THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA*, IN THE VIRGIN ISLANDS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

The best known and most destructive exotic ant species in the US is the red imported fire ant, *Solenopsis invicta* Buren. Recently, this species has been reported from several islands in the West Indies, including St. Croix, US Virgin Islands (USVI) and Guana Island, British Virgin Islands (BVI). In the present study, we report new records of *S. invicta* on St. Croix (13 sites) and the first records of *S. invicta* from 3 other of the Virgin Islands: St. Thomas, USVI (7 sites), St. John, USVI (2 sites), and Tortola, BVI (6 sites). *Solenopsis invicta* appears to be well established in disturbed open environments on all 4 islands. It is important that people in the Virgin Islands and elsewhere in the West Indies, particularly healthcare professionals, are aware of the presence of *S. invicta*, can recognize the symptoms of *S. invicta* stings, and know proper treatments for adverse reactions to the stings, including rare but potentially deadly anaphylactic shock.

Key Words: exotic species, fire ants, *Solenopsis invicta*. Virgin Islands, West Indies

RESUMEN

La especie de la hormiga exótica mejor conocida y muy destructiva en los EEUU es la importada hormiga roja del fuego, *Solenopsis invicta*. Esta especie se ha sido reportado recientemente en varias islas en las Antillas, inclusive S. Croix, las Islas Virgenes de EEUU (USVI) y la Isla de Guana, las Islas Virgenes inglesas (BVI). En el estudio presente, nosotros reportamos nuevos registros de *S. invicta* en el S. Croix (13 sitios) y los primeros registros de *S. invicta* de tres otras de las Islas Virgenes: S. Thomas, USVI (7 sitios), S. John, USVI (2 sitios), y Tortola, BVI (6 sitios). *Solenopsis invicta* aparece ser establecido bien en ambientes abiertos perturbados en las cuatro islas. Es importante que personas en las Islas Virgenes y en otras partes en las Antillas, particulamente profesionales de cuidado medico, están avisado de la presencia de *S. invicta*. Estos profesionales deben de reconocer los síntomas de la picada de *S. invicta*, y saber los tratamientos para reacciones adversas a la picada.

Translation provided by the authors.

The best known and most destructive exotic ant species in the US is the red imported fire ant, *Solenopsis invicta* Buren, which arrived in Alabama by ship from South America sometime before 1945 (Buren et al. 1974). Since then, this predatory ant has spread across the US from Texas to North Carolina in the southeast and California in the west, particularly in open disturbed areas, causing ecological and economic damage (e.g., see Tschinkel 1988, 1993; Allen et al. 2004; Wetterer & Moore 2005). *Solenopsis invicta* is well-known for its powerful sting, which causes a burning sensation in humans, usually followed within one or two days by the appearance of a white pustule. These pustules are diagnostic for the stings of *S. invicta* and other *Solenopsis saevissima* complex fire ants from South America (S. Porter, pers. comm.). The stings of other ants, including the widespread tropical fire ant, *Solenopsis geminata* (Fabricius), do not produce pustules. The venom has hemolytic and neurotoxic properties and may cause allergic responses and result in secondary infections, sepsis, anaphylactic shock, and even death (Prahlow & Barnard 1998; deShazo et al. 2004).

The earliest known West Indian records of *S. invicta* are from Puerto Rico (Buren 1982), where it is now widespread (Torres & Snelling 1997; Davis et al. 2001; RRS & JKW, unpublished data). More recently, *S. invicta* has been reported from numerous other islands in the West Indies (Table 1), including the Virgin Islands, which lie to the east of Puerto Rico. Davis et al. (2001) published records of *S. invicta* from St. Croix, US Virgin Islands (in 1997: Fredensborg National Guard facility, and in 2000; Route 66, 0.8 km east of Route 663) and from Guana Island, a small island north of Tortola, British Virgin Islands (BVI, in 1996). *Solenopsis invicta* closely resembles *S. geminata*, both in appearance and in the pain of its sting.

TABLE 1. EARLIEST KNOWN SPECIMEN RECORDS FOR *SOLENOPSIS INVICTA* ON ISLANDS OF THE WEST INDIES. THE ASTERISK (*) INDICATES DATE PROVIDED BY M. DEYRUP.

Island	Year	Source reference
Puerto Rico	1981	Buren 1982
St. Croix, USVI	1988	present study
San Salvador, Bahamas	1993*	Deyrup 1994
New Providence, Bahamas	1995*	Deyrup et al. 1998
North Andros, Bahamas	1996*	Deyrup et al. 1998
Guana Island, BVI	1996	Davis et al. 2001
Gorda Cay, Bahamas	1997	Davis et al. 2001
Antigua	2000	Davis et al. 2001
Abaco, Bahamas	2000	Davis et al. 2001
Trinidad	2000	Davis et al. 2001
Grand Bahama, Bahamas	2000	Davis et al. 2001
Providenciales, Turks & Caicos	2001	Davis et al. 2001
Berry Islands, Bahamas	2005	M. Deyrup, pers. comm.
St. Thomas, USVI	2005	present study
St. John, USVI	2005	present study
Tortola, BVI	2005	present study

Because *S. geminata* is common throughout the West Indies, the presence of *S. invicta* may be easily overlooked, even by trained entomologists.

In the present study, we examined museum specimens and made field collections to evaluate the distribution of *S. invicta* in the Virgin Islands.

METHODS

JKW searched the ant collection at the US National Museum (USNM) for *Solenopsis invicta* specimens from the Virgin Islands. Between Oct 1991 and Oct 2002, RRS collected ants on Guana Island during several visits (see Snelling 1993, 2003).

From 30 Oct to 21 Nov 2005, JKW collected ants on the 4 largest of the Virgin Islands, the 3 main islands of the US Virgin Islands (St. Croix - 7 d, St. Thomas - 5.5 d, and St. John - 4.5 d), and the main island of the British Virgin Islands (Tortola - 4.5 d). Collection sites included a diversity of disturbed and relatively natural habitats from the coastlines to the mountaintops. We also made a number of other observations concerning *S. invicta* in the Virgin Islands.

RESULTS

The USNM collection had *Solenopsis invicta* specimens from 2 sites in the Virgin Islands, both from St. Croix in 1988: Kingshill and Concordia. These records are earlier than any published records from the Virgin Islands.

RRS did not find *S. invicta* on Guana Island prior to 1996. In Oct 2002, *S. invicta* was common on the south side of the island: on the playa behind White Beach and in the "plantation" area. Forested areas of Guana Island were occupied by *Solenopsis geminata*.

In 2005, JKW collected *S. invicta* from 28 sites in the Virgin Islands: St. Croix (13 sites), St. Thomas (7 sites), St. John (2 sites), and Tortola (6 sites). All sites were in highly disturbed habitats, primarily open grassy areas (Table 2). All sites except one were low elevation (<100 m above sea level; the site at Parasol, St. Croix was 200 m above sea level). JKW collected *S. geminata* at 83 sites in the Virgin Islands: St. Croix (23 sites), St. Thomas (19 sites), St. John (23 sites), and Tortola (18 sites), in a wide variety of disturbed and relatively undisturbed habitats at all elevations.

On St. Croix, Jozef (Jeff) Keularts, an entomologist with the US Cooperative Extension Service, was aware of the presence of *S. invicta* on St. Croix. Lesley Hoffman, Administrative Director at the St. George Village Botanical Garden, St. Croix, related that in Jan 2005, her husband, Robert Hoffman, was stung by *S. invicta* while golfing at the Buccaneer Hotel Golf Course on St. Croix. He was brought to Juan Luis Hospital, where he was treated for anaphylactic shock with adrenaline and antihistamines. He now always carries an auto-injection charged with epinephrine because he was told that a subsequent attack could cause even more severe anaphylactic shock, which could be fatal without immediate treatment. Once stung the body builds up antibodies and subsequent attacks can result in potentially deadly allergic reactions.

On St. Thomas, George Ralish, the superintendent at Mahogany Run Golf Course knew of the presence and threat of *S. invicta* on the course. He has been working to control *S. invicta* on the golf course through spot treatment of nests using two insecticides (Extinguish from Wellmark, active ingredient = 0.5% Methoprene; Varsity from Syngenta, active ingredient = 0.011% Abamectin).

TABLE 2. NEW COLLECTION SITES OF *SOLENOPSIS INVICTA* IN THE VIRGIN ISLANDS (30 OCT TO 21 NOV 2005).

°N	°W	Island	Site	Habitat
17.780	64.770	St. Croix	Salt River, entrance to Gentle Winds	grass lawn
17.759	64.586	St. Croix	Cramer's Park	grass & weeds
17.757	64.817	St. Croix	Parasol; Scenic Dr., 0.5 km E of Rte. 69	grass & weeds
17.740	64.842	St. Croix	Montpellier, by church	grass lawn
17.732	64.813	St. Croix	Upper Love, by church	grass lawn
17.729	64.865	St. Croix	Little La Grange, by Lawaetz Museum	grass lawn
17.720	64.798	St. Croix	Kingshill, UVI	by parking lot
17.717	64.694	St. Croix	Longford, Routes 62 & 85	grass lawn
17.715	64.883	St. Croix	Fredriksted, waterfront park	plantings
17.715	64.830	St. Croix	St George, Botanical Garden	grass lawn
17.702	64.885	St. Croix	Smithfield, south of Cottages by the Sea	grass lawn
17.694	64.891	St. Croix	Hesselberg, south end of Shore Drive	grass lawn
17.694	64.820	St. Croix	Betty's Hope, south of Route 64	scrub forest
18.364	64.923	St. Thomas	Magens Bay, end of Route 35	beach weeds
18.359	64.906	St. Thomas	Lovenlund, Mahogany Run Golf Course	grass green
18.344	64.974	St. Thomas	John Brewer's Bay, UVI	by parking lot
18.344	64.937	St. Thomas	Charlotte Amalie, Griffiths Park	grass & weeds
18.344	64.933	St. Thomas	Charlotte Amalie, Creques Alleys	plantings
18.344	64.930	St. Thomas	Charlotte Amalie, Emancipation Garden	grass lawn
18.339	64.969	St. Thomas	Brewer's Bay, airport	plantings
18.348	64.713	St. John	Coral Bay	baseball field
18.343	64.785	St. John	Caneel Bay, resort	grass lawn
18.447	64.562	Tortola	Josiah's Bay, by hostel	grass lawn
18.425	64.619	Tortola	Road Town, waterfront	weeds
18.425	64.579	Tortola	Paraquita Bay, community college	grass lawn
18.414	64.589	Tortola	Brandy Wine Bay	beach weeds
18.412	64.671	Tortola	Carrot Bay	beach weeds
18.386	64.699	Tortola	Sandy Point, boat yard	grass & weeds

On St. John, the US quarantine office in the main harbor at Cruz Bay had no records of any ants intercepted from in-coming cargo. The personnel there were unaware of any threat posed by pest ant species, including *S. invicta*.

On Tortola, a person visiting a beach complained of white pustules and scars from ant stings he received while working at a boat yard at the Sandy Point. JKW found this entire boat yard heavily infested with *S. invicta*.

DISCUSSION

In the Virgin Islands, *Solenopsis invicta* is now well established on all 4 major islands as well as on Guana Island. Based on specimen records, it appears that *S. invicta* probably arrived in the Virgin Islands in the 1980s, first establishing itself on St. Croix. The first populations of *S. invicta* on the other Virgin Islands may be quite recent, dating from the 1990s and later. It is not surprising that *S. invicta* has spread to St. Croix and the other Virgin Islands, given the large amount of commercial ship traffic to these islands from Puerto Rico and ports in the southeastern US, sites which are heavily infested with *S. invicta*. It seems inevitable that *S. invicta* will soon spread to most other populated islands of the West Indies as well.

Solenopsis invicta poses an important threat not only to terrestrial invertebrates in the Virgin Islands and other West Indian islands, but also to vertebrates. For example, *S. invicta* attacks and kills hatchling sea turtles in Florida (Allen et al. 2001; Parris et al. 2002; Krahe et al. 2003; Krahe 2005), and may pose a similar hazard to sea turtles in the Virgin Islands. The collection site in southwestern Hesselberg, St. Croix, was adjacent to the Sandy Point Wildlife Preserve, an important nesting beach for the endangered leatherback sea turtle, *Dermochelys coriacea* (Vandelli) (Dutton et al. 2005). *Solenopsis invicta* may also represent a threat to already endangered small vertebrates on these islands, including many species of *Anolis* lizards. Finally, it is important that people in the Virgin Islands, particularly healthcare professionals, are aware of the threat of *S. invicta* to humans, can recognize the symptoms of *S. invicta* stings, and know proper treatments for severe adverse reactions to the stings, including rare but potentially deadly anaphylactic shock.

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INVERTEBRATE PREDATORS AND PARASITOIDS OF PLUM CURCULIO, *CONOTRACHELUS NENUPHAR* (COLEOPTERA: CURCULIONIDAE) IN GEORGIA AND FLORIDA

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ABSTRACT

The extent of predation and parasitism on larvae of the plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), was measured independently with several different experimental designs at sites in northern Florida and central Georgia. Experimental manipulation in Monticello, FL, and in Byron, GA, demonstrated equivocal impacts by predation. However, direct observations in Byron, GA, revealed that ants are the dominant invertebrate predators of plum curculio larvae, causing up to 62% mortality. Primary ant predators included *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae) and *Dorymyrmex bureni* (Trager) (Hymenoptera: Formicidae). Predation may be more important later in the season when infested fruit does not abscise and plum curculio larvae must drop to the ground from the trees and spend a considerable time burrowing into the soil. This contrasts with the early season when infested fruit abscise and larvae crawl from the fruit directly into the soil, reducing their exposure to predators. Recorded parasites included *Nealiolus curculionis* (Fitch) (Hymenoptera: Braconidae) and *Cholomyia inaequipes* Bigot (Diptera: Tachinidae). Parasitism, particularly by *N. curculionis*, was common in northern Florida but rare in middle Georgia.

Key Words: *Dorymyrmex bureni*, *Solenopsis invicta*, *Nealiolus curculionis*, *Cholomyia inaequipes*

RESUMEN

El nivel de depredación y parasitismo en contra de *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), fue medido independientemente usando diferentes diseños experimentales en lugares como el norte de Florida y la zona central de Georgia. Manipulación experimental en Monticello, FL, y en Byron, GA, demostró que el impacto de depredación no fue preciso. Sin embargo, observaciones directas en Byron, GA, revelaron que las hormigas son el invertebrado dominante en la depredación de la larva de C. nenuphar, causando hasta 62% de mortalidad en las larvas. Entre las principales hormigas depredadoras se encuentran, *Solenopsis invicta* (Buren) (Hymenoptera: Formicidae) y *Dorymyrmex bureni* (Trager) (Hymenoptera: Formicidae). La depredación es más importante en la temporada tardia, cuando las frutas infestadas no han caido al suelo, por lo tanto las larvas tuvieron que llegar al suelo desde los arboles y pasaron un tiempo considerable tratando de enterarse en el suelo. Contrario a esto, en la temporada temprana cuando las frutas infestadas cayeron al suelo y las larvas pasaron de la fruta al suelo directamente, reduciendo el tiempo que las larvas estuvieron expuestas a los depredadores. Los parásitos reportados incluyen *Nealiolus curculionis* (Fitch) (Hymenoptera: Braconidae) y *Cholomyia inaequipes* (Bigot) (Diptera: Tachinidae). Parasitismo, particularmente por *N. curculionis*, fue común en el norte de Florida pero raro en la zona central de Georgia.

Translation provided by the authors.

The plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), an insect native to North America (Quaintance & Jenne 1912), is the primary direct insect pest of peaches in the Southeastern United States (Horton & Ellis 1989). Adult plum curculios migrate in early spring from overwintering sites in adjacent woods to infest peach orchards (Snapp 1930; Yonce et al. 1995). The females oviposit on young fruit, often causing it to abscise (Quaintance & Jenne 1912;

Snapp 1930). Larvae develop in the fruit and move into the soil to pupate. In the bivoltine southern strain of plum curculio (Chapman 1938), adults emerge from these pupae the same summer to continue the infestation, but their offspring emigrate from the orchard to overwintering sites in adjacent woods or other locations with plenty of leaf litter (Quaintance & Jenne 1912; Snapp 1930; Yonce et al. 1995).

Plum curculio is currently controlled with highly efficacious organophosphate insecticides. The use of these pesticides is being restricted as a result of the implementation of the Food Quality Protection Act. Recent insecticide losses, e.g., methyl parathion, have caused the peach industry in the southeast to seek more sophisticated integrated pest management strategies that take into account the target pest's natural history and biology. These approaches include soil applications of entomopathogenic nematodes and fungi against the larval and pupal stages (Shapiro-Ilan et al. 2002; Shapiro-Ilan et al. 2004; Tedders et al. 1982).

Although predators and parasitoids are important components of integrated management programs for other curculionid pests (Stuart et al. 2003; Stuart et al. 2002; McCoy et al. 2000), little attention has been paid to potential predators or parasitoids of the plum curculio. Field efficacy trials against plum curculio often have variable mortality rates (Shapiro-Ilan et al. 2004; Quaintance & Jenne 1912; Snapp 1930), suggesting that natural sources of mortality may be involved and potentially sources of control. Even though researchers in the northeastern U.S. concluded that natural enemies of the plum curculio are inefficient (Van Driesche et al. 1987) we were interested in surveying predators and parasitoids of plum curculio in central Georgia.

Our objectives were to assess the effects of various natural enemies on the southern strain of plum curculio, *C. nenuphar*, by (1) quantifying the extent of plum curculio mortality attributable to predation and parasitism in northern Florida and middle Georgia, and (2) assaying biological control organisms, such as the fungus, *Beauvaria bassiana* (Bals.) Vuill. (Hyphomycetes) separately and in conjunction with the application of conventional pesticides (e.g., bifenthrin, thiamethoxam, and imidacloprid) to the soil targeting late larval and pupal stages of plum curculio are located. The impacts of these pesticides on potential natural enemies also were assessed.

Materials and Methods

Experiments Conducted at the Southeastern Fruit and Tree Nut Research Laboratory, Byron GA: Predation

At the USDA Southeastern Fruit and Tree Nut Research Laboratory in Byron, GA, (SEFTNRL) we compared the potential emergence under "optimal conditions" to emergence under field conditions in order to estimate overall mortality attributable to abiotic factors and to predators, parasitoids, and pathogens. In May 2004 we exposed 20 virgin female and 20 virgin male plum curculio to 360 green thinning apples (Red Delicious variety) for 2 weeks. Half of these apples were then randomly selected and distributed equally among 6 tilled locations (30 apples/location) at the base of peach trees and within the rows of an unsprayed peach orchard on the grounds of the SEFTNRL. Each tilled location was 0.6 m^2 in area. This orchard had not received pesticide applications in the previous 5 years. Six separate locations in the same unsprayed orchard were tilled and used as negative controls, each receiving 30 uninfested green thinning apples. Each location was covered with a cone emergence trap (Mulder et al. 2000) after 3 weeks. This allowed predators to access the infested apples without interference from the cages but was not enough time for adults to emerge from the soil. Cone emergence cages were monitored daily for the emergence of adults over 60 days. The remaining apples that had been exposed to ovipositing female plum curculio were divided equally among 6 plastic tubs (11.4 L Rubbermaid™ storage box). The infested fruit were placed upon a hardware cloth supported above the bottom of the tub by four 2-cm long corks. The tubs were stored in an environmental chamber at 25° ± 1°C and 50% RH (12:12, L:D) (Amis & Snow 1985). The tubs were monitored daily for the emergence of larvae which were then placed into pupation jars. Pupation jars were 950-ml glass jars 2/3 filled with a moistened mixture of potting soil and vermiculite (2:1) that had been sifted with a 10-mesh sieve to ensure that the soil did not contain insects and covered with a glass Petri dish. Pupation jars were monitored daily for the emergence of adult plum curculio. The number of emerging adult plum curculio was compared between fruits exposed to predators and fruits not exposed to predators using a t-test (SAS 2001).

We monitored plum curculio larvae as they burrowed into the soil, recording any predation we observed. Between Mar 15 and Aug 1, 2003, laboratory-reared plum curculio larvae (Amis & Snow 1985), within 12 h of emerging from green thinning apples, were harvested, taken to the field, and placed singly at random locations on an orchard floor in Byron, GA, between the hours of 8:00 and 20:00. Each larva was observed until the larva buried itself or was carried off by predators. The time interval between setting the larva on the ground and its complete burial or removal by predators was noted. Ant abundance (by species) was measured by counting the number of ants in an area of 0.21 m^2 at random locations in the orchard throughout the summer. An area of 0.21 m^2 was chosen because it was small enough for researchers to survey it intensively. Results were then converted to ants per m^2.

Parasitism

Abscised peaches were collected at Byron, GA, in 2004 and placed on trays with mesh bottoms over a large aluminum funnel (0.3 m high with a slope of 30%). The funnel was positioned over a collection pan so that larvae emerging from the infested fruit could be collected. Larvae were collected daily, enumerated and placed in pupation jars. Pupation jars were monitored daily for 60 d for the emergence of adult plum curculio or parasitoids.

In addition, wild plum fruit, *Prunus angustifolia* Marshall and *P. umbellata* Elliott, infested with plum curculio (as denoted by the distinct oviposition scar, Quaintance & Jenne 1912) were collected from Peach Co., GA, and placed in plastic tubs (11.4-L Rubbermaid™ storage box) and stored, as described earlier in the predation studies. The tubs were monitored daily for the emergence of larvae from fruit. Larvae were collected and placed into pupation jars and were monitored for the appearance of adult plum curculio and/or parasitoids.

One hundred abscised peaches collected from an unsprayed peach orchard at SEFTNRL were placed at each of 5 tilled areas (0.6 m^2) at the base of randomly selected peach trees in the orchard. The peaches were then covered with a cone emergence trap. The cone emergence trap was monitored for 60 d for the emergence of parasitoids historically associated with plum curculio (Krombein et al. 1979).

Experiments Conducted at the North Florida Research and Education Center, Monticello, FL

One thousand peach fruit that had abscised in response to infestation by plum curculio were gathered at the University of Florida, North Florida Research and Education Center (NFREC) in Monticello, FL, in 2003. These fruit were distributed among 10 locations (0.5 m^2) on an orchard floor, so that each location had 100 abscised fruit. Bifenthrin (Talstar® EZ, FMC Corporation, Philadelphia, PA) was applied (1 lb/acre) in a 3.14-m ring around, but not on, infested fruit at 5 locations. Five locations were left untreated as controls. The locations were covered with cone emergence traps and monitored daily for the emergence of adult plum curculio or parasitoids (Krombein et al. 1979).

Parasitoid emergence also was monitored in a separate field trial in 2003 by assaying 5 pesticides applied to the orchard floor and targeting plum curculio larvae. Twenty five sites (1 m^2) were selected and treated with imidacloprid (Admire® 2F, Bayer Crop Sciences, Kansas City, MO) at 1.75 L/ha, bifenthrin (Talstar® EZ, FMC Corporation, Philadelphia, PA) at 1.12kg/ha, thiamethoxam (Platinum®, Syngenta, Greensboro, NC) at 438.07 mL/ha, or *Beauvaria bassiana* (GHA strain, supplied by Emerald Bioagriculture, Butte, Montana) applied at a rate of 10^{14} conidia/ha, or with 2 L of water as a control treatment. All treatments were delivered in 2 L of water from a watering can. One hundred abscised fruit, gathered from the orchard floor, were deposited at each of the 25 sites (5 treatments with 5 replicates).

Results

Southeastern Fruit and Tree Nut Laboratory, Byron GA: Predation

Significantly more adults emerged from apples stored in the incubator (7.5 ± 1.3: mean ± SEM) than from apples stored in the orchard and exposed to predation, disease, and environmental factors (3.8 ± 1.0: mean ± SEM) ($t = 2.75$; $df = 5$; $P = 0.022$). No adult plum curculio emerged from the control plots that contained uninfested apples, indicating that it was likely that all of the curculio emerging in the cone emergence cages in the orchard were from the infested apples and not from plum curculio pupae that were in the soil prior to the experiment.

In total, 268 m^2 were surveyed for ant abundance and 4,038 ants were found. *Solenopsis invicta* Buren (Hymenoptera: Formicidae) comprised 77% of the ants found, *Dorymyrmex bureni* (Trager) (Hymenoptera: Formicidae) comprised 15%, and a *Paratrechina* sp. (Hymenoptera: Formicidae) comprised 8%. There was a mean of 15 (±0.88 SEM) ants of any species in a given m^2. Of these, 12 (±0.85 SEM) were S. invicta, 2 (±0.26 SEM) were *D. bureni*, and 1 (±0.10 SEM) was *Paratrechina* sp.

All 3 ant species were observed capturing and killing larval plum curculio that we had placed on the ground. In total, 229 last instar plum curculio larvae were observed on the orchard floor. Of these, 97 were discovered by *S. invicta*, 26 were discovered by *Paratrechina* sp., and 20 were discovered by *D. bureni*. Eighty six larvae were able to bury themselves before being discovered by ants. On average, all larvae discovered by fire ants were discovered in 13.94 min (SEM = 1.34). All larvae discovered by *Paratrachina* sp. were discovered on average in 9.35 min (SEM = 1.54). All larvae discovered by *D. bureni* were discovered on average in 20.10 min (SEM = 5.67). All larvae discovered by any species of ant were discovered on average in 13.97 min (SEM = 2.49). All larvae that successfully buried themselves did so on average in 15.41 min (SEM = 1.39).

A mean of 4.2 (SEM = 1.2) adult plum curculio emerged from 100 abscised fruit under the 5 cone emergence cages. Only 1 specimen of *Nealiolus curculionis* (Fitch) (Hymenoptera: Braconidae) was detected from the cone emergence cages.

In total, 930 abscised peaches were collected from the orchard floor in Byron, GA. We collected 528 larvae from these fruit and placed them into pupation jars. One specimen each of N. *curculionis* and *Cholomyia inaequipes* Bigot (Diptera: Tachinidae) was reared from these plum curculio.

In total, 1,146 scarred fruit of *P. angustifolia* and 269 of *P. umbellata* were collected. These fruit yielded a total of 546 plum curculio larvae (39% of infested fruit yielded larvae). Two *N. curculionis* were reared from these larvae.

North Florida Research and Education Center,
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A mean of 13.8 (±2.4 SEM) adult plum curculio emerged from 100 abscised fruit in the untreated controls and 12.8 (±2.8 SEM) adult plum curculio emerged from 100 abscised fruit in the locations that received bifenthrin as a ring treatment to prevent entry of fire ants. These results were not different (t = 0.355; df = 4; P = 0.899).

The only parasitoid recovered was *N. curculionis*. The percent of plum curculio infected with this parasite, based on the number of adult plum curculio recovered and the number of adult parasitoids, ranged from 30% to 47%, with an average of 37% (SEM = 2.77) (Table 1). The pesticides assayed did not demonstrate significant control of plum curculio compared to untreated controls, nor did they appear to significantly impact numbers of parasitoids in each treatment (Table 1).

TABLE 1. MEAN NUMBER (±SEM) OF ADULT *CONOTRACHELUS NENUPHAR* AND *NEALIOLUS CURCULIONIS* EMERGING FROM 100 ABSCISED PEACHES COLLECTED IN MONTICELLO, FL.

	Water control (2 L of water)	Imidacloprid at 1.75 L/ha	Bifenthrin at 1.12 kg/ha	Thiamethoxam at 438.07 mL/ha	Talstar® as a fire ant barrier	*Beauvaria bassiana* at 10^{14}conidia/ha	Untreated control
Conotrachelus nenuphar	18.8 (+6.1) a[1]	14.2 (±2.3) a	6 (±2.1) a	11.6 (±0.9) a	12.8 (±2.8) a	14.8 (±3.7) a	13.8 (±2.4) a
Nealiolus curculionis	9 (±3.1) a	11.2 (±3.7) a	4 (±1.1) a	10.4 (±0.9) a	6.2 (±1.2) a	7.8 (±1.8) a	5.8 (±1.6) a

[1]Means followed by the same letter are not significantly different according to a Student-Neuman-Keuls test. For *Conotrachelus* nenuphar,α = 0.05; F= 1.39; P=0.2531; df=28. For *Nealiolus curculionis*, α = 0.05; F = 1.87; P = 0.1210; df = 28.

DISCUSSION

There was a significant reduction in plum curculio mortality when plum curculio were reared indoors, in the absence of natural enemies or adverse environmental conditions, as opposed to those on the orchard floor. However, there was no significant difference between the number of adult plum curculio emerging in areas that had been surrounded with a treatment of bifenthrin and areas that had not received a pesticide treatment to preclude foraging ants. These results suggest a number of scenarios, including the following: (1) predation plays a small role in plum curculio mortality, (2) the pesticide applications used did not preclude foraging ants, or (3) differences in mortality observed between plum curculio reared outdoors and those reared indoors may be attributed to regulated humidity and temperature, consistent environment, and fewer pathogens. Direct field observations reveal that predation by ants alone may be responsible for the mortality of more than 60% of plum curculio larvae attempting to burrow into the soil, with the caveat that placing lab reared plum curculio larvae on the orchard floor is not natural and may exaggerate mortality due to ant predators. Furthermore, there may be a seasonal component to the

effect of predation by ants. Peaches infested earlier in the season are small and usually abscise and drop to the ground when infested with plum curculio larvae (Quaintance & Jenne 1912; Detjen 1938). The larvae continue to develop in the fruit and can burrow directly from the fruit into the soil, probably reducing their chances of being encountered by foraging ants. Peaches infested later in the season do not abscise and larvae must drop from the fruit to the ground. Subsequently, the summer generation of plum curculio in late season peaches may be more susceptible to ant predation. The lack of significant difference between number of adults emerging from infested fruit that were chemically protected from predators and infested fruit that was accessible to predators lends credence to the possibility that larvae moving directly from infested fruit into the soil may suffer less predation than larvae that drop from the tree to the ground.

This is the first quantitative study of the impact of certain predators on plum curculio, although many anecdotal observations have been published (Quaintance & Jenne 1912; Snapp 1930). Plum curculio larvae were monitored in close quarters (within 1 m) for accurate identification of predators. Such proximity to the larvae precluded larger predators, such as birds and carabid beetles, although these may be additional and important sources of mortality. Although *Solenopsis invicta* was not present in central Georgia at the time, Snapp (1930) and Quaintance & Jenne (1912) report that *Dorymyrmex bureni* (reported as *Dorymyrmex pyramica*) was an important predator of larval plum curculio. Quaintance & Jenne (1912) list ground beetles (Coleoptera: Carabidae) and a soldier beetle, *Chauliognathus pennsylvanicus* (De Geer) (Coleoptera: Cantharidae), as important predators of larval plum curculio. Unfortunately, the soldier beetle appears to be in decline in Georgia, perhaps as a result of predation by *S. invicta* (Jenkins & Matthews 2003). Dissections of spadefooted toads, *Scaphiopus* sp., revealed that they are consumers of plum curculio (J. Payne, pers. comm.).

Parasitism was of minimal importance as a mortality factor in middle Georgia but appeared to contribute significantly to the mortality of plum curculio in northern Florida. Though we realize the estimates for parasitism in Florida are necessarily high, the sheer numbers obtained need no statistical differentiation from those obtained in the Byron, GA, studies, considering that of more than 1000 larvae collected from peach and wild plums in central Georgia only 2 yielded parasitoids. It is possible that the *N. curculionis* individuals collected in cone emergence traps in Monticello, FL, had used hosts other than *C. nenuphar*. Indeed, *N. curculionis* is known to use many other hosts (Krombein et al. 1979).

There are a number of parasitoids that have been recorded from plum curculio but that were not found in the current study. These include *Nealiolus collaris* (Brues), *N. rufus* (Riley), *Triaspis kurtogaster* Martin, *Bracon mellitor* Say, *B. politiventris* (Cushman), *B. variablilis* (Provancher) (Hymenoptera: Braconidae), *Tersilochus conotracheli* (Riley) (Hymenoptera: Ichneumonidae), *Patasson conotracheli* (Girault) (Hymenoptera: Mymaridae), *Myiophasia aenea* Wiedemann, *Cholomyia inaequipes* Bigot (Diptera: Tachinidae), and *Pegomyia fusciceps* Zett. (Diptera: Anthomyiidae) (Riley 1871; Quaintance & Jenne 1912; Snapp 1930; Armstrong 1958; Arnaud 1978; Krombein et al. 1979; Tedders & Payne 1986). All of these species, with the exceptions of *T. conotracheli* and *B. politiventris*, have been recorded in Georgia or Florida (Krombein et al. 1979). The vast majority of these parasitoids utilize a variety of other hosts, although many of their hosts are often found in fruit (Krombein et al. 1979).

Historically, percent mortality and percent mortality attributable to parasitism has varied greatly. Quaintance & Jenne (1912) report that the percent of adult plum curculio that emerged from larvae ranged from 9% to 60% with a mean of 32% and that parasitism ranged from 0.7% to 21% with a mean of 8.1%. Snapp (1930) reported that the percentage of adults that emerged from larvae ranged from 1.7% to 18.7% with a mean of 7.4%. Armstrong (1958) reported a range of parasitized plum curculio larvae of 7.5 to 26.6 with an average of 20% parasitized. Even in our study, parasitism varied greatly between the 2 sites and presumably does so from year to year. This broad host range suggests that the abundance of alternate hosts may play an important role in rates of parasitism of *C. nenuphar*.

In summary, variation in mortality of plum curculio is extremely high, as is variation in incidence of parasitism (Quaintance & Jenne 1912; Snapp 1930). The high levels of parasitism observed in Florida are possibly important sources of natural control of plum curculio populations. Further research is needed to elucidate mortality factors and the causes of this variation. Understanding these factors may lead to better pest management strategies.

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ACTIVITY OF *BACILLUS THURINGIENSIS* ISOLATES AGAINST *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

A collection of *Bacillus thuringiensis* isolates plausibly active against coleopteran insects was obtained from the Agricultural Research Service Culture Collection. Each isolate was cultured, spores and δ-endotoxin *cry*stals were pelleted by centrifugation and lyophilized, and the resulting product was incorporated in insect diet for testing against *Diaprepes abbreviatus* neonates. A bioassay method was developed that utilized small amounts of insect diet and *B. thuringiensis* spores and δ-endotoxin to treat single neonates confined to 0.2-mL clear polymerase chain reaction (PCR) tubes. The method was less expensive in terms of labor and materials as compared to previous methods and reduced control losses due to burrowing and aggressive behaviors of *D. abbreviatus* larvae confined together. Of 19 *B. thuringiensis* isolates screened for activity against *D. abbreviatus* with a discriminating dose of 250 ppm spores and δ-endotoxin on diet, 5 were selected for further evaluation in dose-response experiments. *Diaprepes abbreviatus* larvae demonstrated a significant dose response to 4 of the 5 isolates tested. The most active isolates were those that expressed *Cry*ET33 and *Cry*ET34, or *Cyt*2Ca1 proteins. A wild-type *B. thuringiensis* strain that expressed *Cyt*2Ca1 generated the lowest LC_{50} value (50.7 µg/ml) and steepest slope (1.11) based on log10 probit analysis of the data. These *B. thuringiensis* δ-endotoxins may have utility in transgenic approaches to citrus rootstock protection from *D. abbreviatus*.

Key Words: *Diaprepes abbreviatus*, *Bacillus thuringiensis*, *Cry*, *Cyt*, endotoxin, citrus

RESUMEN

Una colección de aislamientos de *Bacillus thuringiensis* posiblemente activos contra insectos del orden Coleóptera fue obtenido de la Colección de Cultivos del Servicio de Investigación Agrícola (USDA, ARS). Cada aislamiento fue criado, las esporas y cristales de endotoxina-δ fueron sedimentados por una centrifugadora y liofilizado (congelado y secado), y el producto resultante fue incorporado en una dieta de insectos para probarlo contra los neonatos (larvas recién nacidas) del *Diaprepes abbreviatus*. Un método de bioensayo fue desarrollado para utilizar cantidades pequeñas de la dieta de insectos, esporas de *B. thuringiensis* y la endotoxina-δ para tratar individualmente los neonatos confinados en frascos claros de 0.2 ml para la reacción en cadena por la polimerasa (RCP). Este metodo fue menos costoso en terminos de mano de obra y materiales comparado con los metodos de control usados anteriormente para reducir las perdidas asociadas al comportamiento minador y agresivo de las larvas juntamente confinadas de *D. abbreviatus*. De los 19 aislamientos de *B. thuringiensis* evaluados con actividad contra *D. abbreviatus* con una dosis de 250 ppm de esporas y endotoxin-δ sobre la dieta, 5 fueron seleccionados para evaluación adicional en experimentos de respuesta de dosis. Las larvas de *Diaprepes abbreviatus* demonstraron una respuesta de dosis significativa en 4 de los 5 aislamientos probados. Los aislados mas activos fueron los que expresaron las proteinas *Cry*ET33 y *Cry*ET34, o *Cyt*2Ca1. Una raza de tipo-silvestre de *B. thuringiensis* que expreso *Cyt*2Ca1 produjó el valor menor de CL_{50} (50.7 µg/ml) y el pendiente empinado (1.11) basado en el análisis de datos usando el probit de log10. Las endotoxinas-δ de *B. thuringiensis* puede ser útiles en un enfoque transgénico para la protección de rizomas citricas contra el *D. abbreviatus*.

The invasive weevil species, *Diaprepes abbreviatus* (L.), has become one of the most damaging insect pests of citrus and nursery crops in Florida since it was first reported in 1964 (Woodruff 1964). Prior to its introduction into the continental United States, *D. abbreviatus* was known to be a serious pest of sugarcane in the Lesser Antilles and is considered the most important pest of agriculture, horticulture, and silviculture in Puerto Rico (Hantula et al. 1987). Efforts of researchers and pest managers to develop an effective long-term management strategy for this pest have been unsuccessful. Apparently, *D. abbreviatus* is not under effective biological control within its putative native range of Puerto Rico and the Lesser Antilles (Lapointe 2004). The lack of natural enemies, combined with the wide host range of this highly polyphagous weevil (Simpson et al. 1996) and its slow subterranean larval development (Lapointe 2000), makes *D. ab-*

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breviatus particularly well adapted to semi-permanent, tropical, and subtropical agroecosystems such as citrus groves. Efforts to identify plant resistance to *D. abbreviatus* within sexually compatible citrus germplasm have been only marginally successful (Bowman et al. 2001; Lapointe & Bowman 2002; Shapiro et al. 2000).

The bacterial entomopathogen, *Bacillus thuringiensis* (Berliner), has been recommended for the control of other insect pests of citrus, particularly those in the order Lepidoptera (Shapiro et al. 1998; Stansly et al. 2006). Although use of *B. thuringiensis* as an applied biopesticide can be an effective control method for some insects, the subterranean feeding habits of *D. abbreviatus* larvae make them difficult to target with a biocontrol agent, such as *B. thuringiensis*, that must be ingested to be effective. It has been suggested that the most appropriate and economically viable method for control of *D. abbreviatus* will be the production of transgenic rootstocks engineered to express exogenous toxins (Lapointe 2004). Al-Deeb & Wilde (2005) reported that transgenic corn, expressing the *Cry*3bb1 toxin from *B. thuringiensis*, was protected from another root-feeding coleopteran, the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Transgenic crops that express *B. thuringiensis* proteins display resistance to some of the most devastating pests of agriculture, yet are virtually safe to nontarget organisms (Betz et al. 2000). A transgenic approach that uses a genetically-engineered citrus rootstock to express a δ-endotoxin active against *D. abbreviatus* is a plausible solution.

Currently, the few *B. thuringiensis* δ-endotoxins known to be active against coleopterans are far outnumbered by known lepidopteran-active toxins. In order to pursue this paradigm toward the development of a transgenic citrus rootstock, *B. thuringiensis* toxins that are active against *D. abbreviatus* larvae must first be identified. One strain of *B. thuringiensis* has been reported to cause mortality of *D. abbreviatus* larvae (Weathersbee et al. 2002), but otherwise *B. thuringiensis* has received minimal attention as a potential biocontrol agent for this pest. We assembled a collection of *B. thuringiensis* isolates that expressed novel δ-endotoxins putatively active against one or more representatives of Coleoptera. This paper presents the results of experiments that determined if any of these toxins were active against *D. abbreviatus* larvae.

Materials and Methods

Source and Culture of *B. thuringiensis* Isolates

Patent databases at the United States Patent and Trademark Office were searched to locate *B. thuringiensis* isolates potentially active against species of Coleoptera. Representative samples of 19 isolates, for which patents had issued (Bradfisch et al. 2005; Donovan et al. 2005; Narva et al. 2005; Rupar et al. 2003; Rupar et al. 2004; Soares et al. 1989), were obtained by request from the curator of the Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois (Table 1). Samples were received as dried pellets sealed in glass ampoules. Growing cultures of each isolate were established following instructions provided with the samples. Briefly, an ampoule was scored with a file and broken. The broken end of the ampoule was flame sterilized, the pellet was removed and then cultured in Luria-Bertani (LB) broth in an incubator shaker. Cultures were stored on LB agar slants at 4°C and in LB broth/glycerol stock solutions at -80°C until needed.

Starter cultures of each isolate were prepared by inoculating 10 mL of LB broth with a loopful of bacterial cells removed from storage. Cultures were grown overnight in 125-mL baffled Erlenmeyer flasks at 27ºC and 150 rpm in an incubator shaker. Then 80 µl of starter culture were added to 100 mL of LB broth in a 500-mL baffled Erlenmeyer flask and grown in the incubator shaker until approximately 90% of the cultured cells had sporulated and autolyzed. Cultures were pelleted by centrifugation (15,000 G) for 15 min at 4°C, washed 3 times with phosphate buffered saline (PBS) containing 0.005% Triton X-100, lyophilized, and weighed. Lyophilized pellets, containing *B. thuringiensis* spores and δ-endotoxin *cry*stals, were stored in 1.5-mL microcentrifuge tubes at -80°C until they were used in the experiments.

Insect Source and Rearing

Neonatal larvae of *D. abbreviatus* were obtained from a laboratory colony maintained at the U.S. Horticultural Research Laboratory, Fort Pierce, FL. Larvae were reared on a commercially-prepared insect diet (Product No. F1675, Bio-Serv, Frenchtown, NJ). Temperature and moisture content of the diet were optimized for larval development according to Lapointe (2000) and Lapointe & Shapiro (1999). Neonate larvae were surface sterilized with a solution of 0.31% sodium hypochlorite and individually inspected to insure only healthy and active larvae were used in the experiments.

Bioassay Method

A bioassay method was developed to test the effectiveness of *B. thuringiensis* isolates on individual *D. abbreviatus* neonates. A clear polymerase chain reaction (PCR) tube containing a small amount of insect diet in the lid was used to hold a single *D. abbreviatus* neonate that could be visually inspected for response to treatments. Monitoring of larvae had not been possible with

TABLE 1. SCREENING OF *B. THURINGIENSIS* ISOLATES AGAINST *D. ABBREVIATUS* NEONATES WITH A DISCRIMINATING DOSE (250 µG/ML) OF LYOPHILIZED, SPORULATED CULTURES IN DIET.

Isolate[a] number	Isolate description	Endotoxins[b] present	Genbank accession[c]	% Mortality ± SE (n=90)	
				Treated[d]	Control
B-21367	recombinant	*Cry*ET33	AAF76375	91.3 ± 3.0*	20.0 ± 10.1
		*Cry*ET34	AAF76376		
B-21365	wild-type	*Cry*ET33	AAF76375	90.3 ± 6.7*	20.0 ± 10.1
		*Cry*ET34	AAF76376		
B-21366	recombinant	*Cry*3Bb2	AAA74198	87.7 ± 4.7*	20.0 ± 10.1
		*Cry*ET33	AAF76375		
		*Cry*ET34	AAF76376		
B-21582	wild-type	*Cyt*2Ca1	AAK50455	81.7 ± 7.5*	11.5 ± 5.9
B-21583	recombinant	*Cyt*2Ca1	AAK50455	52.3 ± 2.9*	0.0 ± 0.0
B-21784	wild-type	*Cry*35Aa2	AAK64561	20.3 ± 8.8	3.0 ± 0.0
		*Cry*34Aa2	AAK64560		
		*Cry*38Aa1	AAK64559		
B-21783	wild-type	*Cry*35Aa2	AAK64561	15.7 ± 5.9	2.0 ± 1.0
		*Cry*34Aa2	AAK64560		
		*Cry*38Aa1	AAK64559		
B-21915	wild-type	*Cry*35Ba1	AAK64566	15.7± 7.0	4.3 ± 1.3
		*Cry*34Ba1	AAK64565		
		*Cry*ET84	AAK64564		
B-21554	wild-type	*Cry*35Ac1	AAG50117	14.3 ± 1.3*	3.0 ± 0.0
		*Cry*34Ac1	AAG50118		
B-21787	recombinant	*Cry*36Aa1	AAK64558	12.0 ± 4.9	1.0 ± 1.0
B-21786	wild-type	*Cry*36Aa1	AAK64558	11.3 ± 3.0*	2.3 ± 2.3
B-21788	recombinant	*Cry*35Ab2	AAK64563	10.0 ± 4.0	3.0 ± 0.0
		*Cry*34Ac2	AAK64562		
B-18765	wild-type	*Cry*5Ba1	AAA68598	8.7 ± 3.0	1.0 ± 1.0
		*Cry*5Ac1	P56955		
B-21916	recombinant	*Cry*35Ba1	AAK64566	7.7 ± 2.9	3.0 ± 0.0
		*Cry*34Ba1	AAK64565		
		*Cry*ET84	AAK64564		
B-21785	wild-type	*Cry*35Ab2	AAK64563	6.7 ± 2.0	0.0 ± 0.0
		*Cry*34Ac2	AAK64562		
B-18243	wild-type	*Cry*5Aa1	AAA67694	5.7 ± 1.3	2.0 ± 1.0
		*Cry*5Ab1	AAA67693		
B-21553	wild-type	*Cry*35Ab1	AAG41672	4.3 ± 1.3	1.0 ± 1.0
		*Cry*34Ab1	AAG41671		
B-18244	wild-type	*Cry*12Aa1	AAA22355	1.0 ± 1.0	0.0 ± 0.0
B-18679	wild-type	*Cry*14Aa1	AAA21516	1.0 ± 1.0	1.0 ± 1.0
		*Cry*35Aa1	AAG50342		
		*Cry*34Aa1	AAG50341		

[a]Isolate numbers were assigned by curators of the ARS Culture Collection, National Center for Agricultural Utilization Research (formerly the Northern Regional Research Laboratory), Peoria, Illinois USA.

[b]Endotoxins labeled *Cry*ET## have not yet been assigned names recognized by the *B. thuringiensis* δ–endotoxin nomenclature committee.

[c]Protein accessions can be retrieved from the National Center for Biotechnology Information, Genbank at http://www.ncbi.nlm.nih.gov/.

[d]Means for *D. abbreviatus* percent mortality in the treated group marked by an asterisk (*) were ($P \leq 0.05$, paired t-test) greater than those for the control group.

older bioassay methods that used larger volumes of medium (soil or diet) because *D. abbreviatus* larvae burrow into the medium, complicating visual inspection.

All items used in the bioassay procedure were sterilized by autoclaving, filtering, or treating with 75% ethanol, and the procedure was conducted in a biological safety cabinet. A stock solution was prepared that contained 5% sucrose and 0.005% Triton X-100 in deionized distilled water. The previously prepared lyophilized pellets of each *B. thuringiensis* isolate were resuspended in

the stock solution and diluted with stock to provide a discriminating dose of spores and δ-endotoxin in diet of 250 ppm (µg/mL) for screening experiments. Dose response assays were conducted with isolates that caused >50% mortality of neonates at the discriminating dose level. Concentrations of 300, 150, 75, and 32.5 ppm of spores and δ-endotoxin in diet were used in the dose-response experiments. Diet treatments for the controls received stock solution only.

Prepared insect diet was liquefied by reheating and 80 µl of diet were pipetted onto the inside surface of the lid of a 0.2-mL clear PCR tube. The diet pellets were dried for 15 min to remove approximately 20 µl of water. *Bacillus thuringiensis* treatments were applied in a volume of 20 µl by pipette to each diet pellet and the pellets were dried for an additional 5 min. Controls were treated equally with stock solution only. A #1 fine camel hair brush was used to place a single *D. abbreviatus* neonate into each PCR tube containing diet and the lid was affixed. The PCR tubes were inverted and placed in a tube rack, covered, placed in a sealed plastic bag with a moist paper towel, and stored in an incubator at 27°C. After 2 weeks, each larva was inspected with the aid of a dissecting microscope and mortality was recorded. There were 3 replications, each containing 30 larvae, for the initial screening of each isolate at 250 ppm. A minimum of 3 replications, each with 30 larvae, was used for each level of treatment in the dose-response experiments.

