton, Editor; Carlos Blanco, Editor for Mexican manuscripts; Wizzie Brown, Past-President; and Allen Knutson, Secretary-Treasurer. The Treasurer's report was distributed, and Knutson explained that the Society opened a new account with BBVA Compass bank to reduce banking fees imposed on authors in Mexico. Thus, in 2019, the Society began to transition transactions from Pavillion Bank to Compass Bank, and as a result two bank statements are summarized in the Treasurer's report. President Campos reported that some authors in Mexico had difficulty completing the banking information and offered to provide an example that could be sent to authors. Knutson proposed a policy for addressing the failure of some authors to pay page charges. This policy would prohibit authors and co-authors on the paper in question from publishing in the *Southwestern Entomologist* if they had unpaid page charges more than 9 months overdue. This will require the authors to complete the Order Form, showing the e-mail of all co-authors, at the time the manuscript is submitted. This policy was approved by the Executive Committee for presentation to the general membership. Editor Pendleton distributed the Editors' report and thanked Carlos Blanco for his service as Editor for Spanish manuscripts. Allen Knutson reported on merchant services fees and details for accepting credit card payments. Some authors ask to pay invoices by credit card, and USDA-ARS policy is pay by credit card. Also, members, especially those in Mexico, prefer to pay membership dues via credit card. Allen Knutson presented a draft proposal on a scholarship program sponsored by the Society of Southwestern Entomologists. The proposal would use income from Bio-One to sponsor four $2,500 scholarships each year. The Executive Committee approved the concept, but many details remain to be determined. In addition to the scholarship program, the Executive Committee discussed and approved using Bio-One funds to reduce page charges. President Campos discussed the value of a manuscript submission and tracking service, such as Allen Press, to the Society. The service was discussed, but no action was taken. There being no further business, President Campos adjourned the meeting.

MINUTES OF THE 2019 ANNUAL MEETING OF THE SOCIETY OF SOUTHWESTERN ENTOMOLOGISTS

The General Business Meeting was held Monday, 15 April 2019 following the Executive Committee meeting and before the annual meeting of the ESA Southwestern Branch at Tulsa, OK. President Campos presided. In addition to the Executive Committee, about 25 members were present. Secretary-Treasurer Knutson distributed the Minutes of the 2018 Annual Meeting, as published in the June 2018 issue of *Southwestern Entomologist*, and a motion was made, seconded, and passed to accept the minutes as published. Secretary-Treasurer Knutson distributed and reviewed the Treasurer's report (see attached). Editor Pendleton presented and reviewed the Editors' report (see attached) and thanked Carlos Blanco, Spanish Editor, for his work with the large number of papers submitted by authors in Mexico. Motions were made, seconded, and passed to accept the Secretary-Treasurer and Editors' reports.

The Secretary-Treasurer reported on efforts to enter into an agreement with a merchant service company to allow the Society to accept payment by credit card. As an example, one company charges 2.10% of each transaction plus a $16.00 monthly fee. Because of the Patriot Act, the company requires a Social Security number for the responsible person. Following further discussion, Knutson was asked to investigate other merchant service companies.

President Campos asked Knutson to report on a policy regarding unpaid page charges. The policy, as approved by the Executive Committee, will provide for contacting an author three times at about 30-day intervals regarding unpaid page charges. If the page charges remain unpaid after 9 months, manuscripts submitted by the author and all co-authors on the paper will not be accepted until the page charges are paid. To make co-authors aware of the policy, the corresponding author must copy by e-mail all co-authors at the time the manuscript is submitted. Also, the corresponding author must complete and return the Order Form before the manuscript can be considered for publication (is sent for review).

President Campos proposed that the Society consider the services of a manuscript submission and tracking service such as Allen Press. Such a service might make it more efficient to submit a manuscript and include automatic notification of manuscript submission and progress to authors and co-authors. This could modernize how the Society manages manuscripts. Following discussion, a motion was made by Justin Talley that a committee be formed to investigate the idea and contact Allen Press about services and costs and furthermore that the committee members shall consist of Editor Bonnie Pendleton, Spanish Editor Carlos Blanco, and the current members of the Editorial Committee. The motion was seconded and passed.

President Campos asked Secretary-Treasurer Knutson to report on his proposal regarding a scholarship program funded by the Society. Knutson reported that additional income generated by BioOne, $30,000-40,000 per year, was expected to continue and the Society could consider reducing page charges and/or using the funds in part to sponsor a scholarship program for students in the ESA

Southwestern Branch and Mexico. A proposal was distributed for awarding four $2,500 scholarships each year, two in the US and two in Mexico. Following further discussion, Justin Talley made a motion that a Scholarship Committee be formed to develop a program and conduct the program such that scholarships could be announced at the 2020 annual meeting. The Executive Committee will appoint the Committee members. The motion was seconded and approved.

President Campos opened discussion on using income from BioOne to reduce page charges. Greg Cronholm made a motion to reduce page charges for members from $40 to $20 per page, with the non-member fee to remain at $45 per page. The motion was seconded by Carlos Blanco and passed.

