Pachycrepoideus vindemmiae (Hymenoptera: Pteromalidae) as a Potential Natural Enemy of Maize-Infesting Ulidiidae

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Pachycrepoideus vindemmiae (Hymenoptera: Pteromalidae) as a potential natural enemy of maize-infesting Ulidiidae

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Abstract

Euxesta annonae Fabricius, *E. eluta* Loew, *E. stigmatias* Loew, and *Chaetopsis massyla* Walker (Diptera: Ulidiidae) are primary sweet corn pests in Florida. Few natural enemies of these flies are known. The pupal parasitoid *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae) was discovered in a laboratory colony of *E. eluta* and *E. stigmatias*, and its potential as a biological control agent was studied. Development times in fresh, chilled, and frozen *E. eluta* pupae were recorded. Fly larvae were allowed to dig into soil to pupate, and pupae covered by 2.5 cm of soil were presented to wasps to determine if *P. vindemmiae* could locate them. Finally, to evaluate susceptibility to insecticides commercially used in ear-stage sweet corn, adult parasitoids were caged on maize leaves treated with chlorpyrifos, methomyl, or zeta cypermethrin for 24 h. *Pachycrepoideus vindemmiae* completed development in normal, chilled, and frozen fly pupae in 15-17 days. None of the fly pupae covered by soil were parasitized. Chlorpyrifos and methomyl residues killed >95% of *P. vindemmiae* within 24 h. Zeta cypermethrin was slower acting, but resulted in 50% mortality after 24 h. Therefore, *P. vindemmiae* does not appear to be well suited as an effective biological control agent of maize-infesting Ulidiidae in sweet corn fields. This is the first known account of this cosmopolitan parasitoid attacking maize-infesting ulidiids.

Key Words: *Pachycrepoideus vindemmia*; *Euxesta eluta*; Ulidiidae; insecticides

Resumen

Un complejo de cuatro especies de Ulidiidae (Diptera) que infestan el maíz son plagas primarias del maíz dulce en la Florida. Se conocen pocos enemigos naturales de estas moscas. El parasitoide de las pupas, *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae), fue descubierto en una colonia de laboratorio de *Euxesta eluta* Loew (Diptera: Ulidiidae) y se iniciaron investigaciones sobre su potencial como agente de control biológico. Se registraron el tiempo de desarrollo en pupas de *E. eluta* frescas, refrigeradas y congeladas. Se les permitió a las larvas de la mosca enterarse en el suelo para pupar, y se presentaron pupas cubiertas por 2.5 cm de suelo a las avispas para determinar si *P. vindemmiae* podría localizarlas. Finalmente, para evaluar la resistencia a los insecticidas utilizados comercialmente en la etapa del elote del maíz dulce, los parasitoides adultos fueron enjaulados sobre las hojas de maíz tratados con clorpirifos, metomilo, o cipermetrina zeta por 24 horas. *Pachycrepoideus vindemmiae* completó su desarrollo en las pupas de la mosca saludables, refrigeradas y congeladas en 15-17 días. Ninguna de las pupas de la mosca cubierta por suelo fueron parasitadas. El Clorpirifos y los residuos de metomilo mataron el > 95% de *P. vindemmiae* dentro de las 24 horas. Zeta cipermetrina fue de acción más lenta, pero resultó en una mortalidad del 50% después de 24 horas. Por lo tanto, *P. vindemmiae* no parece ser muy adecuado como un agente de control biológico efectivo de las moscas Ulidiidae que infestan el maíz en campos de maíz dulce. Este es el primer relato conocido de este parasitoide cosmopolita que ataca las ulidiidas que infestan el maíz.