Data Analyses and Statistics

Data collected from the screening experiments were subjected to the Means Procedure (SAS Institute 1999) to determine means and standard errors for mortality of *D. abbreviatus* neonates exposed to the discriminating dose of each isolate. Paired *t*-tests were conducted using the *T*-test Procedure (SAS Institute 1999) to determine if means for mortalities in treated groups differed from those of control groups. A probability level of 5 percent ($P \leq 0.05$) was considered significant.

Data from the dose-response experiments were adjusted for control mortality by the Abbott (1925) formula and transformed (arcsine) before analyses. Transformed data were analyzed by the General Linear Models Procedure, and differences among treatment level means were determined by Tukey's studentized range test (SAS Institute 1999). Differences among means were considered significant at a probability level of 5 percent ($P \leq 0.05$). Untransformed means are presented in the data tables. Data from isolates that elicited a significant response to treatment were subjected to log10 Probit analyses by the Probit Procedure (SAS Institute 1999) to generate LC_{50} values and slopes of probit lines.

RESULTS

Screening Experiments

Of 19 *B. thuringiensis* isolates screened in diet bioassays against *D. abbreviatus* neonates, 7 caused significantly greater ($P \leq 0.05$, paired *t*-tests) mortality compared with the controls (Table 1). Isolates B-21365, B-21366, and B-21367 containing *Cry*ET33 and *Cry*ET34 toxins caused the highest observed mortalities (90, 88, and 91%, respectively). Isolates B-21582 and B-21583 containing the *Cyt*2Ca1 toxin provided 82 and 52% mortalities, respectively. These five isolates (B-21365, B-21366, B-21367, B-21582, and B-21583) provided meaningful levels of mortality (>50%) and were further evaluated in dose-response experiments.

Dose-Response Experiments

A significant effect of spore and δ-endotoxin dose was observed for 4 of the 5 isolates that were subjected to dose-response experiments against *D. abbreviatus* neonates, including B-21365 ($F = 15.52$; $df = 4, 28$; $P < 0.0001$), B-21367 ($F = 9.46$; $df = 4, 28$; $P < 0.0001$), B-21582 ($F = 33.63$; $df = 4, 8$; $P < 0.0001$), and B-21583 ($F = 56.60$; $df = 4, 16$; $P < 0.0001$). The highest corrected mortality observed in the dose-response experiments was 81% provided by the wild-type isolate B-21582 at a dose of 300 µg spores and δ-endotoxin/ml diet (Table 2). Recombinant isolates B-21367 and B-21583 also elicited good dose-responses with greater than 60% mortality of *D. abbreviatus* larvae observed at the 300 ppm dose. The effect of spore and δ-endotoxin dose on larval mortality was not significant for isolate B-21366 ($F = 1.71$; $df = 4, 8$; $P = 0.2406$). The dose-response obtained with isolate B-21366 was inconsistent, the response data were variable compared to those of the other isolates, and larval mortality obtained at the highest dose remained below 50%. Consequently, isolate B-21366 was not included in subsequent probit analyses.

Results obtained for isolates B-21365, B-21367, B-21582, and B-21583 were examined further by log10 probit analyses to model the effects of spore and δ-endotoxin dose on mortality of *D. abbreviatus* larvae (Fig. 1). The calculated LC_{50} for larvae exposed to B-21365 in diet was 258.3 (95% FL = 130.5-2779) ppm [AI]. The slope of the probit line was 0.65 (SE = 0.23) ($\chi_2 = 8.11$; $df = 1$; $P = 0.0044$) (Fig. 1A). The LC_{50} for larvae exposed to B-21367 was 115.3 (95% FL = 40.4-269.5) ppm [AI] and the slope of the probit line was 0.93 (SE = 0.31) ($\chi^2 = 8.79$; $df = 1$; $P = 0.0030$) (Fig. 1B). The LC_{50} for larvae exposed to B-21582 was 50.7 (95% FL = 28.3-72.1) ppm [AI] and the slope was 1.11 (SE = 0.21) ($\chi^2 = 27.10$; $df = 1$; $P \leq 0.0001$) (Fig. 1C). The calculated LC_{50} for larvae exposed to B-21583 was 174.1 (95% FL = 114.6-361.2) ppm [AI] and

TABLE 2. MORTALITY OF *D. ABBREVIATUS* NEONATES EXPOSED TO DIET TREATED WITH DIFFERING RATES OF LYOPHILIZED, SPORULATED CULTURES OF *B. THURINGIENSIS* ISOLATES.

Dose (µg/ml AI)[a]	% Mortality ± SE [b] by isolate[c]				
	B-21365	B-21366	B-21367	B-21582	B-21583
0.0	6.7 ± 2.2 a	11.1 ± 2.2 a	17.5 ± 3.1 a	7.8 ± 2.9 a	1.3 ± 1.3a
32.5	26.3 ± 6.7 b	21.1 ± 12.8 a	31.3 ± 7.4 ab	40.6 ± 7.0 b	31.9 ± 3.0 b
75.0	39.4 ± 6.8 bc	29.9 ± 3.5 a	40.9 ± 8.7 ab	58.7 ± 6.5 bc	35.0 ± 6.9 b
150.0	40.2 ± 6.3 bc	28.6 ± 6.6 a	50.3 ± 10.0 bc	68.2 ± 7.2 c	44.1 ± 3.3 bc
300.0	52.9 ± 6.8 c	41.8 ± 16.4 a	67.8 ± 7.9 c	80.7 ± 4.3 c	61.6 ± 4.4 c

[a]AI refers to the active ingredient comprising lyophilized spores and δ-endotoxin of *B. thuringiensis* in diet.

[b]Means within a column sharing the same letter were not different ($P > 0.05$, Tukey's studentized range test [SAS Institute 1999]).

[c]Isolate numbers were assigned by curators of the ARS Culture Collection, National Center for Agricultural Utilization Research, Peoria, Illinois.

the slope was 0.79 (SE = 0.0.19) (χ^2= 17.96; df = 1; $P \leq 0.0001$) (Fig. 1D).

DISCUSSION

The subterranean habit, and aggressive behavior when confined together, complicate the evaluation of control measures for *D. abbreviatus* larvae (Lapointe & Shapiro 1999) and result in high levels of control mortality (Schroeder & Sieburth 1997; Quintella & McCoy 1997; Weathersbee et al. 2002). The bioassay method used here provided an efficient means for screening *B. thuringiensis* isolates against *D. abbreviatus* neonates. It was less expensive in terms of labor and materials than methods used in the past. The current method avoids these problems by confining larvae singly on a nominal amount of diet, thereby reducing losses in the control group. Control mortality in these experiments did not exceed 20% and was most often maintained below 10%.

The highest levels of mortality of *D. abbreviatus* in the screening experiments (90, 88, and 91%) occurred when neonates were fed diet containing spores and δ-endotoxin of isolates B-21365, B-21366, and B-21367, respectively. Donovan et al. (2005) has shown these isolates to be active against the larvae of other species of Coleoptera, including the red flour beetle, *Tribolium castaneum* (Herbst), and the Japanese beetle, *Popillia japonica* Newman. Isolate B-21365 is a wild-type *B. thuringiensis* strain that contains genes for *Cry*ET33 and *Cry*ET34 toxins. Isolate B-21367 is a recombinant strain that was engineered to express *Cry*ET33 and *Cry*ET34 toxins. This isolate was derived from a parent *B. thuringiensis* strain that was *cry*stal negative (*Cry*$^-$). Isolate B-21366 also is a recombinant strain engineered to express *Cry*ET33 and *Cry*ET34 toxins, but the parent strain was wild-type and naturally expressed the *Cry*3Bb2 toxin. Although B-21366 expressed an additional δ-endotoxin compared to B-21365 and B-21367, it performed no better against *D. abbreviatus* than those isolates in the screening experiments, and its performance was inconsistent in the dose-response experiments. Perhaps expressing the *Cry*ET33 and *Cry*ET34 toxins in addition to *Cry*3Bb2 added a burden that affected overall toxin expression and the virulence of this strain. Because this isolate produced erratic results it was not further evaluated.

The wild-type isolate B-21365, expressing the *Cry*ET33 and *Cry*ET34 toxins, exhibited the shallowest dose-response curve and highest LC_{50} value of those tested, probably because the highest concentration of B-21365 tested provided 53% larval mortality. The recombinant isolate B-21367 expressing the same toxins displayed a steeper response curve, a lower LC_{50} value, and provided better predictions in the 50% response range. It is unclear why recombinant strain B-21367 apparently produced a more virulent product than the wild-type B-21365 since both expressed the same toxins. Perhaps B-21367 invests less energy into other processes such as spore formation and more into toxin production. Nonetheless, the dose responses displayed by the recombinant strain B-21367 and the wild type B-21365 against *D. abbreviatus* were not statistically different based on overlap of standard errors.

Isolates B-21582 and B-21583 provided 82 and 52% mortalities, respectively, in the screening experiments. Rupar et al. (2004) demonstrated these isolates were active against the larvae of representative species of Siphonaptera: including the cat flea, *Ctenocephalides felis* (Bouché); and Coleoptera: including the southern corn rootworm, *Diabrotica undecimpunctata howardi* Barber; the western corn rootworm, *Diabrotica virgifera virgifera* LeConte; the Colorado potato beetle, *Leptinotarsa decemlineata* (Say); the red flour beetle; and the Japanese beetle. Isolate B-21582 is a wild-type *B. thuringiensis* strain that contains the gene for the *Cyt*2Ca1 toxin, while recom-

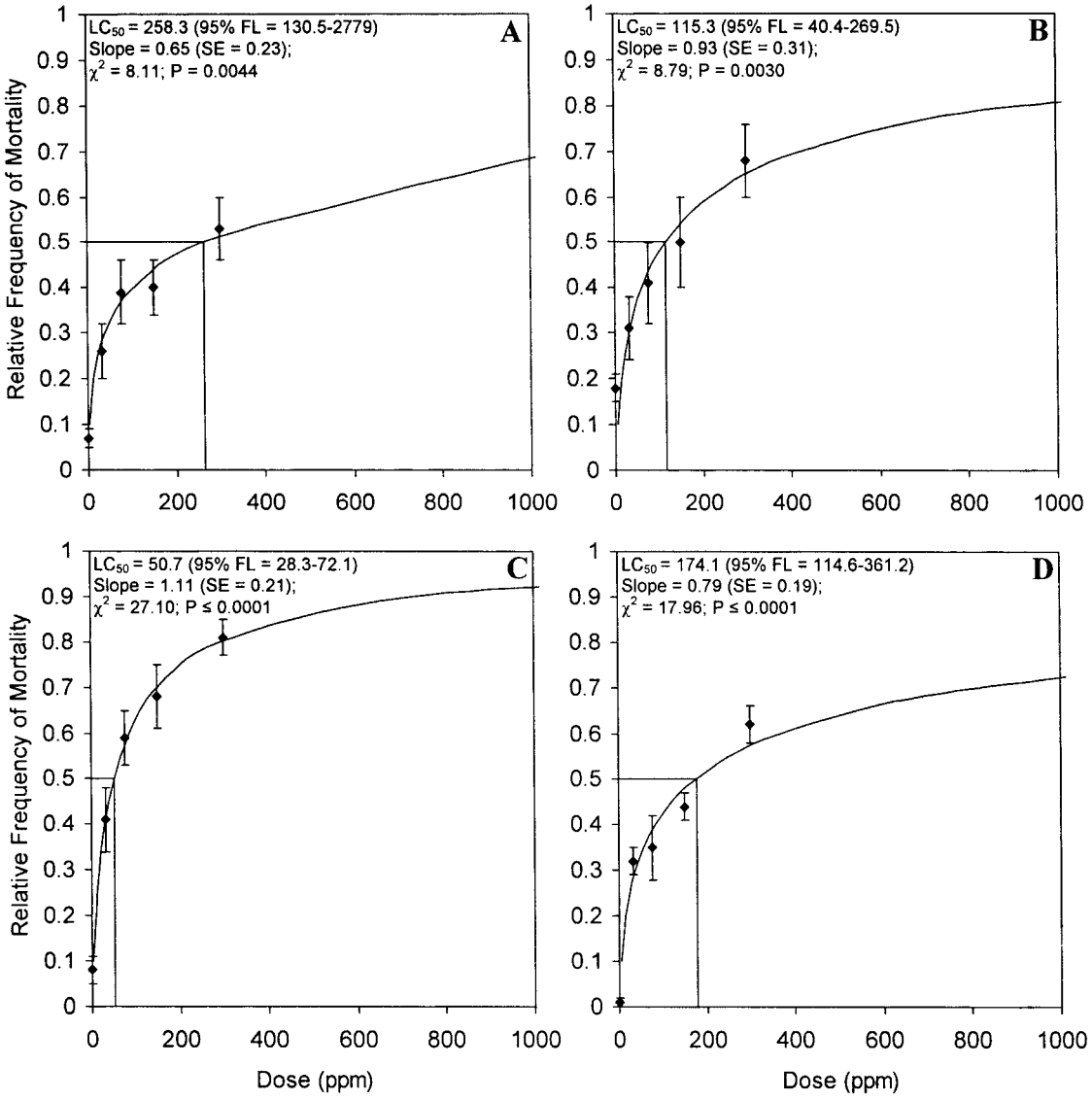

Fig. 1. Observed (♦ ± SE bars) values and predicted (line) dose response curves for *D. abbreviatus* neonates exposed to *B. thuringiensis* spores and δ-endotoxin in diet. LC_{50} values, slopes of log10 probit lines, and χ^2 values are shown for isolates B-21365 (A), B-21367 (B), B-21582 (C), and B-21583 (D).

binant strain B-21583 originally was a *Cry*⁻ *B. thuringiensis* that was engineered to express the *Cyt*2Ca1 toxin. The *Cyt*2Ca1 protein is a δ-endotoxin that fits into a second category, aside from the *Cry* toxins, known as *cyt*olitic (*Cyt*) toxins. The *Cyt* proteins, also known as hemolytic toxins, cause damage to the insect midgut through pore formation and cell lysis much like the *Cry* toxins. Guerchicoff et al. (2001) provides a discussion of the *Cyt* gene family, including similarities and differences with *Cry* genes.

The wild-type isolate B-21582, expressing the *Cyt*2Ca1 toxin, displayed the steepest dose-response curve and lowest LC_{50} value of those tested with the highest confidence in predictions. Though recombinant strain B-21583 also expressed *Cyt*2Ca1, the probit response was not as steep as that for B-21582, the LC_{50} value was greater, and the confidence in predicted values was lower. It appeared in this case that the wild-type isolate B-21582 produced a more virulent product against *D. abbreviatus* than did the recombinant strain B-21583 expressing the same toxin. Perhaps isolate B-21582 produces another, yet undetected product that works in conjunction with the *Cyt*2Ca1 protein to induce mortality in *D. abbreviatus* larvae.

Bacillus thuringiesis products have been widely accepted in agriculture for the control of many insect pests, but only in recent years have strains been discovered that control coleopteran pests. Control strategies that rely on formulated *B. thuringiensis* applications are used world-wide in many crops. Transgenic plant varieties that express *B. thuringiensis* δ-endotoxins have been used in the U.S. for several years and are now gaining international acceptance (Betz et al. 2000). Unfortunately, transgenic approaches to plant improvement are not currently being exploited in some crops that could benefit most from this technology, such as citrus, where a genetically engineered rootstock could be used to alleviate damage caused by *D. abbreviatus*.

These experiments demonstrated that there are *B. thuringiensis* δ-endotoxin genes currently available that could be used to transform citrus for protection against *D. abbreviatus*. The *Cry*ET33, *Cry*ET34, and *Cyt*2Ca1 genes could be expressed together or separately in a citrus rootstock. An appropriately engineered citrus rootstock, if properly managed, has the potential to offer resistance to *D. abbreviatus* throughout the life of the crop.

Because *D. abbreviatus* has a broad host range and is known to feed on other plants within and around citrus groves (Lapointe 2003), the presence of toxin in citrus roots within a grove may be expected to result in increased feeding by larvae on alternative food sources (either wild or intentionally planted) and thus reduce the likelihood of rapid resistance development to the toxin. The use of transformed citrus rootstocks would also avoid concerns associated with the possible effect of pollen from transformed plants, particularly if the gene inserted into the rootstock is constructed to be expressed only in root tissue.

Development of this technology for citrus should not be delayed since introduction of genetically modified crops requires investments of time and money, and years to complete, particularly for slow-maturing crops like citrus. Moreover, an effective control strategy for *D. abbreviatus* is long overdue and alternatives to the proposed genetically-modified citrus rootstock have not been presented.

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RESISTANCE AMONG LANTANA CULTIVARS TO THE LANTANA LACE BUG, *TELEONEMIA SCRUPULOSA* (HEMIPTERA: TINGIDAE)

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ABSTRACT

Lantana lace bug, *Teleonemia scrupulosa* Stål, (Hemiptera: Tingidae) is a primary insect pest of lantana, a landscape plant commonly grown across the southern United States. Twenty-eight cultivars of lantana were evaluated for resistance to lantana lace bug in replicated field plantings. Natural infestations of lantana lace bugs developed in mid-Jul, and were dispersed across all the replicates within 30 d in Dallas, TX. Populations of nymphs and adults were sampled bi-weekly from Sep-Nov 1996. Highest mean populations were present on 'Patriot Desert Sunset' (40.3 nymphs and adults/3-leaf sample/plant), 'Pink Frolic' (20.6) and 'Patriot Sunburst' (19.4). Nineteen of the cultivars exceeded 4 lace bugs per 3-leaf sample. Lace bugs were never detected on 3 cultivars, 'Weeping White', 'White Lightning' and 'Weeping Lavender' during the test period, and 'Imperial Purple', 'Patriot Rainbow' and 'Denholm Dwarf White' had seasonal means of only 0.1 total lace bugs per sample. Cultivars of *L. montevidensis* (K. Spreng.) Briq. (mean of 0.02 lace bugs/3 leaf sample) were highly resistant, whereas many cultivars of *L. camara* L. and *L. hybrida* hort (6.73 and 9.54 lace bugs/3 leaf sample, respectively) were susceptible. Cultivars with gold, red, purple, and white flowers had far fewer lace bugs than did cultivars with either orange/red, yellow, or bicolors of yellow with another color. These results indicate that within most flower colors or bicolors, there exists a range of resistance among the cultivars and usually at least 1 cultivar per color form with resistance to the lantana lace bug.

Key Words: *Lantana montevidensis*, *Lantana camara*, *Lantana hybrida*, host plant resistance, ornamental plants, herbaceous landscape plants

RESUMEN

El chinche de encaje de la lantana, *Teleonemia scrupulosa* Stål, (Hemiptera: Tingidae) es la plaga insectil principal de lantana, una planta de paisaje sembrada comúnmente por todo el sur de los Estados Unidos. Veinte ocho variedades de lantana fueron evaluadas para su resistencia al chinche de encaje de la lantana en replicaciones de siembras de campos. Infestaciones naturales del chinche de encaje de la lantana se desarrollaron a mediados de julio, y fueron dispersados por todas las repeticiones de ensayo dentro de 30 dias en Dallas, Texas. Las poblaciones de las ninfas y adultos fueron muestreadas cada dos semanas desde septiembre hasta el mes de noviembre de 1996. El promedio de las poblaciones mas altas se encontraron en el 'Patriot Desert Sunset' (40.3 ninfas y adultos/por muestra de 3 hojas por planta), el 'Pink Frolic' (20.6) y el 'Patriot Sunburst' (19.4). Diez y nueve de las variedades sobrepasaron los 4 chinches de encaje por muestra de 3 hojas. Los chinches de encaje no fueron detectados en las siguientes 3 variedades, 'Weeping White', 'White Lightning' y 'Weeping Lavender' durante el periodo de la prueba; por otro lado 'Imperial Purple', 'Patriot Rainbow' y 'Denholm Dwarf White' tenian un promedio estacional de solamente 0.1 chinche de encaje por muestra total. Las variedades de *L. montevidensis* (K. Spreng.) Briq. (promedio de 0.02 chinches de encaje/muestra de 3 hojas) fueron altamente resistentes, mientras muchas de las variedades de *L. camara* L. y *L. hybrida* hort (6.73 y 9.54 chinches de encaje/muestra de 3 hojas, respectivamente) fueron susceptibles. Las variedades con flores de color de oro, rojo, morado y blanco tenian mucho menos chinches de encaje que las variedades con flores de color anaranjado/rojo, amarillo, o los de dos colores de amarillo con otros colores. Estos resultados indican que entre la mayoria de un color de flor o de dos colores, existe un rango de resistencia entre variedades y usualmente por lo menos una variedad por color se forma con resistencia al chinche de encaje de la lantana.

Many cultivars of lantana (Verbenaceae) are used as annuals or as herbaceous perennials in containers and hanging baskets, or as a low hedge or as foundation shrubs in urban landscapes. Most cultivated species are native to tropical or subtropical North and South America, but some are native to warmer regions of the Old World. As a landscape plant, lantana is valued for its profuse show of color throughout a long season, often every month of the year in frost-free areas, its drought, heat and salt tolerance, aromatic foliage, and attractiveness to butterflies (Arnold 1999;

Everett 1981; Welch 1989). Two species are commonly used by the landscape industry. *Lantana camara* L. is a robust, more or less prickly shrub that is native to the southern United States and tropical America, whereas *L. montevidensis* (K. Spreng.) Briq. is a trailing or weeping lantana with slender pubescent stems up to 90 cm long or longer (Staff, L. H. Bailey Hortorium 1976; Everett 1981). *Lantana hybrida* hort cultivars is considered to be a hybrid between South American, Mexican, and West Indian species, but some are probably hybrids between the former two species (Everett 1981). *Lantana hybrida* hort cultivars exhibits characteristics of *L. camara* but is far more compact and seldom exceeds 30 cm in height.

Much of the literature on lantana centers on its introduction around the world as an ornamental and its unfortunate escape to become a noxious weed. It has been reported as a weed in 47 countries competing with 14 crops and infesting millions of hectares (Holm et al. 1977). Lantana lace bug, *Teleonemia scrupulosa* Stål (Hemiptera: Tingidae), has been introduced for biocontrol of lantana in over 20 countries, including Australia, India, many countries in Africa, Hawaii, and many island nations around the world (Harley and Kassulke 1971; Julien 1987). Most of the literature (other than taxonomic) on lantana lace bug relates to its introduction and use for biological control.

Across the southern United States, lantana lace bug is a late summer and fall annual pest of lantana cultivated in ornamental plantings. In Texas, as summer temperatures begin to rise and most plants begin to suffer from water stress, lantana plants thrive and flower profusely except where they are under attack by the lantana lace bug. The insect's behavior has been studied in Fiji (Simmonds 1929), India (Kahn 1946; Roonwall 1952) and Australia (Fyfe 1937). The nymphs develop on the underside of the leaves first causing a yellow spotting of the foliage, followed by silver to white bronzing with the leaves eventually browning and dropping from the plant. During nymphal feeding, large patches of black varnish-like droplets of excrement are deposited on the underside of the leaves and the molted skins of nymphs frequently remain attached. Adults are found on the leaves but also feed heavily on the flowers and cause a marked reduction in flowering and seed set (Wilson 1960). The objective of the present study was to evaluate 28 cultivars of lantana that are used in the nursery trade for their resistance or susceptibility to the lantana lace bug.

Materials and Methods

Lantana plants cultivated in 10 × 10 cm pots were planted ≈1 m apart in a series of raised field beds in a randomized complete block design with 6 replications of 1 plant per replicate. Only 3 replicates of several of the cultivars were evaluated (Table 1) due to a shortage of plant material. The highly alkaline (~8.0 pH), poorly aerated clay soil in the beds was amended by thoroughly incorporating a 5.1-cm-thick layer of sphagnum peat. Beds were mulched with a layer (7.5 cm thick) of cottonseed hulls and plants were irrigated thoroughly with soaker hoses every 7-10 d. A 21-7-14 (N-P-K) fertilizer, in which half of the N was formulated for slow-release, was incorporated into the soil mix prior to planting at a rate of 907.2 g / 9.3 m^2. A second application at the same rate of nutrients was applied as a side dressing to the plants in mid-Jul, ca. 8 wk later.

Most of the lantana cultivars were planted on either 15 or 16 May 1996. Due to unavailability of plant material on these dates, 'Patriot Dove Wing' and 'Patriot Honeylove' were not planted until 2 Aug 1996. Cultivars were chosen because of their popularity with growers across Texas and the southwestern United States. Only a few of these cultivars are listed by Howard (1969) in his checklist of lantana cultivars at the Harvard University Arboretum, but many of the cultivars evaluated are recommended for Texas and the Southwest (Brenzel 1997; Perry 1992; Sperry 1991).

Population counts for lantana lace bug were taken every 2 wk beginning 11-12 Sep through 11 Nov 1996, by examining each plant. All plants were examined during a 2-d observation period for each sample date. The overall plant was examined by gently lifting each of the terminal branches and recording the number of nymph and adult lace bugs on 3 leaves with the heaviest infestation. Visual evaluations for leaf bronzing, defoliation, overall loss of plant vigor, and the late summer and fall reduction in flowering were all good visual indicators of cultivars with high lace bug populations.

Data Analysis

Data were analyzed by analysis of variance procedures (ANOVA and GLM) in PC-SAS (SAS Institute 1990) to determine the differences in susceptibility among the cultivars at each observation period. Adult and nymph infestations were analyzed separately to show colonization levels. All count data were transformed as square root of $n + 0.001$ before analysis to stabilize variances. Untransformed means are reported. A Resistance Performance Index = the number of times a cultivar ranked in the top statistical grouping, was calculated for each cultivar as a measure of overall resistance (Engelke et al. 1994).

Results and Discussion

A naturally occurring infestation of lantana lace bugs invaded the replicated lantana planting in mid Jul 1996, and was first detected on plants of the cultivar 'Pink Frolic'. By mid Aug, popula-

TABLE 1. RESISTANCE AMONG LANTANA CULTIVARS TO THE LANTANA LACE BUG (6 REPLICATED FIELD PLOTS) SUMMER, 1996, DALLAS, TX.

		Mean number of nymphs and adults per 3 leaves per plant[2]											
		12 Sep		26 Sep		10 Oct		24 Oct		11 Nov		Mean Total / Plant[3]	Resistance Performance Index[4]
Cultivar	Species[1]	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult	Nymph	Adult		
Weeping White	Lm	0 a [6,*]	0[ns]	0 a	0[ns]	0 a	0 a	0 a	0 a	0 a	0 a	0 a	10
White Lightning[5]	Lm	0 a	0	0 a	0	0 a	0 a	0 a	0 a	0 a	0 a	0 a	10
Weeping Lavender	Lm	0 a	0	0 a	0	0 a	0 a	0 a	0 a	0 a	0 a	0 a	10
Imperial Purple	Lm	0.2 ab	0.2	0 a	0	0 a	0 a	0 a	0 a	0 a	0 a	0.1 a	10
Patriot Rainbow[5]	Lc	0 a	0	0 a	0	0 a	0 a	0.7 a-c	0 a	0 a	0 a	0.1 a	10
Denholm Dwarf White	Lc	0 a	0	0 a	0.2	0.2 a	0 a	0.3 ab	0 a	0 a	0 a	0.1 a	10
Radiation	Lc	4.2 a-d	0.2	0.3 a	0.5	0.3 a	0 a	0.3 ab	0 a	0 a	0 a	1.2 ab	10
Dallas Red[5]	Lc	1.7 a-c	0	2.0 a-d	0.3	1.3 ab	1.0 bc	0.7 a-c	0 a	1.0 a-c	0 a	1.6 ab	9
Gold Mound	Lh	5.3 a-d	0.8	1.8 a-c	0	1.8 ab	0.2 ab	0.3 ab	0 a	0.7 ab	0 a	2.2 ab	10
New Gold	Lh	5.7 a-d	0.2	7.9 a-e	0.7	3.8 a-d	0.2 ab	1.0 a-d	0 a	0.7 ab	0 a	4.0 a-d	10
Lemon Swirl[5]	Lc	15.0 d-g	0	7.0 a-e	0.3	0 a	0 a	1.3 a-e	0 a	0 a	0 a	4.7 a-d	9
Patriot Honeylove[5]	Lc	9.3 a-f	0	1.7 ab	0.3	9.7 d-g	0.3 ab	2.3 b-f	0.7 c	0 a	0 a	4.9 a-d	7
Confetti[5]	Lc	6.7 a-e	0	10.0 b-f	0	7.3 c-f	0 a	3.7 c-f	0.3 a-c	0.7 ab	0 a	5.7 a-e	7
Samantha	Lc	10.2 b-f	0	6.0 a-e	0	6.3 b-e	0 a	4.2 c-f	0.2 ab	4.0 c	0 a	6.2 a-e	6
American Red Bush[5]	Lc	4.3 a-d	1.3	12.3 d-g	0	13.3 e-h	0 a	8.0 fg	0 a	0.3 ab	0 a	7.9 a-f	7
Patriot Fire Wagon[5]	Lc	7.0 a-e	0	13.0 d-g	0.3	11.7 e-h	0 a	7.0 fg	0.4 a-c	2.0 bc	0 a	8.3 b-f	6
Miss Huff[5]	Lc	14.0 d-g	2.0	10.0 b-f	0.3	8.0 c-f	0 a	7.0 fg	0 a	0 a	0 a	8.3 b-f	6
Pink Caprice	Lc	14.5 d-g	0.5	8.0 a-e	0.5	11.2 e-h	0.3 ab	4.5 d-f	0.2 ab	2.7 bc	0 a	8.5 b-f	6
Spreading Sunset	Lh	16.8 d-g	0.2	10.3 b-f	0.2	7.3 b-f	0.2 ab	6.5 fg	0 a	1.8 a-c	0 a	8.7 b-g	6
Patriot Dove Wing[5]	Lc	0 a	0	30.0 f-h	0	15.7 f-h	1.7 c	2.3 b-f	1.0 c	0 a	0 a	10.1 c-g	5
Lemon Drop	Lh	21.3 e-g	0.3	16.2 d-g	0.5	9.0 d-g	0.2 ab	2.8 c-f	0 a	1.5 a-c	0 a	10.4 c-g	6
Silver Mound	Lh	32.0 f-g	0.3	10.8 b-f	0.5	7.3 c-f	0 a	4.0 c-f	0 a	2.2 bc	0 a	11.4 c-g	5
Golden King	Lc	34.2 f-g	1.2	12.3 d-g	0.3	12.3 e-h	0.3 ab	5.2 e-g	0 a	1.8 a-c	0 a	13.5 c-g	6
LSG Red-Orange	Lc	37.7 g	0.2	18.2 d-g	0.8	14.7 e-h	0.2 ab	3.8 c-f	0 a	2.3 bc	0 a	15.6 f-i	5
Irene[5]	Lc	26.7 fg	0.3	36.7 f-h	0	12.3 e-h	0.7 bc	6.0 e-g	0 a	0.7 ab	0 a	16.7 g-i	5
Patriot Sunburst[5]	Lc	3.0 a-d	0	55.3 h	1.0	22.0 h	0.3 ab	11.3 g	0.6 c	3.3 cd	0 a	19.4 hi	5
Pink Frolic[5]	Lc	40.0 g	0.7	28.0 f-h	0.3	20.7 gh	1.0 bc	7.0 fg	2.3 d	2.7 cd	0.3 b	20.6 i	2
Patriot Desert Sunset[5]	Lc	0 a	0	81.7 i	0.3	74.3 i	8.0 d	24.0 h	2.3 d	9.7 d	1.0 c	40.3 j	3

[1]Lantana species in study: Lm = *Lantana montevidensis*; Lc = *L. camara;* Lh = *L. hybrida*.
[2]Mean no. of nymphs or adults per 3-leaf sample per plant for the observation day.
[3]Mean total / plant is the mean of the total of all nymphs and adults for the 5 observation periods.
[4]Resistance Performance Index is the number of times an entry occurred in the top statistical group (highest possible is 10 for 10).
[5]These cultivars were only evaluated in 3 reps, all others had 6 reps.
[6]Analysis was made on square root of $n + 0.001$ transformation of the data: Untransformed means presented.
*Means in a column not followed by the same letter are significantly different by Waller-Duncan k-ratio t-test ($k = 100$) ($P \leq 0.05$): ns = non significant.

tions were also causing damage to foliage of 'Golden King', 'Irene', 'Lemon Drop', 'LSG Red-Orange', 'Silver Mound', and 'Spreading Sunset'. By early Sep, damage was widespread across the planting and relatively consistent across the replicates of the more susceptible cultivars.

The mean total of lace bugs per plant (Table 1) represents the average number of lace bugs (nymph + adult) per 3-leaf sample over the 5 observation periods. Data for 12 Sep showed nymphal development on 21 of the 28 cultivars. Highest populations were present on Pink Frolic (40.0 nymphs/3-leaf sample/plant) and LSG Red-Orange (37.7) whereas no populations of either nymphs or adults were observed on 'Weeping White', 'White Lightning', 'Weeping Lavender', 'Patriot Rainbow', 'Denholm Dwarf White', Patriot Dove Wing, or 'Patriot Desert Sunset'. A significantly lower population of only 0.2 nymphs/3-leaf sample/plant was present on 'Imperial Purple'. By 26 Sep, overall populations had decreased but were still highest on the cultivars that had supported the high populations throughout the season. The highest nymphal populations of 81.7 and 55.3 nymphs/3-leaf sample/plant were present on Patriot Desert Sunset and 'Patriot Sunburst', respectively. Patriot Dove Wing and Patriot Desert Sunset no longer appeared to be resistant as they had during the evaluation 2 wk earlier. By 11 Nov, the populations of lace bugs had declined on most of the susceptible cultivars. A high and damaging level, however, had been present on most of these cultivars throughout the 8-wk evaluation period and many of the cultivars were severely damaged with bronzed leaves and a considerable loss of leaves, flowers, and plant thriftiness. Once a plant was damaged to the extent that bronzed leaves were evident, it remained disfigured throughout the remainder of the growing season.

Lace bugs were never detected on Weeping White, White Lightning, and Weeping Lavender during the test period. Imperial Purple, Patriot Rainbow, and Denholm Dwarf White had mean populations of 0.1 total lace bugs per sample and never exceeded ≤0.7 insects per sample. The Resistance Performance Index shows that in addition to the aforementioned cultivars, 'Radiation', 'Dallas Red', 'Gold Mound', 'New Gold', and 'Lemon Swirl' also ranked either 9 or 10 (out of 10) times in the top statistical groupings. However, 'Patriot Honeylove', 'Confetti', 'Samantha' and 'American Red Bush' were also in the top statistical group for mean total lace bugs per 3-leaf plant sample, but these cultivars sustained significant lace bug populations during Sep and early-Oct and only occurred in the top statistical ranking either 6 or 7 times.

When cultivars are grouped by species and analyzed, the species, *L. montevidensis* (4 cultivars with a mean of 0.02 lace bugs/3-leaf sample) is highly resistant, whereas several of the *L. camara* and *L. hybrida* cultivars were resistant but most of them were susceptible to the lantana lace bug (Table 2). Cultivars of *L. montevidensis* produce either white or purple flowers.

Cultivars were analyzed separately for flower color. A cultivar with two predominant flower colors was analyzed as bicolor for the 2 colors. Cultivars with purple or white flower color had far fewer lantana lace bugs (means of 0.03 and 1.73, respectively) developing on them than did cultivars with other flower colors (Table 3). For the 2 white-flowered *L. camara*, Denholm Dwarf White is resistant while Patriot Dove Wing is a highly susceptible cultivar. A cultivar with low infestations of lace bugs and in the top statistical ranking or resistant was identified for each flower colors except for 2 bicolors, white/yellow and red/yellow (Table 3). Overall, it appears that cultivars with either yellow or yellow bicolor flowers are among the most susceptible to the lantana lace bug. Flower color has been implicated as an indicator of resistance in other ornamental plants. In studies with *Canna* spp., cultivars with red-, orange-, and scarlet-flowers were more susceptible to canna leafroller, *Calpodes ethlius* Stoll than those with yellow- or rose-flowers (Reinert et al. 1983). Also, in studies with oleander, *Nerium oleander* L., susceptibility to oleander caterpillar, *Syntomeida epilais jucundissima* Dyar, was much higher on cultivars with certain flower colors than on those with other flower colors (J. A. Reinert et al. unpublished data). Resistance may not be determined by flower color, but there appears to be a relationship to color, although not independent. Additional work is needed to fully understand what the relationship is between flower color and resistance to lantana lace bug and other insects.

TABLE 2. IMPACT OF SPECIES OF LANTANA ON THE INFESTATION LEVEL OF LANTANA LACE BUG.

Lantana spp.	No.[1]	Range of means for cultivars	Mean total nymphs + adults/3 leaves/plant[2]
L. montevidensis	4	0.0-0.1	0.02 a*
L. camara	19	0.1-40.3	6.73 b
L. hybrida	6	2.2-11.4	9.54 b

[1]No. of cultivars evaluated for each species.
[2]Mean total/plant are the mean of all nymphs and adults per 3 leaves per plant for 5 observation periods.
*Means in column not followed by the same letter are significantly different by Waller- Duncan k-ratio t-test ($k = 100$) ($P \leq 0.05$).

TABLE 3. IMPACT OF THE FLOWER COLOR OF LANTANA CULTIVARS ON THE POPULATION LEVELS OF LANTANA LACE BUG.

Flower color (no.)[1]	Cultivars	spp[2]	Lace bugs per observation period[3]		Mean total lace bugs/3 leaves/5 obs. periods on each flower color[4]
			Highest count	Mean[5]	
Purple (2)[6]					0.03 a
	Weeping Lavender	Lm	0	0 a*	
	Imperial Purple	Lm	0.4	0.1 a	
White (4)					1.73 ab
	Weeping White	Lm	0	0 a	
	White Lightning	Lm	0	0 a	
	Denholm Dwarf White	Lc	0.3	0.1 a	
	Patriot Dove Wing	Lc	30.0	10.1 c-g	
Gold (2)					3.10 bc
	Gold Mound	Lh	6.1	2.2 ab	
	New Gold	Lh	8.6	4.0 a-d	
Red (2)					4.75 bc
	Dallas Red	Lc	2.3	1.6 ab	
	American Red Bush	Lc	13.3	7.9 a-f	
Orange/Red (3)					8.47 cd
	Radiation	Lc	4.4	1.1 ab	
	Spreading Sunset	Lh	17.0	8.7 b-g	
	LSG Red-Orange	Lc	37.9	15.6 f-i	
Pink/Yellow (6)					9.27 d
	Patriot Rainbow	Lc	0.7	0.1 a	
	Patriot Honeylove	Lc	10.0	4.9 a-d	
	Confetti	Lc	10.0	5.7 a-e	
	Pink Caprice	Lc	15.0	8.5 b-f	
	Irene	Lc	36.7	16.7 g-i	
	Pink Frolic	Lc	40.7	20.6 i	
Yellow (6)					10.28 d
	Lemon Swirl	Lc	15.0	4.7 a-d	
	Samantha	Lc	10.2	6.2 a-e	
	Miss Huff	Lc	16.0	8.3 b-f	
	Lemon Drop	Lh	21.6	10.4 c-g	
	Golden King	Lc	35.4	13.5 c-g	
	Patriot Sunburst	Lc	56.3	19.4 hi	
White/Yellow (1)					11.43 d
	Silver Mound	Lh	32.3	11.4 c-g	
Red/Yellow (2)					24.30 e
	Patriot Fire Wagon	Lc	13.3	8.3 b-f	
	Patriot Desert Sunset	Lc	82.0	40.3 j	

[1]Cultivars with two predominant flower colors were analyzed as bicolor for the 2 colors.
[2]Lantana species in study: Lm = *Lantana montevidensis*; Lc = *L. camara;* Lh = *L. hybrida*.
[3]Plants sampled by counting total nymphs and adults per 3 leaves per plant for each of 5 observation periods; highest count per any sample period and mean total count during the test period.
[4]Mean total/plant = mean of the total of all nymphs and adults combined for the 5 observation periods.
[5]Data taken from Table 1.
[6]Number of cultivars with the flower color.
*Means in a column not followed by the same letter are significantly different by Waller-Duncan *k*-ratio *t*-test (k =1 00) ($P \leq 0.05$).

CONCLUSIONS

This information on the range of susceptibility among cultivars within each of the flower color groupings should be of considerable value to commercial growers, retail nurserymen, landscapers, and consumers. The species, *L. montevidensis*, provides resistant purple- (Weeping Lavender and Imperial Purple) or white-flowered (Weeping White and White Lightning) cultivars. Additionally, Denholm Dwarf White is a resistant white-flowered *L. camara* cultivar with a more upright, mounding growth habit. When lantana lace bug was being evaluated as a biocontrol agent in Aus-

tralia, Haseler (1966) observed that it defoliated white-flowered lantana (no species or cultivars given) and caused the plants to die. Our data show both resistance and susceptibility among the white-flowered cultivars. For the other flower color or bicolor groupings, there is a range of susceptibility among cultivars as well, typically with at least 1 cultivar ranking in the top statistical grouping and expressing resistance. For example, in the pink/yellow-flowered group, Patriot Rainbow is resistant, whereas all the other cultivars are susceptible with Irene and Pink Frolic being extremely susceptible. Harley & Kassulke (1971) and Radunz (1971) reported that lantana lace bug showed a preference for red-flowered lantana species, pink-flowered were least preferred, and white and orange showing intermediate damage, but they did not identify cultivars or species. Their statement, and the results presented here, emphasize the need to understand the potential genetic resistance of each cultivar regardless of the flower color. This range of susceptibility among the cultivars within each color grouping should allow the consumer to install landscape plantings of lantana that have an array of flower color but still provide a high level of natural (genetic) protection against this destructive pest.

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ANDROTHRIPS RAMACHANDRAI (THYSANOPTERA: PHLAEOTHRIPIDAE): AN INTRODUCED THRIPS IN THE UNITED STATES

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ABSTRACT

Androthrips ramachandrai Karny is an exotic thrips, assumed to be predacious, and is associated with gall-inducing thrips. It was first reported in the U.S. from FL, and intercepted in CA from Thailand in 2002. We surveyed *Ficus* spp. with *Gynaikothrips*-induced galls in AL, CA, FL, HI, LA, MS, and TX, and document that *A. ramachandrai* is now established in CA, FL, HI, and TX. It probably has been spread by the ornamental horticulture industry. We outline its biology and compare it to a congener *A. flavipes*, a documented thrips predator. *Androthrips ramachandrai* has the potential to be a beneficial biological control agent and a hindrance to weed biological control.

Key Words: predator, invasive species, *Gynaikothrips*, biotic interference

RESUMEN

Androthrips ramachandrai es un trips exótico, que parece ser un depredador, y esta asociado con trips que producen agallas. El trips fue informado por primera vez en los Estados Unidos en el estado de la Florida, e interceptado en California en el 2002 de Tailandia. Nosotros muestreamos las plantas de *Ficus* spp. con agallas inducidas por *Gynaikothrips* en Alabama, California, Florida, Hawaii, Louisanna, Mississippi, y Texas y documentamos que *A. ramachandrai* esta ahora establecido en California, Florida, Hawai, y Texas. Probablemente el trips ha sido dispersado por la industria de horticultura ornamental. Nosotros también describimos su biologia y la comparamos con su congenere *A. flavipes*, un depredador de trips ya documentado. *Androthrips ramachandrai* tiene el potencial para ser un agente de control biológico benéfico y un obstaculo para el control biológico de malezas.

Held et al. (2005) predicted that gall-inhabiting arthropods found in *Gynaikothrips*-induced galls on *Ficus* spp. could be inadvertently transported within the continental U.S. through the ornamental plant industry. *Androthrips ramachandrai* Karny (Thysanoptera: Phlaeothripidae) is known to inhabit the galls of both *Gynaikothrips uzeli* (Zimmermann) and *G. ficorum* (Marchal) (Thysanoptera: Phlaeothripidae) (Takahashi 1934), and is probably being moved throughout the U.S. in shipments of nursery-grown *Ficus*.

The first record of *A. ramachandrai* in the U.S. was of two specimens collected in March 2002, Miami-Dade Co., FL from *Ficus microcarpa* (Moraceae) galled by *G. ficorum* (Nakahara & Edwards 2002). Another collection of *A. ramachandrai* was made from Riverside Co., CA on 02 Aug of the same year (Gaimari 2005). It was intercepted during a federal foreign-quarantine inspection along with *Gigantothrips elegans* Zimmermann (Thysanoptera: Phlaeothripidae) and *Gynaikothrips malabaricus* Ramakrishna on *Ficus* sp. imported from Nong Nooch Tropical Botanical Garden in Thailand (G. Watson, California Department of Food and Agriculture, pers. comm.).

Androthrips ramachandrai was described from India and found in association with the gall thrips *Austrothrips cochinchinensis* Karny (Thysanoptera: Phlaeothirpidae) on *Calycopteris* (=*Getonia*) *floribunda* (Combretaceae) (Karny 1926). Worldwide, *Androthrips* contains 12 species (Mound 2005). *Androthrips flavipes* Schmutz is a known predator of thrips (Ananthakrishnan & Varadarasan 1977; Varadarasan & Ananthakrishnan 1981), and other *Androthrips* species are assumed to be predators, too, but little or nothing is known about their biology.

Androthrips ramachandrai (Fig. 1) is dark brown to black. It can be distinguished from other dark, large phlaeothripids by its large fore femora with a strong, cylindrical tooth near the base followed by a row of small tubercles (Fig. 2). Its tube (abdominal segment X) is almost half the length of *Gynaikothrips* spp., which is easily detectable. It can be separated from other *Androthrips* by the dark middle and hind tibiae (Karny 1926).

Not much is known about the biology of *A. ramachandrai*. It is rare in newly formed galls of *Austrothrips cochinchinensis*. However, as galls mature, *A. ramachandrai* becomes more abun-

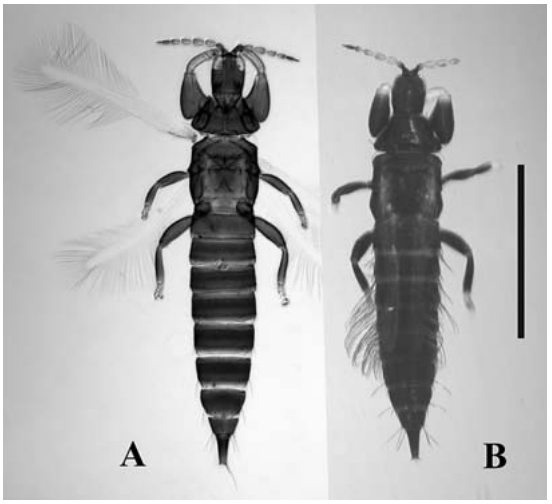

Fig. 1. Dorsal view of *Androthrips ramachandrai* from Riverside Co., CA. (A) Slide mounted, cleared specimen and (B) wet mounted specimen. Scale bar = 1 mm.

dant as the population of *A. cochinchinensis* declines (Ananthakrishnan 1978), which might indicate that *A. ramachandrai* is predacious on the gall-inducing thrips, similar to *A. flavipes* (Ananthakrishnan & Varadarasan 1977).

Currently, *A. ramachandrai* is known from Australia, Costa Rica (L. Mound, pers. comm.), India (Karny 1926), Taiwan (Takahashi 1934), and Thailand (Ananthakrishnan 1978). Herein we report its establishment in the U.S.

The purpose of this paper is to document the currently known distribution of *A. ramachandrai* in the U.S., provide a brief overview from the literature of its biology, and increase the awareness of regulatory and research entomologists in North American to this thrips, which could become economically and ecologically important.

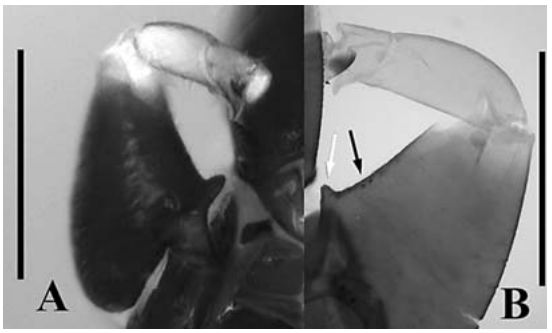

Fig. 2. Fore femur of *Androthrips ramachandrai* from Riverside Co., CA. (A) Wet mounted, right ventral fore femur and (B) slide mounted, cleared left fore femur. The white arrow is pointing to the strong, cylindrical tooth near the base and back arrow to the row of small tubercles. Scale bars = 250 µm.

Materials and Methods

We collected and solicited *Gynaikothrips*-induced galls from the following states in the U.S.: AL, CA, FL, HI, LA, MS, and TX. Galls and contents were collected in the field, preserved immediately in 95% ethanol, and taken to the lab for identification of the thrips. Museum records were requested for *A. ramachandrai* from CA (California Department of Agriculture), FL (Florida State Collection of Arthropods), and TX (Texas A & M University).