President Campos recognized Ed Bynum, representing the ESA SW Branch. Ed Bynum requested that the Society consider sponsoring 50% of the following awards with the cost to the Society of Southwestern Entomologist shown in ( ): Undergraduate Student Achievement Award ($25), Linnaean Games Team Awards ($100), Linnaean Games Team Travel Award ($1000), Linnaean Team 1st Place Plaque ($32), and the Photo Salon Awards ($125.00). A motion was made to approve the Society payment of 50% of these awards with an annual review to continue support. The motion was seconded and passed. It was noted that in 2018 the Society approved to pay 50% of the costs of the ESA SW Branch Early Career Award ($250).

President Campos thanked Bill Ree for maintaining the on-line voting site for electing President-elect, and the Nominations Committee for selecting nominees. The majority of ballots for President-elect were cast for Scott Armstrong. There being no further business, President Campos adjourned the meeting. Incoming President Justin Talley thanked Past-President Campos and presented him with a plaque recognizing his service as President of the Society of Southwestern Entomologists.

Secretary-Treasurer's Report for 2018
1 January 2018 – 31 December 2018

BBVA Compass Bank Account

Account opened October 2017 with deposit of $100.01. Only expense in 2017 was a $3.00 bank fee; no deposits were made. Balance in account on 31 December 2017 was $97.01.

Balance on hand 1 January 2018:	$ 97.01
Income:	
Memberships	$ 280.00
Subscriptions	$ 40.00
Page Charges	$ 32,851.72
BioOne Royalties	$ 40,344.00
Total Income:	$ 73,515.72
Expenses:	
Southwest Printing	$ 6,298.00
AdMail Mailing Service	$ 551.48
Editor Fee	$ 2,250.00
Secretary-Treasurer Fee	$ 2,250.00
Secretary to Editor-Fee	$ 0
Postage	$ 0
Bank Charges	$ 261.30
SW Branch Student Awards	$ 0
Total Expenses:	$ 11,610.78
Transfer to Money-Market Account:	$ 15,000.00
Balance on hand 31 December 2017:	$ 47,001.95

Money-Market Account at BBVA Compass Bank:

Initial deposit August 2018:	$ 15,000.00
Interest (1.489%) through 31 December 2018:	$ 15,065.59

Pavillion Bank Account:

Balance on hand 1 January 2018:	$ 74,129.77
Income:	
Memberships	$ 1,525.00
Subscriptions	$ 624.00
Page Charges	$ 10,359.12
Total Income:	**$** 12,508.12
Expenses:	
Southwest Printing	$ 21,434.90
AdMail Mailing Service	$ 1,570.97
Editor Fee	$ 2,250.00
Secretary to Editor Fee	$ 1,000.00
Secretary-Treasurer Fee	$ 2,250.00
Postage	$ 300.84
Bank Charges	$ 90.00
SW Branch Student Awards	$ 1,175.00
President's Plaque	$ 108.25
Total Expenses:	$ 30,179.96
Balance on hand 31 December 2017:	$ 56,457.93
Total Assets:	**$118,525.47**

As of December 2018, there were 96 members of the Society and 32 institutional subscribers. Sixty-six members requested a printed copy of the journal and 30 members did not. The total number of printed copies for members and subscribers was 98.

As of 1 March 2019, there were 10 unpaid invoices for page charges totaling $4,145. The Society had no outstanding debts.

Respectfully submitted,

Allen Knutson

Allen Knutson
Secretary-Treasurer

Southwestern Entomologist
Editors' Report
2018

A record 156 new manuscripts were submitted in 2018, of which 59 or 37.8% were in Spanish. The 156 manuscripts were 24 manuscripts or 17.9% more than the 134 manuscripts submitted in 2017, and nine manuscripts or 6.1% more than the 147 manuscripts submitted in 2016. Three manuscripts were withdrawn and 23 were rejected, for a total of 16.7%, in 2018.

In 2018, 115 manuscripts (90 regular articles and 25 scientific notes) were published in four regular issues of *Southwestern Entomologist* -- six manuscripts, or 4.9%, fewer than the 121 published in 2017, but four manuscripts, or 3.6% more than the 111 manuscripts published in 2016. Forty-nine (42.6%) of the 115 articles published in 2018 were written in Spanish; 40 of those in Spanish were regular articles and nine were scientific notes. In 2018, 1,082 pages were printed – 4.9% or 56 pages fewer than the 1,138 pages printed in 2017. Of the total 1,082 pages published in 2018, 453 or 41.9% were in Spanish. No Supplement was published in 2018.

We thank Wizzie Brown, Luis A. Rodríguez del-Bosque, and Tom A. Royer for providing photographs for the front covers of the issues of *Southwestern Entomologist* published in 2018. Southwest Printing printed the covers in color for no additional cost. Clear photographs of various arthropods always are needed for covers of the journal.

Carlos Blanco served again as Associate Editor for Spanish-language manuscripts. We thank Carlos for the extra effort required to process Spanish-language manuscripts.

Respectfully submitted,

Bonnie B. Pendleton, Editor
Carlos A. Blanco, Associate Editor

April 2019