Palabras Clave: *Pachycrepoideus vindemmia; Euxesta eluta*; Ulidiidae; insecticidas

Euxesta eluta Loew, *E. stigmatias* Loew, *E. annonae* Fabricius, and *Chaetopsis massyla* Walker (colloquially referred to as silk flies, Diptera: Ulidiidae) are severe primary pests to Florida's 180,000,000 US dollar sweet corn crop (USDA, NASS 2013). *Chaetopsis massyla* has a widespread North American distribution (Steyskal 1965), while the three *Euxesta* species range from Georgia to throughout the Caribbean, and South America (Painter 1955; Frías 1981; Daly & Buntin 2005; Bertolaccini et al. 2010; Goyal et al. 2011). These flies are abundant throughout the year in southern and central Florida. Similar to other ulidiids, they utilize a wide variety of damaged and decaying host plants common in the area, including sugarcane, amaranth, and tomato (Seal & Jansson 1989; Goyal et al. 2012). Unlike other ulidiids, they also oviposit in healthy, undamaged sweet and field corn silk, and ears can be infested with hundreds of larvae (Bailey 1940). Larvae that penetrate through the silk channel disperse into the ear and cob, and feed on kernels, which then turn brown and decay (Barber 1939). Multiple insecticide treatments during the corn reproductive stages are required to produce a marketable crop. In untreated fields, ear infesta-

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tion may reach 90 % (Seal & Jansson 1989). Maggots typically leave the ear to pupate in the soil, but pupae are often found in the silk channel and in emerging tassels of maize plants damaged by fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae).

Laboratory colonies *E. eluta* and *E. stigmatias* (identified using unpublished keys by Ahlmark & Steck (unpublished), were created at the Everglades Research and Education Center in Belle Glade, Florida by rearing larvae out of corn ears and sweep-netting adult flies from corn fields from Jan to Mar 2013. A pupal parasitoid of unknown origin was discovered in the colony on 25 Apr 2013, and investigations were undertaken to examine its potential as a biological control agent of the maize-infesting Ulidiidae.

Materials and Methods

Adult specimens of the parasitoid were reared from *E. eluta* pupae, preserved in 90% ethanol, and sent to the National Museum of Natural History, Washington DC for identification and voucher preservation. Parasitoids were identified to the genus *Pachycrepoideus* (using Bouček & Heydon 1997) and later confirmed as *Pachycrepoideus vindemmiae* Rondani (Hymenoptera: Pteromalidae). Voucher specimens of fly host and parasitoid provided to the NMNH were assigned the following labels: male *P. vindemmiae* as USNMENT00917265 to USNMENT00917269, female *P. vindemmiae* and parasitized *E. eluta* pupae as USNMENT00917255 to USNMENT00917259, male *E. eluta* as USNMENT00917260 to USNMENT00917264, and female *E. eluta* as USNMENT00917250 to USNMENT00917254. Before its identity was confirmed, several experiments were conducted to evaluate acceptable hosts, development rates, and ability to find *E. eluta* pupae in a simulated field condition.

FLY AND PARASITOID COLONY MAINTENANCE

Euxesta eluta adults were maintained in screen cages in a climate controlled room with a temperature setting of 26.7 °C, 40-60% RH and a 12:12 h L:D cycle. The flies were provisioned with sugar water for food and a blended frozen corn diet ovipositional substrate placed into Fisherbrand® glass disposable culture tubes (Thermo Fisher Scientific, Waltham, Massachusetts). Tubes were removed from the colony after 3 days and capped with cotton. Third instar fly larvae leave the diet to pupate in the cotton cap, which was removed to a separate cage for adult emergence. All experiments with *P. vindemmiae* used *E. eluta*, because the *E. stigmatias* colony was destroyed by the parasitoid shortly after its contamination was noted. The parasitoid colony was maintained in a mesh sleeve cage and held at room temperature. Parasitoids were supplied with fly pupae at regular intervals.