Results and Discussion

Ficus galls collected from South Padre Island, Cameron Co., TX by DWH on 24 Aug 2005, from Riverside Co, CA by Chris Hanlon (University of California, Riverside) on 08 Mar 2005, and from Oahu Island, HI by Frank Howarth (Bishop Museum, Honolulu) on 17 Apr 2006 contained specimens of *A. ramachandrai*. Galls from TX were initiated by *G. uzeli* and collected from 7 *Ficus* trees at 2 locations (Table 1). Galls from CA were initiated by *G. ficorum* and collected at 1 location with a total of 258 *G. ficorum* and 21 *A. ramachandrai* (total number of galls not known). Galls from HI were initiated by *G. ficorum* and collected at 1 location with 16 *G. uzeli*. Voucher specimens of *G. ficorum*, *G. uzeli*, and *A. ramachandrai* from CA and TX have been deposited in the USDA, ARS, Systematic Entomology Laboratory, Beltsville, MD; and *A. ramachandrai* from HI have been deposited in the Bishop Museum, Honolulu, HI.

The Florida Department of Plant Industry has at least 44 records of *A. ramachandrai* (2002-2006) from 11 southern counties in Florida (Brevard, Broward, Miami-Dade, Glades, Hillsborough, Lee, Martin, Monroe, Palm Beach, Pinellas, and Sarasota) and from the following plants: *Artocarpus heterophyllus* (Moraceae); *Ficus benjamina* and *F. microcarpa* (Moraceae); *Malvaviscus penduliflorus* (Malvaceae); *Schefflera actinophylla* (Araliaceae); and *Tabebuia heterophylla* (Bignoniaceae).

The California Department of Food and Agriculture has one record of *A. ramachandrai* collected from *F. microcarpa* originating from Irvine Co. and intercepted in Santa Clara Co. on 09 Nov 2004. Galls collected or solicited from AL, LA, and MS did not contain specimens of *A. ramachandrai*, and no further museum records were available for TX.

The 2 records from California are new state records, because the previous record was an intercepted specimen from Thailand (see above). The records from Texas and Hawaii also are new state records.

TABLE 1. GALL INHABITANTS BY SPECIES COLLECTED FROM SOUTH PADRE ISLAND, CAMERON CO., TX, AUGUST 2005.

	No. galls	*Gynaikothrips uzeli*	*Androthrips ramachandrai*	*Montandoniola moraguesi*
			Site 1	
Plant 1	3	15	9	0
Plant 2	3	25	14	0
Plant 3	3	73	3	3 nymphs
Total	9	113	26	3 nymphs
			Site 2	
Plant 1	4	5	34	0
Plant 2	3	12	1	0
Plant 3	3	24	3	0
Plant 4	4	38	0	0
Total	14	79	38	0

Galls were randomly collected from each site, where site 1 was the Convention Center (large landscape plants) and site 2 a local restaurant (containerized plants) on the island.

Our findings from TX (Table 1) might indicate that *Gynaikothrips* populations decline during an increased presence of *A. ramachandrai*. However, further data is needed to substantiate this claim. The trend is consistent with a pattern found by Anathakrishnan (1978) in which populations of *Austrothrips cochinchinensis* decreased as *A. ramachandrai* progressively increased.

Montandoniola moraguesi (Puton) (Hemiptera: Anthocoridae) was present in the galls from TX and HI. This anthocorid is known to feed on gall-inducing thrips and also on *A. ramachandrai* and *A. flavipes* (Dobbs & Boyd 2006). What impact this anthocorid might have on the effectiveness of *A. ramachandrai* in reducing pest thrips populations is not known. Another natural enemy of *Gynaikothrips* is the wasp *Thripastichus gentilei* (del Guercio) (Hymenoptera: Eulophidae), which parasitizes species of *Androthrips* (Loomans et al. 1997). What quantitative impact these 3 natural enemies (individually or together) have on pest-thrips populations and on each other remains unassessed.

Nothing is known about the ecology of *A. ramachandrai*, but some information may be inferred from the better-studied congener *A. flavipes*. When adults of *A. flavipes* enter mature galls of *Arrhenothrips ramakrishnae* Hood (Thysanoptera: Phlaeothripidae), they feed on about 10% of the available prey and deposit eggs in galls near their prey eggs. After hatching, the larvae consume most of the remaining prey and resort to cannibalism. This behavior can occur whether prey is abundant or not and is a limiting factor to its own population growth (Varadarasan & Ananthakrishnan 1981). Typically they feed on the eggs and larvae, but not adults. By the time the larvae pupate, they have devoured almost 80% of available prey (Sureshkumar & Ananthakrishnan 1987). *Androthrips flavipes* develops faster than the galling thrips, which enables the predator to complete its life cycle more quickly than the prey (Varadarasan & Ananthakrishnan 1982). Strangely, the enlarged fore femora of adult *A. flavipes* are not used in subduing prey (Varadarasan & Ananthakrishnan 1982; Sureshkumar & Ananthakrishnan 1987).

Because *A. ramachandrai* feeds primarily on the immature stages of thrips in galls, this does not preclude it from attacking the immature stages of surface feeding thrips. In a laboratory trial, *A. flavipes* readily consumed thrips prey in a Petri dish (Varadarasan & Ananthakrishnan 1981). In FL, where *A. ramachandrai* is well established, there may be possible biotic interference (Reimer 1988) with thrips, particularly phlaeothripids such as *Pseudophilothrips ichini* (Hood) (Thysanoptera: Phlaeothripidae), used for biological control of invasive peppertrees (Cuda et al. 2005). The effectiveness of the weed biocontrol *Liothrips urichi* Karny (Thysanoptera: Phlaeothripidae) released for biocontrol of the weed, *Clidemia hirta* (Melastomataceae), in HI has been reduced by generalist predators (Reimer 1988).

Androthrips ramachandrai is now established in 3 continental U.S. states and HI, but very little is known about its biology or ecology. It is assumed to be predacious and could potentially have an impact on thrips populations, including pests and weed biological control agents. Its presence could cause biotic interference (Reimer 1988) or it could prove to be a successful natural enemy against *Gynaikothrips* spp. on ornamental *Ficus*. We intend this information to increase the awareness of regulatory agents and facilitate the identification of this potentially important thrips.

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LIBURNIA PSEUDOSEMINIGRA (DELPHACIDAE: HOMOPTERA), A NEW AND UNUSUAL PEST OF ST. AUGUSTINEGRASS

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ABSTRACT

No publications have reported delphacid planthoppers (Family Delphacidae) to be turf pests in the United States. In March 2005, a large infestation of the delphacid planthopper, *Liburnia pseudoseminigra* (Muir & Gifford), was found infesting St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze, in a commercial sod farm in southern Florida. Thereafter, a survey was conducted in commercial sod farms in southern Florida to determine the extent of the planthopper infestation in different St. Augustinegrass varieties. The planthoppers were found in low numbers in Floratam, Palmetto, and Seville and were moderately abundant in Bitterblue. However, Classic was clearly the variety supporting large numbers of the planthopper. *Liburnia pseudoseminigra* was described from Florida and apparently is a native species. Virtually nothing was known about its biology prior to its appearance as a pest of St. Augustinegrass in this study.

Key Words: Delphacidae, *Liburnia*, turf, St. Augustinegrass

RESUMEN

No se ha reportado publicaciones de delfacidos saltadores de plantas (Family Delphacidae) como plagas de césped en los Estados Unidos. En marzo del 2005, se encontró una infestación grande del delfacido saltador de plantas, *Liburnia pseudoseminigra* (Muir y Gifford), infestando el césped de San Augustín, *Stenotaphrum secundatum* (Walt.) Kuntze, en una finca comercial de césped en el sur del estado de Florida. Por ello, se realizó un muestreo en fincas comerciales de césped en el sur de la Florida para determinar el alcanze de la infestación del saltador de plantas en variedades diferentes de césped de San Augustín. Se encontraron los saltadores de plantas en números bajos en Floratam, Palmetto y Seville y fueron moderadamente abundante en Bitterblue. Sin embargo, la variedad Classic fue claramente la que apoyo el número mayor de saltadores de plantas. *Liburnia pseudoseminigra* fue descrito del estado de Florida y aparentemente es una especie nativa. Prácticamente no se sabia nada acerca de su biología antes de que apareciera como plaga de césped de San Augustín en este estudio.

St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze lawns are grown throughout the southern United States because of their climatic adaptation and ability to tolerate full sun to moderate shade. Sod production in Florida is a large industry with 37,180 ha (92,950 acres) grown in 2003. Sixty-four percent of the Florida sod acreage was St. Augustinegrass (Haydu et al. 2005). Numerous insects are pests of St. Augustinegrass (Potter 1998; Vittum et al. 1999). However, no publications have reported delphacid planthoppers (Family Delphacidae) to be turf pests on St. Augustinegrass or any other turfgrass in the United States. In March 2005, a sod producer located near Belle Glade, Florida, requested help from the Everglades Research and Education Center (U. of Florida, IFAS) at Belle Glade, Florida in identifying and controlling insect pests in his St. Augustinegrass fields. Upon visiting these fields, large numbers of insects of an unknown type were observed in fields of Classic St. Augustinegrass. Sweep net collections were made and insects sent to Susan Halbert who identified them as a planthopper, *Liburnia pseudoseminigra* (Muir and Gifford) in the family Delphacidae. The identification was confirmed by Dr. Stephen W. Wilson, Central Missouri State University. Specimens are deposited at the Florida State Collection of Arthropods (FSCA) in Gainesville, FL (FSCA# E2005-1134). In 2003, a similar infestation of the planthopper was found in Gainesville, FL in a lawn planted to Classic St. Augustinegrass (FSCA# E2003-1394). However, further study of the insect was not conducted at

that time. Because of the novelty of a delphacid as a turf pest, we pursued research to determine the extent of the infestation in different St. Augustinegrass varieties.

Materials and Methods

The majority of sod production in Florida occurs in southern Florida (Haydu et al. 2005). Our survey was conducted in 2005 at 8 different sod farms in 5 counties (Collier, Desoto, Hendry, Highlands, Palm Beach) in southern Florida to obtain representative samples. Different varieties also were sampled in different areas to determine if the planthoppers were responding to varietal differences. Five fields of Bitterblue, 8 fields of Classic, 10 fields of Floratam, 5 fields of Palmetto, and 4 fields of Seville were sampled. All fields were sampled within a 77-d interval (21 Mar to 6 Jun) to reduce the possibility of seasonal variation affecting planthopper populations. Only fields that had not been treated with an insecticide for at least 1 month and had large numbers of live arthropods present were sampled.

Samples were taken in 5 transects in random locations in each field. Each transect sample consisted of 100 sweeps in a straight line with a 38-cm diam. net. After sweeping, arthropods in nets were bagged and later frozen. Adult and nymphal *L. pseudoseminigra* in samples were counted by microscope examination in a laboratory. Adult and nymphal leafhoppers (Cicadellidae) also were counted, since these are known general turf pests (Potter 1998; Vittum et al. 1999) and this allowed comparisons of the planthopper versus leafhopper abundance in the different varieties. Mean numbers of planthoppers and leafhoppers in the different St. Augustinegrass varieties were compared by the Least Significant Difference (LSD) test (SAS 2005).

Preliminary field observations indicated that planthopper population densities varied among St. Augustinegrass varieties, especially between Classic, with high populations and Floratam, with low populations. Thus, a laboratory study was conducted to determine if population growth on Classic and Floratam corresponded to field observations. Evaluations were conducted with potted Floratam and Classic St. Augustinegrass as a no choice test. Turfgrasses were grown in pots ($6.5 \times 6.5 \times 9.0$ cm deep) filled with a 1:1 mixture by volume of sand and Fafard #2 potting medium (Conrad Fafard, Agawam, MA). All test plants were started from a single double node cutting and all plants were 8 weeks old at the start of the experiment on 9 May, 2005.

Individual plants were placed into a holding cage constructed from two 1 L polypropylene food storage containers. The containers were held together top to top by a coupler made from the lids of the container. The center of the lids was removed and the remaining ring was glued together to form the coupler. The bottom of the top container was removed and replaced with a screen mesh for ventilation. Ten adult *L. pseudoseminigra* were placed in each cage and maintained in a plant growth room. After 38 d, adult planthoppers and nymphs were counted. The 4 treatments were Classic with and without planthoppers and Floratam with and without planthoppers. Five replications (individual plants) were tested. The mean number of planthoppers (nymphs + adults) alive after 38 d in the treatments was separated by using LSD analysis (SAS 2005).

Results and Discussion

Abundance of *L. pseudoseminigra* in different St. Augustinegrass varieties is shown in Table 1. The planthoppers were found in all varieties, but in low numbers in Floratam, Palmetto, and Seville. Planthoppers were moderately abundant in Bitterblue, but not significantly different from the former 3 varieties. This lack of statistical separation from those varieties is partly due to very large variation in planthopper numbers between the 5 Bitterblue fields. Planthoppers averaged < 10/sample in 3 fields and > 100/sample in 2 fields. Examination of morphological characteristics for Bitterblue from the fields showed that the Bitterblue was not a homogenous group. Differences in stigma colors were observed. Preliminary obser-

Table 1. Abundance of *L. pseudoseminigra* in different St. Augustinegrass varieties in southern Florida sod fields.

	Nymphs	Adults	Total
Variety	Mean ± SD	Mean ± SD	Mean ± SD
Bitterblue	66.9 ± 98.8 b	28.4 ± 49.0 b	95.3 ± 145.2 b
Classic	259.2 ± 289.0 a	121.3 ± 208.0 a	380.5 ± 478.3 a
Floratam	0.2 ± 0.8 b	1.5 ± 4.7 b	1.7 ± 5.3 b
Palmetto	13.4 ± 20.8 b	3.5 ± 5.3 b	16.9 ± 25.7 b
Seville	0 ± 0 b	0.4 ± 0.9 b	0.4 ± 0.9 b

Means in a column are not significantly different ($P > 0.05$) when followed by the same letter based on the LSD test (SAS 2005).

TABLE 2. ABUNDANCE OF LEAFHOPPERS IN DIFFERENT ST. AUGUSTINEGRASS VARIETIES IN SOUTHERN FLORIDA SOD FIELDS.

	Nymphs	Adults	Total
Variety	Mean ± SD	Mean ± SD	Mean ± SD
Bitterblue	10.7 ± 16.9 b	124.6 ± 189.2 a	135.2 ± 204.4 a
Classic	22.2 ± 38.7 a	72.7 ± 80.0 b	94.9 ± 107.3 ab
Floratam	10.3 ± 12.6 b	54.0 ± 38.5 bc	64.4 ± 49.3 bc
Palmetto	4.7 ± 4.3 b	28.2 ± 21.8 c	32.8 ± 24.4 c
Seville	2.8 ± 4.0 b	55.0 ± 50.3 bc	57.8 ± 52.5 bc

Means in a column are not significantly different ($P > 0.05$) when followed by the same letter based on the LSD test (SAS 2005).

vations indicated that the Bitterblue with white stigma supported more planthoppers than Bitterblue with lavender stigma (data not shown). However, Classic was clearly the variety supporting large numbers of the planthopper with significantly more nymphs, adults, and total numbers than any other variety.

Field data are corroborated by our potted plant studies. The mean total number of planthoppers/plant after 38 days was 375.8 on Classic initiated with 10 adults versus 0 for the other 3 treatments. Obviously, the 375.8 mean was significantly different ($P < 0.05$) from the other means and shows the high potential population growth of the planthoppers on Classic. These data also show the lack of population growth on Floratam which corresponds to field observations (Table 1).

In field samples, planthopper nymphs of all sizes were found in 4 of the varieties with especially large numbers in Classic and Bitterblue (Table 1). These data show that the planthoppers were reproducing in the fields and not just immigrating into the fields as adults. The planthoppers were found in all 5 counties sampled indicating widespread distribution in southern Florida sod fields.

Abundance of leafhoppers in different St. Augustinegrass varieties is shown in Table 2. As expected, some leafhoppers were found in all varieties. Interestingly, Bitterblue and Classic had the most leafhoppers. These 2 varieties also had the most planthoppers (Table 1). The presence of leafhopper nymphs indicates that reproduction was taking place in the fields and not just adults immigrating into the fields.

Superimposing data from the 2 tables shows that the planthoppers were much more responsive to the different varieties than leafhoppers. For example, planthoppers were 951 times more abundant in Classic than in Seville. In contrast, the maximum variation in leafhoppers occurred between Bitterblue and Palmetto where the former had only 4 times as many leafhoppers as the latter variety. Also, superimposing data from the 2 tables shows that more leafhoppers than the planthoppers were found in every variety except Classic. These data again emphasize that Classic is the variety we tested which is most likely to have problems with the planthoppers. Lastly, as noted earlier, leafhoppers are known pests in turf (Potter 1998; Vittum et al. 1999). Our data show that 4 times as many *L. pseudoseminigra* as leafhoppers were found in Classic, showing that *L. pseudoseminigra* is potentially a greater pest than the known leafhopper pests in at least 1 St. Augustinegrass variety.

Liburnia pseudoseminigra was described (Muir & Gifford 1924) from Florida and apparently is a native species. Virtually nothing was known about its biology prior to its appearance as a pest of St. Augustinegrass in this study.

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HOST SPECIFICITY OF FOUR *PSEUDACTEON* SPP. (DIPTERA: PHORIDAE), PARASITOIDS OF FIRE ANTS IN ARGENTINA (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Several South American species of *Pseudacteon* have been released for biocontrol of red imported fire ants *Solenopsis invicta* in the U.S. Here we provide additional data from host specificity tests on 4 additional candidate species, *P. nocens*, *P. nudicornis*, *P. cultellatus*, and *P. obtusus*, all of which are components of multi-species complexes that occur within Argentinean *Solenopsis* populations. All 4 species were tested with sequential, no choice exposures to the red imported fire ant *S. invicta*, and the tropical fire ant, *S. geminata*. Levels of intrageneric specificity ranged from moderate to high and all 4 species showed greater specificity than some *Pseudacteon* species already approved for release.

Key Words: biological control, parasitism, *Solenopsis geminata*, *Solenopsis invicta*, *Pseudacteon*

RESUMEN

Varias especies de fóridos *Pseudacteon* provenientes de Sur América han sido introducidas en los Estados Unidos para utilizar como control biológico de la hormiga roja de fuego *Solenopsis invicta*. En esta publicación mostramos los resultados de pruebas de especificidad de hospedero realizadas en otros cuatro fóridos, *P. nocens*, *P. nudicornis*, *P. cultellatus* y *P. obtusus* que son parte del grupo de especies normalmente asociadas con poblaciones de *Solenopsis* en Argentina. Las pruebas de especificidad consistieron en la exposición secuencial de las moscas a las hormigas de fuego, *S. invicta* y *S. geminata*. El grado de especificidad de hospedero en las cuatro especies de fóridos varió entre moderado y alto pero fue siempre mayor que el presentado por algunas de las especies aprobadas para liberar como control biológico en los Estados Unidos.

Translation provided by the authors.

After their introduction in the early 20th century, the South American fire ants, *Solenopsis invicta* Buren and *S. richteri* Forel, quickly expanded their range in the United States (US). Today, *S. invicta* (red imported fire ant) occupies the southern states of US and Puerto Rico (Callcott & Collins 1996), is spreading south into Mexico (Sanchez et al. 2005), and is predicted to expand more than 100 km northward in the US due to anticipated climate changes (Morrison et al. 2005). The success of the imported fire ants as invasive species is probably due, in part, to the absence of the natural enemies that have coevolved with this species in South America (Porter et al. 1997). Some enemies left behind include phorid flies of the genus *Pseudacteon* (Diptera: Phoridae) that parasitize and eventually kill ant workers and may likely contribute to decrease population densities to the levels found in their homeland (Porter et al. 1997).

In the US, 3 *Pseudacteon* species, *P. tricuspis*, *P. curvatus*, and *P. litoralis*, have been released as part of the biological control program that seeks to reduce fire ant densities to levels with less ecological and economical impact (Porter & Gilbert 2004). In order to achieve this goal, more species and specific biotypes (geographic distinctive population) are necessary so phorid communities could resemble more closely those that exist in South America. Including more species could, for example, expose ants throughout the day and year, increase the range of workers size exposed, and affect ants in foraging trails as well as disturbed mounds (Pesquero et al. 1996; Orr et al. 1997; Morrison et al. 1997; Folgarait & Gilbert 1999; Folgarait et al. 2003, 2005b). It could offer the possibility of choosing species or biotypes locally adapted to ecological or climatic conditions similar to those where flies are going to be released (Folgarait et al. 2003; Calcaterra et al. 2005: Folgarait et al. 2005a).

Species and biotypes of *Pseudacteon* from Brazil and Argentina have been tested for their host specificity in laboratory conditions (Gilbert & Morrison 1997; Porter & Alonso 1999; Morrison &

Gilbert 1999; Vazquez et al. 2004). Their rates of attack have been low when tested against the North America-native fire ant *S. geminata* with the exceptions of *P. curvatus* and *P. borgmeieri* that exhibit moderate and higher rates, respectively (Gilbert & Morrison 1997; Morrison & Gilbert 1999). However, in spite of those attacks, only *P. curvatus* and *P. obtusus* developed in *S. geminata* but with apparent low success (Porter & Gilbert 2004).

Here we evaluated 4 additional species or biotypes of *Pseudacteon* for their inclination to attack North America-native fire ants, *P. nudicornis* Borgmeier, *P. nocens* Borgmeier, *P. cultellatus* Borgmeier, and *P. obtusus* Borgmeier. We used individuals for a large biotype of *P. obtusus* from the Corrientes province in Argentina instead of the small biotype from Campinas (Brazil) tested before by Morrison & Gilbert (1999). Large females *P. obtusus* in Argentina have a mean body length of 1.395 mm (SD = 0.142 mm, n = 10, P.J.F. unpublished data), about 35% larger than the small Argentinean biotype and the Brazilian *P. obtusus* tested in Morrison & Gilbert (1999) (0.90 ± 0.082 mm, n = 10; 0.91 ± 0.087 mm, n = 4, respective body length of both small biotypes, P.J.F. and L.E.G. unpublished data). Male morphological differences between large and small biotypes have been reported before (Porter & Pesquero 2001). Moreover, recent phylogenetic analysis with 2 mitochondrial and 1 nuclear gene show that both biotypes are genetically distinct and probably constitute different species (Kronforst et al. 2006). Types used for the description of *P. obtusus* were collected in La Plata (Argentina) and have a body length of approximately 1.3 mm (Borgmeier 1925). According to this, flies from the Argentinean large biotype tested here should maintain the name *P. obtusus*.

Materials and Methods

Individuals of 4 species of *Pseudacteon* (Diptera: Phoridae) flies were brought in from Argentina to the quarantine facility at the University of Texas, Brackenridge Field Laboratories (BFL), between June 2003 and April 2004. Flies were either collected directly or were reared as progeny from females attacking *S. invicta* in Argentina. Fly rearing was done in the Centro de Estudios e Investigaciones at the Universidad Nacional de Quilmes (Buenos Aires, Argentina). *P. nudicornis* were collected from the Reserva Ecológica Costanera Sur in Buenos Aires Province (34.37'S and 58.22'W), *P. obtusus* around Mercedes in the Corrientes Province (27. 78'S and 58.05'W), and *P. nocens* and *P. cultellatus* near Brea Pozo in Santiago del Estero province (28.27'S and 63.95'W). Some *P. cultellatus* came in February 2006 from collections by S.D. Porter in Corrientes, Argentina.

Host specificity in these phorid species was tested with the red imported fire ant *S. invicta* and the tropical fire ant *S. geminata* Fabricius, denoted hereafter as exotic and native fire ants, respectively. Exotic and native are adjectives given with regard to the US ant biota. Polygyne colonies (multiple queens) for *S. invicta* were collected from Travis, Williamson, Wharton, La Salle, and Bexar counties in central Texas. Polygyne colonies of *S. geminata* were obtained in Travis, Lampasas, and Mill counties, also in Texas. Colonies were transported to BFL, set in a rearing room at 30°C and 12:12 (L:D) cycle, and fed with frozen crickets, sugar water, and water *ad libitum*.

Female flies were tested upon arrival at the quarantine facility within 3 or 4 days after field collection. When not used in tests, they were kept humid and chilled at ca. 10°C in the dark to keep them alive for longer time. Fly specificity tests were done in plastic flight boxes ($15.5 \times 9.5 \times 5$ cm) (henceforth denoted as arenas), containing either *S. geminata* or *S. invicta*. Arenas were lined inside with Fluon (Polytetrafluoroethylene) to prevent ants from escaping and the top was covered with clear glass. The bottoms of the plastic boxes were covered with a layer of plaster 1 to 2 cm deep. The plaster was moistened every day before the beginning of tests. A small hole covered by a rubber sheet in one of the sides of the arena (about 4 cm high) allowed the introduction of flies. Plastic boxes were used only for 1 ant host species to avoid the occurrence of confounding odor from the other host.

Oviposition rates were recorded in arenas that held 1 to 5 g of unsieved ants of either species (≈500-5000 ants). Approximately half of the workers used had head widths of 0.8 mm or less (0.49 ± 0.06 mm), which is one of the characteristic traits of polygyne fire ant colonies (Morrison & Gilbert 1998). Ants were fed daily with a mealworm and had continuous supply of sugar water and water. One 10 cm diameter × 1.5-cm deep plastic jar lid with a lateral hole was put inside each arena to give the ants a place to hide. This lid was moved manually before introducing the flies and after 10 min within each test to keep ants moving constantly around the arena, facilitating fly attack behavior. Arenas were placed under fluorescent lamps and their internal temperature fluctuated between 26 and 29°C.

Pseudacteon attack behavior and development has been studied elsewhere (Morrison et al. 1997; Porter 1998a). Here, two behaviors were observed as indicators of female attack motivation. 'Approaches' were recorded when flies hovered over the dorsum of an ant and followed it for at least a few seconds. An 'Attack' was designated when females attempted to oviposit in the ant's thorax, which usually immobilizes the ant for a short time.

Two types of sequential no choice tests were designed in order to test for *Pseudacteon* host specificity and to measure rates of attack on *S. invicta*

and *S. geminata*. *Pseudacteon obtusus*, *P. nocens*, and *P. cultellatus* were tested with the first type of tests. Here a single female was introduced into a *S. invicta* arena for a maximum of 15 min to test her motivation to oviposit. Behavior in these arenas varied among individual flies. Only individuals that actively approached and attacked at least 1 ant during those 15 min were considered to be motivated to oviposit and therefore transferred to subsequent arenas. The rate of attacks in this and other arenas was measured as the number of attacks from each female per min during a 5-min period, counted from the first attack. After that, the fly was immediately transferred to an arena containing *S. geminata* for additional 15 min. There, the number of approaches and the attack rate were recorded. In most cases, the fly survived and it was transferred immediately to a second *S. invicta* arena to determine whether it was still motivated to attack. In this third arena flies were observed for maximum15 min or, if they attacked, for 5 min counted from the first attack. The main purpose of this was to test for both handling effects and energy limitations when the flies were transferred between arenas and after some time of active attacks. Individual females were tested only once.

Pseudacteon nocens and *P. nudicornis* were tested in a second type of experimental test. In the former type of tests, some attacks were observed on *S. geminata* ants. The aim of the second type of experiments was to test whether the attacks on the native fire ant were either due to mistakes (after the flies were motivated to attack *S. invicta*) or due to low host specificity. Here 1 female fly was first introduced for 15 min into a *S. geminata* arena and the rate of approaches and attacks recorded. The fly was then transferred to a *S. invicta* arena to test for her motivation to oviposit. If the fly did not attack after 15 min the test was aborted and the female was kept in a cool and dark environment for later testing. If the fly attacked, the rate of attack was measured for 5 min and then it was transferred to the next arena. The final arena for this type of experiments had either *S. geminata* or *S. invicta*. Half of the flies that attacked *S. invicta* were transferred to a different *S. invicta* arena to test for the handling effect and limitations of energy. The other half were transferred to *S. geminata* arenas in order to compare the rate of approach and attacks on this species of ants before and after motivation with *S. invicta*.

The time elapsed between the introduction of the fly in a *S. invicta* arena and the time of the first attack (orientation time) was measured and then compared between the first and second exposure to the exotic fire ant with the non-parametric Mann-Whitney test (STATISTICA for Windows 1999). The mean attack rates, measured as the mean number of attacks per female per min, were compared by the non-parametric Wilcoxon matched pair test (STATISTICA for Windows 1999).

To reduce any effect of minor differences in light or temperature conditions, individual flies started the sequential tests in different *S. invicta* or *S. geminata* arenas. Phorid flies where transferred between arenas with an aspirator similar to the one described in Gilbert & Morrison (1997). They were gently aspirated from one arena to a tube whose bottom was replaced by mesh, and then released immediately to the next arena. Weak and disoriented flies usually walked among the ants after release and were easily killed. To decrease this handling effect, we let the fly go out of the tube at their own pace, which often happens in less than 1 min.

To determine whether flies developed inside the ants attacked, both species of ants were monitored for more than 90 d after their exposure to phorids or until all ants died. Forty days is about the median development period for all the species of phorids tested (Folgarait et al. 2002a). Dead ants were examined for pupae 3 times a week and daily for fly eclosion. Ants were kept in a quarantine room at 27°C and 12L:12D cycle.

RESULTS

Females of the *Pseudacteon* species *P. obtusus*, *P. cultellatus*, and *P. nudicornis* showed high degrees of host specificity for the red imported fire ant *S. invicta*. The former two species were tested with the sequential no choice tests: *invicta-geminata-invicta*, whereas *P. nudicornis* females were exposed to *S. geminata* before being transferred to the motivation arenas with the exotic fire ant. When exposed to *S. invicta* in the motivation arenas, *Pseudacteon* females of these 3 species attacked ants with a rate of about 3 attacks per min (Tables 1 and 2). The same flies were then transferred to *S. geminata* arenas. There, 13% of *P. obtusus*, 46% *P. cultellatus*, and all *P. nudicornis* females were still highly motivated and followed ants displaying the pursuing behavior called 'Approach'. In most cases, flies approached ants after being moved from the *S. invicta* arenas for the first few min, but displayed no activity, afterwards for the rest of the observation period. In spite of those approaches only 1 female of *P. obtusus*, 4 *P. cultellatus* (Table 1) and none of *P. nudicornis* (Table 2) attacked *S. geminata* ants. Fig. 1 shows the percentage of the females exposed to *S. geminata* that attacked those ants, and compares the percentages with those obtained for *P. curvatus*, *P. tricuspis*, and *P. litoralis*, species of phorid flies currently used for biocontrol of exotic fire ants in the US.

Females of the 2 *Pseudacteon* species that attacked *S. geminata*, *P. obtusus* and *P. cultellatus*, however did not attempt to oviposit more than twice, about 10% of the number of attacks per female on *S. invicta*. There were not enough females attacking both *S. geminata* and *S. invicta*

TABLE 1. APPROACH AND ATTACK RATES OF *PSEUDACTEON NOCENS*, *P. OBTUSUS*, AND *P. CULTELLATUS* IN THE FIRST TYPE OF SEQUENTIAL HOST SPECIFICITY TESTS.

	P. nocens	*P. obtusus*	*P. cultellatus*
Initial exposure to *S. invicta* (motivation)			
No. of individuals attacking	61	8	24
Attack rate	3.27 ± 3.20 (44)	3.47 ± 2.3	4 ± 2.51 (20)
Exposure to *S. geminata*			
No. individuals approaching/No. exposed	48/61	1/8	11/24
Approach rate	8.85 ± 12.88	0.2	1.36 ± 1.58
No. of individuals attacking/No. exposed	22/61	1/8	4/24
Attack rate	0.62 ± 0.44	0.4	0.2 ± 0
Final exposure to *S. invicta*			
No. individuals attacking	36/54	4/6	14/21
Attack rate	3.55 ± 3.07	1.76 ± 2.14	3.85 ± 2.18

Attack and approach rates are given as the mean ± SD of rates calculated from independent females. Rates are the number of attacks or approaches per min after 5 or 15-min exposure respectively. In 1 experimental period flies were transferred to *S. geminata* arenas immediately after they attacked *S. invicta* twice. In those cases rates of attack in the motivation arena were not calculated. Therefore when the number of flies used for mean calculations were different than total number of individuals attacking, this number is written in brackets.

to do statistical tests on this subset of flies. Nevertheless, when all females were considered, numbers of attacks per female per min to the native fire ants were always lower than those to *S. invicta* (Table 1).

After being exposed to the native fire ants, females were subsequently transferred to *S. invicta* arenas to test for handling effects and energy limitations. Flies attacking in this last step of the test demonstrated that lack of attraction to *S. geminata* was not due to either the disturbance of being relocated or due to lack of energy. More than 60% of the *P. obtusus*, *P. cultellatus*, and *P. nudicornis* females attacked in the second *S. invicta* arenas (Tables 1 and 2), but they did it in a lower rate than when first exposed to these ants ($Z = 1.98$, $P = 0.05$, Wilcoxon matched pair test, $n = 15$, data from the 3 species combined). *Pseudacteon nudicornis* first and second exposure to *S. geminata* showed that more flies hovered over ants after being motivated to attack in *S. invicta* than they did otherwise. In addition, rates of

TABLE 2. APPROACH AND ATTACK RATES OF *PSEUDACTEON NOCENS* AND *P. NUDICORNIS* IN THE SECOND TYPE OF SEQUENTIAL HOST SPECIFICITY TESTS.

	P. nocens	*P. nudicornis*
Initial exposure to *S. geminata*		
No. individuals approaching/No. exposed	2/10	1/6
Approach rate	2.17 ± 1.46	0.6
No. of individuals attacking/	0/10	0/6
Attack rate	0	0
Initial exposure to *S. invicta*		
No. of individuals attacking	10/10	6/6
Attack rate	4.92 ± 2.74	3.16 ± 1.48
Subsequent exposure to *S. geminata*		
No. individuals approaching/No. exposed	4/4	3/3
Approach rate	5.05 ± 6.17	3.91 ± 2.3
No. of individuals attacking/No. exposed	1/4	0
Attack rate	0.2	0
Subsequent exposure to *S. invicta*		
No. individuals attacking	4/4	1/1
Attack rate	2.7 ± 1.83	2.8

Attack and approach rates are given as the mean ± SD of the rates calculated from independent females. Rates are the number of attacks or approaches per min after 5 or 15-min exposure, respectively.

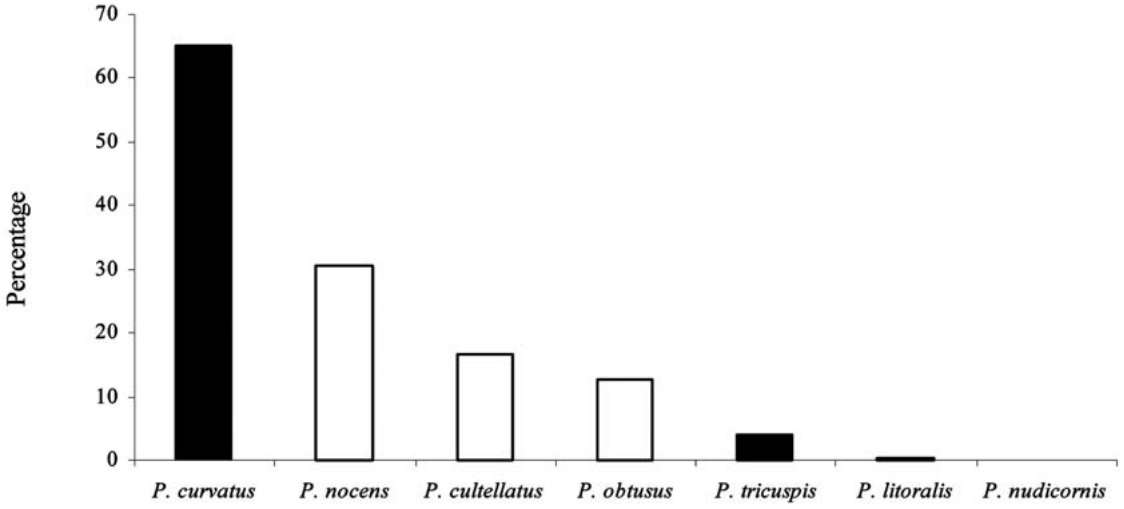

Fig. 1. Percentage of *Pseudacteon* females that attacked *S. geminata* ants in host specificity tests. *Pseudacteon nocens* percentage was calculated using both types of sequential tests. Value for *P. nudicornis* is zero. Percentages for *P. curvatus*, *P. tricuspis* and *P. litoralis* (bars in black), species currently released in the US for biocontrol of exotic fire ants, are data from Gilbert and Morrison (1997).

approach in both arenas were different, with 1 female approaching the native fire ants 10 times in the first exposure and 3 females approaching 94, 25, and 57 times in the second trial (Table 2).

Pseudacteon nocens was tested using both the sequential test: *invicta-geminata-invicta* and the tests where flies were exposed to *S. geminata* first (Tables 1 and 2). *Pseudacteon nocens* host specificity for *S. invicta* was lower than that seen in the other *Pseudacteon* species described above. From the 61 females exposed to *S. geminata* in the first type of tests, 78% approached and 36% attacked these ants at least once (Table 1).

The behavior of *P. nocens* females was very similar in the second type of tests. Those showed that, once motivated in *S. invicta* arenas, *P. nocens* females readily approached and some proportion of them attacked the native fire ants (Table 2). In contrast, females exposed first to *S. geminata* exhibited little motivation to approach and none of the 10 females exposed attacked (Table 2). The majority of females first introduced into *S. geminata* arenas flew to the top of the arena or stood most of the time on the box wall ignoring the presence of ants. The same females, when moved to motivational *S. invicta* arenas, were attracted to the ants as soon as 26 s after introduction (2.19 minutes ± 2.54, mean ± SD orientation time). Flies that were then moved to another *S. invicta* arena started to attack as soon as 7 s after being transferred (1.74 ± 2.09 mean ± SD orientation time). In contrast with the first exposure to the native fire ants, those flies transferred from motivation *S. invicta* to *S. geminata* arenas started approaching ants very quickly, and 1 was observed attacking (Table 2). Rates of approach in both *S. geminata* arenas were different, with a lower rate in the first exposure (although statistic tests were not done because of the small sample size). Females hovered on top of ants 17 and 48 times in the first and 34, 107, 7, and 50 times in the second exposure to *S. geminata*.

Considering both types of tests, out of the 71 *P. nocens* females exposed to *S. geminata*, 31% attempted to oviposit in those ants (Fig. 1). Nevertheless, the mean number of attacks per female per min to the native fire ants was very low and comparable to numbers found for the other *Pseudacteon* species tested here (Table 1). Females of *P. nocens* that attacked both species of ants did so in a significantly reduced rate in *S. geminata* than in the exotic fire ant ($Z = 3.15$, $P = 0.002$, $n = 17$, Wilcoxon matched pair test). In contrast, for this phorid species, neither, the attack rates or the orientation times differed in their first and second exposure to *S. invicta* ($Z = 1.62$, $P = 0.11$, $n = 30$ and $Z = 0.02$, $P = 0.98$, $n = 19$, Wilcoxon matched pair test for rates of attack and orientation time respectively).

In the choice tests only about 40% (17-66%) of females initially tested attacked at least 1 *S. invicta* ant in the motivation arena and were transferred to subsequent arenas. We did not use these percentages to compare to those in subsequent exposures to *S. invicta* because several causes besides lack of motivation contributed to the low percentage of females useful for the specificity tests. In most cases, for example, after released, flies were killed by ants in the arena or died prematurely due perhaps to causes related to their transport from the field to BFL.

From the 69 oviposition attempts observed in *S. geminata* by *P. nocens*, only 3 pupae developed.

One pupa was found in the native fire ant attacked by *P. cultellatus*. No adult flies emerged from these pupae. No pupae were observed in *S. geminata* colonies attacked by *P. obtusus*. Nevertheless, because of the limited sample size, the possibility of larval development in *S. geminata* by the phorid species studied here cannot be eliminated. In addition, attempts to rear those phorid species in *S. invicta* were largely unsuccessful at the time when the tests were done. For example, only 1.5 pupae per female were produced from 349 *P. nocens* that attacked *S. invicta* repeatedly for few hours (during a 5-month period), and only 58% of those pupae developed successful to adults.

Discussion

The species of *Pseudacteon* tested here showed high and moderate degrees of host specificity. Low percentages of *P. obtusus*, *P. cultellatus*, and none of the *P. nudicornis* females attacked *S. geminata* even though they were already motivated to attack *S. invicta*. Those females that attacked *S. geminata*, however, did so very infrequently (no more than 2 attacks in 15 min). In contrast, 31% of *P. nocens* females attempt to oviposit in *S. geminata* after being exposed to exotic fire ants. These attacks, though, could be attributed to mistakes because flies only did so after exposure to their usual host, *S. invicta*, while none of the females attempted to oviposit on *S. geminata* if exposed to this ant first. Even though one third of the tested *P. nocens* females attacked the native fire ants, they did so at about 1/6 the frequency they did in *S. invicta* arenas. This lower rate of attack can not be attributed only to handling effects and energy limitations because the frequencies of attacks in *S. invicta* arenas before and after exposure to *S. geminata* were not different.

Despite of the small sample size of females of *P. obtusus*, *P. cultellatus*, and *P. nudicornis* used, our results of host specificity are similar to those for other species of *Pseudacteon* reported elsewhere (Gilbert & Morrison 1997; Porter & Alonso 1999; Morrison & Gilbert 1999; Folgarait et al. 2002b; Vazquez et al. 2004). They add to the body of evidence that indicates that *Pseudacteon* species from South America are highly specific in the *S. saevissima* group of fire ants. It is also becoming apparent that some of these flies may mistakenly approach and even try to oviposit on other hosts. However, it seems that *Pseudacteon* offer a low risk to native species in the *S. geminata* group if introduced as biocontrol agents of red and black imported fire ants (Porter & Pesquero 2001; Porter & Gilbert 2004).

The species studied here have many characteristics that made them worth considering for further evaluation for classical biocontrol. They showed high to moderated degrees of host specificity to *S. invicta*, in all cases greater than those exhibited by species already released in the US. They attacked and developed successfully in both exotic fire ant species *S. invicta* and *S richteri* (Folgarait et al. 2002a, 2002b, 2005b, 2006) but with the exception of *P. obtusus* (Porter & Gilbert 2004) none of them have been reported to develop in native fire ants. In addition, development times and temperature conditions have been thoroughly studied for laboratory rearing and they show high rates of success (Folgarait et al. 2002a, 2002b, 2005b, 2006).

Finally, these 4 species of *Pseudacteon* could enhance in many ways the effect of the species already released as biocontrol agents of exotic fire ants. *Pseudacteon obtusus* and *P. nudicornis*, for example, are known to be attracted to workers in foraging trails in addition to disturbed mounds (Orr et al. 1997; Folgarait et al. 2005b). In contrast, *P. tricuspis*, *P. litoralis*, *P. curvatus* (species already introduced to U.S.), *P. cultellatus*, and *P. nocens* are found more frequently in disturbed mounds, mating flights or when ants engage in fights, likely cued by alarm pheromones (Orr et al. 1997; Folgarait et al. 2002a; Morrison & King 2004; Folgarait et al. 2006).

Fly size varies considerably among *Pseudacteon* species and is positively related with the range of worker sizes they parasitize (Morrison et al. 1997). *Pseudacteon cultellatus*, *P. nudicornis*, and some *P. nocens* are small flies that attack smaller than average sized workers (Folgarait et al. 2002a, 2006). This could be especially favorable in regions were polygyne fire ant colonies with low worker mean sizes predominate. These *Pseudacteon* species could complement parasitism by the already introduced small species *P. curvatus*, or replace it in regions not favorable for this species. *Pseudacteon cultellatus*, *P. nudicornis*, and *P. curvatus* frequently coexist in phorid communities in Argentina (Folgarait et al. 2005a). *Pseudacteon obtusus* (large biotype) and some *P. nocens*, on the other hand, are big flies that coexist with *P. tricuspis* in Argentina and probably will complement their effect over imported fire ants in the U.S.

Therefore, the species of *Pseudacteon* studied here are good candidates for further evaluation for introduction to the U.S. for fire ant biocontrol. They (1) show high and moderate specificity for *S. saevissima* group species of fire ants, (2) are widely distributed in their homeland, and probably locally adapted to several climatic conditions that can be matched in the southern US, (3) can be reared in the laboratory, and (4) exhibit different sizes and host searching strategies, therefore complementing the species of phorids already released as biocontrol.

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LIQUID BORATE BAIT FOR CONTROL OF THE ARGENTINE ANT, *LINEPITHEMA HUMILE*, IN ORGANIC CITRUS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

A liquid bait delivery system containing borate was evaluated for controlling the Argentine ant, *Linepithema humile* (Mayr), in an organic citrus orchard. Two concentrations of disodium octaborate tetrahydrate (1% and 0.5%) were tested in 500-mL capacity bait stations placed at the base of trees. Both concentrations significantly reduced ant activity over the 11-wk duration of the test when compared with controls. However, the 1% concentration of borate significantly reduced ant activity up to 76 m away from the treatment, whereas the 0.5% did not. Compared to ant control with contact insecticides, the bait delivery system uses less insecticide and is more target-specific, reducing environmental contamination.

Key Words: Citrus, Argentine ant, borate, liquid bait delivery system, organic, homopterous pests, bait station

RESUMEN

Un sistema de distribución de líquidos conteniendo boratos fue evaluado por el control de la hormiga argentina, *Linepithema humile* (Mayr), en una huerta de árboles cítricos. Dos concentraciones de disodium octaborate tetrahydrate (1% y 0.5%) fueron probadas en estaciones de comida puestas cerca de los troncos de los árboles. Comparado con los controles, las dos concentraciones redujeron significativamente la actividad de las hormigas durante las 11-semanas del experimento. No obstante, la concentración 1% del borato produjo una reducción significante en la actividad de las hormigas hasta una distancia de 76 metros, mientras que la solucion 0.5% de borato no tenia ese efecto. Comparado con el control de hormigas con insecticidas de contacto, nuestro sistema de distribución usa menos insecticida y es dirigido específicamente a la hormiga; y de esa manera reduce la contaminación ambiental.

Translation provided by the authors.

Argentine ants became a serious pest in citrus shortly after their introduction into the United States in the late 1800s, most likely offloaded from ships transporting coffee from Brazil into the port of New Orleans (Newell & Barber 1913). As early as 1918, a researcher in Louisiana reported trapping 1,307,222 Argentine ant queens and collecting 1,150 gallons of workers and brood over a one-year period in a 19-acre citrus grove (Horton 1918). In 1905 they were reported in southern California, and by 1908 they had spread through the citrus growing regions as far north as San Francisco (Vega & Rust 2001).

Colonies of Argentine ants have tremendous capacity for growth and expansion due to numerous queens (typically 15 to every 1000 workers) and their ability to undergo colony multiplication by fission (Aron 2001; Majer 1993). Colony fission or budding eventually creates a network of interrelated nests that form a cooperative unit, which sometimes extends over an entire habitat. The flow of food in these supercolonies is decentralized, moving in many directions depending on the needs of the individual colonies. This behavior is known as dispersed central place foraging (Holway & Case 2000; McIver 1991).

These large cooperative units channel their energy into foraging and colony growth, and by the sheer number of ants produced out-compete native species for limited resources. Their populations can reach astronomical proportions, as for example, in a citrus grove in San Diego County, California, it was estimated that from 50,000 to 600,000 ants ascended each tree daily in order to tend homopterans (Markin 1967).

Argentine ants tend a variety of homopterans in citrus including the citrus mealybug, *Planococcus citri* Risso, spirea aphid, *Aphis spiraecola* Patch, wooly whitefly, *Aleurothrixus floccossus* (Maskell), and brown and black soft scales, *Coccus hesperidum* L. and *Saissetia oleae* Olivier, respectively. These phloem-feeding homopterans excrete honeydew, which is the primary food of Argentine ants (Markin 1970). The ants guard this resource tenaciously by protecting the homopterans from parasites and predators and consequently interfere with biological control programs. The outcome of this trophobiotic associa-

tion is an increase in populations of both ants and homopterans. (See reviews of this topic in Bartlett 1961; Flint et al. 1991; and Gulla 1997).

Moreno et al. (1987) demonstrated that by controlling Argentine ants in citrus, the wooly whiteflies and citrus mealybugs were reduced in number by their natural enemies. They applied residual insecticides (chlorpyrifos or diazinon) as a barrier on the trunk or on the ground around skirt-pruned trees. Recently, however, growers have reduced their use of broad spectrum insecticides, and as a consequence ant populations have increased, and there is a growing demand for selective pesticide baits (Martinez-Ferrer et al. 2003).

In previous research in commercial citrus groves, Klotz et al. (2003, 2004) obtained significant reductions of Argentine ant populations using liquid baits (25% sucrose-water) with ultra-low concentrations (1×10^{-4}%) of fipronil or thiamethoxam.The purpose of this study was to test borate in a sugary solution for Argentine ant control in citrus. If effective, then organic growers would have a means of reducing Argentine ant populations in citrus. This is especially significant considering the limited options for ant control available to organic growers.

Materials and Methods

Test Site and Experimental Design

An organic citrus grower in Fallbrook (Rainbow Valley Orchids, San Diego County, California) provided us with an orange grove, which we partitioned into 21 plots, each consisting of 3 rows by 5 trees, and measuring 12.2×15.2 m. The rows were 6.1 m apart and trees within rows 3.0 m apart. Each plot was a minimum of 20 m from adjacent plots. This buffer zone was set up in order to mitigate any treatment effects from neighboring plots due to the movement of toxicant through the ant population.

A randomized block design was used consisting of 7 blocks of 3 treatments. Each block consisted of plots with similar ant activity based on a pretreatment survey (see monitoring below) in order to reduce variability due to differences in the initial ant activity.

Monitoring Plots

To estimate ant activity in each plot, we monitored 3 trees in the center row of each plot with sucrose-water monitors. Monitored trees were never on the edge of the plot. Due to missing trees in some plots, several plots had less than 3 trees to monitor. The monitors consisted of 50-mL plastic centrifuge tubes (Fisher Scientific, Pittsburgh, PA) filled with 25% sucrose-water. The cap on the monitor had a 2-cm hole drilled in its center and was screwed down over a 6-cm square piece of Weedblock (Easy Gardener, Waco, TX), a perforated plastic material with many tiny holes. The monitors were inverted and taped to tree trunks so that trailing ants could feed on the sucrose-water. To correct for evaporative water loss in monitors, a 50-mL tube was filled with 25% sucrose-water, inverted, and suspended on a string from a tree branch in the grove. The string was coated with Stikem Special (Seabright, Emeryville, CA) to prevent ants from feeding on this tube. The tube and monitors were left on the trees for 24 h and consumption of sucrose-water by the ants was obtained by correcting for evaporative water loss. Consumption of sucrose-water from these monitors indicated the number of ant visits, with each mL consumed corresponding to about 3300 ant-visits (Reierson et al. 1998). Estimates of ant activity were made in all plots before treatments and on a weekly basis for 11 wk after treatment (wk 6 and 10 were skipped).

Monitoring Transects

At the end of the 11-wk study we also monitored sucrose-water consumption along a series of transects in order to determine how far the toxic baits were having an effect. Each transect extended ≈76 m out from a baited plot into surrounding untreated areas (i.e., some treatment plots were adjacent to parts of the grove that we did not use for plots). Beginning in the middle of the treated plot, monitors were placed in trees at ≈6 m intervals along the transect. Monitors were left out for 24 h, and then collected to measure the consumption of sucrose-water by the ants. As described in the procedure for monitoring plots, a tube was also used to correct for evaporative water loss.

Treatments

Gourmet Liquid Ant Bait (Innovative Pest Control Products, Boca Raton, FL) containing 1% disodium octaborate tetrahydrate (DSOBTH) was used. One of the treatments consisted of bait applied at full strength (1% DSOBTH) and the other diluted with deionized water to half strength (0.5% DSOBTH). The liquid bait was delivered in 500 mL capacity KM AntPro Stations (KM AntPro, LLC, Nokomis, FL). Stations were placed on the ground at the base of every other tree in the treatment plots, staggering the placement between rows of trees. In case of a missing tree, the bait station was placed where the tree should have been. Thus, there were 7 or 8 stations per plot, making a total of 105 stations used in the study. Stations were checked weekly and refilled when necessary. During the monitoring procedure the stations were closed to prevent ants from feeding on them and potentially attracting them away from the monitors, thereby reducing our estimate of ant numbers at the monitors. In addition to the

2 bait treatments we had a third treatment consisting of control plots, which were not baited.

Statistical Analyses

Examination of the plot data with histograms and probability plots to assess normality showed that a square root function, rather than a logarithmic transformation, more closely approximated normality. Therefore, to compare the treatments and controls over time we did a repeated measures ANOVA (Systat 2004) on the square root (X + 1) transformation of sucrose-water consumption for the 11 post-treatment wk that we monitored. In this analysis we were interested in the Between Subjects (Treatments) effects, which is equivalent to comparing the grand means of the treatment profiles over the 11 wk. Each monitor is compared with itself over time, giving a mean value for each monitor and a grand mean for each treatment. We also did separate ANOVAs for each data period with the transformed data. For all the ANOVAs the blocking variable was used to remove variability due to differences in initial ant numbers in the plots and the remainder, or MSE, was used for tests of significance.

For the transect data originating in baited plots, consumption of sucrose-water was plotted against distance and pooled for each treatment. A linear regression analysis was performed on these pooled data for each treatment (Systat 2004).

Results

Table 1 shows a summary of the results. One wk post-treatments, ant visits to the monitors in treatment plots were significantly less than in the controls, with reductions of 54 and 47%, respectively, for the 0.5% and 1% DSOBTH. In the second wk the respective reductions were 68% and 70%. However, consumption in the control plots also began to decline in the second wk and was not now significantly different from the treatments. From wk 7 through 11 the consumption of sucrose-water in the 1% DSOBTH was again significantly lower than in the control. Consumption of sucrose-water in the 0.5% DSOBTH treatments was significantly lower than controls only in wk 1 and 8.

The grand means of the mean consumption of sucrose-water for each treatment, ignoring the pretreatment values, were obtained by finding the mean of each monitor over the 11 post-treatment wk and averaging these means within each treatment. These grand means showed overall reductions from pre-treatment values in sucrose consumption by 76, 52, and 48%, respectively, for the 1% DSOBTH, 0.5% DSOBTH, and the controls. The differences between the grand means were tested for significance by looking at the Between Subjects (Treatments) part of a repeated measures ANOVA (Systat 2004) for the 11 post-treatment wk on square root (X + 1) transformed grand means. The treatment (df = 2, 48; F = 12.5) and blocking (df = 6, 48; F = 4.5) effects were both significant ($P < 0.001$). A follow-up comparison of the grand means with Tukey's HSD test showed that sucrose-water consumption for both the 1% and 0.5% DSOBTH bait treatments were lower than the controls ($P < 0.001$ and $P < 0.01$, respectively), but not different from one another ($P > 0.25$).

Eleven transects of sucrose-water consumption vs. distance were completed. Five of these received the 0.5%, and 6 the 1.0%, DSOBTH treatments. The regression analysis of the 1% DSOBTH bait transects (Fig. 1a) was highly significant ($P < 0.001$), whereas it was not significant for transects from plots treated with 0.5% DSOBTH bait ($P > 0.25$, Fig. 1b).

Table 1. Mean[1] consumption of sucrose-water (g) as a measure of ant abundance.

	Pretreat.	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 7	Wk 8	Wk 9	Wk 11
0.5% DSOBTH	25.0 (2.48) a	11.6 (1.77) b	8.0 (1.28) a	7.5 (1.20) a	3.2 (0.73) a	6.9 (1.27) a	16.4 (3.23) a	6.8 (0.76) b	3.4 (0.60) ab	16.4 (1.60) a
% reduction	—	53.5	67.8	70.0	87.1	72.2	34.3	72.9	86.5	34.4
1.0% DSOBTH	29.3 (4.17) a	13.8 (2.95) b	8.7 (1.97) a	7.3 (1.51) a	5.1 (1.07) a	3.7 (0.84) b	6.7 (1.16) b	5.6 (0.39) b	2.5 (0.65) b	6.9 (1.11) b
% reduction	—	46.8	70.4	75.1	82.5	87.5	77.0	80.8	91.5	76.6
CONTROL	29.4 (3.94) a	27.8 (5.87) a	16.0 (3.21) a	11.4 (2.21) a	6.6 (1.60) a	5.4 (0.90) ab	19.0 (1.82) a	9.7 (0.96) a	6.4 (1.53) a	15.2 (2.74) a
% reduction	—	9.3	45.4	61.3	77.7	81.4	35.2	67.0	78.1	48.2

[1]Means (± SE). In each column values followed by the same letter are not significantly different ($P > 0.05$), with Tukey's HSD test performed on square root (X + 1) transformed data; untransformed means are shown above. Blocking variable was used in the ANOVAs to reduce the error variability, thereby increasing the power of the treatment statistics. DSOBTH = disodium octaborate tetrahydrate. n = 19 for all treatments, except for Wk 1, where n = 16 for the Control and the 1% DSOBTH. % reduction = % reduction from pretreatment values.

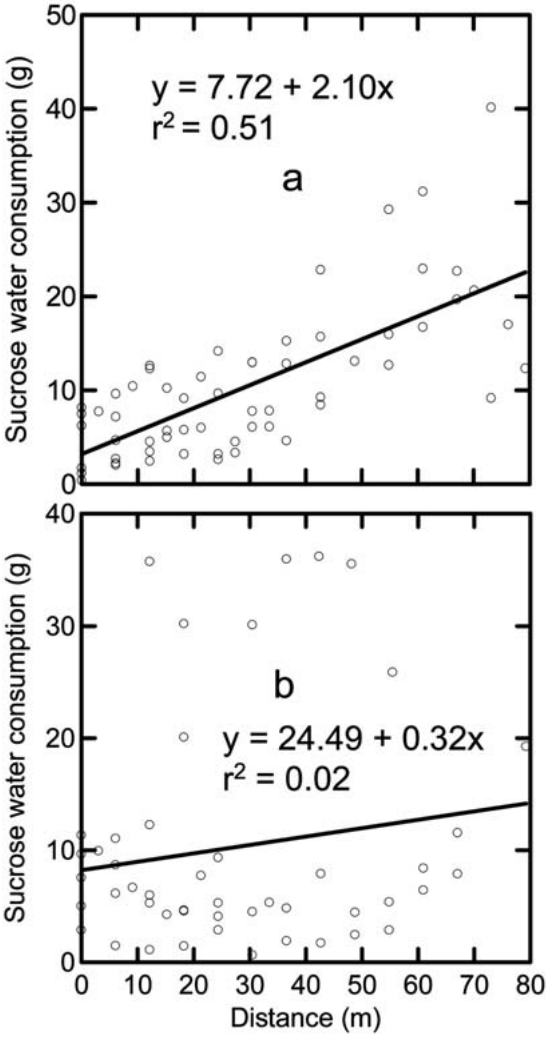

Fig. 1. Pooled data of regressions of sucrose-water consumption (g) vs. distance (m) from treated plots. (a) 1% DSOBTH (6 transects, n = 60), and (b) 0.5% DSOBTH (5 transects, n = 48).

Discussion

As described above, ant numbers in control plots began to decline 2 wk after treatments. To test the hypothesis that the treatments could influence control plots, we sampled ants along transects starting at a treatment plot and going into untreated parts of the citrus grove. For the treatments with the 1% DSOBTH the significant regression analysis shows an effect at least 70 m away from the treatment. In spite of the control plots being within the active space of the treatment plots, we were still able to show overall differences between treatments and controls. The regressions suggest that these differences would be higher if the control plots were further from the treatments.

Various non-chemical and chemical methods have been developed for Argentine ant control in citrus (Vega & Rust 2001). In the early 1900s in Louisiana, traps consisting of wooden boxes containing decaying vegetable matter were set out in groves to attract colonies of ants during the winter (Newell & Barber 1913). The warmth of the decomposing organic matter was thought to attract the ants, which moved into the boxes where they were treated with an insecticide such as carbon bisulfide. Another early method involved flooding orchards in order to force the ants into a concentrated area where they were treated with scalding water or kerosene (Newell & Barber 1913). Tree banding with a mixture of sulfur and sticky material was also a recommended treatment (Woglum & Neuls 1917). More modern banding techniques incorporate Stikem + repellents such as farnesol (Shorey et al. 1992), or controlled-release chlorpyrifos (James et al. 1998). Although effective, these methods have generally not been adopted by growers because they are labor intensive (Rust et al. 2003).

A more practical means of control is the application of broad-spectrum residual insecticides. Chlordane, for example, was the standard treatment for ant control in citrus in the mid twentieth century, until its use was prohibited by the Environmental Protection Agency (EPA) in 1980 (Moreno et al. 1987). Organophosphates, such as chlorpyrifos and diazinon, replaced the chlorinated hydrocarbons and are still being used today for ant control. However, their use is being phased out in urban environments, and growers are also reducing their applications of these chemicals for ant control (Martinez-Ferrer et al. 2003).

Baits offer several advantages over residual insecticides. First, with regard to efficacy, baits exploit the recruitment and food-sharing behaviors of ants to spread a toxicant throughout the colony. In the case of Argentine ants, baits have the added benefit of being spread among nests due to transfer of foods and movement of ants in this unicolonial species. For example, Markin (1968) estimated that >50% of the worker population was exchanged among neighboring nests in 5 d. In contrast to baits, residual insecticides kill ants on contact, mostly the aboveground foragers, which are readily replaced with colony reserves.

Second, in comparison to residual insecticides there is far less active ingredient in baits and particularly when contained, as in bait stations, there is reduced environmental contamination. Indeed, the degree of environmental protection provided by bait stations convinced EPA that certain expensive data requirements could be waived, making future registration of these innovative technologies much more likely (Klotz et al. 2004).

In previous tests in urban settings, we reduced Argentine ant populations by 80% using 0.5% boric acid in 25% sucrose-water (Klotz et al.

1998). Adopting our techniques, Daane et al. (2006) used the same bait in grape vineyards and significantly reduced Argentine ant populations at one of two sites where it was tested. Over the course of their 3-year study Daane et al. developed better dispensers and more effective deployment patterns for liquid baits leading to more consistent reduction in ants, and significantly less mealybugs and crop damage. In comparison, the standard treatment with chlorpyrifos for ants in vineyards had little or no long-term impact on the ant densities (Daane et al. 2006).

We believe that monitoring transects as was done in this study may provide valuable information for determining rates of application as well as concentration of active ingredient. For example, after 11 wk of exposure to the 1.0% DSOBTH bait there was significant reduction of ants up to 76 m away from the treated plots. On the other hand, the 0.5% DSOBTH bait did not have an effect over this same distance. A likely cause for this difference in efficacy is due to dilution of the bait toxicant by trophallaxis. Rust et al. (2004) showed that in the case of borates there is a relatively narrow range of concentrations that are effective, and that trophallaxis can readily dilute a toxicant to a sublethal dose. This dilution effect is magnified in the high population densities of Argentine ants that are found in some citrus groves. In lighter infestations as in the urban setting mentioned above, 0.5% boric acid bait was sufficient.

Based on previous research in commercial settings (Klotz et al. 2004), a baiting program for a heavy infestation of Argentine ants in organic citrus might start with 55 bait stations per hectare and 1% borate solution. Only half the number of bait stations would be used the following year, since there is significant carry-over of reduced populations from one season to the next (Klotz & Rust 2002). The number of stations might even be further reduced, but the amount is yet to be determined by future research.

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INSECT HERBIVORE FAUNAL DIVERSITY AMONG INVASIVE, NON-INVASIVE AND NATIVE *EUGENIA* SPECIES: IMPLICATIONS FOR THE ENEMY RELEASE HYPOTHESIS

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ABSTRACT

The enemy release hypothesis (ERH) frequently has been invoked to explain the naturalization and spread of introduced species. One ramification of the ERH is that invasive plants sustain less herbivore pressure than do native species. Empirical studies testing the ERH have mostly involved two-way comparisons between invasive introduced plants and their native counterparts in the invaded region. Testing the ERH would be more meaningful if such studies also included introduced non-invasive species because introduced plants, regardless of their abundance or impact, may support a reduced insect herbivore fauna and experience less damage. In this study, we employed a three-way comparison, in which we compared herbivore faunas among native, introduced invasive, and introduced non-invasive plants in the genus *Eugenia* (Myrtaceae) which all co-occur in South Florida. We observed a total of 25 insect species in 12 families and 6 orders feeding on the six species of *Eugenia*. Of these insect species, the majority were native (72%), polyphagous (64%), and ectophagous (68%). We found that invasive introduced *Eugenia* has a similar level of herbivore richness as both the native and the non-invasive introduced *Eugenia*. However, the numbers and percentages of oligophagous insect species were greatest on the native *Eugenia*, but they were not different between the invasive and non-invasive introduced *Eugenia*. One oligophagous endophagous insect has likely shifted from the native to the invasive, but none to the non-invasive *Eugenia*. In summary, the invasive *Eugenia* encountered equal, if not greater, herbivore pressure than the non-invasive *Eugenia*, including from oligophagous and endophagous herbivores. Our data only provided limited support to the ERH. We would not have been able to draw this conclusion without inclusion of the non-invasive *Eugenia* species in the study.

Key Words: biological invasion, endophagous insect, herbivore fauna, introduced species, invasive species, non-invasive species, oligophagous insects

RESUMEN

La hipótesis de escape del enemigo (HEE) ha sido frecuentemente utilizada para explicar la naturalización y extensión de especies introducidas. Una de las ramificaciones de la HEE es que las plantas invasoras soportan un grado de herbivorismo menor que el de las especies nativas. La mayor parte de los estudios empíricos para analizar la HEE han implicado compariciones de dos-vías entre la especie invasora y su contraparte nativa del área de invasión. Estos análisis serían de mayor relevancia si los mismos también incluyeran especies no nativas que fueran no invasoras. Estas especies, independientemente de su abundancia e impacto, podrían tener una reducido fauna herbivora y por tanto experimentar un grado menor de daño. En este estudio nosotros usamos una comparación de tres vías en la cual se compara las fauna herbivoras de especies nativas, especies invasoras introducidas y especies introducidas no invasoras del género *Eugenia* (Myrtaceae) del Sur de La Florida. Observamos un total de 25 especies de insectos en doce familias y seis órdenes alimentandose sobre seis especies de *Eugenia*. Entre éstos, la mayoría son nativos (72%) polifagos (64%) y ectofagos (68%). Nosotros encontramos que especies invasoras introducidas de *Eugenia* tiene niveles similares de riqueza de herbívoros que los de las especies nativas e introducidas no invasoras. Sin embargo el número y el porcentaje de insectos oligofagos fue mayor en las especies nativas, aunque estas diferencias no fueron significativas entre las especies introducidas in-

vasoras y no invasoras de *Eugenia*. Uno de los herbívoros oligofago y endofago es probable que haya cambiado desde la especie nativa a la invasora, pero ninguno de éstos a la especie no invasora de *Eugenia*. En resumen, la especie invasora de *Eugenia* ha encontrado la misma, o quizás mayor, presión por parte de herbivoros que la especie no invasora de Eugenia, incluyendo oligofagos y endofagos. Nuestros datos indican un apoyo muy limitado para la HEE. Nosotros no habriamos podido llegar a esta conclusión al menos que hubieramos incluido la especie no invasora de *Eugenia* en nuestro estudio.

Translation provided by the authors.

The enemy release hypothesis (ERH) states that introduced invasive species are successful because they left their co-evolved natural enemies behind. This idea makes intuitive sense and is the theoretical foundation of classical biological control. It is one of the most cited explanations for the undesired success of introduced invasive species worldwide (Williams 1959; Crawley 1997; Maron & Vilà 2001; Keane & Crawley 2002). Although empirical studies testing the ERH on invasive plants are limited in number (Maron & Vilà 2001; Keane & Crawley 2002; Liu & Stiling 2006) and vigor (but see Schierenbeck et al. 1994; Wolfe 2002; Siemann & Rogers 2003; DeWalt et al. 2004), there have been several syntheses to test the predictions stemming from ERH during the last decade (Maron & Vilà 2001; Keane & Crawley 2002; Colautti et al. 2004; Liu & Stiling 2006). One consensus generated from these syntheses and other more recent empirical studies is that the total number of insect herbivores, and the numbers of endophagous and oligophagous herbivores, are all reduced on introduced invasive species compared with conspecific populations in the native range or on co-occurring native congeners (Keane & Crawley 2002; Colautti et al. 2004; Hinz & Schwarzlaender 2004; Torchin & Mitchell 2004; Liu & Stiling 2006). In addition, a modification of the ERH, which states that it is the escape from specialist insects (including endophagous species) that allow the introduced plants to be successful, has received increasing support (Wolfe et al. 2004; Joshi and Vrieling 2005; Stastny et al. 2005; Mitchell et al. 2006).

All the empirical studies reviewed above were performed in one of two ways: first, insect herbivore diversity, load, or insect herbivore impact either on invasive plants in native vs. introduced ranges was examined (e.g., Wolfe 2002; DeWalt et al. 2004), or second, the same comparisons were made between invasive plants and their native counterparts in the new region (Schierenback et al. 1994; Agrawal & Kotanen 2003; Siemann & Rogers 2003). The latter approach is not a direct test of the ERH. Rather, it tests a ramification of the ERH that invasive introduced plants sustain less insect herbivore pressure than their native counterparts. However, all introduced plants, regardless of their abundance or impact, may support a reduced insect herbivore fauna and experience less damage simply because plants tend to lose their associated insect herbivores during the introduction (Colautti et al. 2004) and it takes time, on the ecological and/or evolutionary scale, for a new population to acquire its insect herbivore fauna (Strong et al. 1984). Testing the ERH would be more meaningful if such studies also included introduced plants which do not become invasive, or so-called innocuous species (Colautti et al. 2004; Levine et al. 2004). However, few studies have included introduced non-invasive plants (but see Mitchell & Power 2003; Cappuccino & Carpenter 2005; Carpenter & Cappuccino 2005).

A three-way comparison of insect herbivore faunas in a system in which congeneric native, introduced invasive, and introduced non-invasive (innocuous) plants that co-occur in the same region can provide insightful information on the validity of the ERH. If release from natural enemies is important in determination of the success of an introduced plant species, one would expect that invasive introduced plants escape more from herbivore pressure than do non-invasive introduced plants. One question of particular interest is whether there have been any shifts of oligophagous and/or endophagous herbivores from the native to the introduced plant congeners, and if such shifts occur more onto the non-invasive than to the invasive congeners. Endophagous herbivores are of interest because an internal feeding niche is likely to be correlated with dietary specialization (Frenzel & Brandl 1998). Plants that are closely related phylogenetically (i.e., congeners or confamiliers), as used in many ERH tests, offer a good chance to detect host shifts by herbivores to the introduced plants because herbivore host choice is often determined by plant relatedness.

In this study, we compared insect herbivore faunas among native (two species), invasive (one species), and non-invasive (three species) of *Eugenia* growing in South Florida. The *Eugenia* spp. studied here are small-medium sized trees native to Florida and Central-South America (Wunderlin & Hansen 2003; Ruehle et al. 1958). We predict that (1) the total number of herbivore species will be (a) greater on the native *Eugenia* species than on the introduced invasive and non-invasive congeners; and (b) greater on the introduced non-invasive *Eugenia* than on the introduced invasive congener; (2) the number and proportion of oligophagous and endophagous herbivores will be (a) greater on the native *Eugenia* species than on the

introduced invasive and non-invasive congeners; and (b) greater on the introduced non-invasive *Eugenia* than on the introduced invasive congener, and (3) fewer herbivores, particularly oligophagous and endophagous herbivores, will be shared between the native *Eugenia* and the introduced invasive *Eugenia* than between the native and introduced non-invasive *Eugenia*. The first portions of the first two predictions are comparable to predictions made by the usual two-way (native vs. introduced invasive plants) comparisons. For ERH to be supported in the current three-way testing system, the second portion of the prediction should be validated. We believe this study represents the first known comparison of herbivore funna on native, invasive, and innocuous species of the same genus in the same geographic location.

Material and Methods

Study Plants

Eugenia uniflora L. (Surinam cherry), *E. aggregata* Kiaersk. (cherry of the Rio Grande), *E. brasiliensis* Lam. (grumichama), and *E. luschnathiana* Klotzsch (pitomba) are all large shrubs or small trees with potentially animal-dispersed fleshy fruits that were introduced to south Florida from Brazil in the late 1800s or early 1900s for home garden fruit and ornamental purposes (Ruehle et al. 1958; Martin et al. 1987). *Eugenia uniflora* is a common hedge plant in South Florida, probably due to its robust and rapid growth. Since its introduction, *E. uniflora* has escaped cultivation and invaded hammocks (evergreen broad-leaved forests) in South Florida, growing side by side in some areas with 2 native congeners, *E. axillaris* (Sw.) Willd. (white stopper) and *E. foetida* Pers. (Spanish stopper) (Gann et al. 2001) (Table 1). The other 3 introduced *Eugenia* spp. still remain in cultivation in many public and private gardens and nurseries.

Study Sites

We carried out most of our sampling at two subtropical hammocks in Broward County where *E. axillaris* (native), *E. foetida* (native), and *E. uniflora* (invasive) co-occur: Hugh Taylor Birch State Park (hereafter referred to as Birch Park), and the Bonnet House Museum and Garden (Hereafter referred to as Bonnet House). Subtropical hammocks in South Florida are evergreen, broad-leaved forests composed predominantly of trees common to the Bahamas and Greater Antilles (Snyder et al. 1990). They occupy limestone outcroppings that are elevated, rarely inundated, and relatively fire-free. In hammocks of both Birch Park and Bonnet House, the canopy trees are primarily composed of *Bursera simaruba* (L.) Sarg. (gumbo-limbo), *Coccoloba unifera* L. (seagrape), *Krugiodendron ferreum* (Vahl) Urb. (black iron wood), and *Ficus aurea* Nutt. (strangler fig). The understory is dominated by *E. axillaris*, *E. foetida*, and *E. uniflora*. Sandy soil is characteristic of both sites.

For the introduced non-invasive *E. aggregata*, *E. brasiliensis*, and *E. luschnathiana*, we located up to 14 individuals per species in 4 research, public, and private gardens in Miami Dade and Broward, 2 adjacent counties in South Florida. These gardens include University of Florida, Tropical Research and Education Center, the Fruit and Spice Park, Plantation Heritage Park, and the Fairchild Tropical Garden. These plants are referred to as cultivated aggregata, cultivated brasiliensis, and cultivated lushnathiana (Table 1). In addition, as a control for potential site related differences between these gardens and the natural subtropical hammocks, we also sampled 9, 10, and 28 individuals, respectively, of *E. axillaris* (native), *E. foetida* (native), and *E. uniflora* (invasive) at the above gardens. These individuals were referred to as cultivated axillaris, cultivated foetida, and cultivated uniflora. Sampling frequencies for the cultivated plants were the same as for the wild populations mentioned above.

Determination of Insect Herbivore Faunas

Four and two 5×3-m^2 plots were established at the Birch Park and the Bonnet House, respectively, for herbivore faunal surveys on wild populations of *E. axillaris*, *E. foetida*, and *E. uniflora* (Table 1). We tagged a total of 182, 202, and 97 wild plants of various sizes of *E. axillaris*, *E. foe-*

Table 1. Summary of the study system, including the number of plants sampled (*n*). Plants that grow in gardens are cultivated.

Plant species	Status	Growing habitat in south Florida (*n*)
E. axillaris	Native	Natural hammocks (182) and garden (9)
E. foetida	Native	Natural hammocks (202) and garden (10)
E. uniflora	Introduced invasive	Natural hammocks (97) and garden (28)
E. aggregata	Introduced non-invasive	Garden (9)
E. brasiliensis	Introduced non-invasive	Garden (14)
E. lushnathiana	Introduced non-invasive	Garden (10)

tida, and *E. uniflora*, respectively. All these plants were visited every other month during the dry season (Oct to Apr) and monthly during the wet season (May to Sep) from Jan to Dec 2004. Larval and adult insects were hand caught and brought back to the lab for rearing, specimen preparation, and identification. For fruit and seed feeders, we collected random fruit samples from 3-10 trees and 20-100 fruits per tree, depending on availability. Some non-rotten fruits on the ground directly beneath the trees were also included in the samples. Unidentified fruit/seed feeders were reared to maturity for identifications. We sent unknown specimens to specialists in the USA for identification. Information on insect immigration status (i.e., native or exotic) and diet breadth were provided by these insect specialists when possible. Insects were classified as native or exotic, oligophagous or polyphagous, and endophagous or ectophagous feeders. Oligophagous refers to insects which feed only on plants of 1 family while polyphagous indicates herbivores that feed on more than 1 family. Insects were "very important" if they were seen in every census, or were seen to cause 10% or more of leaf or seed damage on average in at least 1 census (Liu, unpublished data). Insects were "important" if they were seen in more than 1 census but caused less than 10% leaf or seed damage. Herbivores were "not important" if they were seen only once during the entire study period or caused very little plant damage. Determination of % damage to plants depended on the nature of the insect. For example, the % damage by a leaf miner was determined by counting the % of leaves with mines, while the % damage by a chewing caterpillar was by counting the % of leaves chewed.

Data Analyses

In addition to the identity of the herbivores, the number of total insect herbivore species on each *Eugenia* species, the number and percentage of native insect herbivores, the number and percentage of endophagous vs. ectophagous feeders, and the number and percentage of oligophagous vs. polyphagous feeders were determined. The differences in these percentages among the native (average among the 2 species), invasive and non-invasive (average among the 3 species) plants were determined with chi-square tests (Zar 1984) in SPSS 13.0 (SPSS, Chicago, Illinois, USA). Because there may be differences in the herbivore fauna between wild and cultivated populations of the same species as the latter are in artificial settings, 2 sets of the chi-square tests were performed. One was a two-way test that included wild native plants and wild introduced invasive plants. The other was a three-way test that included cultivated native, invasive, and non-invasive plants. We also determined the number of herbivores, particularly oligophagous and/or endophagous, shared between the native, invasive and non-invasive plants. Samples from the two natural area sites were pooled because they had identical herbivore fauna for the three wild *Eugenia* populations. Samples from the four garden sites were pooled because all gardens did not have adequate sample sizes for among site comparisons.

Results

We observed, collected, and reared a total of 25 insect species in 12 families and 6 orders feeding on the 6 species of *Eugenia* during the 1-year sampling period (Table 2). Among them, the majority were native (72%), polyphagous (64%), and external feeders (68%). There were 7 additional uncommon species of Lepidoptera reared from bagged branches of various *Eugenia* spp. that were not included in the results because herbivory by these species was not confirmed. The native wild *Eugenia* species had higher numbers of herbivore species than the wild introduced *E. uniflora* and most cultivated *Eugenia*. The only exception was that the cultivated *E. uniflora* had more herbivore species than the native *Eugenia* (Fig. 1A).

The introduced invasive and non-invasive *Eugenia* recruited fewer oligophagous insect herbivores than the native *Eugenia* (Fig. 1A). The difference in proportions of herbivore diet breadth (oligophagous vs. polyphagous) among the cultivated native, invasive, and non-invasive *Eugenia* was marginally insignificant (Pearson $\chi^2 = 5.76$, $df = 2$, $P = 0.056$). The difference in herbivore diet breadth was not statistically significant between the wild native *Eugenia* and wild invasive *Eugenia* (Pearson $\chi^2 = 1.94$, $df = 1$, $P = 0.163$). In addition, the proportions of herbivore feeding site (endophagous vs. ectophagous) were not different between the wild native *Eugenia* and wild invasive *Eugenia* (Pearson $\chi^2 = 0.003$, $df = 1$, $P = 0.960$), or among the cultivated plants (Pearson $\chi^2 = 1.91$, $df = 2$, $P = 0.385$) (Fig. 1B). Separate analyses (not reported here) incorporating the excluded uncommon Lepidoptera yielded similar results. Finally, all introduced *Eugenia* species attracted more exotic insect herbivores than the native *Eugenia* plants (Fig. 1C). However, the differences in the proportion of native herbivores were not significant between the wild native *Eugenia* and the wild invasive *Eugenia* (Pearson $\chi^2 = 1.02$, $df = 1$, $P = 0.311$), and among the cultivated native, invasive, and non-invasive *Eugenia* (Pearson $\chi^2 = 0.76$, $df = 2$, $P = 0.683$) (Fig. 1C).

The native *Eugenia* shared a total of 6 generalist herbivores, 4 with the invasive *Eugenia*, 4 with the non-invasive *Eugenia*, and 2 (the weevil *Diaprepes abbreviatus* L. and a kerriid scale *Paratachardina lobata* Chamberlin) with both kinds (Table 2). Among the shared herbivores, only 1 native weevil (*Artipus floridanus* Dietz) fed on the inva-

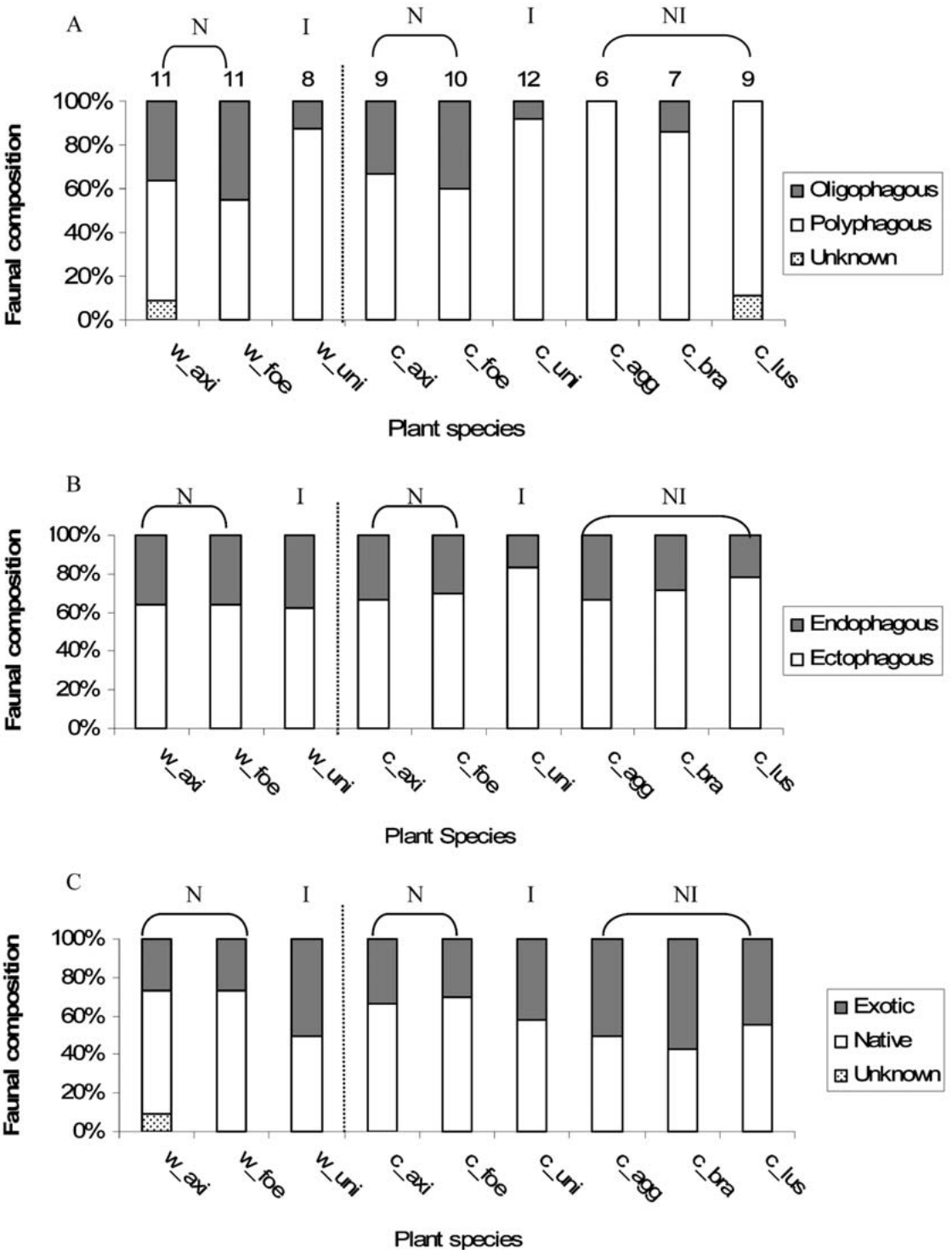

Fig. 1. Percentage of herbivore species found in different categories on six wild and/or cultivated *Eugenia* species in South Florida. The vertical dash lines separate wild plants from cultivated ones, with the former on the left. W_axi = wild *E. axillaris*, w_foe = wild *E. foetida*, w_uni = wild *E. uniflora*, c_axi = cultivated *E. axillaris*, c_foe = cultivated *E. foetida*, c_uni = cultivated *E. uniflora*, c_agg = cultivated *E. aggregata*, c_bra = cultivated *E. brasiliensis*, c_lus = cultivated *E. lushnathiana*. "N" indicate native plants, "I" the introduced invasive plant, and "NI" the introduced non-invasive plants. Numbers on top of the bars are the total number of herbivore species found.

TABLE 2. HERBIVOROUS INSECT SPECIES FOUND ON SIX WILD AND/OR CULTIVATED *EUGENIA* SPECIES IN SOUTH FLORIDA. NATIVE *EUGENIA* SPECIES ARE IN BOLD AND INVASIVE *EUGENIA* ARE IN ITALICS. W_AXI = WILD *E. AXILLARIS*, W_FOE = WILD *E. FOETIDA*, W_UNI = WILD *E. UNIFLORA*, C_AXI = CULTIVATED *E. AXILLARIS*, C_FOE = CULTIVATED *E. FOETIDA*, C_UNI = CULTIVATED *E. UNIFLORA*, C_AGG = CULTIVATED *E. AGGREGATA*, C_BRA = CULTIVATED *E. BRASILIENSIS*, C_LUS = CULTIVATED *E. LUSHNATHIANA*, POLY = POLYPHAGOUS OR GENERALIST. OLIGO = OLIGOPHAGOUS OR SPECIALIST. ENDO = ENDOPHAGOUS, ECTO = ECTOPHAGOUS. — DOES NOT OCCUR, + NOT IMPORTANT, ++ IMPORTANT, +++ VERY IMPORTANT. UNID = UNIDENTIFIED. ? INDICATES UNKNOWN OR UNCERTAIN INFORMATION.

Insect species	Origins[b]	Diet breadth[b]	Feeding nich[b]	Guild /plant parts	Occurrence on *Eugenia* species								
					w_axi	**w_foe**	*w_uni*	**c_axi**	**c_foe**	*c_uni*	c_agg	c_bra	c_lus
Coleoptera													
Curculionidae													
Anthonomus alboannulatus Boheman	Native	Oligo	Endo	Seed	++	+++	—	—	—	—	—	—	—
Anthonomus irroratus Dietz	Native	Oligo	Endo	Seed	—	—	—	+++	++				
Atractomerus punctipennis Gyllenhal	Native	Oligo	Ecto	Leaf	+	—	—	—	—	—	—	—	—
Artipus floridanus Horn	Native	Poly	Ecto	Leaf, root?	+	+	+	+	+	+	—	—	—
Diaprepes abbreviatus L.	Exotic	Poly	Ecto	Leaf, root?	++	++	++	+	+	+	+	+	+
Myctides imberbis Lea	Exotic	Oligo	Ecto	Leaf, fruit?	—	—	—	—	—	++	—	+	—
Myllocerus undatus Marshall	Exotic	Poly	Ecto	Leaf, root?	+	+	+++	++	++	++	—	—	—
Pheloconus hispidus LeConte	Native	Poly	Endo	Seed	—	—	++	—	—	++	++	++	++
Nitidulidae													
Lobiopa insularis Castlenau[a]	Native	Poly	Ecto	Fruit flesh	—	—	—	—	—	++	++	++	++
Epuraea luteolus Erichson[a]	Native	Poly	Ecto	Fruit flesh	—	—	—	—	—	++	++	++	++
Diptera													
Cecidomyiidae													
Dasineura eugeniae Felt	Native	Oligo	Endo	Leaf, fruit galler	+++	+++ (fruit only)	—	++	++	—	—	—	—
Stephomyia eugeniae Felt	Native	Oligo	Endo	Leaf galler	—	+++	—	—	—	—	—	—	—
Tephritidae													
Anastrepha suspense Loew[a]	Exotic	Poly	Endo	Fruit flesh	—	—	+++	—	—	+++	++	++	+++
Hemiptera													
Coccidae													
Pulvinaria psidii Maskell	Native	Poly	Ecto	Stem and leaf	—	—	—	++	—	—	—	—	—
Flatidae													
Melormenis basalis Walker	Exotic	Poly	Ecto	Leaf	—	—	—	—	—	—	—	—	+
Kerriidae													
Paratachardina lobata Chamberlin	Exotic	Poly	Ecto	Stem	++	+	+	++	++	++	+	++	++

[a]Herbivores with little fitness consequences because they only consume fleshy parts of the fruit without damaging the seed.
[b]unknown cases are assumed to be native, polyphagous, and external feeders for the chi-square tests.

TABLE 2. (CONTINUED) HERBIVOROUS INSECT SPECIES FOUND ON SIX WILD AND/OR CULTIVATED *EUGENIA* SPECIES IN SOUTH FLORIDA. NATIVE *EUGENIA* SPECIES ARE IN BOLD AND INVASIVE *EUGENIA* ARE IN ITALICS. W_AXI = WILD *E. AXILLARIS*, W_FOE = WILD *E. FOETIDA*, W_UNI = WILD *E. UNIFLORA*, C_AXI = CULTIVATED *E. AXILLARIS*, C_FOE = CULTIVATED *E. FOETIDA*, C_UNI = CULTIVATED *E. UNIFLORA*, C_AGG = CULTIVATED *E. AGGREGATA*, C_BRA = CULTIVATED *E. BRASILIENSIS*, C_LUS = CULTIVATED *E. LUSHNATHIANA*, POLY = POLYPHAGOUS OR GENERALIST. OLIGO = OLIGOPHAGOUS OR SPECIALIST. ENDO = ENDOPHAGOUS, ECTO = ECTOPHAGOUS. — DOES NOT OCCUR, + NOT IMPORTANT, ++ IMPORTANT, +++ VERY IMPORTANT. UNID = UNIDENTIFIED. ? INDICATES UNKNOWN OR UNCERTAIN INFORMATION.

Insect species	Origins[b]	Diet breadth[b]	Feeding nich[b]	Guild /plant parts	Occurrence on *Eugenia* species								
					w_axi	**w_foe**	*w_uni*	**c_axi**	**c_foe**	*c_uni*	c_agg	c_bra	c_lus
Psyllidae													
Katacephala tenuipennis Tuthill	Native	Oligo	Ecto	Leaf	—	+++	—	—	+++	—	—	—	—
Lepidoptera													
Gracillariidae													
Chilocampyla dyariella Busck	Native	Oligo	Endo	Leaf miner	++	++	+?	++	+	—	—	—	—
Tortricidae													
Ancylis sp.	Native	Poly	Ecto	Leaf tier young leaves	—	+++	—	—	+++	—	—	—	+++
Platynota flavedana Clemens	Native	Poly	Ecto	Leaf tier	—	—	+	—	—	+	—	—	—
Sparganothis lentiginosana Walsingham	Native	Poly	Ecto	Leaf tier young leaves	—	—	—	—	—	+	—	—	—
Strepsicrates smithiana Walsingham	Native	poly	Ecto	Leaf tier young leaves	+++	+++	—	+++	+++	—	—	—	—
Orthoptera													
Acrididae													
Stenacris vitreipennis Marshall	Native	Poly	Ecto	Leaf	—	—	—	—	—	+	—	—	—
Unid. Acrididae	Native?	Poly	Ecto	Leaf	+	—	—	—	—	—	—	—	—
Thysanoptera													
Phlaeothripidae													
Elaphrothrips sp.	Native	Poly?	Endo	Leaf galler	++	—	—	—	—	—	—	—	++

[a]Herbivores with little fitness consequences because they only consume fleshy parts of the fruit without damaging the seed.
[b]unknown cases are assumed to be native, polyphagous, and external feeders for the chi-square tests.

sive *Eugenia*, while two native insects (*Ancylis* sp. and *Elaphrothrips* sp.) fed on the non-invasive *Eugenia*. The insect that caused substantial damage on the invasive *Eugenia* was an exotic weevil (*Myllocerus undatus* Marshall), while the insect that caused substantial damage on the non-invasive *Eugenia* was a native moth (*Ancylis* sp.). The native *Eugenia* also likely shared a specialist insect (a leaf blotch mining moth, *Chilocampyla dyariella* Busck) with the invasive congener (Table 2). However, it was not clear if the leaf miners were able to complete their development in *E. uniflora* leaves, because these incidents were rare and we were not able to rear any adults.

Discussion

Prediction 1—there will be greater numbers of herbivore species on native *Eugenia* than on introduced species.

There is limited evidence supporting our first prediction in relation to herbivore species richness on native vs. introduced non-invasive *Eugenia* because the cultivated native species had more insect herbivore species than 2 of the 3 introduced non-invasive species. This is consistent with the results found in a study comparing insect herbivore fauna between a native *Pinus* and a co-occurring introduced non-invasive congener (Lindelöw & Björkman 2001). There also was only limited support for the prediction in relation to the native vs. introduced invasive species in this study because the native *Eugenia* species had more insect herbivore species than the introduced invasive *Eugenia* in the wild, but not in cultivation. In the only other similar study (Bürki & Nentwig 1997), comparing the herbivore fauna between populations of the native *Heracleum sphonylium* L. and the co-occurring introduced invasive congeners, *H. mantegazzianum* Simmier & Levier, there was an equal number of insects associated with both plant species.

Furthermore, contrary to the second part of our first prediction that the invasive *Eugenia* should have a smaller number of herbivore species than the non-invasive congeners, the invasive *Eugenia* (*E. uniflora*), wild or in cultivation, had greater numbers of insect herbivore species than all 3 non-invasive *Eugenia*. This result is the opposite to that reported in a study on plant pathogens (Mitchell & Power 2003), in which the authors found that more invasive plants tended to have fewer pathogens. Nevertheless, differences in herbivore richness were small among the *Eugenia* species studied here. In addition, there is always the possibility that high number of herbivore species may not translate into high damage level (Liu, unpublished data).

Prediction 2—There will be greater numbers of oligophagous and endophagous herbivore species on native *Eugenia* than on introduced species.

The data support the first part of our second prediction that native *Eugenia* species should have the highest number and percentage of oligophagous insect herbivores. However, the statistical results should be interpreted with caution due to the small number of insect species on each *Eugenia* species. Our result is consistent with 1 congeneric native vs. introduced species comparison (Bürki & Nentwig 1997), but differs from another (Lindelöw & Björkman 2001). In addition, the native plants had higher number of internal feeders even though the percentage of endophagous herbivore species was not different between the native and introduced *Eugenia*. However, in contrast to the second part of our second prediction, the invasive *Eugenia* had as many or more oligophagous and/or endophagous feeders than non-invasive introduced *Eugenia*. No other studies were found to compare the number of oligophagous and endophagous insects between invasive and non-invasive plants.

Prediction 3—Fewer herbivores will be shared between native *Eugenia* and invasive *Eugenia* than between native *Eugenia* and non-invasive *Eugenia*.

The third prediction that native *Eugenia* should share fewer specialist and endophagous herbivores with invasive *Eugenia* than with non-invasive *Eugenia* was not supported by the data. While native *Eugenia* shared no oligophagous or endophagous herbivores with non-invasive *Eugenia*, they likely shared a leaf miner with *E. uniflora* (the invasive introduced *Eugenia*). However, because the blotch mines were only found on the wild individuals, it is possible that the host shift occurred after *E. uniflora* had invaded the natural areas. In addition, because the mines occurred at such a low rate the biotic resistance from this miner should be small. Host sharing by oligophagous herbivores largely depends on the taxonomic closeness of the host plants (Strong et al. 1984). A phylogeny of the genus *Eugenia* may help to explain and predict the shifts of specialists from the native to the introduced congeners.

No leaf galls were observed on any of the introduced *Eugenia* species in this study whereas one specialist galling fly, *Eugeniamyia dispar* Maia et al. (Diptera, Cecidomyiidae) (Maia et al. 1996) was found on *E. uniflora* in its native range. All introduced *Eugenia* studied here have probably escaped specialist insects that may be found in their native ranges. The lack of specialist insect attack may lead to a shift in plant resource allocation to growth (Blossey & Nötzold 1995; Siemann & Rogers 2001, Wolfe et al. 2004) and/or defense to generalist herbivores (Joshi & Vrieling 2005).

Native *Eugenia* plants in cultivation have a less diverse insect fauna than those in the wild, probably due to the differences in time since population establishment (Strong et al. 1984). Cultivated populations tend to be much younger and

have less time to acquire insect fauna. Pesticide treatment in some horticulture or agriculture situations also may cause a decrease in herbivore fauna. However, all cultivated *Eugenia* individuals sampled in this study were not treated directly with pesticides (Jonathan H. Crane of TREC, Micheal Davenport of FTBG, Chris Rollins of FSP, personal communications). Nevertheless, our analyses and discussions are mostly limited to faunal comparisons among different species of the same source (wild or cultivated).