PARASITOID DEVELOPMENT AND HOST SEARCHING

Parasitoid development was evaluated by first caging *E. eluta* pupae with *P. vindemmiae* adults. Four groups of 5 *E. eluta* pupae were exposed to groups of 3 wasps of mixed sex for 2 h in 20-dram polystyrene vials (Thornton Plastic Co, Salt Lake City, Utah) in the climate controlled fly colony. To determine whether *P. vindemmiae* would accept alternatives to fresh live fly pupae for the purposes of mass production, refrigerated and frozen pupae were also presented to the parasitoids for testing. Four groups of 5 pupae were refrigerated for 4 days at 4 °C and 2 groups of 5 pupae were frozen prior to the 2-h exposure period. Pupae were checked 4 times a day for 3 weeks.

The majority of silk fly larvae leave maize plants to pupate within the soil under field conditions; however, the actual position of these pupae within the soil profile has never been documented. For this parasitoid to be effective in controlling the flies, they must locate and parasitize pupae and escape the soil as an adult. First, fly pupation depth was evaluated by suspending tubes containing unknown numbers of third instar *E. eluta* larvae horizontally for 3 days over a 15 cm^2 tray containing 5.5 cm of Dania-muck soil (organic Histosol found throughout the Everglades Agricultural Area) taken from a sweet corn field. At the end of the 3 days, soil was carefully scraped with an index card and the larval and pupal depths were recorded. The ability of the parasitoid to locate and parasitize the fly pupae was evaluated by covering 4 groups of 5 fly pupae to the average depth determined from the test above and exposing them to groups of 3 parasitoid adults for 24 hours. Buried pupae were held, covered by soil, for four weeks to allow for any potential parasitioid emergence from the pupae.

INSECTICIDE BIOASSAYS

Insecticide assays were performed in the laboratory to determine how *P. vindemmiae* might be affected by grower practices in sweet corn fields. Sweet corn leaf sections (5-cm long) were excised from pre-tassel-push (whorl-stage) plants and sprayed with high label rates (for fall armyworm or ulidiids) of either chlorpyrifos (Lorsban Advanced®, Dow AgroSciences LLC, Indianapolis, Indianapolis) at a rate of 208 g a.i./ha (1.2 L/ha), zeta cypermethrin (Mustang®, FMC Corporation, Philadelphia, Pennsylvania) at a rate of 56 g a.i./ha (0.3 L/ha), or methomyl (Sinon Corporation, Taichung Hsien, Taiwan) at a 312 g a.i./ha rate (1.75 L/ha). Treatments were applied using a Generation III Research Track Sprayer (DeVries Manufacturing, Hollandale, Minnesota) fitted with an 8003 TeeJet XR flat fan nozzle (Spraying Systems Co., Wheaton, Illinois) and calibrated to deliver a volume of 187 L/ha. One hour after treatments were applied, treated leaves were placed on moist 7.0 cm diam filter paper (Whatman®, Whatman International Ltd., Maidstone, England) within 7.0 cm diam polystyrene plastic dishes (1 leaf section per dish). Twelve to 18 adult parasitoids (2 to 5-days-old) were aspirated from the colony and sealed within these dishes to evaluate survivorship to insecticide exposure. Parasitoid survivorship was evaluated at 1, 4, and 24 hours post introduction. Parasitoids that did not move when repeatedly probed were recorded as dead, while those displaying uncoordinated twitching were recorded as displaying sublethal effects. Treatments were replicated 4 times.

STATISTICAL ANALYSIS

The proportion of dead wasps, wasps exhibiting sublethal effects, and healthy wasps was calculated. Residuals were not normally distributed, even after data transformation. Therefore, non-parametric Kruskal-Wallis tests were performed on *P. vindemmiae* response to insecticide residues for each observation time using SAS JMP® v. 8.0 (SAS Institute 2009). Data were weighted according to the number of wasps in each replicate to account for replicate differences. The Tukey-Kramer HSD was used to compare means ($\alpha = 0.05$).