A result that is not related to the ERH testing but nonetheless interesting is the composition of native vs. exotic herbivores on the 3 categories of *Eugenia* plants. The native herbivores constituted about half of the insect herbivore fauna acquired by the introduced *Eugenia*. The numbers of exotic insects attacking the native, invasive, and non-invasive plants are similar (3-5 on each plant species). Since most of these exotic herbivores came from continents other than Central and South America, where the introduced *Eugenia* are native, it is unlikely that these exotic herbivores were associated with the exotic *Eugenia* in its native range. We did not observe any native insect herbivores having more importance on the introduced than on the native *Eugenia* plants. In contrast, it appeared that an exotic weevil (*M. undatus*), a new comer from Sri Lanka (Schall 2000), fed more heavily on *E. uniflora* (the invasive *Eugenia*) than on other congeners (Liu, personal observations). In addition, the only exotic oligophagous weevil (*Myctides imberbis,* an Australian native) found in this study also was observed on *E. uniflora* more than on the native or the non-invasive *Eugenia*. Together, our data suggested that the exotic herbivores provided as much, if not more, herbivore pressure as the native insects to the introduced *Eugenia*. Our finding was different from that of a recent study which found that the native herbivores, mostly vertebrates, suppressed introduced plants, whereas exotic herbivores, also mostly vertebrates, promoted exotic plants (Parker et al. 2006).

In summary, data on herbivore faunal diversity of *Eugenia* species provided limited support to the ERH. It is likely that other factors contribute to the success of *E. uniflora*. If we did not include the non-invasive *Eugenia* species in the study and only compared the herbivore fauna between the native *Eugenia* and invasive *Eugenia*, we would have thought that release from the insect herbivores was an important factor in the success of *E. uniflora*. We did not include pathogens, also recognized as natural enemies, in this study. Future study should take advantage of this unique three-way system to examine the effects of pathogens and other competing but non-exclusive hypotheses to help explain the success of *E. uniflora*. For example, competitive interactions of introduced invasive *Eugenia* vs. native co-occurring plants and non-invasive introduced *Eugenia* vs. native plants could be examined. The three- way comparison could also be used to examine the importance of relative seed numbers (the propagule pressure hypothesis (Williamson 1996), which states that the species with the greater number of propagules will be the most invasive). *Eugenia uniflora*, is much more abundant than the non-invasive Eugenia because it has long been used as a hedge plant, and probably produces more potentially invasive seeds.

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PAPILIO DEMOLEUS (LEPIDOPTERA: PAPILIONIDAE): A NEW RECORD FOR THE UNITED STATES, COMMONWEALTH OF PUERTO RICO

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ABSTRACT

We report the first record of the citrus pest *Papilio demoleus* Linnaeus collected near Guánica in the United States, Commonwealth of Puerto Rico, in March 2006.

RESUMEN

Reportamos la primera ocurrencia de la mariposa asiática *Papilio demoleus* en Puerto Rico qua fue coleccionado alrededor de Guánica, Puerto Rico, en marzo, 2006.

Translation provided by the authors.

Papilio demoleus L., commonly known as the lime or citrus swallowtail, is found throughout southern Asia (Corbet & Pendlebury 1992, cited in Guerrero et al. 2004) where it is a commercially important pest of citrus. In recent times it has expanded its range into new areas of the Old World following the introduction and cultivation of citrus (Matsumoto 2002). More recently, Guerrero et al. (2004) documented the presence of *P. demoleus* in the eastern Dominican Republic on the island of Hispaniola; the first confirmed report of this species in the Americas. Eastwood et al. (2006) subsequently reported that *P. demoleus* had spread across much of the Dominican Republic and, using molecular data, were able to trace its provenance and confirm the pest status of the introduced population. To date it has not been recorded from any other locality in the Western Hemisphere (although there is a dubious record from California (Tilden 1968, cited in Guerrero et al. 2004). Here, we report the collection of 1 specimen (female) of *P. demoleus* in a residential enclave within the Guánica Dry Forest Reserve in Puerto Rico (Fig. 1). It was 1 of 3 specimens observed alighting and possibly ovipositing on an ornamental lime tree (*Citrus aurantifolia* Swingle) in a residential garden.

Collection data are UNITED STATES: COMMONWEALTH OF PUERTO RICO, Municipality of Guánica. 4.III.2006. Nicholas T. Homziak. 7.1 km south and east of town of Guánica on Rte 333 (from junction with Rte 116) to right turn at Hoya Hondo, then 1 km south. (17 degrees, 57.0 minutes North; 66 degrees, 52.6 minutes West). Elevation: near sea level. The identity of the specimen was confirmed by Rod Eastwood (Griffith University, Brisbane, Australia, personal communication). The residential area is located along a slight coastal ridge; largely cleared of the original Subtropical Dry Forest. The low area behind the ridge is dominated by introduced drought-tolerant legumes on poorly draining saline soils. There is commercial citrus production in the nearby region of Yauco.

Guerrero et al. (2004) suggested that the lime swallowtail was likely to disperse rapidly away from its initial point of introduction in the eastern Dominican Republic. *Papilio demoleus* is recognized as a major pest of citrus throughout most of its Old World range, causing significant economic losses (Agribusiness Information Centre of India, 2005; Malaysian Tropical Fruit Information System 2004; Pakistan Agricultural Research Council 2003). Based on its dispersal and life history characteristics documented in Asia, *P. demoleus* is likely to expand and become a serious citrus pest throughout the Caribbean and adjacent mainland locations. Our collection of a specimen from Puerto Rico indicates that it is expanding its range across a much wider area, with potentially serious economic implications for regional citrus production in the Caribbean and Florida.

Papilio demoleus has a history of successful dispersal and range extensions throughout Asia. Found throughout Southeastern Asia (Commonwealth Institute of Entomology 1979), it has extended its range across mountain ranges, deserts, and other inhospitable terrain to become a major citrus pest in India (Agribusiness Information Centre of India, 2005), Pakistan (Pakistan Agricultural Research Council 2003), Iraq (Larsen 1977, cited in Eastwood et al. 2006) and the Middle East (Farid 1987; Badawi 1981). From South

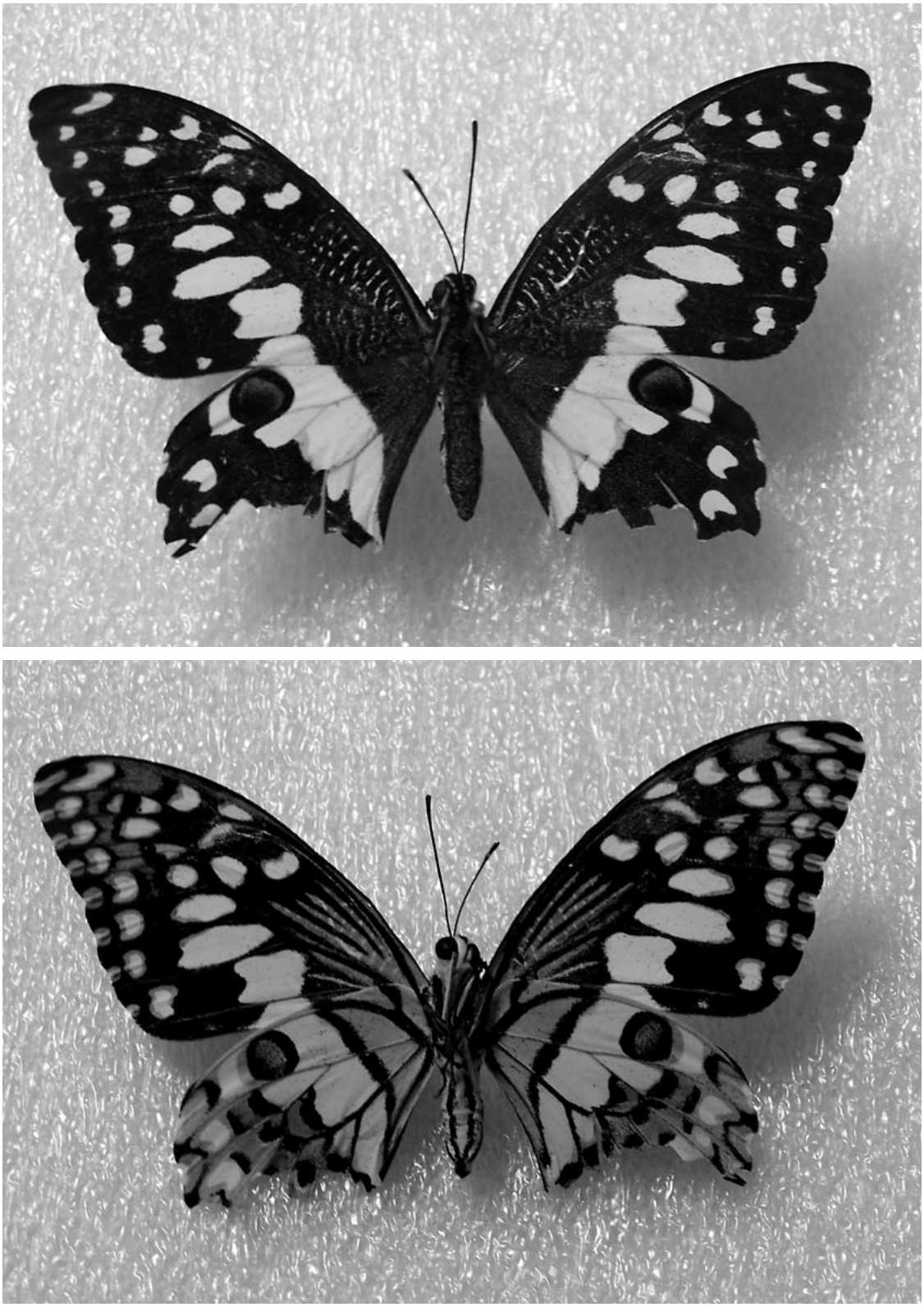

Fig. 1. *Papilio demoleus* from Guánica, Puerto Rico, (a) dorsal view, (b) ventral view.

and East Asia it has extended its range into the Indo-Pacific, dispersing throughout the islands of Indonesia (Dunn 1999; Matsumoto 2002; Moonen 1991) to New Guinea (Moonen 1999) and Australia (Smithers 1978; Williams et al. 1998). With this capacity for successful migration and range extension, *P. demoleus* is likely to rapidly expand its range beyond Hispaniola to include most islands in the Caribbean and adjacent mainland areas, including Florida.

Papilio demoleus has the potential to become a pest because it shows rapid population growth under favorable circumstances (Bhan & Singh 1997; Chatterjee et al. 2000; Pathak & Rizvi 2003; Radke & Kandalkar 1988). *Papilio demoleus* can have 5 broods per year in warm temperate China (Chen et al. 2004). Under ideal experimental conditions in India, Pathak & Rizvi (2003) reported generation time for *P. demoleus* to be just over 30 d.

Dispersal ability and the capacity for rapid population growth make *P. demoleus* a potentially serious pest throughout the Caribbean with significant economic impact. Citrus is an important agricultural commodity in most of the Caribbean. It is already in decline in several countries because of pests and diseases (Donovan 2002). In a review of the Caribbean citrus industry, Donovan (2002) reports that citrus production contributes significantly to income generation, foreign exchange earnings, employment, food security, economic diversification and growth in the region. Citrus production and associated manufacture are important contributors to the GDP of most Caribbean island nations.

Based on estimates from the Caribbean Cooperative Citrus Association, 52,000 persons are employed in the industry across CARICOM, generating over US$61 million dollars in foreign exchange earnings. Instead of crop value, production data may be more informative because most Caribbean citrus is produced for domestic markets. Production for CARICOM countries in 2001 was estimated to be 510,000 metric tons, 520,000 metric tons for Cuba, 70,200 metric tons for the Dominican Republic and 27,000 metric tons for Haiti. Countries of the Organization of Eastern Caribbean States (OECS) also produce significant amounts of citrus. Most production is by small farmers for domestic consumption. Small citrus farms (less than 5000 boxes per year) make up between 93 and 98 percent of all CARICOM farms and supply 42 percent of citrus fruits. Because the citrus industry is critical to the economic survival of many small farmers, this group would be most affected by the spread of *P. demoleus* in the Caribbean.

In 2002, the value of the Florida citrus crop exceeded $1.5 billion; the U.S. total (Florida, California, Arizona, and Texas) was more than $2.6 billion (National Agricultural Statistics Service 2004). The introduction of *P. demoleus* could have a significant economic impact on production and profitability of the industry. While advanced pest management tools are more readily available in the US than in the wider Caribbean region, growing insect resistance to microbial and other control strategies (Narayanan 2005) may leave the industry vulnerable to this new pest species.

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TWO NEW SPECIES OF *CERATOPHYSELLA* (COLLEMBOLA: HYPOGASTRURIDAE) FROM KOREA

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ABSTRACT

Two new species of the genus *Ceratophysella* from Korea, *Ceratophysella biclavata* **n. sp.** and *Ceratophysella platyna* **n. sp**. are described and illustrated. *Ceratophysella biclavata* differs from the closely related species *Ceratophysella sigillata* (Uzel 1891) by the shape of antennal bulb on antennal segment IV, the number of clavate tenent hairs and the number of granules between p_1 upon abdominal segment V. *Ceratophysella platyna* resembles *Ceratophysella denticulata* (Bagnall 1941) and *Ceratophysella communis* (Folsom 1898), but distinctly differs from the latter by the shape of tenent hairs. A key to the identification of the Korean species of *Ceratophysella* is included. In addition, the known species *Hypogastrura gracilis* (Folsom 1899) is described and recorded for the first time from Korea.

Key Words: *Hypogastrura*, Poduromorpha, Arthropleona, springtail, Apterygota, South Korea

RESUMEN

Dos nuevas especies del género *Ceratophysella* de Korea, *Ceratophysella biclavata* **sp. n.** y *Ceratophysella platyna* **sp. n**. son descritas e ilustradas. *Ceratophysella biclavata* se distingue de la especie cercana *Ceratophysella sigillata* (Uzel, 1891) por la forma del bulbo antenal en el segmento IV de la antena, el número de setas adhesivas clavadas y el número de los gránulos entre p_1 en el segmento V del abdomen. *Ceratophysella platyna* se parece a *Ceratophysella denticulata* (Bagnall, 1941) y *Ceratophysella communis* (Folsom, 1898), pero difiere claramente de estos por la forma de las setas adhesivas. Una clave para la identificación de las especies koreanas de *Ceratophysella* es incluida. También se adjunta la especie conocida *Hypogastrura gracilis* (Folsom, 1899) la cual es descrita y registrada por primera vez en Korea.

The family Hypogastruridae is common, widespread, and has cosmopolitan distribution containing approximately 659 world species in about 40 genera. The genus *Ceratophysella* also with worldwide distribution is one of the largest genera in the family, with more than 108 known species (Bellinger et al. 2006). Their habits were noted by Hopkin (2002), who stated that they often form enormous swarms on roads, glaciers, snow, and on the surfaces of puddles. Individuals in the swarms all leap together in the same direction using the orientation of the sun to navigate. They have small expandable sticky sacs on their antennae that help them adhere to the substrate when they land after a jump to stabilize them (Hopkin 2002).

Eight species of the genus *Ceratophysella* occur in Korea. Yosii & Lee (1963) recorded *C. communis* (Folsom 1897), Lee (1974) added 4 species, *C. liguladorsi* Lee, 1974, *C. sinetertiaseta* Lee, 1974, *C. armata* (Nicolet 1841) and *C. duplicispinosa* Yosii, 1954. Later Thibaud & Lee (1994) added the species, *C. bengtssoni* (Agren 1904), and Lee & Kim (1995, 2000) recorded 2 species, *C. dolsana* Lee & Kim, 1995, *C. denticulata* (Bagnall 1941). We add here 2 new species of the genus *Ceratophysella* and 1 species of the genus *Hypogastrura* as additions to the Korean fauna.

The purpose of this paper is to describe 2 new species and to provide an identification key to the species of *Ceratophysella* from Korea. Lee & Kim (1995) described *C. dolsana* as a new species, but there is no description of the genus in their work. Most authors regarded *dolsana* as belonging in the genus *Hypogastrura* (Bellinger et al. 2006; Thibaud et al. 2004). However, we include it in the key of *Ceratophysella*, primarily on the basis of long p_2 seta on thoracic segments II-III and on the shape of mucro in holotype and paratypes. Morphological abbreviations used in this paper are as follows: Ant. I-IV: antennal segments I-IV; Th. I-III: thoracic segments I-III; Abd. I-VI: abdominal segments I-VI; seta *a* and *b*: seta *a* and *b* among the 7 dorsal sensory setae of Ant. IV; $a_{1,\ 2}\ldots$: setae 1, 2 . . . of the anterior row counted from the "middle line"; $m_{1,\ 2}\ldots$: setae 1, 2 . . . of the middle row, counted from the "middle line"; $p_{1,\ 2}\ldots$: setae 1, 2 . . . of the posterior row, counted from the "middle line".

MATERIALS AND METHODS

Material was collected from 3 localities in Korea. Either an aspirator for direct collection or a Tullgren apparatus for extracting specimens was used. Collembola were fixed in 90% ethanol. Marc André I and II solutions were used to clear and

prepare specimen slides (Massoud 1967). KOH solution (10%) was used for rapid de-coloration. To prepare permanent slides, glycerine was placed along the cover glass edge to prevent the slide medium from drying. All type specimens are deposited in the Insect Collection of Biology Education Department, Chonbuk National University, Jeonju, Korea.

Ceratophysella biclavata, **new species**

Description (Fig. 1). Body length 1,110-1,400 µm (1,200 µm long in holotype). Color dark brown or blackish brown on whole body except inter-segmental portions and the ventral side. Body cylindrical, being narrower abruptly at Abd.V (Fig. 1A). Head length 220 µm in holotype. Antenna shorter than head, 0.9 in ratio to head; ratio of length of antennal segments I:II:III:IV is 5:5:6:4. Ant. IV with a simple apical bulb and a closely associated small papilla, a socket seta and some weak setae (Fig. 1B), and with 7 dorsal sensory setae of which seta *a* and *b* thickened. Eversible sac between Ant. III and Ant. IV distinctly developed. Ant. III organ with 2 short sensory and 2 guard sensilla (Fig. 1D). Mandible with 4 apical teeth (Fig. 1F). Eyes 8 + 8, eye patch with 3 setae. Postantennal organ (PAO) consists of 4 peripheral tubercles, about 1.2-1.5 times as long as the diameter of the nearest ocelli, with anterior lobes distinctly larger than posterior and with a small accessory tubercle (Fig. 1C). Tenent hairs 2, 2, 2 with distal end weakly clavate. Unguis elongate, with an inner tooth and a pair of lateral teeth. Unguiculus setaceous and with broad, rounded basal lamella (Figs. 1I-K). Ventral tube with 4 setae on each half. Tenaculum with 4 + 4 barbs without setae (Fig. 1E). Dens dorsally finely granulated and with 7 setae, 4 of them thicker than the others, about twice as long as mucro. Mucro apically rounded and with well developed outer lobe, anterior margin modified to form a tooth-like thickening from which a thin lamella extends basally (Fig. 1H). Abd.V with a granulated medial stripe, granules not modified, but arranged rather regularly. Mostly 11-13 granules lying between the p_1 seta on Abd.V (Fig. 1L). Anal spines 1/2-2/3 as long as inner unguis and about 2-2.5 times as long as papillae. On Abd. VI, a_1 shorter than anal spine including anal papilla (Fig. 1G).

Chaetotaxy. Area verticalis confluent with area occipitalis and with 2 + 2 setae. Th. I with 3 + 3 setae in a row. Th. II and III composed of 3 rows of setae, lacking m_2 p_2 a macrosetae and p_4 the sensory seta. Abd. I-III with 2 rows of setae, p_2 a macroseta and p_5 the sensory seta. Abd. IV with 3 rows of setae, lacking a_2, m_2 and m_3; p_1 longer than p_2 and p_3. Abd. IV setae often asymmetric in position. Abd. V with 2 rows of setae, p_1 longer than p_2, a_2 lacking and p_3 sensory seta (Fig. 1M).

Type Materials

Holotype: Female, Temple Jeongamsa, Gacheon-ri Dongmyeong-myeon Chilgok-gun, Gyeongsangbuk-do Province, collected from litter soil layer of the forest near stream. 24-X-2004, collection no. 204-21. Paratypes: 2 males and 3 females, same data as holotype.

Etymology. The specific name is derived from the number and shape of tenent hairs in each leg.

Remarks. The present species is very similar to *C. sigillata* (Uzel 1891), and redescribed by Babenko et al. (1994), in chaetotaxy of thorax and abdomen, in shape of mucro and basal lamella of unguiculus and in shape of seta on dens. However, they can be separated easily by differences in the shape of antennal bulb on Ant. IV, the number of tenent hairs on each leg and in the number of granules between p_1 upon Abd.V. Number of granules between p_1 of Abd. V is 20-25 in *C. sigillata* and 11-13 in the present new species. Also, the present species differs from *C. sigillata* by the strongly developed eversible sac (weakly developed in *C. sigillata*) and the absence of hook-like sensilla upon fourth antennal segment (Table 1).

Ceratophysella platyna, **new species**

Description (Fig. 2). Body length 1,200-1,400 µm (1,200 µm long in holotype). Body dark brown with blue pigment scattered over dorsum of segments in the form of irregular transverse bands (Fig. 2A). Head length 270 µm in holotype. Antenna shorter than head, 0.8 length of head; ratio of length of antennal segments I:II:III:IV is 3:4:5:6. Fourth antennal segment with a simple apical bulb and a closely associated protective papilla, giving a bilobed appearance to the antennal apex; lacking ventral file, but with 11-13 relatively long straight setae and seven clear blunt setae (Figs. 2B, E). Eversible sac between Ant. III and IV distinctly differentiated. Left mandible with 5 apical teeth and right with 4 apical teeth (Figs. 2D, H). Postantennal organ with 4 peripheral tubercles, a small accessory tubercle, anterior lobes strikingly larger than posterior and about 1.5 times as long as nearest ocelli. Eye patch with 8 ocelli on each side (Fig. 2C). Unguis slender, slightly curving distally, with 1 inner tooth on internal lamella. Unguiculus pointed and with a basal lamella tapering into a filament, almost ½ as longer internal lamella of unguis. Tenent hairs 1, 1, 1 almost as long as outer unguis and truncate to feebly clavate (Fig. 2G). Ventral tube with 3 + 3 setae. Tenaculum with 4 + 4 barbs. Dens about twice as long as mucro, with 7 posterior setae, without basally enlarged angled setae (Fig. 2F). Outer unguis 1.5 times as long as mucro. Mucro 0.8-0.9 times as long as anal spines. Body setae all smooth and slender. Integument

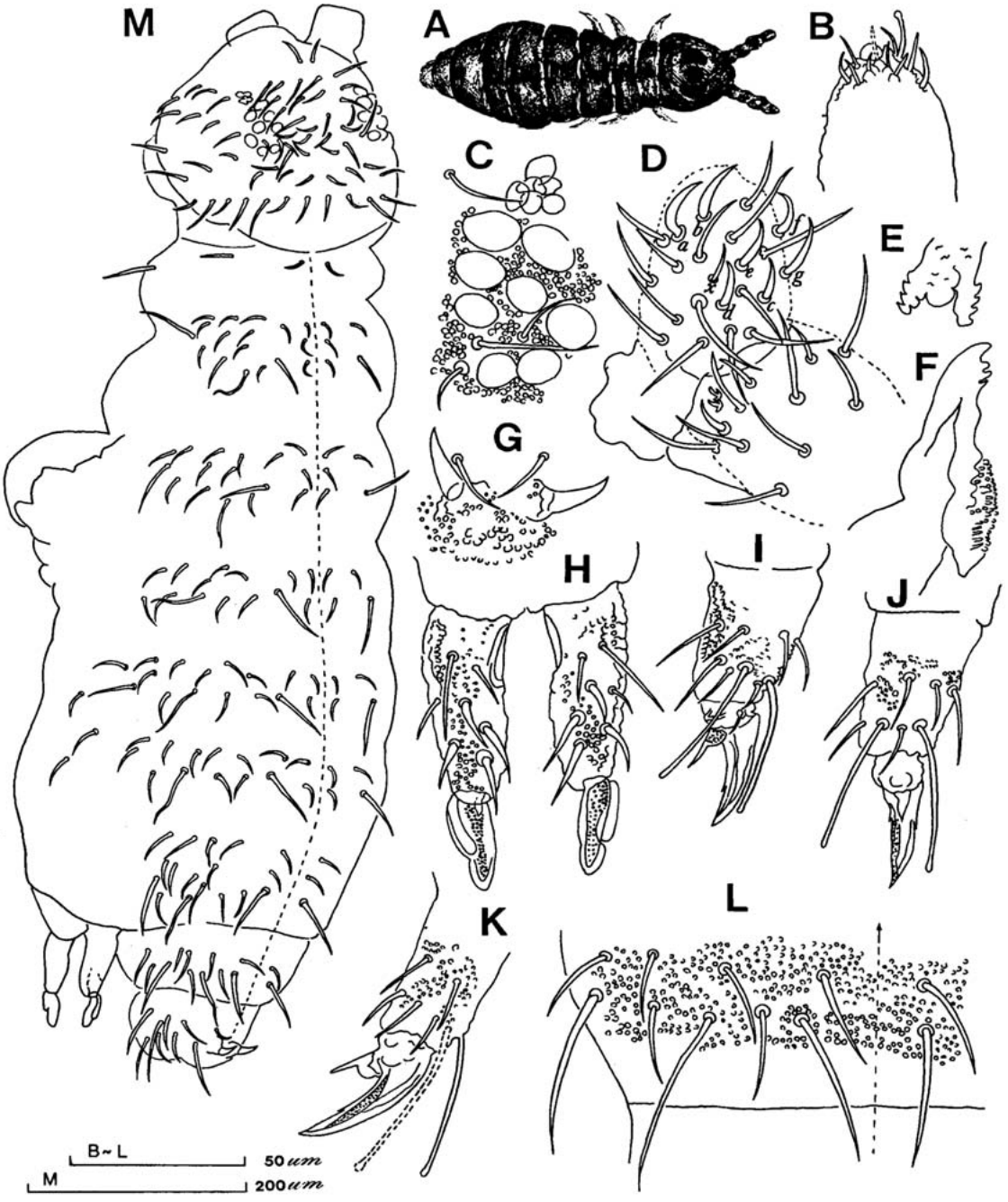

Fig. 1. *Ceratophysella biclavata* n. sp. A. Habitus. B. Apical view of antenna IV segment. C. Postantennal organ (PAO) and 8 ocelli. D. Dorsal view of antenna III, IV segments and the expandable sac between antennal segment III and IV. E. Tenaculum. F. Mandible. G. Anal spine. H. Dorsal view of mucro and dens. I. First leg. J. Second leg. K. Third leg. L. Abdomen V segment. M. Dorsal chaetotaxy of body.

moderately granular. Granular stripe on Abd. V arranged regularly, 9-12 granules lying between the p_1 setae on Abd. V (Fig. 2I). Fovea lying between the p_1. Anal spines slender, on unusually large contiguous papillae. On Abd. VI, a_1 nearly as long as anal spine including anal papilla (Fig. 2J).

Chaetotaxy. Area verticalis confluent with area occipitalis and with 2 + 2 setae. Th. I with 3

TABLE 1. DIAGNOSTIC CHARACTERS FOR *CERATOPHYSELLA SIGILLATA* AND *CERATOPHYSELLA BICLAVATA* N. SP.

Species/Character	*C. sigillata*	*C. biclavata* n. sp.
The number of clavate tenent hairs	1, 1, 1	2, 2, 2
Ant. IV antennal bulb	a simple apical bulb	a simple apical bulb and a closely associated small papilla
The number of granules between p_1 upon Abd. V	20-25 grains	11-13 grains
Eversible sac	weakly developed	strongly developed
hook-like sensilla upon Ant. IV	Present	absent

+ 3 setae in a row. Th. II and III with 3 rows of setae, m_2 and m_3 absent, p_2 a macroseta and p_4 the sensory seta. Abd. I-III with 2 rows of setae, without m-seta, with a_2', p_2 a macroseta and p_5 the sensory seta. Abd. IV with 3 rows of setae, a_1 slightly laterally dislocated, a_2, m_2 and m_3 absent, p_2 longer than p_1 and p_5 the sensory seta. Abd. V with 2 rows of setae, without a_2', p_1 longer than p_2, a_2 lacking and p_3 the sensory seta (Fig. 2K).

Type Materials

Holotype: Male, 700 m a.s.l., Mt. Moacksan, Gui-myeon, Wanju-gun, Jeollabuk-do Province, collected from the leaf litter under snow, 14 Feb 2004, collection no. 204-01-1. Paratypes: 2 males and 2 females, same data as holotype.

Etymology: The specific name, *platyna*, refers to the shape of body in this species.

Remarks: This species is characterized by the presence of an antennal bulb and the shape of tenent hairs. In many respects this species resembles *C. pratorum* of *C. boletivora*-group from North America (Christiansen & Bellinger 1998), but they differ in chaetotaxy. The present species is a member of Gisin's A type (Gisin 1947) with p_2 seta longer than p_1 seta on Abd. IV ($p_1 > p_2$ in *C. pratorum*). The antennal bulb clearly separates *C. platyna* **n. sp.** from *C. boletivora* and *C. biloba* of *C. boletivora*-group. Also, the present species is closely related to palaearctic species *C. annae* described by Babenko (1994), but is distinguished by the darker body colour, the presence of eversible sac and having 7 dorsal sensilla setae on Ant. IV (*C. annae* has 6). Chaetotaxy of the present species is similar to *C. communis* (Folsom) from Korea (Lee 1974; Lee & Thibaud 1975) by the presences of the a_2' seta on Abd. I-III, the absence of the a_2' seta on Abd. V, but it is separated from the latter in the shape of tenent hairs and the number of granules between p_1 upon Abd. V. It also has the same number of granules between p_1 upon Abd. V with cosmopolitan *C. denticulata* (Bagnall 1941) (Yosii 1962; Lee & Kim 2000). However, this new species is distinctly different from *C. denticulata* and *C. communis* in the shape of tenent hairs (Table 2).

Hypogastrura gracilis (Folsom, 1899),
new record

Diagnosis (Fig. 3). Body length 1,500-1,900 µm (1,700 µm long in holotype). Color grey or blackish brown on whole body except only intersegmental portions and the ventral side. Body laterally swollen at Abd. II and III, being gradually narrower toward posterior end (Fig. 3A). Head length 310 µm in holotype. Antenna longer than head, ratio 1.1 to head length; ratio of length of antennal segments I:II:III:IV is 12:13:18:30. Fourth antennal segment with a distal, slightly trilobed end-bulb and a number of socket setae, with 3 weak setae each on a slightly differentiated, small subapical papillae (Figs. 3C, E). Third antennal segment organ of 2 small rods in a shallow groove accompanied by 2 curved setae. Labrum with 4/5, 5, 4 setae, their distal row very weak. Labral margin with 4 rounded tubercles (Fig. 3H). Postantennal organ of 4 peripheral tubercles, with or without a small accessory tubercle, subequal to nearest ocelli (Figs. 3B, D). Eyes 8 + 8, on black patches. Unguis of all legs subequal, relatively small, dorsally carinate and with 1 inner tooth near the distal end. Unguiculus setaceous and reaching three-quarters of the distance from base to apex of unguis. Basal half with lamella on the inner side apically arcuate. Tenent hairs 2, 3, 3 rather thick and conspicuously swollen at apex. Median tenent hairs larger than others and above the level of others on the second and third legs (Fig. 3F). Ventral tube with 4+4 setae. Tenaculum with 3 + 3 barbs. Dens almost smooth dorsally with 7 setae, about 4 times as long as mucro. Mucro strongly compressed bilaterally and somewhat blade-shaped (Fig. 3G). Mucro 3.7-5.5 (mostly 4) times as long as anal spines. Outer unguis 1.3-1.8 times as long as mucro. Anal spines 0.25 times as long as inner unguis and subequal to anal papillae. All body setae short and fine.

Chaetotaxy. Th. I with 3+3 setae in a row. Th. II and III composed of 3 rows of setae, p_4 a little longer than others, sensory seta on Th. II without m_3 seta and Th. III without m_2, m_3, a_3 setae. Abd.

Fig. 2. *Ceratophysella platyna* n. sp. A. Habitus. B. Ventral view of antenna IV segment. C. Postantennal organ (PAO) and 8 ocelli. D. Left mandible. E. Dorsal view of antenna IV segment. F. Dorsal view of mucro and dens. G. First leg. H. Right mandible. I. Abdomen V segment. J. Anal spine. K. Dorsal chaetotaxy of body.

TABLE 2. DIAGNOSTIC CHARACTERS FOR *CERATOPHYSELLA PLATYNA* N. SP.

Species/Character	*C. denticulata*	*C. communis*	*C. platyna* n. sp.
Shape of tenent hair	acuminate	acuminate	clavate
The number of granules between p_1 upon Abd. V	9-12 grains	20 grains	9-12 grains
a_2' seta on Abd. V	present	absent	absent

I-III bearing two rows of setae, p_2 a macroseta and p_5 the sensory seta. Abd. IV with three rows of setae and p_4 sensory seta. Abd. V bearing 2 rows of setae, p_1 longer than p_2, and p_3 the sensory seta (Fig. 3I).

Material Examined. Numerous specimens collected from soil samples taken from mixed forest floor at Bisugumi, Dongchon, Hwacheon-eup, Hwacheon-gun, Gangwon-do Province. 15 Nov 2003, collection no. 203-27. Numerous specimens collected from litter of natural mixed forest consisting of coniferous and broad-leaved trees 300 m a.s.l., at the foot of Mt. Obongsan Gui-myeon Wanju-gun Jeollabuk-do Province. 10 Dec 2005, collection no. 205-33.

Remarks. This specimen generally correlates with the descriptions by Yosii (1960) from Japan. Some minor differences are observed, however, in the fourth antennal segment setae, in the presence or absence of accessory tubercle, in the position of the median tenent hair on the second and third legs. In addition, the present material is shown to have some local variation as compared to the original description. More extensive collections must be examined to determine whether this is a geographically variable species or a group of several similar species. The present species resembles *H. bulba* Christiansen & Bellinger 1980 of the *viatica* group in the trilobed antennal bulb. But it differs somewhat from *H. bulba* in the length ratio of mucro and dens, the number of tenent hairs on each leg (2, 3, 3 or 3, 3, 3 in *H. bulba*), and relative length of anal spine to anal papilla. Also, this species is similar to *H. tullbergi* (Schäffer 1900), but differs in the absence of spine-like setae on the apex of the third antennal segment.

Distribution. Japan, Korea (new record).

DISCUSSION

The species of *Ceratophysella* are characterized by having a well developed unguiculus and a spoon-shaped mucro with a lateral lamella. Posterior arms of postantennal organ are large, and seta m_2 on thoracic segment II is absent. In Japan, about 12 species are recorded (Furuno et al. 2000; Tamura 2001). Three species are known to occur in China (Zhao et al. 1997).

The taxonomic status of the members of genus *Ceratophysella* have been described by several researchers world-wide (Yosii 1960, 1962; Bourgeois & Cassagnau 1972; Bonet et al. 1973; Christiansen & Bellinger 1998; Babenko et al. 1994; Thibaud 2004). According to Yosii (1960, 1962), 3 species-groups are recognized in the genus *Ceratophysella*: *communis*, *armata*, and *denisana*-groups. The *communis*-group has the chaetotaxy of Gisin's A type (1947), which seta p_2 on Abd. IV larger than p_1 and is represented by *C. denticulata* Bagnall 1941 in Europe. The chaetotaxy of *armata*-group represents Gisin's B type (1947), which seta p_2 on Abd. IV smaller than p_1. Chaetal arrangement of *Ceratophysella biclavata* **n. sp.** is typical for the *armata*-group in the chaetotaxy of Abd. IV. *Ceratophysella platyna* **n. sp.** is clearly different from *armata*-group in the chaetotaxy of Abd. IV, where seta p_2 is longer than p_1 and p_3. Microsetae and macrosetae of the species weakly differentiated, but some setae as p_2 on Th. II and III, p_2 on Abd. I-IV and p_1 on Abd. V are longer than others, thus indicating the *communis*-group of chaetotaxy, that is Gisin's A type. *Ceratophysella platyna* **n. sp.**, commonly forms enormous swarms under leaves covered with snow.

In the present study, 2 new species and 1 newly recorded species are recognized in Korea. As result of this study, the Korean faunal list of Hypogastruridae consists of 28 species in 6 genera.

KEY TO 10 SPECIES OF *CERATOPHYSELLA* FROM KOREA

1. Fourth abdominal segment with seta p_1 longer than seta p_2 2

—. Fourth abdominal segment with seta p_1 shorter than seta p_2 8

2. Fourth abdominal segment with seta p_2 and seta p_3 short, sensory seta p_5 3

—. Fourth abdominal segment with seta p_2 short and seta p_3 long, sensory seta p_4 7

3. Fifth abdominal segment, an integumentary process "languette" present . . . . . *liguladorsi*

—. Fifth abdominal segment, an integumentary process "languette" absent 4

4. Dens with bladder-like swelling. . . . . *bengtssoni*

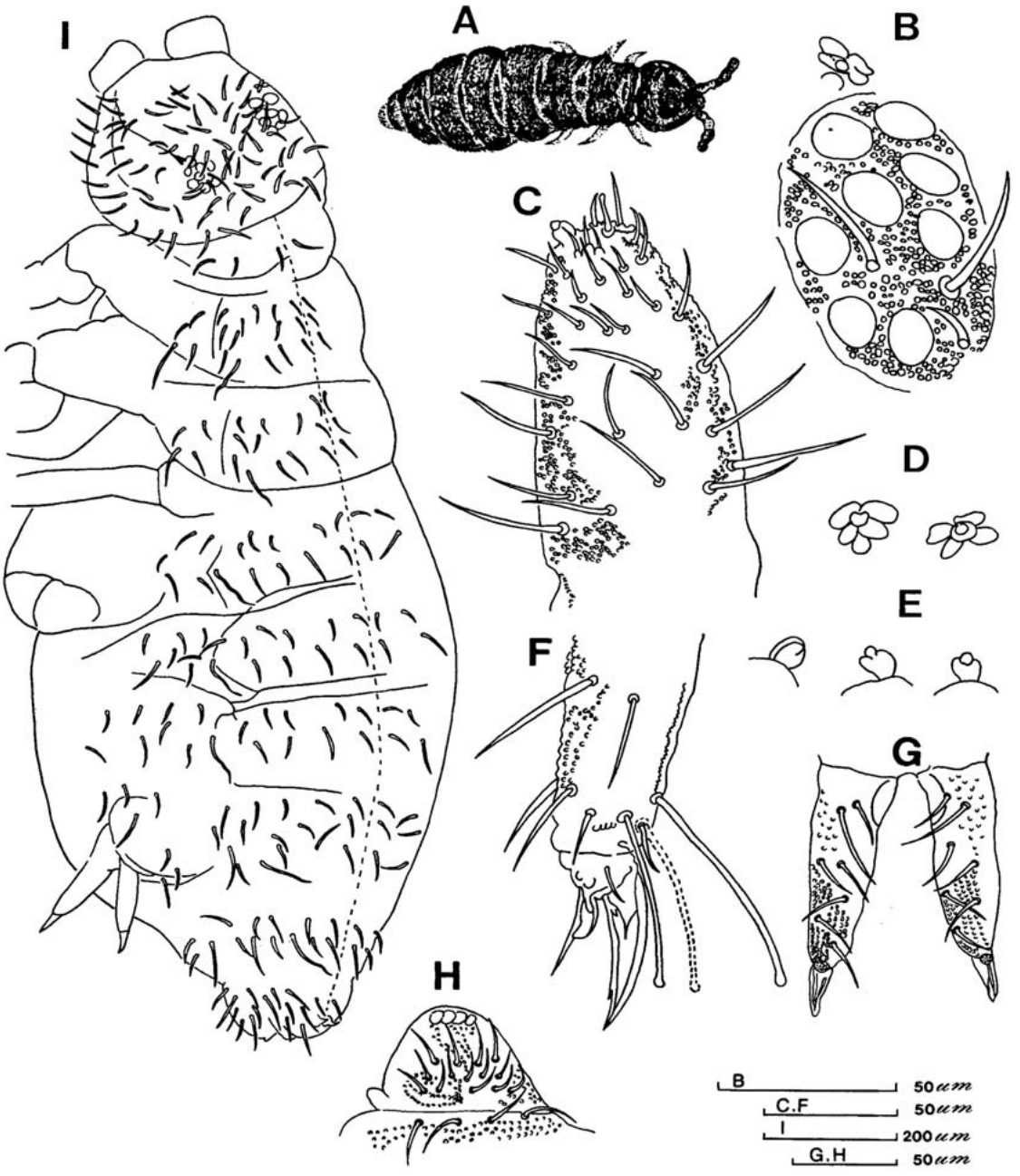

Fig. 3. *Hypogastrura gracilis*. A. Habitus. B. Postantennal organ (PAO) and 8 ocelli. C. Dorsal view of antenna IV segment. D. Various types of postantennal organ (PAO). E. Various types of fourth antennal segment apical bulb. F. Second leg. G. Dorsal view of mucro and dens. H. Labrum. I. Dorsal chaetotaxy of body.

—. Dens without bladder-like swelling 5

5. Fourth antennal segment with conspicuous ventral "file", tenent hair acuminate . . . . . *armata*

—. Fourth antennal segment without conspicuous ventral "file", tenent hair clavate or truncate 6

6. p_6, p_5 and p_4 sensory setae upon Abd. I-III, Abd. IV and Abd. V, respectively. Tenent hairs 1, 1, 1 and apical bulb of fourth antennal segment trilobed . . . . . *dolsana*

—. p_5, p_5 and p_3 sensory setae upon Abd. I-III, Abd. IV and Abd. V, respectively. Tenent hairs 2, 2, 2 and apical bulb of fourth antennal segment unilobed . . . . . *biclavata* **n. sp.**

7. Two spines present in the position of p_1 setae on Abd. *V*. . . . . *duplicispinosa*

—. Two spines absent in the position of p_1 setae on Abd. V . . . . . *sinetertiaseta*

8. Abd. V with a_2' setae; tenent hair acuminate; 9-12 granules between p_1 upon fifth abdominal segment . . . . . *denticulata*

—. Abd. V without a_2' setae 9

9. Tenent hair acuminate; 20 granules between p_1 upon fifth abdominal segment . . . . . *communis*

—. Tenent hair clavate or truncate; 9-12 granules between p_1 upon fifth abdominal segment . . . . . *platyna* **n. sp**.

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INTRASPECIFIC COMPETITION FOR RESOURCES BY *ORMIA DEPLETA* (DIPTERA: TACHINIDAE) LARVAE

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ABSTRACT

Ormia depleta is a parasitoid of pest mole crickets in the southeastern United States. From 2 to 8 larvae of *O. depleta* were placed on each of 368 mole cricket hosts and allowed to develop. The weights of the host crickets, number of larvae placed, number of resulting pupae, and the weights of those pupae were all factored to determine optimal parasitoid density per host under laboratory rearing conditions. Based on larval survival and pupal weight, this study indicates that 4-5 larvae per host is optimal for laboratory rearing.

Key Words: biocontrol, *Scapteriscus*, parasitoid, superparasitism

RESUMEN

Ormia depleta es un parasitoide de grillotopos en el sureste de los Estados Unidos. Entre 2 y 8 larvas de *O. depleta* se colocaron en 368 grillotopos huéspedes y se dejaron madurar. El peso de los huéspedes, el número de larvas de *O. depleta* colocadas, el número de pupas resultantes y el peso de las pupas fueron usados para determinar la densidad optima de parasitoides en cada huésped para ser usadas en la reproducción de este parasitoide en el laboratorio. Nuestros resultados muestran que entre 4 y 5 larvas por cada grillotopo es la densidad optima para la reproducción en el laboratorio de este parasitoide.

Translation provided by the author.

Ormia depleta (Wiedemann) is a parasitoid of *Scapteriscus* spp. mole crickets, imported pests of turf and pasture grasses in the southeastern United States (Frank et al. 1998). Female flies are phonotactic to the call of the male *Scapteriscus* spp. crickets (Fowler 1987; Fowler & Garcia 1987; Walker et al. 1996). *Ormia depleta* was originally collected from Piracicaba, Brazil, for use as a biocontrol agent against *Scapteriscus* spp. mole crickets and was first released in 1988 (Frank et al. 1996). Since then, it has established in at least 38 counties in Florida, and in some it has suppressed mole cricket populations (Parkman et al. 1996).

Ormia depleta can be a difficult organism to maintain in a laboratory colony. One of the factors that makes it difficult to rear lies in the variable and generally low proportion of gravid females obtained under the current laboratory rearing protocol. For example, a colony of 100 individuals may in 1 generation produce 20 gravid females and in the next only 1 or 2 or even zero (R. Hemenway, Dept. Entomology and Nematology, University of Florida, personal communication). Therefore, it is necessary to determine the best way to use the number of planidia available in any 1 generation to produce the maximum number of healthy pupae to start the next generation. This must also be balanced with the expense of rearing the mole cricket hosts, which are very labor intensive to maintain. Current laboratory protocol requires hand inoculation of 3 planidia under the posterior margin of the pronotum of each host (R. Hemenway, Dept. Entomology and Nematology, University of Florida, personal communication). Fewer planidia per host may increase the chances of survival by reducing competition and subsequently producing larger pupae. This would, however, require more hosts to produce enough pupae to maintain the colony. Inoculating hosts with more planidia may increase the number of pupae and reduce the cost associated with host rearing, but superparasitism should be avoided to minimize consequences associated with production of pupae and adults with reduced fitness.

Previous research with *O. depleta* showed that there was no relationship between the number of planidia used to inoculate the host and the number of pupae produced (Fowler 1988), but my preliminary research suggested that higher numbers of pupae could be produced than previously recorded. Additionally, Fowler & Martini (1993) found a weak correlation between host size and the weights of the flies produced. In the present experiment, the host-parasitoid relationship was also examined to determine (1) whether host weight should be a selecting factor and (2) to determine whether an increase in the number of pupae produced per host could be achieved without sacrificing the survivability or vigor of the larvae due to superparasitism. In addition to varying the

number of planidia applied to each host, the weights of the host mole crickets were measured during inoculation to see whether larger hosts could provision more parasitoids. These factors were examined to determine their effect on the number of pupae produced, the mean weight of those pupae, and the survivability of the larvae to the pupal stage.

Materials and Methods

During the maintenance of the laboratory colony of *O. depleta*, *S. abbreviatus* Scudder from the University of Florida mole cricket rearing lab were individually weighed and inoculated with varying numbers of *O. depleta* planidia. The weights of the hosts ranged from 0.54-1.59 g and the weights of the hosts were not considered in determining the number of planidia used to inoculate each individual. The number of planidia per host ranged from 2-8, with most of the mole crickets being inoculated with 3, 4, or 5 planidia. These numbers were favored because they are the numbers most frequently used in the routine maintenance of the colony. Host crickets were randomly assigned to particular numbers of planidia. The numbers of mole crickets inoculated with 2, 3, 4, 5, 6, 7, and 8 planidia were 12, 108, 110, 52, 43, 32, and 11, respectively. Each mole cricket was then returned to an individual 20-dram (90-mL) plastic vial filled with moist sand, and the larvae were allowed to develop for 12 d at a room temperature of ~26°C. At that time, the pupae were collected and weighed. Statistical analysis was performed with the general linear model procedure (SAS Institute 2001). Regression analysis was used to determine the effect of the number of planidia on pupal production, the mean pupal weight, and the survivability. Additionally, the weights of the host mole crickets were analyzed to determine their effect on the survivability of the planidia used. Where applicable, the differences between the means were determined by Duncan's multiple range test (SAS Institute 2001). Regression analyses were conducted to determine the relationships between each of these factors (SAS Institute 2001).

To determine the effect that host mole cricket weight had on planidia survival, the number of pupae produced and the mean weights of those pupae and mole cricket weights were rounded to the nearest 0.1 g to place them into weight classes. Additionally, weight classes which had only 2 or fewer samples were eliminated. In this case, the smallest weight class, 0.70 g (n = 2) and the 2 largest weight classes, 1.5 g (n = 2) and 1.6 g (n = 2) were eliminated from the statistical analysis. The survival of the planidia on hosts in the remaining weight classes were analyzed by ANOVA PROC GLM (SAS Institute 2001).

Results

The mean number of pupae produced relative to the number of planidia used is shown in Fig. 1. There is an increase in the number of pupae produced as the number of planidia increases (F = 15.77; df = 360; $P < 0.0001$) and significant differences between the means of the treatments. The regression analysis (Fig. 2) supports this trend and indicates an increase of 0.41 pupae for each increase in planidia (F = 83.77; $P < 0.0001$; r^2 = 0.19).

Fig. 3 shows the survival of planidia grouped by the number of planidia placed on each host. ANOVA is significant for the model (F = 2.57; df = 360; $P < 0.02$). Fig. 4 is the regression analysis of the same data set (F = 9.16; $P < 0.002$; r^2 = 0.03), indicating an approximate 3% reduction in survival for each increase in the level of planidia density.

The analysis of the number of planidia used as it affected the mean weight of the pupae produced was found to be significant at the 0.10 level, but not at the 0.05 level by ANOVA (F = 2.06; df = 360; P = 0.06). There was some significance among the means as indicated by the letters over the bars in Fig. 5. The regression analysis for the mean weights of pupae produced as a function of number of planidia inoculated per host is in Fig. 6 (F = 8.33; $P < 0.004$; r^2 = 0.02) and indicates a reduction in the mean weight of the pupae of 2.2 mg for each additional planidium.

The effect that host mole cricket weight had on the number of pupae produced was not significant when analyzed by ANOVA (F = 1.06; df = 361; P = 0.39). The effect of host mole cricket weight on the survivability of the larvae was significant (F = 2.12; df = 361; P = 0.05). The effect of host mole cricket weight on mean weight of the pupae produced was highly significant (F = 3.49; df = 361; $P < 0.002$) (Fig. 7). The regression analysis can be seen in Fig. 8 (F = 20.62; $P < 0.0001$; R^2 = 0.05).

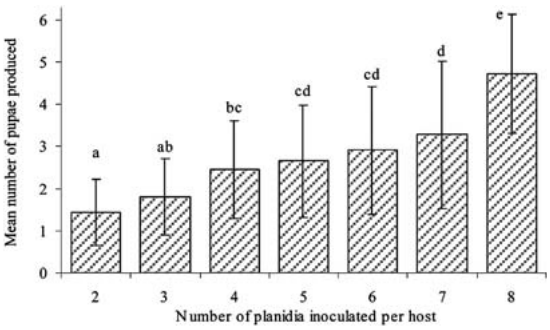

Fig. 1. The effect of planidia density used to inoculate mole crickets on the number of pupae produced (error bars indicate standard deviation, significantly different means indicated by letters over bars as determined by Duncan's procedure, α = 0.05).

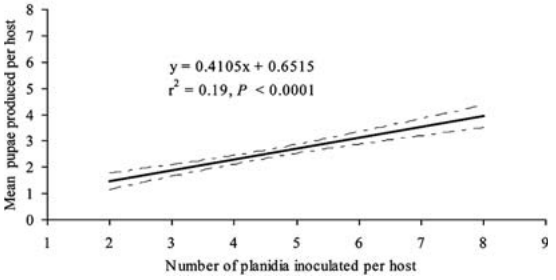

Fig. 2. The effect of number of planidia used to inoculate mole crickets on the number of pupae produced; regression analysis with 95% confidence intervals.

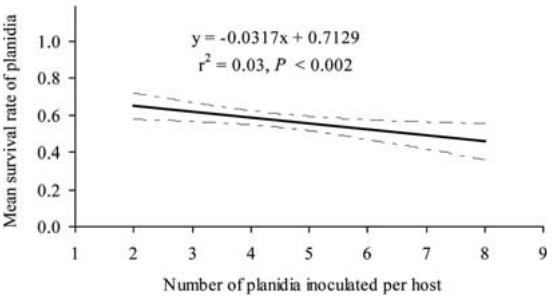

Fig. 4. The effect of number of planidia used to inoculate mole crickets on the survival rate of the larvae to the pupal stage; regression analysis with 95% confidence intervals.

DISCUSSION

The number of planidia used to inoculate host mole crickets as well as the weight of those mole crickets are important factors to the rearing of *O. depleta* in the laboratory. Although these data do not clearly dictate a specific protocol that should be used, they do provide a framework that would allow anyone rearing *O. depleta* to structure an inoculation protocol specific to their needs. When large numbers of planidia are available with only a few possible hosts, the data suggest that inoculating mole crickets with more planidia would increase the production of pupae. Too many, however, would result in reduced pupal size. At times when fewer planidia are available and maximum survivability is required, inoculating 2 or 3 planidia per host would be more effective. Alternatively, if larger pupae are desired, reducing the number of planidia per host along with using larger hosts would achieve the desired goal. Therefore, the current method of inoculating 3 planidia per host is less efficient than inoculating 4 or 5, because there is no significant reduction in pupal size, but there is a significant increase in the number of pupae produced. The reduction in size that results from the use of 8 planidia, or possibly more, would likely be detrimental to the colony of flies. Furthermore, these data only show a reduction in larval survival, they do not indicate other negative factors that may be associated with reduced size. Future research may be needed to determine whether individuals developing from heavily parasitized hosts show any reduction in longevity, ability to mate, or in fecundity as well as how the reduction in size of a generation may affect the size or fitness of future generations of flies.

Due to the flies' phonotactic search method for hosts and the solitary nature of the adult mole crickets, it would seem advantageous for the flies to maximize the number of offspring per host. Under field conditions, however, the mean number of *O. depleta* larvae found within trapped *Scapteriscus* hosts is less than 2 (Amoroso 1990).

The closely related *O. ochracea* Bigot, a parasitoid of *Gryllus* spp. crickets, has an optimal laboratory clutch size of 4-5 larvae per host, but under field conditions only deposit 1.7 ± 1.0 (SD). larvae (Adamo et al. 1995). There must be some ecological advantage to depositing fewer larvae than what would appear to be the optimal number.

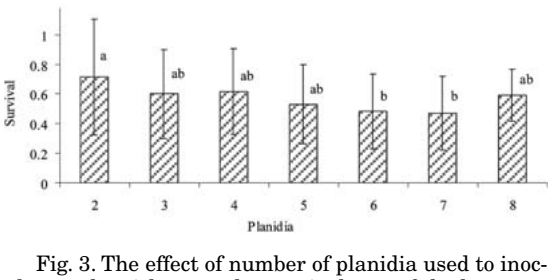

Fig. 3. The effect of number of planidia used to inoculate mole crickets on the survival rate of the larvae to the pupal stage (error bars indicate standard deviation; significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$).

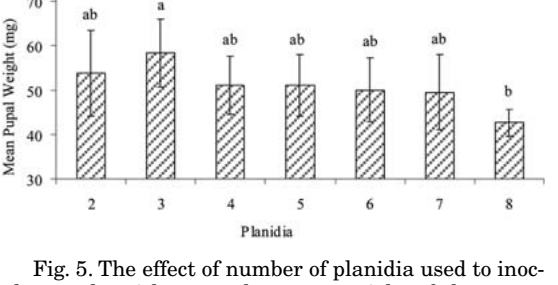

Fig. 5. The effect of number of planidia used to inoculate mole crickets on the mean weight of the pupae produced (error bars indicate standard deviation; significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$)

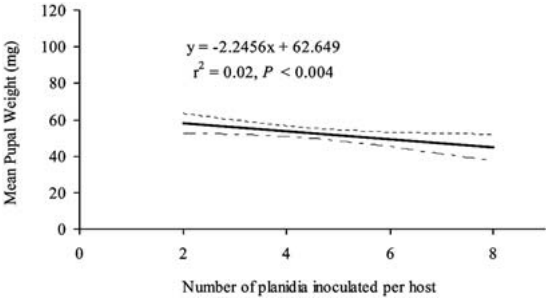

Fig. 6. The effect of number of planidia used to inoculate mole crickets on the mean weight of the pupae produced; regression analysis with 95% confidence intervals.

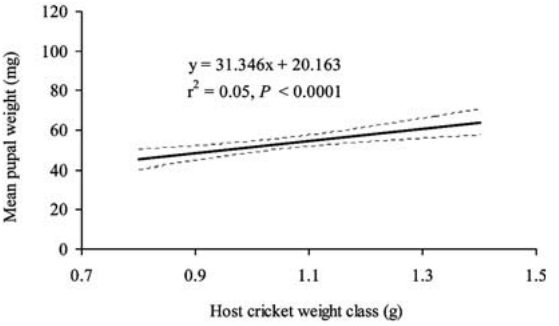

Fig. 8. The effect of host cricket weight class on the mean weight of the pupae produced; regression analysis with 95% confidence intervals.

It may be that *O. depleta* does not suffer from any shortage of hosts. Mole crickets are certainly abundant and calling during certain times of the year, but at other times seemingly unavailable. *Ormia depleta* may be able to find non-calling mole crickets in other ways, or there may be alternative hosts. Adamo et al. (1995) concluded that host availability was not a likely factor in determining the number of larvae deposited on hosts by *O. ochracea*. Another possibility is that *O. depleta* is responding to a factor in the field that is greatly reduced in the laboratory, such as mortality of the hosts. Under laboratory conditions, mole crickets suffer little disease and no predation. Higher host mortality in the field may make it advantageous for parasitoid offspring to be located in multiple hosts and subsequently reduce the effects on their population due to host predation. This hypothesis is somewhat strengthened by the fact that *O. depleta* does not deposit eggs, but planidia larvae, so the female's investment in parasitizing a host is already greater than that of an egg layer. Another laboratory factor that should be considered is hand-inoculating. The mole crickets that are hand-inoculated are unable to protect themselves in any way and have no opportunity to use any natural defenses such as brushing of planidia or retreating underground.

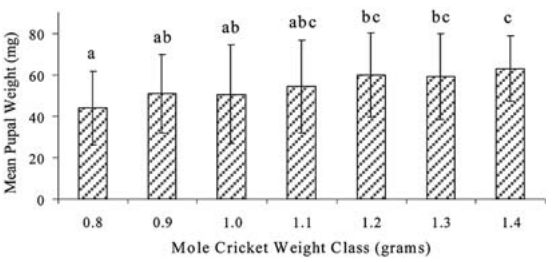

Fig. 7. The effect of host cricket weight on mean pupal weight (error bars indicate standard deviation; significantly different means indicated by letters over bars as determined by Duncan's procedure, $\alpha = 0.05$).

This type of grooming has been observed in *Gryllus* spp. crickets after an encounter with *O. ochracea* (Adamo et al. 1995).

The final reason for the low numbers of larvae found in field-captured hosts may be that there is a reduction in fitness caused by the high numbers of larvae used in this experiment. Reduced size is the easiest type of fitness reduction to observe, but many others may be at work. It may be that, due to competition, certain key resources are not available in sufficient amounts for the flies reared under superparasitoid conditions for the resulting adult flies to develop, mate, locate hosts, or reproduce properly. Many physiological deficiencies may result from superparasitoidism, and they may not be obvious either externally, or immediately (Waage & Ng 1984). These possibilities still remain for future research.

ACKNOWLEDGMENTS

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PESTICIDE SUSCEPTIBILITY OF *CYBOCEPHALUS NIPPONICUS* AND *RHYZOBIUS LOPHANTHAE* (COLEOPTERA: CYBOCEPHALIDAE, COCCINELLIDAE)

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ABSTRACT

The susceptibility of the predatory beetles *Cybocephalus nipponicus* Endrödy-Younga and *Rhyzobius lophanthae* Blaisdell to 6 pesticides commonly used for treating cycad aulacaspis scale, *Aulacaspis yasumatsui* Takagi, was tested. Three concentrations (half field rate, field rate, and twice field rate) of each pesticide were tested against both beetle species with a coated glass vial bioassay. Nearly 100% mortality in both beetle species occurred at all concentrations when treated with methidathion, dimethoate, and malathion. Insecticidal soap, fish oils, and imidacloprid were much less toxic. At one-half the field rate, *C. nipponicus* had 66% mortality with insecticidal soap, 76% mortality with imidacloprid, and 83% mortality with fish oil. At one-half the field rate, *R. lophanthae* had 43% mortality with insecticidal soap, 63% mortality with imidacloprid, and 46% mortality with fish oil. Mortality rate for each beetle species rose with increasing concentration of each pesticide and the soap and oil were the least toxic of all pesticides tested.

Key Words: biocontrol, coated glass vial bioassay, predatory beetle, toxicity test

RESUMEN

Se investigó la susceptibilidad de los escarabajos depredadores *Cybocephalus nipponicus* Endrödy-Younga y *Rhyzobius lophanthae* Blaisdell a seis pesticidas comunmente usados en el control de la escama de las cícadas, *Aulacaspis yasumatsui* Takagi. Se probaron tres concentraciones (mitad de la tasa recomendada en el campo, la tasa recomendada en el campo, y doble la tasa recomendada en el campo) de cada pesticida contra cada especies de escarabajo, usando frascos de vidrio aplicado para bioensayos. La mortalidad en ambas especies de escarbajos fue casi 100% a todas las concentraciones de metidatión, dimetoato, y malatión. Jabón insecticida, aceite de pescado, e imidacloprid fueron mucho menos tóxicos. A la mitad de la tasa recomendada en el campo, los niveles de mortalidad de *C. nipponicus* fueron 66% con jabón insecticida, 76% con imidacloprid, y 83% con aceite de pescado. A la mitad de la tasa recomendada en el campo, los niveles de mortalidad de *R. lophanthae* fueron 43% con jabón insecticida, 63% con imidacloprid, y 46% con aceite de pescado. La tasa de mortalidad por cada especie de escarabajo aumentó con mayores concentraciones de cada pesticida y el jabón y aceite de pescado fueron los menos tóxicos de todos los pesticidas probados.

Translation provided by the authors.

Beetles of the families Coccinellidae and Cybocephalidae are the most economically important groups of predators of diaspidid scales in the world (Blumberg & Swirski 1982). *Cybocephalus nipponicus* Endrödy-Younga (Cybocephalidae) and *Rhyzobius lophanthae* Blaisdell (Coccinellidae) are commonly used as biological control agents for many armored scale pests. *Rhyzobius lophanthae* has been established in Florida since the 1930s (according to specimen label data in the Florida State Collection of Arthropods). *Cybocephalus nipponicus*, misidentified as *Cybocephalus binotatus* Grouvelle, was recently released in south Florida in an effort to control the cycad aulacaspis scale (CAS), *Aulacaspis yasumatsui* Takagi (Homoptera: Diaspididae) (Anon. 1998; Howard et al. 1999; Howard & Weissling 1999). CAS is the most economically damaging scale to cycads that the state of Florida has ever seen (Hodges et al. 2003). Although *C. nipponicus* is present in Hawaii (Heu & Chun 2000), *R. lophanthae* is usually suggested as the better control agent of CAS (Heu et al. 2003; A. Hara, personal communication). In both places, CAS has continued to spread and multiply. A more promising approach to controlling CAS would be one using integrated pest management (IPM). In this manner, a combination of pesticides and biological control would be used to combat CAS.

There has been some success controlling CAS with various pesticides. Oils, either an ultra-fine horticultural oil or a product containing fish oils, seem to be the most effective chemical control method (Hodges et al. 2003). This is not surprising given that oils have long been used to control armored scale insects. The oil not only covers the insects and suffocates them but also covers the surface of the plant making it difficult for crawlers to settle onto the plant (Howard & Weissling 1999). Soaps are quite popular with homeowners; but they must be applied frequently, in some cases once a week (personal observation). The effective application of pesticides for control of CAS is difficult due to the scale's tendency to heavily infest the abaxial surface of leaves, a site difficult to spray (Howard & Weissling 1999). In the case of *Cycas revoluta* Thunberg (Cycadaceae), the architecture of the plant itself, with the margins of the leaflets curling down and inward to form an arch on the abaxial surface of the leaflet, makes foliar treatments inefficient (Hodges et al. 2003). Frequent or "as needed" applications of oils seems to be the most effective technique for controlling CAS, and by mixing oil with contact pesticides such as malathion, even greater scale mortality can be achieved (Hodges et al. 2003). Systemic pesticides such as dimethoate and contact pesticides like methidathion have yielded mixed results, being very effective in some instances and completely ineffective in other cases (Hodges et al. 2003). Imidacloprid used as a soil drench can be very effective, but Howard & Weissling (1999) found that this product had to be mixed at very high concentrations to be effective. This product can also be used as a foliar spray.

The reproductive biology of *C. nipponicus* makes it a good biological control agent. Alvarez & Van Driesche (1998) found that, at low scale densities, *C. nipponicus* was able to maintain its populations and maintain populations of euonymus scale, *Unaspis euonymi* (Comstock), and San Jose scale, *Quadraspidiotus perniciosus* (Comstock), in check. In the presence of greater scale densities, *C. nipponicus* will increase egg production accordingly. With a total life cycle from egg to adult only taking around 44 days (Smith & Cave 2006), it is conceivable that 5-6 generations could be produced every year in Florida. *Cybocephalus nipponicus* is available commercially in the U.S. market.

Rhyzobius lophanthae is an exceptional biological control agent because of its high fecundity, lack of parasitoids, absence of diapause, and resistance to low temperatures especially in the immature stages (Rubstov 1952; Smirnoff 1950; Stathas 2000). Female *R. lophanthae* are able to lay hundreds of eggs in a lifetime (Stathas 2000). *Rhyzobius lophanthae* also seems to be able to resist extreme heat, but Atkinson (1983) found that adult *R. lophanthae* could not survive for long at 42°C. *Rhyzobius lophanthae* is also available commercially in the U.S. market.

This study was conducted to determine the susceptibility of *C. nipponicus* and *R. lophanthae* to 6 pesticides commonly used in the control of CAS. Given the established presence of both predators on cycads in south Florida and their commercial availability, it is very important to learn what effects the commonly used pesticides against CAS will have on them. This information is vital for development of IPM programs aimed at controlling CAS.

Materials and Methods

Insects

Adult *R. lophanthae* were reared at, and purchased from, Rincon-Vitova Insectaries (Ventura, California). Adult *C. nipponicus* also were purchased from Rincon-Vitova but were reared by Philip Alampi Beneficial Insect Laboratory, New Jersey Department of Agriculture. Both beetle species were maintained in Plexiglas cages at 25°C and 80% relative humidity prior to testing. All life stages of CAS were provided as a food source.

Food was not provided during testing because of the very small size of the beetles (1 mm in width and 2.5 mm in length). The beetles could have conceivably perched on the food source for long periods of time, never coming into contact with the walls of the treated vial. Preliminary studies indicated that a 24-h period without food would not unduly stress the beetles. On average, untreated *C. nipponicus* survived for 8-9 d (n = 30) and untreated *R. lophanthae* lived for 5-6 d (n = 30) before dying of starvation. Cotton used to stopper the vials was soaked in water to prevent dehydration.

Bioassays with the Coated Glass Vial Method

A coated glass vial method (Plapp 1971; Amalin et al. 2000; Snodgrass 1996; Snodgrass et al. 2005) was used to determine the chemical susceptibility of adult *R. lophanthae* and *C. nipponicus* to 6 pesticides used to control CAS (Howard et al. 1997; Howard & Weissling 1999; Weissling et al. 1999; Hodges et al. 2003; Emshousen & Mannion 2004). This is a very effective method for testing the chemical susceptibility of small arthropods (Amalin et al. 2000) such as *R. lophanthae* and especially *C. nipponicus* because of its extremely small size. The 6 pesticides tested were fish oil emulsion (Organocide®), insecticidal soap (Garden Safe, Inc.), imidacloprid (Provado®), malathion (Spectracide, Inc.), methidathion (Supracide®), and dimethoate (Cygon®). The fish oil and insecticidal soap were purchased as commercial grade, while the imidacloprid (99% purity), malathion (98% purity), methidathion (98.6% purity), and dimethoate (98.7% purity) were purchased as the technical grade from Chem Service (West Chester, PA).

All pesticides were dissolved in acetone, except the insecticidal soap, which does not dissolve in acetone. Instead, the insecticidal soap was dissolved in 95% ethanol. The fish oil was shaken in a paint shaker after being placed in acetone in order to break the oil into fine globules. Each pesticide was separated into 3 dilutions: field rate, twice field rate, and one-half field rate. The field rate was taken from label data for each pesticide as directed for use against scale insects. A small amount (0.5 mL) of the pesticide working solution was dispensed into 20-mL scintillation vials. Concentrations of active ingredient for the working solution and the amount of active ingredient residue within the vials can be seen in Table 1. Vials were hand rotated until the acetone or ethanol completely evaporated leaving an insecticidal residue on the inner surface. Vials treated with only acetone or ethanol, as well as untreated vials, were used as controls. A single beetle was placed into a treated vial. All beetles had emerged from pupae within the previous 14 d. Vials were sealed with cotton soaked in water allowing the beetles to drink. Vials were placed upright in a ventilated cabinet with a fume hood and at a constant temperature of 25°C and 80% relative humidity for 24 h. For each treatment of 10 beetles, 5 females and 5 males were used. Each treatment of 10 beetles was replicated 3 times for each dosage. All trials were carried out the same day that the pesticide was applied to the vials.

Mortality of beetles was determined immediately after the 24-h period. A beetle was considered dead if it was not moving or could not right itself. Percent mortality was measured as the proportion of 30 beetles dead after a 24-h exposure to the pesticides.

Statistical Analyses

All descriptive statistics were generated in EXCEL (Microsoft 2000). The mortality rates for each pesticide were compared by the Student-Newman-Keuls mean separation test (SAS Institute 2001).

TABLE 1. FIELD RATES (1X) FOR EACH PESTICIDE USED.

Insecticide	Working solution (µg*AI/mL)	Insecticide residue (µg*AI/cm^2)
Organocide®	47000	8.29
Insecticidal Soap®	512300	27.71
Imidacloprid	106	2.40
Methidathion	233	5.26
Dimethoate	305	6.91
Malathion	1990	45.07

*AI = Active Ingredient.

RESULTS

Of the 6 pesticides tested on adult *C. nipponicus* and *R. lophanthae*, 3 (methidathion, dimethoate, and malathion) caused >90% mortality at all concentrations, while the other 3 (fish oil, insecticidal soap, and imidacloprid) were less toxic but still caused very high mortality (Tables 2 and 3).

Effects of Pesticides on *C. nipponicus*

Cybocephalus nipponicus was extremely susceptible to all pesticides. The three least toxic pesticides were imidacloprid, insecticidal soap, and fish oil (Table 2). There were significant differences ($P < 0.05$; Table 4) in mortality between concentrations among these 3 pesticides. Fish oil was not only toxic to the beetle, but due to its very small size, *C. nipponicus* would often get trapped in small globules of oil, eventually dying from suffocation.

Effects of Pesticides on *R. lophanthae*

Rhyzobius lophanthae was more tolerant than *C. nipponicus* to the experimental pesticides, although mortality rates were high for this species, too. The 3 least toxic pesticides to *R. lophanthae* were imidacloprid, insecticidal soap, and fish oil (Table 3). There were significant differences in

TABLE 2. PERCENT MORTALITY OF *CYBOCEPHALUS NIPPONICUS* PER 30 INDIVIDUALS EXPOSED. X = FIELD RATE.

	% Beetle mortality			
Pesticide	at 0X	at 0.5X	at 1X	at 2X
Organocide®	—	83	100	96
Insecticidal Soap®	—	66	86	96
Imidacloprid	—	76	93	100
Methidathion	—	100	100	100
Dimethoate	—	100	96	100
Malathion	—	93	100	100
Control (Acetone)	0	—	—	—
Control (Ethanol)	0	—	—	—
Control (No coating)	0	—	—	—

TABLE 3. PERCENT MORTALITY OF *RHYZOBIUS LOPHANTHAE* PER 30 INDIVIDUALS EXPOSED. X = FIELD RATE.

	% Beetle mortality			
Pesticide	at 0X	at 0.5X	at 1X	at 2X
Organocide®	—	46	83	100
Insecticidal Soap®	—	43	76	96
Imidacloprid	—	63	80	100
Methidathion	—	100	100	100
Dimethoate	—	100	96	100
Malathion	—	93	90	96
Control (Acetone)	0	—	—	—
Control (Ethanol)	6	—	—	—
Control (No coating)	0	—	—	—

survivorship between concentrations of these 3 pesticides (Table 4). *Rhyzobius lophanthae*, about twice the size of *C. nipponicus*, had much less difficulty traversing oil globules on the surface of the vials.

DISCUSSION

In the present study, a significant difference ($P < 0.05$) was observed between mortality in the control and that of even the lowest pesticide concentration. This sensitivity to pesticides makes an IPM approach to the control of CAS quite difficult. Unfortunately, most of the success in chemically controlling CAS has involved very toxic pesticides often being used at higher than recommended doses (Howard & Weissling 1999; Weissling et al. 1999).

The high mortalities experienced by *C. nipponicus* and *R. lophanthae* are not unexpected. Nakao et al. (1985) found that all 18 species of Coccinellidae inhabiting Japanese citrus groves were severely affected by the application of pesticides, including methidathion and dimethoate. They also found that *Cybocephalus gibbulus* Erichson, one of the most common scale predators found in Japanese citrus groves, was virtually eliminated by long-term pesticide use. Oils have proven to be the most effective pesticides used against many plant-sucking pests, while maintaining the natural enemy populations. Erkiliç & Uygun (1997) found that oils were much less toxic to *Cybocephalus fodori minor* (Endrödy-Younga) and *Chilocorus bipustulatus* (Linnaeus) than was methidathion. In fact, they went as far as saying that methidathion should not be used in IPM programs.

In natural conditions, the predatory beetles may not be in contact with the pesticide for as long as the exposures in this experiment. However, *C. nipponicus* and *R. lophanthae* are uniquely suited for life in chemically-treated environments. Both beetle species place their eggs underneath the scale cover and at least part of larval development takes place beneath the armored scale, allowing the beetles some protection from both the elements and pesticides (Smirnoff 1950; Alvarez & Van Driesche 1998; Stathas 2001). In Greece, Katsoyannos (1984) found that *C. fodori* was able to survive in pesticide-treated

TABLE 4. STUDENT-NEWMAN-KEULS TEST SHOWING RANKED VALUES OF MORTALITY OF ADULT *CYBOCEPHALUS NIPPONICUS* AND *RHYZOBIUS LOPHANTHAE* BY 4 DOSES OF IMIDACLOPRID, INSECTICIDAL SOAP, AND ORGANOCIDE.[1]

	Dose	Imidacloprid	Organocide®	Insecticidal soap
C. nipponicus	0.0X	2.0 a	2.0 a	2.0 a
	0.5X	5.5 b	5.3 b	5.3 b
	1.0X	8.5 c	8.6 c	8.3 c
	2.0X	10.0 c	10.0 c	10.3 c
R. lophanthae	0.0X	2.0 a	2.0 a	2.0 a
	0.5X	5.6 b	5.0 b	5.3 b
	1.0X	7.3 b	8.0 c	7.8 c
	2.0X	11.0 c	11.0 d	10.8 d

[1]Means within columns with the same letter are not significantly different based on Student-Newman-Keuls mean separation test, $P = 0.05$.

fruit orchards. In date palm plantations in Israel, Kehat et al. (1974) found that, while all coccinellids in a chemically-treated plantation died, species of *Cybocephalus* survived.

For some pesticides, it is apparent that from these tests, the lower the concentration of the pesticide, the lower the mortality. However, these tests were conducted in a laboratory environment wherein the test subjects were in constant contact with the pesticide for 24 h. A whole host of factors, such as humidity, UV degradation, evaporation, and precipitation, will influence pesticide activity in the field. Nevertheless, whenever possible, insecticidal soaps and fish oils should be used. While many homeowners use various types of soaps to treat CAS, this method requires treatment every 7 to 10 d, thus increasing exposure of the beetles to the pesticide. If more toxic pesticides must be used, then applying them to "hot spots" rather than broadcast spraying may protect the scale predators from complete annihilation. This type of selective spraying may also protect other entomophagous insect populations from being decimated (Kuznetsov 1997). The results of these laboratory experiments yield some baseline data from which more research in the field can be conducted.

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HYMENOPTERAN PARASITOIDS OF *ANASTREPHA* FRUIT FLIES (DIPTERA: TEPHRITIDAE) REARED FROM DIFFERENT HOSTS IN YUCATAN, MEXICO

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ABSTRACT

In order to carry on the detection and species inventory of hymenopteran parasitoids associated with fruit flies, we examined various tropical fruits growing at the Southern region of Yucatan. During a yearly cycle (Jun 2000 to Jun 2001), 9 host fruit species (including some varieties) were collected by 2 different methods. The first method involved weekly collection of ripened fruits that were transported to the laboratory ("Fruit-Lab"); and the second method was collection of fruits placed on the ground below the tree canopy ("Fruit-Beds"), and which remained in the field for two weeks, after which they were transported to the laboratory. Fruits obtained were counted and weighed, and the recovered pupae were quantified for each sample. As a whole, we sampled 4,470 fruits (850.8 Kg) from the 9 host plant species and varieties, which were infested by 5 fruit fly species: *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), *A. striata* Schiner, and *A. fraterculus* (Wiedemann). The average parasitism in all samples was 3.69% represented by 11 hymenopteran species as follows: Braconidae, *Doryctobracon areolatus* (Szépligeti), and *Opius bellus* (Gahan); Figitidae, *Aganaspis pelleranoi* (Brethes), *Aganaspis* sp., *Odontosema anastrephae* Borgmeier and *Odontosema* sp.; Diapriidae, *Coptera haywardi* (Oglobin); Chalcididae, *Dirhinus* sp.; Pteromalidae, *Spalangia endius* Walker; Eurytomidae, *Sycophila* sp.; and Perilampidae, *Euperilampus* sp. On the basis of results in differences among samples for parasitism rates, fruit fly parasitoid, and fruit fly host plant, parasitoid assemblages are analyzed and discussed.

Key Words: parasitism, fruit flies, host plants, natural enemies

RESUMEN

Con el propósito de realizar la detección e inventario de especies de parasitoides asociados con moscas de la fruta, se examinaron diversos frutos tropicales cultivados en la región Sur del estado de Yucatán. Durante el ciclo anual comprendido entre junio de 2000 a junio de 2001, se estudiaron nueve especies y variedades de frutos de la región, empleando dos métodos de colecta: el primero se realizó por medio de la colecta semanal de frutos maduros transportados al laboratorio ("Fruit-Lab"); y el segundo mediante la recolección de camas de frutos ("Fruit-Beds") colocados en el suelo bajo la cobertura de los árboles, los cuales permanecieron por dos semanas, y posteriormente trasladados al laboratorio. En ambos casos, los frutos fueron contados y pesados, además de la cuantificación de pupas recuperadas en cada muestra. En total se recolectaron 4,470 frutos (850.8 Kg) de las nueve especies y variedades de plantas hospederas, las cuales resultaron infestadas por cinco especies de moscas de la fruta: *Anastrepha ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), *A. striata* Schiner, y *A. fraterculus* (Wiedemann). La proporción de parasitismo en todas las muestras fue de 3.69% representado por 11 especies de himenópteros de las siguientes familias: Braconidae, *Doryctobracon areolatus* (Szépligeti), y *Opius bellus* (Gahan); Figitidae, *Aganaspis pelleranoi* (Brethes), *Aganaspis* sp., *Odontosema anastrephae* Borgmeier, and *Odontosema* sp.; Diapriidae, *Coptera haywardi* (Oglobin)); Chalcididae, *Dirhinus* sp.; Pteromalidae, *Spalangia endius* Walker; Eurytomidae, *Sycophila* sp.; and Perilampidae, *Euperilampus* sp.. Con base en estos resultados, se analizan y discuten las diferencias entre los índices de parasitismo, así como entre los ensambles mosca- parasitoide y planta hospedera-parasitoide.

Translation provided by the authors.

Diverse regional studies in Latin America have addressed the incidence of native parasitoids of the genus *Anastrepha* in countries such as Guatemala (Eskafi 1990), Costa Rica (Jirón & Mexzon 1989), Colombia (Yepes & Vélez 1989; Carrejo & González 1999), Venezuela (Katiyar et

al. 1995; Boscán & Godoy 1996; García & Montilla 2001), Brazil (Canal et al. 1995; Leonel et al. 1995; Guimarâes et al. 1999; Aguiar-Menezes et al. 2001), and Argentina (Ovruski 1995; Ovruski et al. 2004, 2005).

Previous studies have stated that as many as 18 parasitoid species of *Anastrepha* have been recorded in Mexico, including the exotic species *Diachasmimorpha longicaudata* (Ashmead) and *Aceratoneuromyia indica* (Silvestri), both of which have been considered as established (Ovruski et al. 2000). However, at least 6 other exotic species have been introduced into Mexico for control of *A. ludens* and *A. obliqua* (Jiménez-Jiménez 1955, 1956, 1963).

Inventories of native parasitoids of *Anastrepha* fruit flies have been conducted in commercial orchards at Morelos and Chiapas (McPhail & Bliss 1933; Baker et al. 1944; Aluja et al. 1990), but also in wild environments associated with native fruit fly hosts in Nuevo León (Plummer & McPhail 1941; González-Hernández & Tejada 1979), Veracruz (Hernández-Ortiz et al. 1994; López et al. 1999), and Chiapas (Aluja et al. 2003). Inventories have not been done in many other fruit growing regions of Mexico.

Anastrepha ludens (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wiedemann), *A. striata* Schiner, *A. fraterculus* (Wiedemann), *A. ampliata* Hernández-Ortiz, and *A. pallens* (Coquillett) have been recorded from the state of Yucatan (Hernández-Ortiz et al. 2002). The first 4 species are significant pests in fruit crops in Mexico and most of the Neotropics (Hernández-Ortiz & Aluja 1993). Fruit fly control in Yucatan has generally involved use of pesticides (CESVY 2000), and very little is known of the native hymenopteran parasitoid communities. An earlier regional study showed the presence in Yucatan of certain Opiinae (Braconidae) that potentially parasitize *Anastrepha* species, including *Doryctobracon* Ender, *Utetes* Foerster, and *Opius* Wesmael (Delfín-González & León 1997), although sampling methods in that study were not focused on host collection. Thus, specific relationships between *Anastrepha* and braconid species remain unknown.

The present study focuses on the search for and inventory of parasitoids that attack *Anastrepha* species, as well as determination of the relationships between fruit flies, host plants, and parasitoids in the fruit growing region of southern Yucatan, which mainly consists of mixed orchards of citrus, mango, sapodilla, guava, and red mombin.

Materials and Methods

The study was carried out in mixed commercial orchards in the Yaax-Hom Fruit Unit, 5 km from the Lol-Tun archaeological site, Oxkutzcab municipality, in southern Yucatan (20°18'N, 89°42'W). Surrounding native vegetation is semievergreen tropical forest (Flores & Espejel 1994). Collection of fruit samples occurred from Jun 2000 to Jun 2001, and included 9 host plant species during their fruit-growing seasons: sour orange, *Citrus aurantium* L. (Aug 2000 to Jan 2001); Valencia orange, *C. sinensis* (L.) Osbeck var. valenciana (Oct 2000 to Mar 2001); Ruby grapefruit, *C. paradisi* MacFad (Jul 2000 to Jan 2001); star apple, *Chrysophyllum cainito* L. (Jan to Mar 2001); mango, *Mangifera indica* L. c.v. cordoba, criollo, pico de loro, and manglova (Mar to Jul 2001); sapodilla, *Manilkara zapota* (L.) P. Royen (Sept to Dec 2000); mamey sapote, *Pouteria sapota* (Jacq.) H. Moore & Stearn (Apr to Jul 2001); guava, *Psidium guajava* L. (Jun to Sep 2000; Febr , April to Jun 2001); and red mombin, *Spondias purpurea* L. c.v. San Juan, tuxpana, and chi-abal (Apr to May 2001).

Fruits were sampled during the fruiting season of each host plant, according to availability of mature fallen fruits under the trees by means of 2 different methods as follows:

(1) Fruit-Lab Samples. Fruits were weekly sampled, placed in 20-liter containers with a substrate of soil from the collection site, covered with wire mesh and topped with a fine-mesh screen to prevent contamination. Samples were taken to the laboratory where they were counted, weighed, and reviewed daily. The recovered pupae were separated in small plastic containers for adult fly and parasitoid emergence.

(2) Fruit-Bed samples. This method was implemented once a sufficient amount of fruits were available. Collected fruits were arranged in "fruit-beds" under the tree canopy, consisting of a plastic tarp covered with soil, containing a known number of fruits previously weighed. "Fruit-beds" remained in the field for 2 weeks and were observed. All pupae recovered were taken to the laboratory in small plastic containers for adult fly and parasitoid emergence.

Percent of parasitism (PP) was recorded as PP = a/(a + b) 100, where a = Number of recovered parasitoids; and b = Number of emerged adult flies in each sample (Steck et al. 1986). Correlation analysis (Statistica 1999) was used to compare mean fruit weight of host sampled (calculated as the $\text{Log}_{(10)}$ of fruit weight), infestation index (calculated as the number of larvae/Kg fruit), and percentage of parasitoids recovered in each sample.

Specimens of fruit flies and parasitoids were determined by VHO and HDG, respectively. Voucher specimens are deposited in the Insect Collections (IEXA) of the Instituto de Ecologia (Xal-

apa, Veracruz), and in the Regional Entomological Collections (CERUY) of the Universidad Autónoma de Yucatán (Mérida, Yucatán). Botanical samples were identified by personnel of the Botanical Department of the UADY and deposited in the Herbarium of this institution. Botanical nomenclature is based on Terrel et al. (1986), and parasitoid nomenclature follows Ovruski et al. (2000).

Results

Altogether, 4,470 fruits (850.8 kg) from 9 host species (including 4 mango varieties and 3 red mombin varieties) were examined and found to be infested by 5 *Anastrepha* species. All the citrus hosts (*C. aurantium*, *C. sinensis*, and *C. paradisi*) were infested by *A. ludens*, and 1 specimen of *A. serpentina* was recovered from ruby grapefruit and 2 from sour orange. A single specimen of *A. fraterculus* was found in sour orange. The hosts of the family Sapotaceae (*C. cainito*, *P. sapota* and *M. zapota*) were only infested by *A. serpentina*, and all *S. purpurea* varieties were infested by *A. obliqua*. The mango varieties (*M. indica*) were infested by *A. ludens* (53.4%) and *A. obliqua* (45.9%), and 2 specimens of *A. serpentina* were recovered. The guava fruits (*Psidium guajava*) were infested by *A. fraterculus* (84.2%) and *A. striata* (15.8%).

In total, 12,929 larvae and pupae were recovered from the sampled fruits. Although the number of fruits collected by each sampling method were equivalent, the "Fruit-Lab" samples exhibited a higher degree of infestation (2,227 fruits, with 8,511 recovered pupae), than that left in the "Fruit-Bed" samples (2,243 fruits, with 4,418 recovered pupae). The highest infestation indices per host were observed in *P. guajava* (103.2 larvae/Kg), *S. pupurea* (all varieties with 83.3 to 44 larvae/Kg), *C. cainito* (40.4 larvae/Kg), *P. sapota* (29.6 larvae/Kg), and *C. aurantium* (22.5 larvae/Kg). The lowest infestation rates occurred in *M. indica* (all varieties with 15.9 to 0.3 larvae/Kg), *M. zapota* (15.7 larvae/Kg), *C. sinensis* (4.2 larvae/Kg), and *C. paradisi* (3.1 larvae/Kg). Sample sizes in some of these low-infestation hosts were relatively small. In total, 9,223 fruit fly viable pupae were recovered during the study, which produced 8,883 adult flies and 340 parasitoid specimens. Average parasitism of all fruit flies was 3.69% (Table 1).

The recovered parasitoids included the following 11 species: the larval-pupal parasitoids *D. areolatus* (Szépligeti) and *Opius bellus* (Gahan) (Braconidae); *Aganaspis pelleranoi* (Brethes), *Aganaspis* sp., *Odontosema anastrephae* Borgmeier and *Odontosema* sp. (Figitidae); and the pupal parasitoids *Coptera haywardi* (Oglobin) (Diapriidae), *Dirhinus* sp. (Chalcididae), and *Spalangia endius* Walker (Pteromalidae). In addition, 2 other parasitoid species in the genera *Sycophila* sp. (Eurytomidae) and *Euperilampus* sp. (Perilampidae) were recorded for the first time in *Anastrepha*.

Relationships between fruit fly-parasitoids among samples showed that *A. ludens* was attacked in *Citrus* spp. by 5 parasitoids, which accounted for 29.3% of overall species, while in *M. indica* only 2 parasitoid species were recorded with 0.6%. In this sense, *A. obliqua* was parasitized in *Spondias purpurea* by 5 parasitoid species (16.7%); *A. serpentina* was attacked by 5 parasitoids (25.6%) infesting 3 hosts of the family Sapotaceae; and the *Psidium guajava* fruits infested by *A. striata*/*A. fraterculus* were parasitized by 8 species (27.8%).

Odontosema anastrephae was found in 7 host plant species representing 43.2% of all recovered parasitoids with highest proportions in *Psidium guajava* and *Citrus aurantium*. *Coptera haywardi* represented by 16.2% of parasitoids was found in 6 hosts; *Doryctobracon areolatus* (14.2%) was present in 4 hosts, particularly in *C. cainito*; and *Spalangia endius* only accounted for 6.5% of the overall recorded parasitism, but it was found in 4 different fruit hosts (Table 2).

Parasitism observed between 2 sampled collections revealed that specimens recovered from "Fruit-Bed" samples were higher than those recovered from the "Fruit-Lab" samples with 68.5% and 31.5%, respectively. In this sense, species as *C. haywardi*, *O. anastrephae*, *S. endius*, and *Dirhinus* sp. were dominant in "Fruit-Beds" accounting for 65% of all parasitoid specimens. On the contrary, the dominant species observed in "Fruit-Lab" samples were *D. areolatus*, *Sycophila* sp. and *Euperilampus* sp., which accounted for 21.2%. Table 3 shows the proportions of parasitoids by hosts obtained from each sampling method.

Correlation analysis between average fruit weight (Log Fruit Weight) and the infestation index (Mean Larvae/kg Fruit) were significant (r = -0.695; P = 0.005), indicating that as average weight increased in the different fruit species, the degree of infestation in the sample decreased. In contrast, there was not a significant correlation between the average fruit weight and the percentage of parasitism (r = -0.090; P = 0.758), and no correlation between infestation index and the percentage of parasitism among samples (r = 0.270; P = 0.350).

Discussion

All parasitoid species reported here are first records for *Anastrepha* in Yucatan. No previous published records exist in literature of the genera *Sycophila* sp. (Eurytomidae) and *Euperilampus* sp. (Perilampidae) as parasitoids in *Anastrepha* (Ovruski et al. 2000). In this sense, *Eurytoma sivinskii* Gates & Grissell (Eurytomidae) was recently described attacking field populations of

TABLE 1. HOST PLANT SAMPLED AND RECOVERED FRUIT FLY PUPAE AND PARASITOIDS OF *ANASTREPHA* SPECIES IN YUCATAN MEXICO. *MANGIFERA INDICA*: 1 = VAR. CORDOBA; 2 = VAR. CRIOLLO; 3 = VAR. PICO DE LORO; 4 = VAR. MANGLOVA; *SPONDIAS PURPUREA*: 1 = VAR. SAN JUAN; 2 = VAR. TUXPANA, 3 = VAR. CHI-ABAL.

Host plant	Fruit sampled	Total fruit weight (Kg)	Mean fruit weight (Kg)	Infestation (larvae/Kg)	Total pupae recovered	Pupae viable	Flies emerged	Parasitoids emerged	Parasitism %
C. aurantium	558	90.40	0.162	22.50	2037	1630	1578	52	3.19
C. sinensis	732	138.60	0.189	4.24	587	438	414	24	5.48
C. paradisi	251	124.40	0.496	3.15	392	269	245	24	8.92
Ch. cainito	200	22.80	0.114	40.40	918	514	447	67	13.04
M. indica 1	325	64.70	0.199	2.80	179	110	108	2	1.82
M. indica 2	29	4.60	0.159	15.90	73	67	67	0	0.00
M. indica 3	225	87.00	0.387	0.33	29	23	23	0	0.00
M. indica 4	234	105.90	0.453	1.20	127	81	81	0	0.00
Ma. zapota	454	83.60	0.184	15.70	1310	1084	1072	12	1.11
Po. sapota	92	72.80	0.791	29.60	2157	1940	1932	8	0.41
Ps. guajava	442	26.80	0.061	103.20	2765	1773	1679	94	5.30
S. purpurea 1	716	26.80	0.037	83.30	2232	1188	1133	55	4.63
S. purpurea 2	138	1.75	0.013	44.00	77	73	72	1	1.36
S. purpurea 3	74	0.65	0.009	70.80	46	33	32	1	3.03
All samples	4470	850.8	0.232	31.2	12929	9223	8883	340	3.69

TABLE 2. PARASITOID SPECIES EMERGED BY HOST FRUIT SPECIES UNDER 2 DIFFERENT SYSTEMS OF COLLECTION. *M. INDICA*; 1 = VAR. CORDOBA; *S. PURPUREA*; 1 = VAR. SAN JUAN; 2 = VAR. TUXPANA; 3 = VAR. CHI-ABAL. ACRONYMS FOR PARASITOID SPECIES ARE AS FOLLOWS: DAR = *D. AREOLATUS*; OBEL = *O. BELLUS*; CHAY = *C. HAYWARDI*; APELL = *A. PELLERANOI*; ASP = *AGANASPIS* SP.; OANAS = *O. ANASTREPHAE*; OSP = *ODONTOSERNA* SP.; SPEND = *S. ENDIUS*; DSP = *DIRHINUS* SP.; SYSP = *SYCOPHILA* SP.; ESP = *EUPERILAMPUS* SP.

Hosts	Parasitoids	Oar	Obel	Chay	Apell	Asp	Oanas	Osp	Spend	Dsp	Sysp	Esp	Totals	% by sample
C. aurantium	Fruit Lab	0	0	0	1	0	1	1	0	0	0	0	3	0.9
	Fruit beds	0	0	8	0	0	38	0	3	0	0	0	49	14.4
C. sinensis	Fruit Lab	0	0	0	0	0	1	0	0	0	0	0	1	0.3
	Fruit beds	0	0	6	0	0	10	0	7	0	0	0	23	6.8
C. paradisi	Fruit Lab	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	Fruit beds	0	0	1	0	0	23	0	0	0	0	0	24	7.1
Ch. cainito	Fruit Lab	30	0	0	0	0	12	0	0	0	0	0	42	12.4
	Fruit beds	5	0	7	0	0	13	0	0	0	0	0	25	7.3
M. indica 1	Fruit Lab	0	0	0	0	0	1	0	0	0	1	0	2	0.6
	Fruit beds	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Ma. zapota	Fruit Lab	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	Fruit beds	0	0	9	0	0	3	0	0	0	0	0	12	3.5
S. purpurea 1	Fruit Lab	5	0	0	0	0	0	0	0	0	8	16	29	8.5
	Fruit beds	0	0	0	0	0	0	0	10	15	1	0	26	7.7
S. purpurea 2	Fruit Lab	0	0	0	0	0	0	0	0	0	1	0	1	0.3
	Fruit beds	0	0	0	0	0	0	0	0	0	0	0	0	0.0
S. purpurea 3	Fruit Lab	1	0	0	0	0	0	0	0	0	0	0	1	0.3
	Fruit beds	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Po. sapota	Fruit Lab	0	0	0	0	0	0	0	0	4	0	4	8	2.3
	Fruit beds	0	0	0	0	0	0	0	0	0	0	0	0	0.0
Ps. guajava	Fruit Lab	6	2	0	7	1	3	1	0	0	0	0	20	5.9
	Fruit beds	1	0	24	5	0	42	0	2	0	0	0	74	21.7
Total specimens	Fruit Lab	42	2	0	8	1	18	2	0	4	10	20	107	31.5
	Fruit beds	6	0	55	5	0	129	0	22	15	1	0	233	68.5
% parasitism	FLab + FBeds	14.1	0.6	16.2	3.8	0.3	43.2	0.6	6.5	5.6	3.2	5.9	340	100

TABLE 3. RELATIONSHIP FRUIT FLY-PARASITOID SPECIES RECOVERED FROM ALL SAMPLED HOSTS EXPRESSED IN PERCENTAGES.