Results

DEVELOPMENT AND HOST SEARCHING

A total of 10 *P. vindemmiae* emerged out of 15 healthy fly pupae (not chilled or frozen) between 369 (15.4 days) and 429 h (17.9 days) after exposure of *E. eluta* pupae to adult parasitoids. One group of 5 pupae were not parasitized and excluded from the data. Of the 20 refrigerated pupae exposed to parasitism, 15 were successfully parasitized and adults emerged between 14 and 17 days after exposure to the pupae. Adult flies emerged out of the remaining pupae, indicating that *E. eluta* pupae can survive prolonged refrigeration. Of the 10 frozen fly pupae presented to adults for oviposition, 4 ultimately resulted in parasitoid wasp emergence between 14 and 16 days after exposure to the wasps.

Twenty six *E. eluta* pupae and nine *E. eluta* larvae were recovered from the soil 3 days after being placed beneath diet tubes containing late instar maggots. Mean pupal depth was 2.4 cm and mean larval depth was 2.7 ± 0.4 cm. Overall mean depth of recovered *E. eluta* larvae and pupae was 2.5 ± 0.2 cm below the soil surface. No parasitoids were observed emerging from the buried pupae. Upon soil examination, no parasitoids that may have emerged from pupae were detected in the soil. Most of the fly pupae successfully completed development and flies were observed in the soil and above the soil. None of the dead pupae appeared to have been parasitized.

There were significant treatment effects on the proportion of dead wasps after 1 h (χ^2 = 12.8, df = 3, *P* = 0.005), 4 h (χ^2 = 14.2, df = 3, *P* = 0.003), and 24 h exposure (χ^2 = 14.2, df = 3, *P* = 0.003) to dried insecticide residues on leaves. There were significant treatment effects on the proportion of wasps exhibiting sublethal effects only after 24 h of exposure (χ^2 = 8.3, df = 3, *P* = 0.04). There were significant treatment effects on the proportion of surviving wasps after 1 h (χ^2 = 13.4, df = 3, *P* = 0.004), 4 h (χ^2 = 14.8, df = 3, *P* = 0.002), and 24 h (χ^2 = 14.7, df = 3, *P* = 0.002) post exposure. Chlorpyrifos and methomyl resulted in rapid mortality (Table 1). Zeta-cypermethrin was slower to affect *P. vindemmiae*, but after 24 h nearly 50% of wasps were dead or incapacitated. None of the wasps exposed to the control water-treated leaves died.

Discussion

This is the first report of *P. vindemmiae* attacking pupae of *E. eluta* and *E. stigmatias*, and also the first report of a parasitoid attacking these flies in the United States. *Pachycrepoideus vindemmiae* is most commonly reported as a larval-pupal/pupal ectoparasitoid of cyclorrhaphous Diptera (12 families; Carton & Sokolowski 1994; Aluja et al. 1998), but is also known to be a facultative hyperparasitoid (Van Alphen & Thunnissen 1984; Wang & Messing 2004). Two other *Euxesta* parasitoids, a *Spalangia* pupal parasitoid (Hymenoptera: Pteromalidae) and *Dettmeria euxestae* Borgmeier (Hymenoptera: Eucoilidae), a larval parasitoid, have been recorded to parasitize silk fly pupae found in sweet corn ears in Sinaloa, Mexico and in Brazil and Argentina, respectively (Valicente 1986; Bertolaccini et al. 2010; Báez et al. 2012).

Table 1. Mean ± SEM[1] percentage of parasitoids affected by high label rate leaf residues of zeta cypermethrin, chlorpyrifos, and methomyl at several different periods of exposure.

Insecticide	Dead	Sublethal effects[2]	Alive
1-h exposure			
Water control	0 b	0 a	100 a
Zeta-cypermethrin	6.7 ± 6.7 b	6.7 ± 6.7 a	86.7 ± 7.7 a
Chlorpyrifos	89.8 ± 4.2 a	10.2 ± 4.2 a	0 b
Methomyl	78.8 ± 14.5 a	10.8 ± 8.1 a	10.5 ± 7.3 b
4-h exposure			
Water control	0 c	0 a	100 a
Zeta-cypermethrin	15.7 ± 3.9 b	11.8 ± 5.7 a	72.6 ± 7.7 b
Chlorpyrifos	100 a	0 a	0 c
Methomyl	95.8 ± 4.2 a	4.2 ± 4.2 a	0 c
24-h exposure			
Water control	0 c	0 b	100 a
Zeta-cypermethrin	38.2 ± 7.2 b	8.6 ± 3.3 a	53.2 ± 10.1 b
Chlorpyrifos	100 a	0 b	0 c
Methomyl	98.6 ± 1.4 a	1.4 ± 1.4 ab	0 c

[1]Means in the same column and exposure time followed by different letters are significantly different at α = 0.05 (Tukey Kramer HSD).

[2]Sublethal effects were counted as wasps exhibiting uncoordinated twitching and could not right themselves.

The developmental period of this parasitoid in ulidiid pupae (15.4 – 17.8 days) was similar to reported development times at room temperature in *Piophila casei* L. (Diptera: Piophilidae) (17.5–18.5 days, min 15 days) (Crandell 1939) and *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) pupae (18–19 days at 25 °C) (Nøstvik 1954). Furthermore, our observations of successful development from refrigerated and frozen pupae support earlier observations of *P. vindemmiae* developing from frozen *P. casei* and *Musca domestica* L. (Diptera: Muscidae) pupae (Crandell 1939; Rueda & Axtell 1987), although a lower proportion of frozen pupae were attacked in our study. These data indicate it should be easy to produce the parasitoid in large numbers.

The success of a natural enemy depends on its ability to locate and attack hosts in the field. Previously, the soil depth of *E. eluta* pupation in the field was unknown. We observed *E. eluta* pupating at a mean soil depth of 2.5 cm in our study, but no parasitoids emerged from any soil-covered pupae in our trial. *Pachycrepoideus vindemmiae* also did not dig in soil to locate buried tephritid pupae buried between 1.0 and 7.0 cm (Guillén et al. 2002). Most *E. eluta* that move to the soil to pupate appear to be safe from *P. vindemmiae* attack, although parasitism of pupae buried at shallower soil depths < 1.0 cm could be further evaluated.

Sweet corn is sprayed intensively with insecticides during the tassel push through ear development period to control ulidiid flies, *S. frugiperda*, and *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae). If pyrethroids were sprayed in the fields, then it is possible that *P. vindemmiae* might be able to find some pupae before becoming intoxicated. After this period, fields are frequently sprayed with pyrethroids and methomyl until harvest. Their susceptibility to insecticides indicates that parasitoids would not survive well in sweet corn fields until after harvest, when growers stop their insecticide programs.

Other habitats within southern Florida may provide more suitable reservoirs for *P. vindemmiae* from which they could parasitize maize-infesting ulidiid species. While the majority of these ulidiid larvae pupate in the soil, some pupate in exposed positions on emerged corn tassels, or on dried silk inside and outside of the silk channel. Ulidiid pupae are more frequently recovered in field corn silk rather than sweet corn silk, and field corn in the area is normally not widely treated for Lepidoptera larvae or flies. In addition to maize, these fly species also complete development within partially exposed insect tunnels in stems, and in decomposing fruits of many other crops that may not receive insecticides, particularly in agronomic crops and abandoned fruiting vegetable fields (Goyal et al. 2012). *Pachycrepoideus vindemmiae* might still attack exposed silk fly pupae in such situations, including in *Diatraea saccharalis* (Fabricius) (Lepidoptera: Crambidae) tunnels in surrounding sugarcane fields (e.g., > 400,000 acres surrounding sweet corn in southern Florida) and in mechanically-damaged amaranth and grass weeds. Additional studies are planned to evaluate the suitability of such exposed ulidiid pupae for successful parasitism by *P. vindemmiae*.

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