Fruit fly	*A. ludens*	*A. ludens* / *A. obliqua*	*A. obliqua*	*A. serpentina*	*A. striata*/ *A. fraterculus*	Parasitism %
Hosts	*Citrus* spp.	*M. indica*	*Spondias purpurea*	*Ch. cainito, P. sapota, M. zapota*	*P. guajava*	All hosts
Parasitoids						
Odontosema anastrephae	21.4	0.3	0.0	8.2	13.3	43.2
Odontosema sp.	0.3	0.0	0.0	0.0	0.3	0.6
Aganaspis pelleranoi	0.3	0.0	0.0	0.0	3.5	3.8
Aganaspis sp.	0.0	0.0	0.0	0.0	0.3	0.3
Doryctobracon areolatus	0.0	0.0	1.8	10.3	2.1	14.2
Opius bellus	0.0	0.0	0.0	0.0	0.6	0.6
Coptera haywardi	4.4	0.0	0.0	4.7	7.1	16.2
Spalangia endius	2.9	0.0	2.9	0.0	0.6	6.4
Dirhinus sp.	0.0	0.0	4.4	1.2	0.0	5.6
Sycophila sp.	0.0	0.3	2.9	0.0	0.0	3.2
Euperilampus sp.	0.0	0.0	4.7	1.2	0.0	5.9
Parasitism %	29.3	0.6	16.7	25.6	27.8	100.0

A. obliqua in Mexico (Gates & Grissell 2004). The eurytomids also occur as parasites in Cynipidae, Pteromalidae, Eurytomidae, Tanaostigmatidae, and Agaonidae (Grisell & Schauff 1990; DiGiulio 1997), and members of the family Perilampidae are hyperparasitoids of Ichneumonidae (Darling 1997). However, since the tephritid pupae were separated from the fruit and counted before adult emergence, these may be cases of hyperparasitism. These results should be further investigated.

The genus *Dirhinus* (Chalcididae) has been reported as a pupal parasite in Brachycerous Diptera widely distributed throughout the world tropics, with 3 known species in the USA (Burks 1947), and about 15 native species yet to be studied in regions ranging from Indiana (USA) to central Argentina (Boucek 1992). Unpublished data for Mexico indicate the presence of at least *D. buscki* (Crawford), *D. schwarzi* (Crawford), *D. texanus* (Ashmead), and *D. giffardii* (Silvestri) (data provided by Alejandro González-Hernández and Serguei Triapitsyn), although there are probably 1 or 2 more species with cosmopolitan distribution (Robert A. Wharton, Texas A & M University, personal communication).

Dirhinus giffardi is the unique species reported attacking fruit flies in the Neotropics, a native western African species introduced in Israel around 1950 (Podoler & Mazor 1981), and in Latin American countries of Puerto Rico (1935-1937), Costa Rica (1955), Peru (1960), Colombia (1970), and Bolivia (1971), and in Florida, USA (1977-1979) (Ovruski et al. 2000). In Mexico, it has been introduced in the states of Morelos and Oaxaca (Jiménez-Jiménez 1956), however there is no evidence that it is established in these regions. The *Dirhinus* species reported in this paper is very similar to *D. schwarzi* and *D. giffardii*, representing an undescribed species, and a new record of a native parasitoid for *A. obliqua* and *A. serpentina*.

The exotic species *Diachasmimorpha longicaudata* and *Aceratoneuromyia indica* were not recorded during the present study, but both have been documented as established and as having significant parasitism indices in Costa Rica (Wharton et al. 1981) and Mexico (Aluja et al. 1990) respectively, though both these studies were only concerned with coffee and mango orchards. *Spalangia endius* is a remarkable record, since it has been recorded from *Anastrepha* in Florida, though rarely reared from tephritids (Ovruski et al. 2000).

The majority of the published papers on *Anastrepha* parasitoids indicate that *D. areolatus* (Braconidae) is the most important native parasitoid species, having the highest parasitism indices in the Neotropical region in countries such as Mexico (Hernández-Ortiz et al. 1994; López et al. 1999), Guatemala (Eskafi 1990), Costa Rica (Jirón & Mexzon 1989), Colombia (Yepes & Velez 1989; Carrejo & González 1999), Venezuela (Katiyar et al. 1995), Brazil (Canal et al. 1995; Leonel et al. 1995; Aguiar-Menezes & Menezes 1997; Aguiar-Menezes et al. 2001), and Argentina (Ovruski et al. 2004, 2005).

On the basis of our results, *O. anastrephae* (Figitidae) is the dominant species occurring in 7 host plants attacked by 5 *Anastrepha* species. This species is considered a koinobiont parasitoid of *Anastrepha* larvae (Ovruski et al. 2000),

though most of the recovered specimens were found in the Fruit-Bed samples, particularly from *Citrus* species and guava accounting for 87.7%. Such differences in the parasitism indices may be related to parasitoid biological factors, such as the ability of *O. anastrephae* to reach their host larvae by entering wounds in fruit located on the ground (Sivinski et al. 1997, 2000).

Comparisons between fruit weight and infestation rates among different hosts showed that the number of larvae was larger in small fruits but decreased as fruit size increased. This coincides with results observed for *A. suspensa* in Florida (USA), when fruit sizes and infestation indices were compared for 6 host species (Sivinski 1991).

Previous hypothesis on parasitism levels have been attributed in part to physical difficulties in locating immature stages within large fruits (Sivinski 1991). However, our comparisons between fruit weight of 14 hosts and the parasitism rates of the 11 parasitoid species showed no correlation. This may be due to the fact that more sample sizes are needed in order to test this hypothesis, or that the native parasitoid community has only become recently adapted to certain exotic fruit species included in our analysis, such as *Citrus* spp. and *M. indica*.

The low level of parasitism (3.69%) observed in this study is probably due to orchard management practices, in which destruction of fallen fruit and periodic pesticide use (CESVY 2000), could have a negative impact on parasitoid populations. Similar studies carried out in Brazil reported similar species diversity and levels of parasitism (Uchôa-Fernandes et al. 2003). Based on the parasitoid species diversity that attack the *Anastrepha* fruit flies in Yucatan, further studies need to be focused on the biology and ecology of certain native parasitoids such as *O. anastrephae*, *C. haywardi*, and *D. areolatus* as promising biological control agents.

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INCIDENCE OF *LYTTA UNGUICULARIS* (COLEOPTERA: MELOIDAE) ON HYBRID AZALEAS, *RHODODENDRON* SPP., IN THE GREAT SMOKY MOUNTAINS NATIONAL PARK

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The genus *Lytta* (Coleoptera: Meloidae) contains approximately 69 species found in the Nearctic (Pinto & Bologna 2002), primarily in the United States and Mexico. Although this genus contains approximately 17% of all known species of meloids in the Nearctic (n = 410) (Pinto & Bologna 2002), little is known about many of these species of blister beetles. Several *Lytta* species are extremely uncommon in collections and have not been seen in decades (Pinto & Bologna 1999). One colorful species, *Lytta unguicularis* (LeConte, 1866), has a metallic green or blue body and orange legs and has been documented in Illinois (type locality), Alabama, North Carolina, and Tennessee (Downie & Arnett 1996; Selander 1960). More specifically, its known distribution ranges from eastern Alabama to Illinois and northeast to the Smoky Mountains in eastern Tennessee and western North Carolina. This large beetle, with a maximum length of 25 mm, is uncommon in museum collections and is known from fewer than 70 specimens from 9 documented locations (Selander 1960). The larval hosts of *L. unguicularis* are unknown; however, other species of *Lytta* are known to parasitize the nests of native bees, where larvae feed on provisions and possibly on immature bees. Larval hosts include immatures of Apoidea, particularly Anthophoridae, Megachilidae, Halictidae, and Colletidae (Pinto & Bologna 1999; Bologna & Pinto 2002). Adults reportedly feed on the flowers and foliage of Rosaceae and Ericaceae, including peach, rose, and mountain laurel, and have been collected on azalea (Selander 1960).

During a study to identify pollinators of a hybrid swarm of azalea, *Rhododendron arborescens* (Pursh) Torrey, *R. viscosum* (L.) Torrey, and *R. cumberlandense* Braun, in the Great Smoky Mountains National Park, a small population (<50) of adult *L. unguicularis* was observed on flowers and foliage of hybrid azaleas. This report is the first documentation of this species in the Great Smoky Mountains National Park since the 1950s and early 1960s based on museum and park collections and other records (Selander 1960). In 1958, adult *L. unguicularis* were collected previously on azalea in the Park (Selander 1960). Sherman (1913) reported thousands of beetles on peach, rose, and mountain laurel at a site in Blowing Rock, North Carolina, from 8 to 25 Jun 1901. He stated that they consumed the blossoms of the mountain laurel and leaves of peach. Selander (1960) suggested that the somewhat gregarious nature of Meloidae, including *Lytta* species, serves to maintain the adult beetles near nesting sites of host bees.

In our study, *L. unguicularis* was found on azalea plants growing along the northern margin of one of the balds in the Great Smoky Mountains National Park. The origin of the grassy balds may have been natural; however, their present flora is partially an artifact of human interference, such as animal grazing, lumber harvesting, and fire prevention (Lindsay 1977; Lindsay & Bratton 1979). The specific identity and location of this bald cannot be provided because of low numbers of individuals and the sensitivity of the site, but interested persons can contact the Inventory and Monitoring Coordinator of the Great Smoky Mountains National Park for additional information. This bald, similar to one of the many grassy balds that occur only in the Southern Appalachian Mountains, is currently maintained by personnel with the Great Smoky Mountains National Park. The bald is home to a hybrid swarm of multicolored azaleas, with flowers ranging in color from red, orange, pink, yellow, to white, and many of these flower colors and forms are not found on other balds. Most of the insects visiting flowers of these hybrid azaleas were bees in the families Andrendidae, Halictidae, and Apidae. The nests of some of these families of Hymenoptera are hosts of larvae of other species of *Lytta* (Pinto & Bologna 1999).

Adult beetle activity was observed on only 5 or 6 azaleas located on the north side of the bald on 15 and 18 Jun 2000 between 10 AM and 3 PM. This observed activity coincides with Selander (1960), who reported that the seasonal incidence of this species was from 2 May to 4 Jul. Adults were observed to feed on the blossoms and foliage of azalea on each sampling date, but the extent of this feeding was not quantified. Mating also was observed on each date. Representative male and female specimens were collected into individual 3½ dram vials and taken to the laboratory, where they were sexed, pinned, labeled and identified. Specimens included 1 ♂ and 1 ♀ collected on 15 Jun 2000 and 11 ♂ and 6 ♀ collected on 18 Jun 2000. Voucher specimens were deposited in the

University of Tennessee Insect Museum, the University of California Riverside Museum, the Florida State Collection of Arthropods (Gainesville), the Museum of the Great Smoky Mountains National Park, and with the Coleoptera Taxonomic Working Group at the Louisiana State Arthropod Museum.

Only 15 species of meloids including two species of *Lytta* (*L. unguicularis* and *L. aenea* Say) are recorded in the Checklist of Coleoptera Known from Great Smoky Mountains National Park (http://www.lsuagcenter.com/Inst/research/departments/arthropodmuseum/smokieschecklist.htm, 25 Jan 2006); this database is maintained in support of the ATBI (All Taxa Biological Inventory) Project in the Great Smoky Mountains National Park. Although *L. unguicularis* is listed as previously found in the Park, no detailed source collection information was provided (Selander 1960). Thus, this research contributes to the known distribution and host records of this uncommonly collected species and may encourage researchers to learn more about the biology and life history of this little known species.

Summary

This report documents the occurrence of *L. unguicularis* on hybrid azaleas in the Great Smoky Mountains National Park, representing the first time it has been recorded in the Park since the late 1950s and early 1960s. The infrequent collections of a relatively large and conspicuous beetle in a reasonably well-known and visited area suggests that its populations may be limited. Certain other species of *Lytta* in the western U.S. have been identified as 'species of concern' by the U.S. Fish and Wildlife Service (Halstead & Haines 1992), and *L. unguicularis* in the Great Smoky Mountains National Park may represent a similar case. Additional research is necessary to more fully define the population density, dynamics, and status of *L. unguicularis* in this geographical area.

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FECUNDITY OF THE SISAL WEEVIL, *SCYPHOPHORUS ACUPUNCTATUS* (COLEOPTERA: CURCULIONIDAE), ON *POLIANTHES TUBEROSA* (LILIALES: AGAVACEAE)

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The sisal weevil *Scyphophorus acupunctatus* Gyllenhal breeds in economically important agave varieties in México, including *Agave tequilana* Weber, *Agave fourcroides* Lemaire, and *Agave salmiana* Otto ex Salm Dick. These weevils also are associated with tuberose, *Polianthes tuberosa* L. in Morelos, México, (Camino et al. 2002). Although not fully understood, mating and oviposition apparently occur in the subterranean bulbs of the plants. Larvae develop inside the bulb where they make galleries. The last instars migrate to the fibrous periphery of the bulb and construct cocoons from fiber and mud.

Camino et al. (2002) reported tuberose as a new host and outlined damage the weevil causes in cultivated *P. tuberosa*. Solís et al. (2001) mention that *S. acupunctatus* is active throughout the year with overlapping populations. This is consistent with Waring and Smith (1986), who point out that it is a multivoltine species associated with wild and cultivated agaves. Adults drill holes in the base of the plant, causing mechanical damage and facilitating the entry of microorganisms that decompose the plant tissues. Ramírez (1993) reported that the adults of *S. acupunctatus* were most frequently found between the base of the leaves and the main root of the henequen. The weevil prefers mature plants and abandoned plantations. Adults can be detected every month of the year, but are more abundant in the rainy season. The adult's favorite habitat is the inferior stratum of the agave, with oviposition occurring in moist tissues of rotten leaves or in the base of the leaves (Lock 1969). The adults are rarely found on recently planted specimens. Copulation usually occurs on the rotten shafts of plants (Lock 1969; Hill 1983). In the field larvae of *S. acupunctatus* feed on *P. tuberosa* bulbs until completing

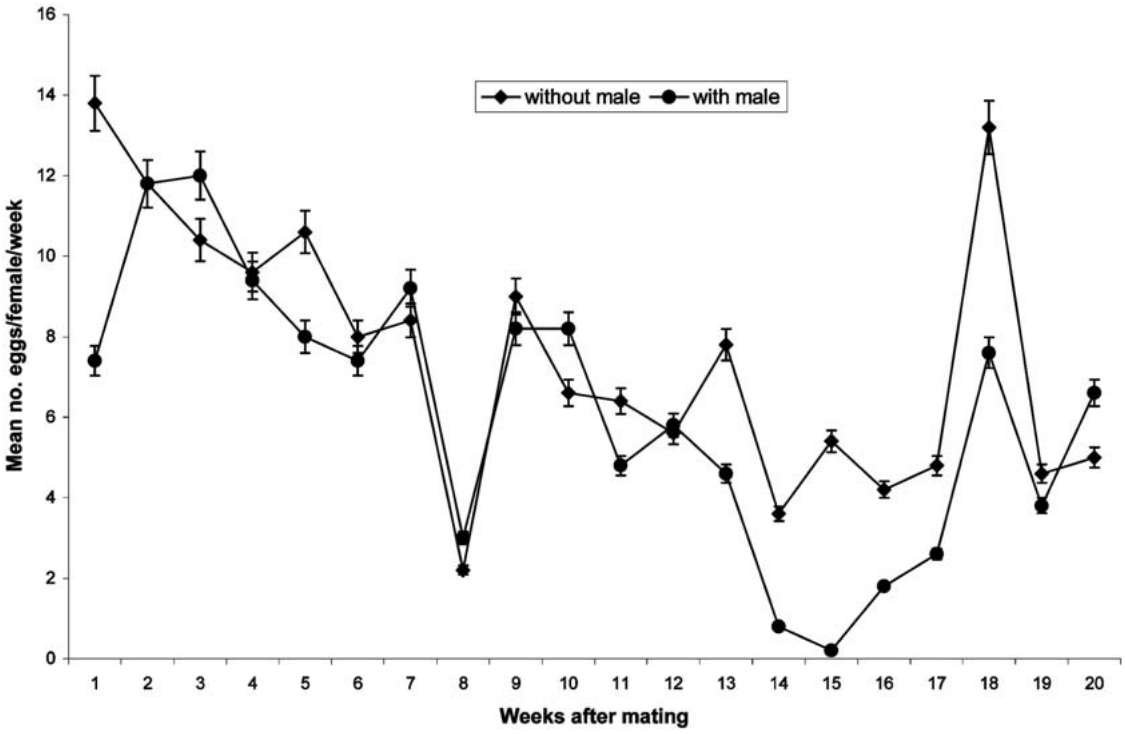

Fig. 1 Mean (±SEM) weekly egg production by newly-emerged *S. acupunctatus* female (n = 20) confined with and without males.

their larval development (pers. obs.). They pupate inside the bulb, from which the adult emerges. There are no published references about the fecundity and fertility of *S. acupunctatus*: However, we did find a report for *Rhynchophorus cruentatus* indicating that the average fecundity of the field female is of 26 ± 15 eggs (Giblin-Davis et al. 1989).

In this study we made observations on fecundity of *Scyphophorus acupunctatus* females confined with or without males, using tuberose bulbs as an ovipositional substrate. In Oct 2001, larvae and cocoons were harvested in the field from infested tuberose or in the laboratory from tuberose bulbs (*P. tuberosa*). Larvae and cocoons were placed individually in covered 100-mL plastic cups with moistened tissue paper (Giblin-Davis et al. 1989) and were stored at 29°C until adult emergence. One male and one female at 14 days post-emergence were placed in a 60-mL covered plastic container with moistened tissue and were stored at 29°C, 60% RH. One test was with confined females and males, and in a second test males were removed after 24 h and a thin slice (5-10 mm; 5-15 g wet weight) of tuberose was added. All containers were placed in an environmental chamber (Presicion, incubator 818, mod. FFU20FCACWO18, Electrolux home products, USA) at 29°C with photoperiod of 11:13 (L:D) 60% RH. Tuberose slices usually were replaced every day. The tests were repeated 4 times with 5 females per test (20 females total). Slices removed from containers were carefully dissected and eggs were removed. The tuberose bulb slices were inspected and changed daily for the duration of the experiment (Oct 2001-Feb 2002) until mortality began. During 2 tests, eggs were separated from tuberose bulb slices and placed in petri dishes (60 × 15 mm) with wet filter paper, sealed with parafilm, and stored at 29°C. Neonate larvae were inspected daily and dead ones were removed. The number of eggs and of larvae that emerged were recorded daily and were converted to eggs laid per female per week. The data were analyzed by Student-Newman-Keul means separation procedure in the Sigma Stat program. The Pearson's correlation test was applied to determine if the male's presence influenced the fecundity of females.

As an index of fecundity, the number of eggs oviposited and egg viability was recorded (Figs. 1 and 2). The two curves parallel each other, with greater oviposition and egg viability in the first

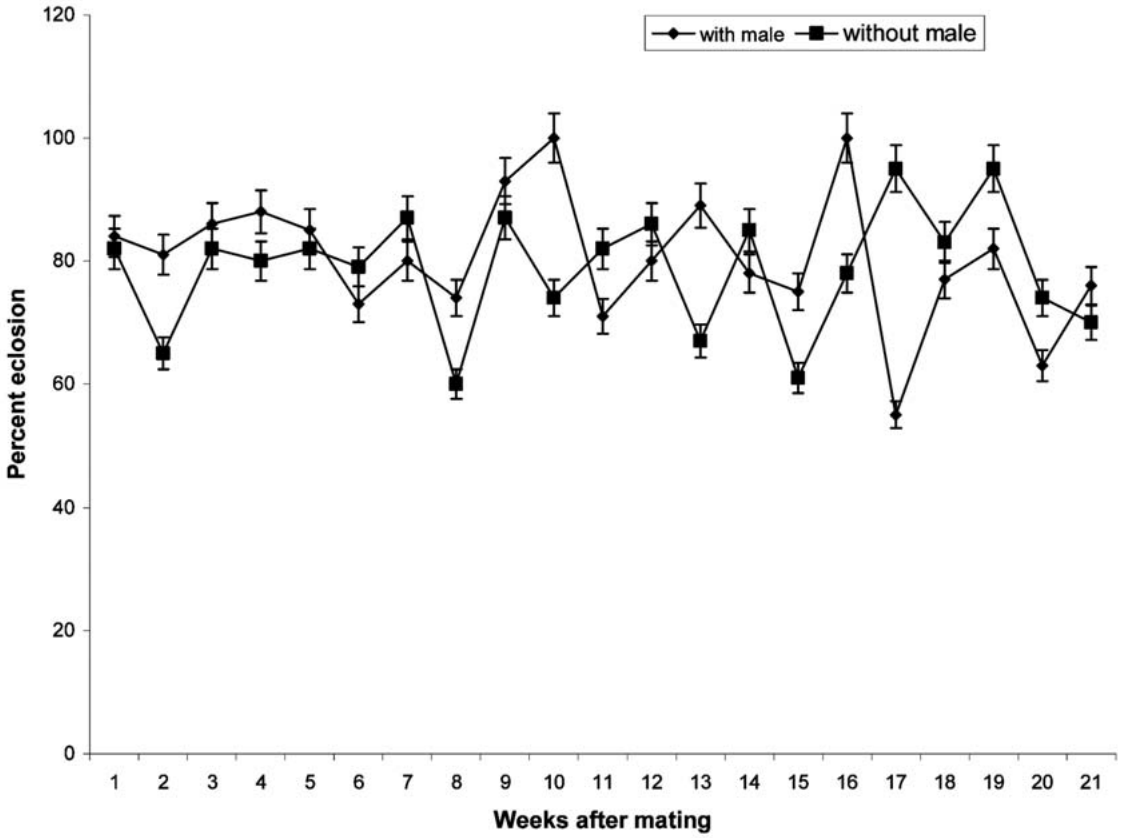

Fig. 2 Percent hatching (eclosion) of eggs produced by newly-emerged *S. acupunctatus* females (n = 20).

weeks. Fecundity was analyzed by ANOVA. We were unable to demonstrate a significant difference between the number of eggs for the group of females with males and that of females without males (P = 0.429). Nor were we able to demonstrate a significant difference between the two groups with regard to the viability of the eggs.

The effect of the different treatments doesn't depend on the time it is presented. We were not able to detect a statistically significant interaction between treatment and time (week) (P = 0.055). These results suggest that the presence of males does not significantly affect oviposition or egg viability, but these two factors are affected by the age of the adults, resulting in a general trend of diminishing number of eggs and decline in viability over time, with an intervening cyclical increase and decrease.

Figs. 1 and 2 suggest the existence of a cyclical pattern of oviposition and egg viability with a variable periodicity. This could be due to the reproductive physiology of the females or the existence of a mechanism of population self-regulation, as described by Padmanaban and Sathiamoothy (2001) for the banana tree borer *Odoiporus longicollis* Olivier. This reduces the number of eggs deposited as the borers become more frequent on their host, indicating the existence of a spacer pheromone, which may deter oviposition by females of the same species. Koppenhofer (1993) observed that females of the banana weevil, *Cosmopolites sordidus* Germar, laid an average of 2.7 eggs/week in rhizome and 0.7 eggs/week in banana pseudo stem in the laboratory, and oviposition declined in high populations. However, Gold and Messiaen (2000) found that the oviposition rate of *C. sordidus* is one egg per week. Adair et al. (1999) found that, under laboratory conditions, females of *Diaprepes abbreviatus* L., a weevil pest of citrus fruits, deposited approximately 60 masses of between 30 and 260 eggs each, with an average of 5000 eggs during their lifetime. The results suggest that females store enough sperm in their spermatheca to fertilize eggs for 20 wk, making multiple copulation unnecessary. The females may have the capacity to select the sperm to fertilize the eggs, as mentioned by (Córdoba 2000). In some cases the female is discriminatory in fertilization of her eggs, and can even avoid using the last male's sperm (Siva & Hooper 1996).

Summary

As an index of fecundity, the number of eggs deposited by females, both with and without males, and egg viability (proportion of eggs hatched) on tuberose bulbs were measured. The results showed that the presence of males does not affect the number of eggs deposited or the viability of eggs.

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GENETIC ANALYSIS OF BREEDING STRUCTURE IN LABORATORY-REARED COLONIES OF *RETICULITERMES FLAVIPES* (ISOPTERA: RHINOTERMITIDAE)

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Primary reproductives, or kings and queens, within *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) colonies suppress sexual maturation of their offspring (Lüscher 1961). In the absence of this influence, immature individuals may differentiate into replacement reproductives (neotenics) (Pickens 1932; Esenther 1969; Howard & Haverty 1980; Thorne 1996). Snyder (1920) speculated that these neotenic individuals may leave the main nesting area with a small group of workers in order to establish distinct bud nests. To evaluate whether colonies containing neotenics would establish distinct daughter or bud nests within a network of physically separated but linked food resources, we provided laboratory colonies with 3, equal-volume food resources linked by 1-m sections of tubing. Termites were permitted to forage and move among the locations. After 20 months, workers were sampled from each of the 3 resources. Microsatellite analyses were performed to determine whether subpopulations within the resources exhibited distinct genotypic frequencies.

In 1993, incipient *R. flavipes* colonies were established in the laboratory with pairs of sibling alates collected from dispersal flights in Prince George's County, Maryland, USA (Thorne et al. 1997). In 2000, 13 of these colonies were transferred to their own three-resource feeding networks (Long et al. 2006 in press). All of these colonies retained their kings; 9 "queenright" colonies also contained a queen. In 4 "queenless" colonies, the founding queen had been replaced by at least 1 neotenic female 2-6 years prior to this experiment (Long et al. 2003).

Here we present data from Colony 1, a queenright colony (for simplicity, a single, representative sample is discussed), and the 4 queenless colonies (Colonies 2-5). DNA was extracted from 60 workers per colony, with 20 workers pulled from each food resource. Preparation and analysis of DNA followed Vargo (2003). Individuals were genotyped at seven microsatellite loci: *Rs 16, Rs 33, Rs 62, Rf 1-3, Rf 5-10, Rf 15-2,* and *Rf 24-2*. Twenty-one alleles were identified (Table 1); loci contained an average of 3 alleles. Average heterozygosity was 0.54 (0.31-0.90), a value comparable with those observed in North Carolina field populations (Vargo 2000; DeHeer & Vargo 2004).

Worker genotypes in the queenright colony and 3 of the 4 queenless colonies (Colonies 1-4) were consistent with those from simple families. However, locus *Rf 24-2* in Colony 5, which contained 14 neotenic females, contained 3 alleles in 5 genotypic classes; 4 homozygous genotypes were scored at *Rs 33*. Both scenarios are possible only if at least 3 and 4 parents, respectively, contribute to the offspring. Genotype frequencies alone cannot indicate exactly how many parents contribute.

Significant deviation from expected, homogeneous genotype frequencies for each locus were evaluated by a *G*-based test of differentiation among the subpopulations and then summed for an overall estimate of significance (Genepop 2004; Raymond & Rousset 2004). Only Colony 5 showed evidence of significant differentiation in genotype frequencies among the resources ($P < 0.0001$, $df = 12$).

The non-uniform distribution of Colony 5's alleles across the three-resource network suggests that differentiation may have a spatial component, either in offspring production or preferred distribution (i.e., associations of closest kin). At 2 loci, alleles or genotypes were not observed in all resources: at *Rs 33*, alleles *259* and *267* were missing in two resources, and the genotype *196*/*106* at locus *Rf 24-2* was absent from 1 of the sites.

In Colony 5, the resource in which workers harbored 2 unique alleles also contained the king, all 14 neotenic sisters, and all of colony's eggs and instars 1-3. Travel and mark-recapture data indicate that worker exchange occurred among all 3 sites throughout the colony's tenure in the three-resource network (Long 2005). Although the co-habitation of all reproductives does not suggest nest budding in this case, genetic isolation of a subset of workers that maintain constant contact with less genetically differentiated individuals lends support to the hypothesis that physical or functional budding can occur without complete isolation from nestmates (Thorne et al. 1999).

Our results provide a rare opportunity to evaluate the response of queenless colonies to a foraging arena consisting of physically separated but linked food resources. Even after 20 months, 3 of

TABLE 1. NUMBERS OF EACH GENOTYPE FOUND AMONG *R. FLAVIPES* WORKERS SAMPLED FROM 5 COLONIES. COLONY 1 WAS QUEENRIGHT; THE OTHERS WERE HEADED BY AT LEAST 1 NEOTENIC FEMALE. TWENTY-ONE ALLELES WERE IDENTIFIED AT 7 LOCI. MISSING DATA (—) INDICATE EITHER NON-SCORABLE PCR PRODUCT FOR THAT LOCUS OR THAT THE LOCUS WAS NOT SEQUENCED FOR THAT COLONY.

	Colony				
Locus genotypes	1[a]	2	3	4	5
Rs 16					
305/305	27		60	60	38
305/295	30	30			17
295/295		29			
Rs 33					
259/259	39	16	60	60	4
267/259		32			5
255/255					21
263/255					17
263/263					7
267/267	18	10			1
Rs 62					
315/315	57	60	21	19	55
319/319			9	9	
319/315			29	32	
Rf 1-3	—				
236/221			16		22
224/224			17		
224/221			16		
236/224			8		
224/218				4	
236/218					7
221/218		25			13
221/221					17
218/218		33			
245/245				2	
245/224				8	
245/218				7	
Rf 5-10	—				
153/153		59	60	31	24
153/147				25	33
Rf 15-2					
235/232		31	30	36	23
235/235	57		29	24	22
232/232		29			10
Rf 24-2					
106/106	12		30	1	10
196/106		31	30		6
169/106	12			15	20
169/169	12			27	6
196/169					16
199/106	21				
196/196		28			
199/169				16	

[a]Sixty workers were examined from each colony. Failure of individual samples to yield readable data account for discrepancies between these totals and the number of genotypes presented.

the 4 queenless colonies were genetically homogeneous. The genotypes sampled from the fourth queenless colony, which contained 14 female neotenics, indicate that genetic differentiation had begun to develop among the resources.

SUMMARY

Thirteen laboratory-reared *R. flavipes* colonies were housed in 3-resource foraging arenas for 20 months. Four of these colonies were queenless, having lost their founding queen 2-6 years prior. Microsatellite analysis performed on workers sampled from each resource allowed each colony to be classified as either a simple or an extended family and to examine the queenless colonies for evidence of genetic differentiation among the 3 linked feeding resources.

F-statistics (Wright 1921) and relatedness coefficient (*b*) (Pamilo 1984) were generated with Genetic Data Analysis software (Lewis & Zaykin 2001) with notational conventions of Thorne et al. (1999) and Bulmer et al. (2001). Among the 4 queenless colonies, F_{IT} = 0.52 (*c.i.* 0.37-0.65), F_{CT} = 0.59 (*c.i.* 0.48-0.69), F_{IC} = -0.17 (*c.i.* -0.27-0.08), and b = 0.78. These results are not significantly different from values predicted for an inbred colony with 2 female neotenics and a single male (Thorne et al. 1999). F_{IT} = 0.52 and b = 0.78 indicate marked inbreeding in this laboratory population. The founding of these colonies by probable siblings undoubtedly accounted for a portion of this observed loss of heterozygosity, but regional variation in levels of inbreeding may have also contributed (Reilly 1987; Bulmer et al. 2001; Vargo 2003). F_{IC} = -0.17 suggests an intermediate loss of heterozygosity within each colony, but not existence of differentiated bud nests. F_{CT} = 0.59 in this laboratory population indicates relatively high contrast between colonies.

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EFFICIENCY OF *HETERORHABDITIS BACTERIOPHORA* (NEMATODA: HETERORHABDITIDAE) ON *ANASTREPHA SERPENTINA* (DIPTERA: TEPHRITIDAE) LARVAE UNDER LABORATORY CONDITIONS

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The sapote fruit fly, *Anastrepha serpentina* (Wiedemann), sometimes called the tropical fruit fly, is an important species in Mexico because its larvae infest sapote (*Calocarpum* spp.), mammee [*Pouteria sapota* (Jacq.) Moore & Stearn], sapodilla (*Achras zapota* L.), willowleaf lucuma (*Lucuma salicifolia* Hbk.) and related fruits (Aluja 1994). Infestations in tree-ripe fruits frequently are so high that in parts of the country where these fruits are grown, especially in Veracruz, the growers do not allow them to mature on the trees, but pick them green and ripen them artificially to avoid infestation. Mammee tree is native to Central America and southern Mexico and it is becoming important as an exotic fruit in international commerce. For this reason, the sapote fruit fly is part of the pest management program of the National Campaign Against Fruit Flies (CNCMF, after its Spanish acronym) (Reyes et al. 2000). Unfortunately, its control is mostly based on the use of chemical insecticides, applied either on the foliage to control adults or on the soil to control larvae or newly emerged adults. Consequently, new control alternatives are being explored, such as natural products and biological control agents, which may at least partially substitute for the chemical insecticides. This is an important strategy due to the growing interest in organic agriculture.

The entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar) is a natural soil dweller that parasitizes a number of insect species. Infection occurs through the insect's natural apertures such as the mouth, spiracles, or anus (Woodring & Kaya 1988). Once in the host hemocoel, the nematode releases its symbiotic bacterium *Photorhabdus* spp., which causes a rapid and lethal septicemia. This allows the growth and reproduction of the nematode for one or more generations. Due to its lethal efficiency, *H. bacteriophora* may become an important regulation factor for several insect populations whose larvae co-exist within the soil. This includes several species of fruit fly larvae (Tephritidae) whose susceptibility to nematode infection has been demonstrated previously (Beavers & Calkins 1984; Lindegren & Vail 1986; Lindegren et al. 1990; Lezama-Gutiérrez et al. 1996; Gazit et al. 2000; Toledo et al. 2001, 2005, 2006). In this report we present evidence on the infectivity of *H. bacteriophora* to third instars of *A. serpentina* under laboratory conditions.

Sapote fruit fly larvae were obtained from the mass rearing facility at Moscafrut Plant (SAGARPA-IICA), located in Metapa de Domínguez, Chiapas, México. They were reared on artificial diet, following the procedure and conditions described by Domínguez et al. (2000). The nematode was originally collected in Costa Rica with wax moth (*Galleria mellonella* L.) soil traps from a warm, rainy region, described by Castillo & Marbán-Mendoza (1996). The nematode was reared by infecting wax moth larvae, and infective juveniles (IJ) were collected in White traps (Woodring & Kaya 1988). IJs were quantified and working concentrations were adjusted to 800 IJ/mL in sterile, distilled water. Suspensions were stored at 10 ± 2°C until further use (Woodring & Kaya 1988).

Bioassays were performed on late, mature third instars of the sapote fruit fly with infection units made from PVC pipes 5 cm long and 5 cm in diameter (19.63 cm^2 surface). Each unit was filled with 70 g of sandy soil (96% sand, 3% clay, 1% lime, 0.18% organic matter, and adjusted to 6.6 pH), previously sieved (mesh 18), autoclaved, and adjusted to 15% mixture (weight/volume). A total of 25 larvae was added to each unit. Larvae immediately crawled into the soil (<10 min). The nematode IJ concentrations tested were 0, 6, 13, 25, 51, 76, 102, 127, and 178 IJ/cm^2 soil, added in 1 mL suspension and uniformly distributed on the soil surface. Infection units were incubated at 26 ± 1°C, 70 ± 5% RH, and L12:D12 photoperiod for 7 d. After this period, soil was sieved to separate larvae and pupae, and mortality was quantified under a dissecting microscope to verify nematode infection. To estimate an LC_{50}, a total of five replicates was performed and data were subjected to Probit analysis (SAS Institute 1992), in which statistical requirements were fulfilled as described by Ibarra & Federici 1987.

Once an LC_{50} was estimated, a simple test on the dispersion of mortality was performed by testing the LC_{50} and three times the LC_{50}, under the

same bioassay conditions. A total of five replicates was carried out and statistical difference was analyzed by Student's *t* test (Steel & Torrie 1993).

The LC_{50} of *H. bacteriophora* infective juveniles tested on late third instars of the sapote fruit fly was estimated at 36.0 ± 5.4 IJ/cm^2 (n = 491; $\chi^2 = 3.6$; Y = 3.00 + 1.28 X), within highly precise fiducial limits (26.7-46.4). The LC_{95} was estimated at 686 IJ/cm^2. The negative control never showed infection and the natural mortality was always around 2%, with >90% adult emergence. In the dispersion of mortality test, although mortality caused by the LC_{50} and three times the LC_{50} (108 IJ/cm^2) showed a statistically significant difference (t = -3.5; df = 4; P = 0.001), actual mortality barely increased, ranging only from 42.4 ± 2.0% at LC_{50} to 54.5 ± 2.7% at three times the LC_{50}. These results indicate that, in spite of the low number of IJs required to kill 50% of the larval population, a much larger number of nematodes is required to kill a significant proportion of the insect population. According to these results, approximately 700 IJ/cm^2 are necessary to obtain significant control levels, which is close to the estimated LC_{95}.

This is the first report on the susceptibility of sapote fruit fly larvae to *H. bacteriophora* under laboratory conditions. Based on our finding this nematode can be considered a potential biological control agent for this pest, and the results should be corroborated under field conditions. The test was conducted on third instars because it is the only larval stage that may be in contact with the soil under natural conditions. The highest tolerance to nematode infection occurs in the 3rd instar, as observed in other *Anastrepha* species (Toledo et al. 2005). However, the invasive ability of the nematode varies not only among the different species but also between strains of the same species, as observed when different species and strains of nematodes were tested against *A. suspensa* (Beavers & Calkins 1984).

Laboratory tests on the infectivity of nematodes are important because they are performed under controlled, optimum conditions. The interaction host/parasite is tested without the influence of other factors that may be found in the field. It is known that *H. bacteriophora* moves easily in the soil, showing a high ability to find hosts at different soil depths (Campbell et al. 1996). However, its performance can be severely hampered by some soil factors such as texture, pH, humidity, and possibly other factors. In general, these factors influence the failure or success of these control agents when tested under field conditions (Portillo-Aguilar et al. 1999). Fruit fly larvae also are influenced by these factors (Eskafi & Fernández 1990; Jackson et al. 1998; Alyokhin et al. 2001), and especially the soil compactness (Aluja 1994), which can influence nematode infectivity (Portillo-Aguilar et al. 1999).

Moisture is another important factor. IJs of *H. bacteriophora* are more infective in sandy-clay soils with 15% moisture (Toledo et al. 2006), while in sandy soils the optimum is at 10% moisture (Toledo et al. 2005). Slightly higher or lower moisture levels drastically decrease its infective efficiency. High moisture content in a sandy soil may slow down the IJ's movement, due to an excess of water between the particles, while a low moisture content may limit the search for hosts. The effect of humidity may vary among the nematode species. The nematode *Steinernema riobrave* kept its infectivity to the Mediterranean fruit fly larvae in a sandy-clay soil at humidity levels ranging from 3 to 20% (Gazit et al. 2000).

Heterorhabditis bacteriophora has shown its potential as a biological control agent in the field, against other fruit fly larvae (Toledo et al. 2006). A field test on the sapote fruit fly is feasible, and should be followed by an analysis of economical and practical viability.

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Summary

The infectivity of the entomopathogenic nematode *Heterorhabditis bacteriophora* was tested on third instars of the tropical fruit fly, *Anastrepha serpentina*, under laboratory conditions. An LC_{50} was estimated at 36.0 ± 5.4 IJ /cm^2 of sandy soil, adjusted to 15% humidity, with 5-cm-deep infectivity units. Significant amounts of nematodes are required to obtain satisfactory control levels, as shown by 3× the LC_{50} value. This is the first report on the susceptibility of the tropical fruit fly larvae to *H. bacteriophora*. Potential of this nematode as a biological control agent of this pest should be corroborated under field conditions.

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SURVEY FOR POTENTIAL PREDATORS OF THE ELONGATE HEMLOCK SCALE IN TENNESSEE AND NORTH CAROLINA

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The elongate hemlock scale, *Fiorinia externa* Ferris (Hemiptera: Diaspididae) (EHS), is an invasive insect from Japan (Takagi 1963) that feeds on the needles of eastern hemlock, *Tsuga canadensis* (L.) Carriere. This diaspidid often co-exists with the exotic hemlock woolly adelgid, *Adelges tsugae* Annand (Hemiptera: Adelgidae), throughout the northern United States (McClure 2002), where they cause extensive damage to eastern hemlock and threaten to disrupt forest composition. EHS invaded eastern hemlocks in the southern Appalachian range and has become well established in western North Carolina and eastern Tennessee. Heavily-infested trees were discovered in Mar 2004 in two urban areas located at Tyson Park and Lynnhurst Cemetery, Knoxville, TN (Buck et al. 2005; Lambdin et al. 2005), where little is known about the natural enemies of this introduced pest. Several species of predators including *Atractotomus magnicornis buenoi* (Mercet) and *Phytocorus* sp. (Heteroptera: Miridae), *Conwentzia pineticola* Enderlin (Neuroptera: Coniopterygidae), and *Chilocorus stigma* (Say) and *C. kuwanae* Silvestri (Coleoptera: Coccinellidae) have been recorded to feed on EHS in other areas of the United States (Davidson & McComb 1958; McClure 1977). In addition, the parasitoid *Encarsia citrina* (Craw) (Hymenoptera: Aphelinidae) has been reported to be an important mortality factor of EHS (McClure 1978, 1979, 2002). To better understand the population dynamics of EHS, a survey was designed to identify its potential predators in eastern Tennessee and western North Carolina.

Populations of potential predators were sampled with beat sheets and branch extractions of eastern hemlock at 2 urban and 2 forest sites. Urban sites were located at Lynnhurst Cemetery (Knoxville, TN) and at Biltmore Estate (Asheville, NC). Forest sites were located at Bays Mountain Park (Kingsport, TN) and at Biltmore Estate (Asheville, NC). All sites were sampled from Sep 15, 2004 to Apr 28, 2006 except at the Bays Mountain Park site, which was sampled from Aug 29, 2005 to Apr 10, 2006. Each urban or forest site was arranged into 5 blocks with 3 trees sampled monthly per block for predators.

Predators were collected with a beat sheet (75 cm × 75 cm) by striking one branch 3 times from each of the 4 cardinal directions per tree. The beat sheet was scanned for predators, and when discovered, they were placed into a glass vial (6 dram), labeled (date, site, block, tree, and direction) and transported to the laboratory for processing. Data (date, site, tree number, species, number of specimens per species, location and developmental stage of the predators collected) recorded from the 4,380 beat sheet samples were subjected to Kruskal-Wallis Test with SPSS 14.0 for Windows. We extracted 2 branch samples (30 cm) from each of 5 trees per block, and placed them into separate, labeled "Ziploc" bags to observe predators and the impact of their feeding upon the scale insect in the laboratory. Data recorded included the date of collection, site, number of EHS from 100 needles per sample, and the number and location of EHS damaged by predators. Predator damage (defined as any injury or mutilation to the scale test or body) was determined from EHS specimens taken from 3,600 branch samples throughout the study period.

Six predaceous species (*C. stigma*, *Conwentzia* nr. *pineticola* Enderlein, *Harmonia axyridis* Pallas, *Rhyzobius lophanthae* (Blaisdell), *Scymnillus horni* (Gordon), and *Scymnus loweii* Mulsant) were collected and identified from EHS-infested eastern hemlock. A total of 504 specimens consisting of 347 adults and 157 larvae was obtained from beat sheet sampling. Experiments are underway to assess the ability of these field-collected predators to feed on EHS. The only other scale insect species encountered was the native hemlock scale, *Abgrallaspis ithacae* (Ferris) (Hemiptera: Diaspididae), comprising less than 0.02% of the scale insect fauna at the sites. Two of the coccinellid species (*S. horni* and *R. lophanthae*) represent new state records for Tennessee, while collections of *S. loweii* represent new county records for eastern Tennessee. Except for *S. horni*, the remaining predators were previously collected and identified in North Carolina (Kathleen Kidd, personal comm.). The native species *S. horni* is common to the forests of the eastern United States (Robert Gordon, personal comm.). The coccinellids *C. stigma*, *R. lophanthae*, and *S. horni* are primary predators of scale insects, while *S. loweii*, *H. axyridis*, and *Coniopterix* sp. appear to be more generalist predators feeding primarily on aphids, scale insects, and mites.

Lowest numbers of specimens were collected for the species *S. loweii*, *Conwentzia* sp., and

H. axyridis. Percent damage to EHS field-collected samples was 9.8% for Biltmore Estate urban, 9.7% for Biltmore Estate forest, 6.2% for Lynnhurst Cemetery urban, and 4.7% for Bays Mountain Park forest sites, respectively. Although predators were found at urban and forest sites, the number of species and species combinations differed per site.

More predator specimens for each species were found in urban areas (n = 55, df = 3, $H < 0.05$) with *R. lophanthae*, *C. stigma*, and *S. horni* comprising the dominant species. *Scymnillus horni* was the only species found at the Bays Mountain Park site, and *S. loweii* was only found at the Lynnhurst Cemetery site. Although more specimens of *S. horni* were found on the south side of the tree and higher numbers of *R. lophanthae* were found on the north side of the tree, no differences (n = 80, df = 3, $H > 0.05$) for direction were noted for direction preference among the species.

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Summary

Six predators from EHS-infested eastern hemlocks were collected in eastern Tennessee and western North Carolina. *Rhyzobius lophanthae* and *S. horni* are known to feed on scale insects as their primary food source, and both represent new state records for Tennessee. The most dominant species collected were *S. horni*, *R. lophanthae*, and *C. stigma*, respectively. The natural predator abundance does appear sufficient to significantly reduce the heavy populations of EHS now present on eastern hemlocks within the region. However, augmentation of their numbers along with the use of the parasitoid *E. citrina* or other more host specific parasitoids offer the potential of suppressing pest populations in both forests and urban landscapes.

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GEOGRAPHIC RANGE EXPANSION OF *BOREIOGLYCASPIS MELALEUCAE* (HEMIPTERA: PSYLLIDAE) TO PUERTO RICO

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The Australian tree *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae) was introduced into South Florida (U.S.) by horticulturists during the late 1800s (Dray 2003). Nearly 100 years later, *M. quinquenervia* was widely recognized as a pernicious invader of wetland systems in the Florida Everglades (Browder & Schroeder 1981; Woodall 1981, 1982), due in part to the tree's competitive superiority over most native vegetation (Turner et al. 1998). Current estimates of geographic distribution suggest that the invasive tree now occupies approximately 200,000 ha of graminoid/herbaceous wetlands, including portions of the Everglades National Park (Turner et al. 1998).

A classical weed biological control program targeting *M. quinquenervia* was initiated in 1986, with expectations that introduced herbivores would limit invasion and complement conventional control tactics (Balciunas et al. 1994). The curculionid weevil *Oxyops vitiosa* Pascoe (Coleoptera: Curculionidae) was the first candidate selected for quarantine-based host specificity testing (Purcell & Balciunas 1994) and, once deemed environmentally safe, was released in South Florida during 1997 (Center et al. 2000; Pratt et al. 2003).

The second herbivore introduced for biological control of *M. quinquenervia* in Florida was the melaleuca psyllid, *Boreioglycaspis melaleucae* Moore. Host range studies demonstrated that the insect completes its development only on a small group of species in the *Melaleuca* genus (Wineriter et al. 2003), of which there are no native representatives in the New World. Based on this narrow host range, the psyllid was permitted for release in South Florida during the spring of 2002 (Pratt et al. 2004). Both adults and nymphs feed on expanding buds and leaves but nymphs also exploit mature, fully expanded leaves as competition for preferred feeding sites increases. Initial field data indicate that feeding by psyllids induces leaf senescence, eventually resulting in mortality of coppicing stumps and seedlings (Morath et al. 2006; Franks et al. 2006). Psyllids also rapidly disperse from release points, spreading on average 4.7 km/yr but ranging as high as 10 km/yr (P. D. Pratt, unpublished data). Following establishment, common garden experiments confirmed that feeding and development by the melaleuca psyllid was restricted to *Melaleuca* species, as predicted in quarantine-based host range testing, and so it posed no threat to native or economically important species (P. D. Pratt unpublished data). In response to observed impacts of the psyllid, federal, state, and county agencies initiated a redistribution campaign for *B. melaleucae* in 2003. Over 1 million individuals have been redistributed to nearly 100 locations in South Florida since 2002.

In addition to its occurrence in Florida, *M. quinquenervia* has been planted throughout much of the Caribbean (Serbesoff-King 2003). In Puerto Rico, for instance, it was planted islandwide in public parks, promenades, and along certain highway medians and green areas from the 1970-90s (Angleró 1960; Pratt et al. 2005). Not surprisingly, the extensive use of *M. quinquenervia* as an ornamental in Puerto Rico enabled it to naturalize in ecologically sensitive wetlands, including the Tortuguero Lagoon Natural Reserve (Pratt et al. 2005). The implementation of chemical controls for invasive populations of the tree on the island is currently underway. The use of biological controls, which have been very effective in Florida, were considered less suitable for Puerto Rico due to the small size of the infested areas and possible conflicts of interest. Conservationists in Puerto Rico are interested in halting continued invasion of the tree in wetlands, although public policy as to how to address ornamentally planted trees has yet to be determined. More importantly, the biological control agents approved for introduction into Florida have not been evaluated as to their propensity to oviposit and develop on Caribbean species of Myrtaceae. Liogier (1994) cites 30 species in the family Myrtaceae that are native to the island of Puerto Rico and these were not included in initial host testing for the biological control agents described above. Pratt et al. (2005) indicated that additional representatives from the Puerto Rican Myrtaceae and closely related economically important flora must be tested as possible hosts prior to introducing the natural enemies.

In Jan 2006, however, the psyllid *B. melaleucae* was observed on leaves of *M. quinquenervia* trees growing near the San Juan Airport, Puerto Rico. A survey of the island was conducted in Apr 2006 to determine the geographical distribution of *B. melaleucae* on the island. This was accomplished by traveling E, W, and S on primary roads while stopping every 10-20 km to search for

M. quinquenervia trees. Once encountered, trees were examined by 3 observers for 15 min each to detect psyllid presence and estimate feeding damage and proportion of trees infested. Feeding damage was assessed on a 5-point scale based on a visual estimation of percentage of the suitable foliage destroyed by psyllid feeding as follows: 0 = no damage; 1 = <25% destroyed; 2 = 26 to 50%; 3 = 51 to 75%; 4 = 76 to 100% destroyed.

Identification of *B. melaleucae* was confirmed by Susan Halbert (Florida Department of Agriculture and Consumer Services) and voucher specimens were deposited in the Florida State Collection of Arthropods (E2006-2142-201). Surveys indicated that *B. melaleucae* was distributed widely on the island, except for the west coast where no psyllids were found on *M. quinquenervia* trees near Aguadilla and Cabo Rojo (Fig. 1). Damage was greatest (level 3) near the San Juan Airport and Rio Piedras but decreased with increasing distance from the greater San Juan area (ANOVA $df = 3, 14; F = 3.71; P = 0.0460$). The proportion of trees infested exhibited a similar trend, with fewer trees per site harboring psyllids as the distance from San Juan increased (linear regression $df = 1, 14; F = 1.94; P = 0.0742$).

The discovery of *B. melaleucae* in Puerto Rico raises several questions regarding pathways of introduction. First, where was the point of introduction on the island? If we assume that increased damage and infestation levels are positively correlated with time, then *B. melaleucae* was likely established in the greater San Juan area prior to other locations. The subsequent dispersal and its current distribution underscores the long range host-finding abilities of *B. melaleucae* under highly fragmented populations of its host. The psyllid had successfully located isolated *M. quinquenervia* trees, for instance, <30 m from the ocean (Arecibo) as well as within canopies of 3 trees growing at 800 m elevation.

Florida, as compared to Australia, is the most logical origin of the Puerto Rican psyllid population based on proximity and frequency of transportation. On-going genetic analyses may help elucidate the country of origin for the Puerto Rican populations. Considering the widespread occurrence of *M. quinquenervia* among the Caribbean islands, one introduction pathway may include unassisted inter-island dispersal from Florida, through the Bahamas or Greater Antilles to Puerto Rico. Hurricanes may facilitate the long range dispersal of insects through the Caribbean (Drake & Farrow 1988). This line of reasoning, however, is not supported by recent surveys of *M. quinquenervia* in the northern Bahamian islands (Grand Bahama, New Providence, and Andros) where *M. quinquenervia* is abundant but where *B. melaleucae* was not detected despite a recent hurricane (Hurricane Wilma, Oct. 2005) that crossed South Florida prior to making landfall on Grand Bahama (Pratt unpublished data). San Juan lies approximately 1660 km southeast of Miami whereas New Providence is about 300 km east and Grand Bahama is only about 130 km northeast. Thus, if the psyllid were dispersing on its own or through the agency of hurricanes, it should reach the more proximate Bahama Islands first. A more probable explanation is that *B. melaleucae* was introduced, either accidentally or intentionally, to Puerto Rico. Human activities play an important role in accidental insect invasions, with the most common introduction pathways including international transportation of airplane luggage and cargo (Kiritani & Yamamura 2003). Considering the frequent transport of tourists and cargo between South Florida and Puerto Rico, the premise that *B. melaleuca* was inadvertently carried or "hitchhiked" to the island remains a plausible explanation. Of greater concern, however, is the possibility that the *B. melaleucae* may have been intentionally smug-

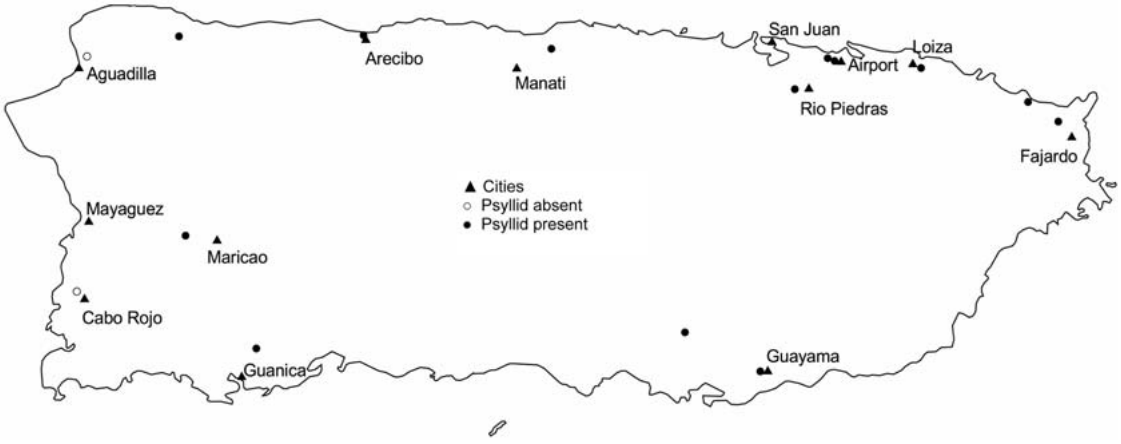

Fig. 1. Geographical distribution of *B. melaleucae* in Puerto Rico.

gled into Puerto Rico to aid in control efforts of *M. quinquenervia*. While the invasion pathway of *B. melaleucae* remains uncertain, the occurrence of the melaleuca psyllid in Puerto Rico draws attention to the potential for movement of biological control agents far beyond their intended range. For this reason, biological control programs must consider risks to the flora of neighboring regions, especially if these regions harbor populations of the target.

Both host range testing and post release field studies indicate that development of *B. melaleuca* is restricted to *M. quinquenervia* and closely related congeners, and is therefore unlikely to pose a threat to the flora of Puerto Rico. However, host specificity studies were based on the flora and particularly Myrtaceae of Florida, which is less diverse than that of Puerto Rico. Seven genera in the family Myrtaceae, for instance, were not tested as possible hosts during quarantine testing. Additional laboratory and field monitoring of *Gomidesia*, *Marlierea*, *Myrcia*, *Myrciaria*, *Myrtus*, and *Siphoneugenia* would provide additional information as to the potential host range in Puerto Rico.

Summary

The Australian psyllid *Boreioglycaspis melaleucae* is a specialized herbivore of *Melaleuca quinquenervia* and other closely related congeners. *Boreioglycaspis melaleucae* was discovered in Puerto Rico feeding on naturalized and ornamentally planted *M. quinquenervia* trees. The psyllid is widely distributed on the island except for the western coast. It is unlikely to harm native plant species but will impact ornamental landscape plantings of *M. quinquenervia*.

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CONTROL OF PEST MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE) IN BAHIAGRASS PASTURES WITH THE NEMATODE *STEINERNEMA SCAPTERISCI* (RHABDITIDA: STEINERNEMATIDAE)

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Mole crickets, *Scapteriscus* spp., cause nearly $100 million annual losses in revenue to cattle producers in south-central Florida, with about 50% of that loss due to reduced forage and hay production and 50% due to need for pasture renovation (Adjei et al. 2003). Mole cricket damage to pasture and turf grasses principally is caused by the tawny mole cricket *S. vicinus* Scudder feeding on roots, and by shallow tunneling (galleries) by both *S. vicinus* and the southern mole cricket, *S. borellii* Giglio-Tos (Walker & Ngo 1982; Hudson 1985). Damage first appears as yellow patches of grass that later turn brown and die. In areas of high population densities of mole crickets, the surface soil layer is honeycombed with numerous galleries and the ground feels spongy when stepped on. Heavily damaged bahiagrass (*Paspalum notatum* Fluegge) has virtually no root system and plants are easily pulled from the soil by cattle as they graze or walk. Treatment of a large cattle pasture in Florida to control mole crickets is prohibitively expensive and impractical. No insecticide is registered by EPA for mole cricket control in pastures. Consequently, we wanted to see if natural dispersal of mole crickets infected with the entomopathogenic nematode *Steinernema scapterisci* Nguyen & Smart (1990) would reduce the population of mole crickets and ameliorate the damage caused to a bahiagrass pasture. The nematode functions as a biopesticide (Leppla et al. 2004) that kills and reproduces in *Scapteriscus* mole crickets. From each mole cricket cadaver, some 50,000 infective juveniles enter the soil, usually establishing a population and functioning as a classical biological control agent (Hudson et al. 1988). Our experiment was designed to determine whether applying nematodes in strips that covered 12.5%, 25%, or 50% of a plot would result in controlling mole crickets in a larger area through natural dispersal of nematodes by mole crickets.

We collected pre-treatment data on pasture condition and on mole cricket populations by installing 6 linear pitfall traps (Lawrence 1982) in Jun 1997 on a 10-ha bahiagrass pasture in south-central Florida (A.D. Combee Ranch, Polk County, FL). The soil in the area is mostly EauGallie fine sand (USDA, 1990). The total number of mole crickets, *S. vicinus* and *S. borelli*, captured in each trap was counted once every week from Jul 1997 through Aug 2000 before any nematodes were released in the pasture. Traps were emptied, cleaned, and reset after each weekly collection. The total numbers of mole crickets trapped in the 3 years before the nematode was applied were 3456 in 1997 (Jul-Dec), 5112 in 1998, and 5347 in 1999, indicating a heavy and damaging population. Mole crickets caught in the traps were examined in Jun, Jul, and Aug, 2000, in order to determine whether any were already infected with *S. scapterisci*. None of 666 mole crickets trapped and examined was infected with the nematode, and we concluded that the nematode was not present.

The condition of the bahiagrass over the 10-ha pasture was evaluated in May 1997, 1998, 1999, and 2000 as the percentage of the pasture covered by (1) green bahiagrass and (2) yellow and dead bahiagrass, weeds, and bare ground by using a 1-m^2 quadrat subdivided into 100 small squares (Adjei et al. 2003). The quadrat was tossed randomly to 5 locations on the pasture.

On 7 Sep 2000, we established 3 blocks of plots, with each block containing 4 subplots each 41.3 m $\times$ 97.3 m, arranged near each other in a rectangular pattern (Fig. 1). Three of the plots in each block were subdivided into 16 strips, each 6.08 m wide and 41.3 m long. In 1 plot 8 alternate strips (50% of plot area) were treated with the infective juvenile stage of *S. scapterisci* in a water suspension by injecting the nematodes into the soil about 1.5 cm with a modified slit seeder. In another plot, only 4 strips (25% of plot area) received nematodes, and in the third plot only 2 strips (12.5% of plot area) received nematodes (Fig. 1). The fourth plot in each block served as a control and received no nematodes. Nematode dosages were based on overall coverage with 2.5 billion infective juveniles per hectare in the equivalent of 935 L water per ha. The injection slits were closed with press wheels mounted behind the seeder.

Before treatment, 6 pitfall traps were installed in each plot, 3 in nematode-treated strips and 3 in untreated strips in the treated plots. For the untreated controls, 6 traps in each plot were arranged similarly to those in the treated plots (Fig. 1). The total number of mole crickets captured in each trap was counted weekly from Sep 2000 through Dec 2002. Traps were emptied, cleaned, and reset after each weekly collection. Periodic assessment of nematode infection of trapped mole crickets was done twice each month from Oct 2000 through Jun 2002, and from Oct

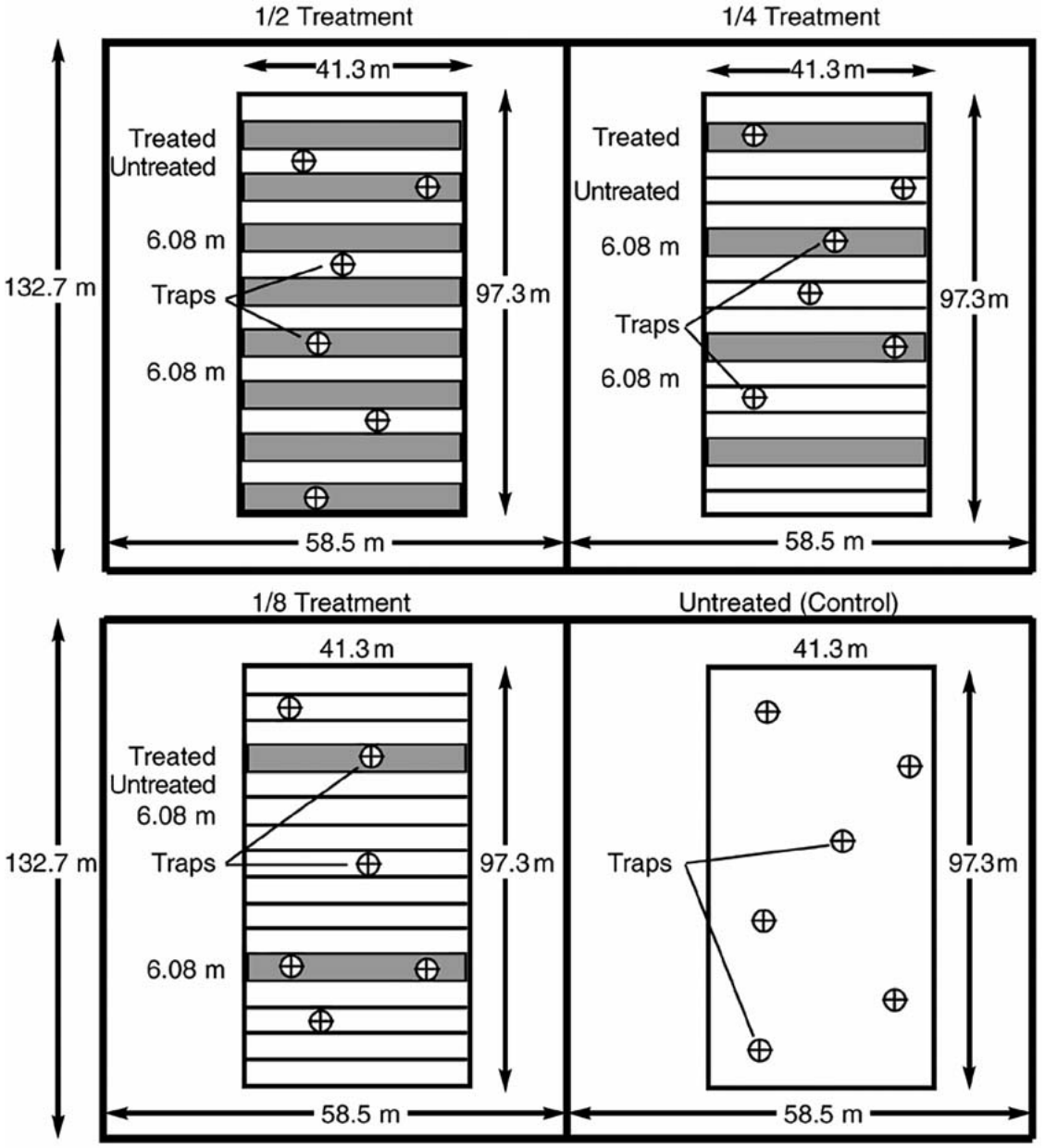

Fig. 1. One of 3 blocks (replicates) with 3 treated plots and a control plot. Treatment plots were subdivided into 16 strips, each 6.08 m wide, as indicated by the shaded and unshaded areas in the diagram. *Steinernema scapterisci* were applied to 8, 4, or 2 strips per plot to provide coverage of 50%, 25%, or 12.5% of the plot area, respectively. The control plot received no nematodes. Each of the 4 plots in a block had 6 pitfall traps to capture mole crickets. Captured crickets were held to determine if nematodes emerged from the dead crickets. The 3 blocks covered a 10-ha pasture.

2002 through Mar 2003. Trapped adult mole crickets were placed individually in vials and kept for 14 d to await possible emergence of infective juveniles from the cadavers. Emerging nematodes were identified as *S. scapterisci* by methods described by Woodring & Kaya (1988). Only adults were examined because the adults are more susceptible to infection than nymphs (Hudson & Nguyen 1989). The condition of the bahiagrass was evaluated in May 2001, 2002, and 2003 as for the pretreatment evaluation with the quadrat tossed randomly to 5 locations on each plot.

The weekly captures of mole crickets and monthly numbers of adults infected with nema-

todes were analyzed statistically by the GLM procedure (SAS 1999), with treatment as main plot, treated vs. non-treated strips as subplot, and year and week/month as sub-subplot and sub-sub-subplot in time, respectively. Following a significant *F*-test in the analysis of variance, treatment means were separated by Tukey's Studentized Range Test. Average plot ratings were analyzed statistically as a split plot experiment, with treatment as main plot, and year as split plot in time. Sources of variation examined in the statistical analysis of variance included replications, treatment, treatment × replication (error a) year, year × treatment and residual (or error b) effects.

After *S. scapterisci* was applied in Sep 2000, the total numbers of mole crickets trapped in 2000, 2001, and 2002 were 3,251, 3,326, and 676, respectively. The number trapped in 2002 was 79.2% less than the number trapped in 2000. In 2002, in plots with 12.5%, 25%, and 50% of the area treated, the average percentage of infected mole crickets was 89%, 84%, and 86%, respectively. This indicated that treating 12.5% of the area was as effective as treating 50% of the area, thus, potentially saving 75% of the purchase of the nematodes and application cost. Within treated plots, no differences ($P > 0.60$) between treated strips vs. non-treated strips were observed on any sampling date, and strips had no interaction with the other variables**.** The percentage of infected mole crickets in the control plots was significantly less than in all treatment plots in Apr 2001, but thereafter, with the exception of May and Jun 2002, there were no significant differences between treated and control plots because an average of 41% of the crickets trapped in control plots were infected with *S. scapterisci*. It is highly probable that movement of infected mole crickets from the treated plots into the control plots occurred because the control plots were very close to the treated plots (approximately 18 m from a treated plot). March and April are the months of maximum activity by adult mole crickets in central Florida, and most likely it was during these months in 2001 that infected mole crickets dispersed to both untreated strips in the treated plots, and to the control plots.

At the beginning of the study in 1997, the pasture showed classic symptoms of damage by mole crickets (Walker & Ngo 1982; Hudson 1985). Patches of yellow and dead grass, bare ground, and weed growth covered 58% of the area in 1997, 61% in 1998, 65% in 1999, and 67% in 2000. After *S. scapterisci* was applied, the damaged area declined to 51% in 2001, 18% in 2002, and 4% in 2003, and concomitantly, the bahiagrass coverage increased to 49% in 2001, 82% in 2002, and 96% in 2003 (Fig. 2). We conclude that strip application of the nematodes is a satisfactory method of dispersing the nematode and is significantly less expensive than broadcast application of nematodes.

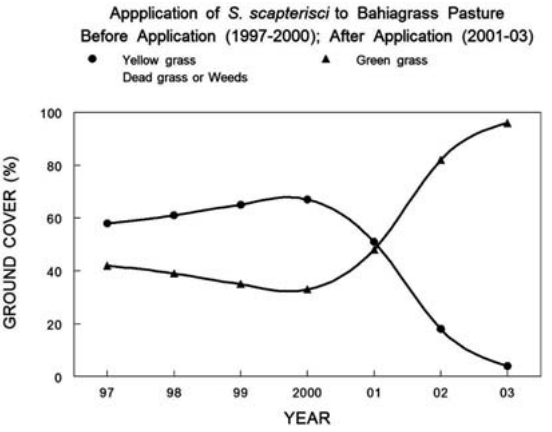

Fig. 2. Condition of a 10-ha bahiagrass pasture monitored annually from 1997-2000 (before application of *Steinernema scapterisci)* and from 7 Sep 2000-2003 (after application of *S. scapterisci*.

Because the control plots became infested by the nematode from the treated plots within 7 months after application, it is obvious that control plots must be much farther from treated plots to prevent infestation. How far control plots should be from treated plots requires further research.

Applications of *S. scapterisci* reduce populations of *Scapteriscus* mole crickets (Parkman et al. 1994), and once *S. scapterisci* is established, the nematodes persist for years in Florida pastures (Frank et al. 1999; Parkman et al. 1993a). Over time, *S. scapterisci* applied to Florida pastures should spread widely as a classical biological control agent.

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SUMMARY

The entomopathogenic nematode, *Steinernema scapterisci*, applied in strips to a 10-hectare bahiagrass pasture reduced populations of mole crickets, *Scapteriscus* spp., by 79.2% over a 3-year period. Bahiagrass cover increased from 33% to

96% in the same time period. Strip applications were much less expensive than treating the entire pasture, but equally effective in providing biological control of mole crickets.

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RANGE EXPANSION OF THE FIRE ANT DECAPITATING FLY, *PSEUDACTEON TRICUSPIS*, EIGHT TO NINE YEARS AFTER RELEASES IN NORTH FLORIDA

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Pseudacteon tricuspis Borgmeier (Diptera: Phoridae) was the first decapitating fly species released in the United States as a biological control agent against imported *Solenopsis* fire ants. Early releases were made in and around Gainesville, FL on several occasions between Jul 1997 and Nov 1999. The flies originated from collections made in Jaguariúna, State of São Paulo, Brazil in 1996 (Porter & Alonso 1999; Porter et al. 2004). Release methods varied and flies were either introduced into the field as adult flies or as immatures in parasitized fire ant workers. By the fall of 2001, the decapitating flies had expanded 35-60 km from the release sites, and occupied approximately 8100 km^2 (Porter et al. 2004). A survey was conducted in late 2005 and early 2006 to determine the current extent of the range of *P. tricuspis* resulting from the original release in Gainesville, FL.

The distribution of decapitating flies in the field was sampled on grassy areas and fields along roads with low traffic volume, where frequent stopping and sampling could be accomplished safely. Fire ant mounds were disturbed to cause the ants to come to the soil surface. On most occasions we used a modified cattle prod to electrocute the fire ants (Barr & Calixto 2005). This causes them to release alarm pheromone (Vander Meer et al. 2002), which apparently attracts the flies (Morrison & King 2004). Flies were detected by closely inspecting areas around disturbed ants for hovering flies. Flies were aspirated with a modified double chamber aspirator with an external collecting tube for easy removal. Flies were either identified in the field with a 10× hand lens, or brought into the lab for identification under dissecting or light microscope. The number of mounds inspected varied depending on the number of people involved and the availability of fire ant nests. Typically, at each survey location, 2-4 people observed between 5 and 20 nests total. Monitoring for flies was done when air temperatures were greater than 20°C. Usually observations were restricted to the period between 10 AM and 4 PM.

If no flies were observed, the total observation period was 30-45 min at one location. In locations where the decapitating flies were present, observations were interrupted as soon as one or more decapitating flies were confirmed. In these cases, the observation period may have been no longer than a few min. Survey sites were chosen in locations where fire ants were abundant. Geographical coordinates of the surveyed locations were determined with GPS equipment (GPS V, Garmin International, Inc., Olathe, KS), and survey locations were mapped by ArcGIS (ESRI, Redlands, CA).

In Nov 2005, *P. tricuspis* were observed in East-Central Florida in Seminole Co. near Sanford, FL, at a distance of approximately 145 km from the release sites around Gainesville (Fig. 1). This represents an average expansion rate of approximately 26 km/year since the fall of 2001. In the northeast direction from the release sites, flies were observed up to 275 km away, close to the town of Richmond Hill, just south of Savannah, GA. This represents an average expansion rate of 57 km/year since fall 2001. Northwest of Gainesville, *P. tricuspis* were observed in summer 2005 in northeast Jefferson Co. and also in Georgia up to Adel, approximately 200 km from the release sites in Florida (35 km/year expansion since 2001). Toward the Florida panhandle, flies were observed in Leon Co. up to the western border of Calhoun Co. on Route 20. Survey in the western direction was stopped because flies observed at these western sites could have been derived from a decapitating fly release near Dothan, AL.

These rates of expansion are approximately 20-180% faster than the respective rates observed in 2001 (Porter et al. 2004). Currently the flies occupy about 86,500 km^2 compared to the 8,100 km^2 in the fall of 2001. Higher rates of expansion could simply be the result of larger populations of flies or evolutionary selection of flies that disperse farther. Decapitating flies are expanding northward at higher rates than they are to the south, but the reason for this is uncertain (Porter et al. 2004). As the decapitating flies move further north, they will eventually encounter colder temperatures and shorter growing seasons which will permit fewer generations of flies per year and probably result in a slower rate of northward expansion.

Several successful releases have been made in South Florida, including in Bonita Springs (1999) and Sarasota, FL (2002). Either site could be responsible for the fly population found in Arcadia, FL in Nov 2005, approximately 100 and 70 km away, respectively. Releases in Dothan, AL (2000) may explain the flies found in Calhoun Co. in the Florida Panhandle, but recent field surveys have not recovered flies around Dothan (L. C. Graham,

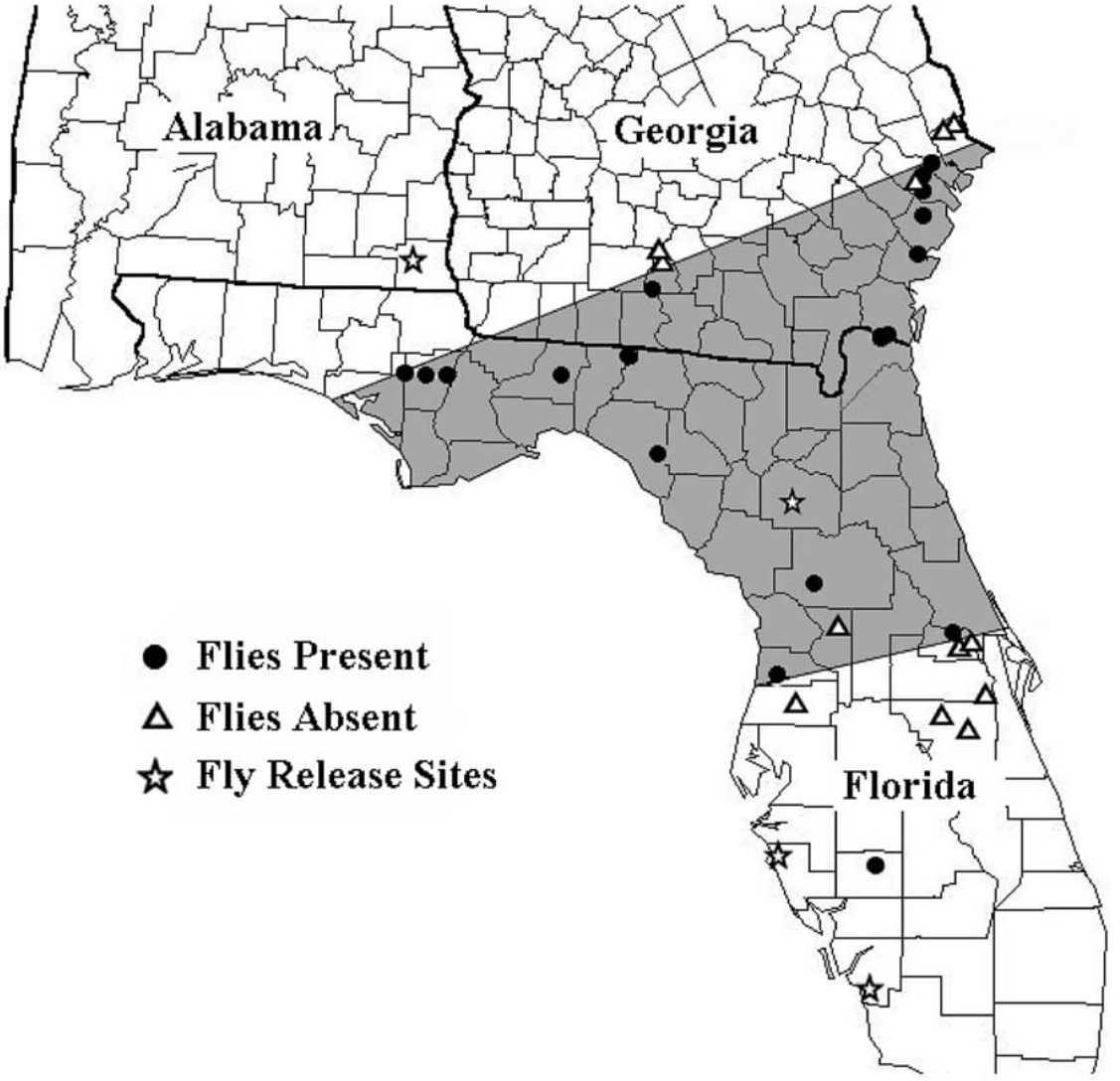

Fig. 1. Range of the fire ant decapitating fly *Pseudacteon tricuspis* 8-9 years after releases in North Florida.

Auburn University, personal communication). Flies found in Calhoun Co. also could have originated in Gainesville, FL, based on the rate of expansion up the east coast of Georgia. Considering the observed expansion rates and the additional successful releases of *P. tricuspis* in southern Florida and elsewhere in the state, it is likely that almost all of Florida will have resident populations of *P. tricuspis* in the next 3 to 5 years.

Researchers have not been able to measure an impact of *P. tricuspis* on fire ant populations (Morrison & Porter 2005). However, the decapitating flies work as a species complex in South America, with each fly species taking advantage of a portion of the niche available for fire ant parasitoids (e.g., different ant sizes, habitats, attack times, etc.). Consequently, several additional species of flies, and perhaps other natural enemies may be necessary for measurable impacts on the US fire ant population (Porter 2000). As other species of the decapitating flies are released and expand their range, possibly at rates comparable to those reported here and elsewhere (Graham et al. 2003), the impacts of these parasitoids on the fire ant populations may become clearer.

We are grateful to Mr. David Milne, Ms. Rebecca Blair, and Ms Damali Kelly for good eyes, great attitude, and efforts in locating fire ant mounds and decapitating flies in the field. We thank Drs. David Oi (USDA-ARS) and Fudd Graham (Auburn Univ.) for critical reviews and comments that helped improve this manuscript. The

use of trade, firm, or corporation names in this publication are for the information and convenience of the reader, and does not constitute an official endorsement or approval by the United States Department of Agriculture or the Agricultural Research Service of any product or service to the exclusion of others that may be suitable.

SUMMARY

In the fall of 2005 and the spring of 2006, the fire ant decapitating fly *Pseudacteon tricuspis* (Diptera: Phoridae) was observed 145-275 km out from the original release sites in northern Florida, occupying an area of about 86,500 km^2. Average expansion rates of 26-57 km/year were considerably faster than previous observations. Considering these rates and other releases, we estimate that almost all of the state of Florida will harbor resident populations of *P. tricuspis* in the next 3 to 5 years.

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THE PREDOMINANCE OF *DIATRAEA FLAVIPENNELLA* (LEPIDOPTERA: CRAMBIDAE) IN SUGAR CANE FIELDS IN THE STATE OF ALAGOAS, BRAZIL

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Brazil, the third largest producer of cane sugar in the world, is responsible for approximately 330,000 tons per year or 25% of the total world production (IBGE, 2004). While the state of Alagoas, in north-eastern Brazil, ranks as number three in terms of agricultural and industrial productivity of sugar cane in the country, the potential yield is diminished through damage by pests, mainly insects of the genus *Diatraea* (Lepidoptera: Crambidae). Two species of *Diatraea* predominate in Brazil, namely, *D. saccharalis* Fabricius 1974 and *D. flavipennella* Box 1931. The former is widespread throughout the country while the second is restricted to Alagoas and a few other states in the north-eastern region (Guagliumi, 1972/73). Studies carried out in Alagoas during the 1970s and 1980s (Risco et al. 1975) indicated that *D. saccharalis* prevailed (70.12%) over *D. flavipennella* (29.88%). According to a survey conducted in 1985 by the Entomology Sector of PLANALSUCAR (PLANALSUCAR 1985), however, an inversion of this situation commenced in some areas of the state and *D. flavipennella* (89.80%) showed preponderance over *D. saccharalis* (10.20%).

Knowledge of the frequencies of occurrence of these two species is of considerable importance to the sugar cane industry because the damage caused by these pests leads to significant loss of yield. Thus, a 1% change in the level of infestation by *D. saccharalis* gives rise to a reduction of 2.5 kg of sugar per ton of cane collected (Gallo et al. 2002). Because the data regarding the prevalence of the two pests are dated, we have conducted a new survey of the incidence of *Diatraea* species in the sugar cane plantations of Alagoas. Eight different edaphic and climatic areas in Alagoas were selected for assessment, encompassing the agricultural estates belonging to the sugar cane factories Cachoeira, Cansanção de Sinimbu, Marituba, Santo Antônio, Seresta, Sumaúma, Terra Nova, and Triunfo (Fig. 1). The study was conducted between Sep 2003 and Feb 2004 during the initial phase of sugar cane cultivation and subsequent growth of the culture. Larvae of *Diatraea* species were collected from severely infested sugarcane plants found in the 8 locations mentioned. The plants were selected on the basis of observable damage to the apical buds and infiltration of larvae into the culms, both of which may lead to the penetration of phytopathogenic micro-organisms and subsequent disease provoking sucrose breakdown. Identification of the species collected was performed on the basis of the morphological characteristics of the larvae as described previously by Guagliumi (1972/73) and Mendonça (1996).

The total number of larvae collected from all sampling areas was 3341, of which 78 specimens (2.33%) were *D. saccharalis* and 3263 (97.67%) were *D. flavipennella* (Table 1). In all 8 locations studied, the number of specimens of *D. flavipennella* randomly collected was far greater than that of *D. saccharalis*, the latter being completely absent in 3 areas. The results clearly demon-

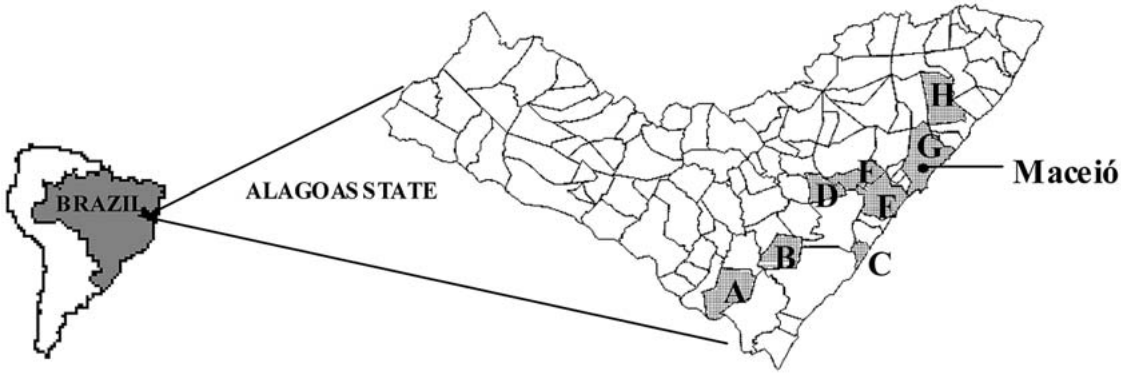

Fig. 1. The location sites of specimens of *Diatraea* spp. in sugar cane fields in Alagoas, Brazil: A—Marituba; B—Seresta; C—Cansanção de Sinimbu; D—Triunfo; E—Sumaúma; F—Terra Nova; G—Cachoeira and H—São Luís do Quitunde.

TABLE 1. NUMBER OF SPECIMENS OF *DIATRAEA* SPP. COLLECTED IN 8 SUGAR CANE FIELDS IN THE STATE OF ALAGOAS, BRAZIL, DURING THE PERIOD OF SEP 2003 AND FEB 2004.

Sampling sites (estates associated with sugar cane factories)	Number of specimens identified (% of total)	
	D. saccharalis	*D. flavipennella*
Cachoeira	0 (0)	57 (100)
Cansanção de Sinimbu	33 (3.82)	831 (96.18)
Marituba	3 (1.95)	151 (98.05)
Santo Antônio	19 (7.51)	234 (92.49)
Seresta	16 (6.11)	246 (93.89)
Sumaúma	7 (0.47)	1475 (99.53)
Terra Nova	0 (0)	141 (100)
Triunfo	0 (0)	128 (100)
Totals: Specimens studied—3341	78 (2.33)	3263 (97.67)

strate that there has been an inversion in the prevalence of the 2 insect species during the last 30 years in Alagoas. Thus, whereas Risco et al. (1975) reported that *D. saccharalis* was the predominant (93.56%) species present in the fields belonging to the Seresta factory during the 1970s, our investigation shows that this species currently comprises only 6.11% of the population and that *D. flavipennella* predominates. Moreover, during the period 1975/1976, *D. saccharalis* constituted 10.69% of the larvae population present in the fields belonging to the Triunfo factory (Risco et al. 1975), whereas the present results show that the larval population consists exclusively of *D. flavipennella*. The inversion of species dominance may be associated with the fact that the methods adopted for the biological control of *D. saccharalis*, i.e., integrated pest management (IPM) involving the manual collection of larvae, introduction of resistant varieties of sugarcane, and the use of the larval parasitoid, *Cotesia flavipes* (Hymenoptera: Braconidae) (Arencibia et al. 1997; Setamou et al. 2002; Baker et al. 1992), were not efficient in reducing the infestation level of *D. flavipennella* in the field. Furthermore, the increase in intensity and irregularity of the rainy season experienced in the last decade may favor *D. flavipennella* over *D. saccharalis*.

SUMMARY

The occurrence of insects of the genus *Diatraea* (Lepidoptera: Crambidae) in sugar cane fields was investigated in 8 different edaphic and climatic areas of the state of Alagoas, Brazil, during the period of Sep 2003 and Feb 2004. The randomly sampled insect population consisted of *D. saccharalis* (2.33%) and *D. flavipennella* (97.67%), indicating that there has been an inversion in the prevalence of the 2 insect species during the last 30 years in the state of Alagoas. These results will serve as a basis for further studies concerning the establishment of appropriate methods for the control of *D. flavipennella*, perhaps by using its natural predator *Cotesia flavipes* (Hymenoptera: Braconidae) or through the entrapment of females by using the specific female sex pheromone.

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FIRST RECORD OF *RETICULITERMES FLAVIPES* AND *RETICULITERMES HAGENI* IN OREGON (ISOPTERA: RHINOTERMITIDAE)

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The majority of pestiferous subterranean termites in North America belong to the endemic genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Reticulitermes flavipes* (Kollar), the eastern subterranean termite, is the most economically important (Su 1993) and widespread termite (Austin et al. 2005a) in the United States. Existing taxonomic studies provide information on only 1 *Reticulitermes* spp. in Oregon, *R. hesperus* (Banks), the western subterranean termite, which is the most common termite pest species found from southern British Columbia to central California (Snyder 1954; Weesner 1965). Distribution studies on Pacific Northwest species of *Reticulitermes* have been addressed by Castle (1928) and Light & Pickens (1934). We report herein findings of two unreported *Reticulitermes* spp. (*R. flavipes* and *R. hageni*) from Oregon.

Soldiers, if available, and worker termites were collected from a total of 79 different colonies from 34 locations in Oregon located in the following counties: Clackamas, Coos, Jackson, Klamath, Lane, Linn, Marion, Multnomah, Polk, Umatilla, Washington, and Yamhill by our own collection efforts, by Pest Management Professionals (PMPs), and through the 2002 national termite survey. A 428-bp region of the mt-DNA 16s rRNA gene was amplified by PCR from 79 samples consisting of 1 worker from each colony and subjected to DNA sequencing per Szalanski et al. (2003).

Two *R. flavipes* soldier specimens collected from a colony at a collection site in Keizer, OR were identified morphologically, applying keys of Scheffrahn & Su (1994) and by evaluating soldier labra (Hostettler et al. 1995), and confirmed genetically via sequence data from a worker specimen (Szalanski et al. 2003). A worker specimen from a collection site in Salem, OR was identified genetically from sequence data as *R. hageni*. Soldiers were not collected from this colony and morphological identification was not performed. Both Keizer and Salem are located within 50 km from Portland, OR.

Reticulitermes flavipes is the most widely distributed *Reticulitermes*, and is found in the entire eastern region of North America as far as Ontario, Canada, and south to Florida (Snyder 1954; Weesner 1965). The known western distribution of the species extends through the central plains to the Rocky Mountains and down to Monterrey, Mexico (Banks & Snyder 1920; Snyder 1954; Weesner 1965; Messenger 2003). Austin et al. (2005a) reported the first occurrence of *R. flavipes* in California and Nevada, extending its distribution westward. The presence of *R. flavipes* in western states has subsequently been independently verified (Su et al. 2006; Tripodi et al. 2006). The *R. flavipes* 16S rRNA haplotype was FF (GenBank Accession D2001958), which is predominately found in the eastern United States (Austin et al. 2005a). Because this is outside of the previously known distributions of both *R. flavipes* and *R. hageni* (Banks & Snyder 1920; Snyder 1954; Weesner 1965; Messenger 2003; Austin 2005a), eastern introductions to Oregon from anthropogenic sources are implicated.

Because *R. flavipes* is a primary pest of structures in the United States (Austin et al. 2005a) and around the world (Scheffrahn et al. 1999; Austin et al. 2005b; Su et al. 2006), assessment of this pest should be carefully evaluated to determine whether its establishment in western Oregon will compete with *R. hesperus*, the dominant species of western Oregon (Szalanski et al. 2006). Likewise, it remains unknown whether these will be further dispersed or compete with eastern Oregon *Reticulitermes* species (Szalanski et al. 2006) as destructive pests in the future.

Reticulitermes hageni has been depicted as occurring throughout the southeastern United States, with distributions from the central Missouri valley expanding westward towards southeastern Kansas, south towards the Texas-Louisiana border areas (Light & Pickens 1934; Austin et al. 2006). Flight records of *R. hageni* (Banks) alates suggest that its western limit occurs around Kansas City, Kansas (Krishna & Weesner 1970; Hungerford 1935). *Reticulitermes hageni* has the least amount of genetic diversity among *Reticulitermes* spp. in North America, and is represented by only four haplotypes in North America (A.L.S., unpublished data). It has significantly retarded flight dispersal relative to other congeners (JWA, personal observation) and would likewise support the lack of genetic diversity and higher levels of inbreeding (Vargo & Carlson 2006). The *R. hageni* sample found was haplotype H1 (GenBank Accession AY257235), which has been observed throughout its known eastern Nearctic range (JWA unpublished). The presence of these *Reticulitermes* spp. in Oregon in the absence of native enemies will be interesting to

evaluate for their future impacts on urban and natural environments.

Funds for this project were made available by the City of New Orleans Mosquito and Termite Control Board.

SUMMARY

A survey of *Reticulitermes* spp. in Oregon has resulted in the first report of 2 eastern Nearctic species, *R. flavipes* and *R. hageni*. These occurrences should be closely monitored and considered in future studies which attempt to delimit *Reticulitermes* species from Oregon in order to avoid misidentifications and future confusion about residing native *Reticulitermes* populations there.

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BOOK REVIEW

LEHANE, M. J. 2005. The Biology of Blood-sucking Insects. Cambridge Univ. Press, Cambridge, U.K. 2nd edition, xi + 320 pp. Paperback, ISBN 0-521-54395-9, $60.00 [Also hardback, ISBN 0-521-83608-5, $120.00].

The insect fauna adapted to the blood-sucking lifestyle arguably contain the most important arthropods affecting human history. More important than the pain and distress caused by their blood-feeding habits, the disease-causing organisms transmitted to humans or livestock have caused incalculable distress throughout history. Considerable effort is being directed to eliminate many of the most damaging vector-borne diseases and advances in molecular biology may soon allow for this long sought after goal. The development of integrated pest management principles specifically tailored to medical and veterinary entomology will greatly aid in this process. The progress in biology, ecology, behavior, toxicology and a host of other disciplines are melding, thereby creating an opportunity to rid the world of the scourge of human malaria, dengue, onchocerciasis, and perhaps sleeping sickness and nagana. In particular, the opportunity for disease relief and ultimately economic development in Sub-Saharan Africa holds great promise.

In this second edition, Lehane updates his 1991 edition with considerable information on feeding preference, host location, ingestion and management of the blood meal, host insect interactions, and parasite transmission. The greatest change in this version is the incorporation of the tremendous progress resulting from advances in molecular biology.

An effective delivery is provided with a topic-based outline, rather than by a traditional insect classification design. Within these topics, Lehane uses a comparative technique to illustrate the different modifications utilized by insects. Initially, the focus is on the modifications made by the insects to successfully adapt to a blood-sucking lifestyle. These include the blood-feeding habit, mouthpart adaptations, host location, and blood-meal processing, not a simple task. Considerable effort is made to delineate the variety of theories as to how and perhaps why diverse groups of insects made these adaptations independently. Throughout the book the text is enhanced by the addition of useful tables, figures and images to support the author's presentation.

Although our knowledge of the blood feeding insects has expanded considerably, collective knowledge is still greatest for only a few species. In fitting with the presentation-by-topic style, Lehane eases the presentation by discussing the groups of insects by behavioral methodology, including temporary ectoparasites, such as tabanids, mosquitoes and blood-feeding bugs, permanent ectoparasites, such as lice and the sheep ked; and periodic ectoparasites, such as many of the fleas and the Pupipara. This approach has benefits and drawbacks; however, this style fits well into the overall presentation. By the time students would be ready for the information provided in this book, they should already have a firm understanding of the taxonomic relationship, as well as the behavioral reasons for this grouping.

The book opens with a short chapter on the importance of blood-sucking insects, followed by a discussion on the evolution of the blood-sucking habit. Lehane includes a discussion on the theories that best describe the shift to blood-feeding, such as having a close association with a host or having pre-adapted piercing mouthparts. These introductory chapters are supplemented by a presentation on feeding preferences and host location, information that is becoming increasingly important as researchers continue to develop ways to prevent attack, thereby preventing disease transmission. The rationale on host choice and host specificity provides insight as to the eventual vector capabilities of various groups. Behavior associated with appetite driven searching behavior and host finding and orientation techniques is very intriguing.

As is the primary focus of this book, several chapters specific to the handling of the blood meal and the interrelationships of the host, vector, and pathogen follow. The ingestion of the blood meal presents a precarious situation for many insects. The variety of approaches utilized by the insects is presented individually by major taxa. A discussion of the host response to probing and blood feeding, the resultant challenge facing and response utilized by the insects provides a solid understanding of a fascinating but often-overlooked interrelationship. Chapter 6 is one of the most interesting and deals with the management of the blood meal. This chapter approaches the discussion based on the structure (with or without diverticula) and method of blood meal processing (batch or continuous), resulting in four approaches to handling a blood meal. Lehane illustrates the impact the blood meal can have on gonotrophic concordance and nutrition. A discussion of host hormone impacts is highlighted by a description of the rabbit flea, *Spilopsyllus cuniculi* (Dale) feeding on the European rabbit, *Oryctolagus cuniculus.*

A discussion of host-insect interactions covers insect distribution on the host, the morphological specializations developed by insects, host immune responses and salivary contents, host be-

havioral responses, and the impact of insect density on feeding success. This important chapter outlines the adaptations by the lice, fleas, and keds and includes specific discussions of the wingless lifestyle, tarsal and other locomotory changes and adaptations to support physiological processes, such as water loss. The host-based adaptations to avoid or mitigate blood-feeding insects includes sections on responses to insect saliva, such as increased sensitivity as well as behavioral responses including aggregation by cattle, grooming by mice, and defensive movements by birds subjected to mosquito bites.

Certainly the greatest damage caused by blood feeding insects is their capacity to serve as pathogen vectors. This chapter opens with a discussion of transmission routes that students of the science will find invaluable. Included is a table listing many of the important pathogens with their associated major vectors, hosts, and geographic distribution. The discussion on vector-parasite specificity flows well into the section on the origin of vector-parasite relationships. Although this book is written largely from the insect perspective, a discussion is included on the strategies employed by parasites for locating both a vector and a vertebrate host. An often-overlooked component of vector ecology, the parasite impact on the vector, including vector immune mechanisms is presented.

The final chapter of the book encompasses an overview of the major families involved with the blood feeding strategy. This section will be most useful to students, and should be considered a starting point for their understanding of these important groups of insects.

As medical entomologists continue to make progress in utilizing molecular tools to aid in the disruption of pathogen transmission and disease manifestation, the knowledge of the interactions between the host, insect, and pathogen have and will continue to increase. The book contains an extensive citation listing and a highly useful index. Although the book is written in both a clear and concise fashion, students entering the field would have benefited by a glossary.

The book is an invaluable tool for undergraduate and graduate students entering the fields of medical and veterinary entomology, ecology, behavior, epidemiology, human and veterinary medicine, as well as others. I found the up-to-date information very useful, as should others who teach about these highly important pests.

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