SIX ALIEN APHID SPECIES (HEMIPTERA: APHIDIDAE) RECORDED FOR THE FIRST TIME FROM SOUTH AMERICA

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ABSTRACT

Six aphid species: *Saltusaphis scirpus* Theobald, *Myzocallis boerneri* Stroyan, *Macrosiphoniella absinthii* (Linnaeus), *Macrocrosiphoniella abrotani* (Walker), *Macrosiphoniella pseudoartemisiae* Shinji and *Macrosiphoniella tapuskae* (Hottes & Frison) are recorded for the first time in South America. They were all collected in Argentina. Comments for each species and identification keys for Myzocallidina and *Macrosiphoniella* known in South America are given.

Key Words: Hemiptera, Aphididae, Neotropical, alien species

RESUMEN

Se citan por vez primera para Sudamérica seis especies de pulgones: *Saltusaphis scirpus* Theobald, *Myzocallis boerneri* Stroyan, *Macrocrosiphoniella abrotani* (Walker), *Macrosiphoniella absinthii* (Linnaeus), *Macrosiphoniella pseudoartemisiae* Shinji y *Macrosiphoniella tapuskae* (Hottes & Frison). Todas ellas recogidas en la Argentina. Se presentan comentarios de cada especie y claves de identificación para las especies de Myzocallidina y de *Macrosiphoniella* conocidas en Sudamérica.

The distribution of most aphid species (Hemiptera: Aphididae) is limited to holarctic territory. Only those belonging to the subfamilies Greenideinae, Lizeriinae, Neophyllaphidinae, Pterasteriinae, and Spicaphidinae (with approximately 150, 33, 12, 5, and 13 species, respectively) and most of the subfamily Hormaphidinae (with approximately 180 species) are found in gondwanic territories. The species in the remaining subfamilies are characteristic of holarctic territories with some penetration in neighboring Oriental, Ethiopian, or Neotropical regions. The number of species in the subfamilies Calaphidinae, Eriosomatinae, Chaitophorinae, Lachninae, Saltusaphidinae (with, respectively, 332, 319, 165, 124, and 68 species, according to Eastop 1998) and the large subfamily Aphidinae (with around 2800 species) recorded in one or another southern territory is limited or very low. Some of these species may be considered as natives of these territories due to the evolution into lineages of holarctic origin (see Nieto Nafría et al. 2002 for several South American macrosiphins). Many others, the alien species, inhabit these territories due to direct introduction from holarctic populations.

The introduction of these species could be the result of (1) natural invasions during recent geological periods, as in the case of species colonizing (especially high regions) northern Neotropical and northern Oriental regions and the suboriental border of the Ethiopian region; and (2) human activities and displacements, which, in the case of South America date to no more than to 500 years. In most cases, however, this period of time is no more than 100 or 150 years, when traffic to and from other parts of the world increased and journeys lasted much shorter periods of time (Remaudière et al. 1985; Dixon 1998; Blackman & Eastop 2000; Rapoport 2000). The exotic component of aphid fauna in continental Argentina is considerable, accounting for just over 75% according to the latest review (Ortego et al. 2004), and is still growing.

MATERIALS AND METHODS

In this paper we follow the classification of the family Aphididae used by Remaudière & Remaudière (1997), completed by Quednau (1999), with the nomenclatural adaptations by Nieto Nafría et al. (1998a).

RESULTS AND DISCUSSION

After studying specimens collected during recent years in the Andean Argentinian strip we recorded another six alien aphid species, also recorded for the first time in South America.

New Data for the subfamiliy Saltusaphidinae:

The subfamily Saltusaphidinae Baker, 1920 is of holarctic origin, though there is currently one exclusive species in Chile (Quednau 1990):

Thripsaphis (*T.*) *unciniae* Quednau, 1990. A few species have been recorded in other regions (Australia, New Zealand, sub-Saharan Africa) as a result of human activities and displacements (Eastop 1986). Saltusaphidinae live on species of the family Cyperaceae or more unusually, Juncaceae, Poaceae, or other monocotyledonous species. They are generally very globose or flattened aphids, sometimes elongate and even bacilliform, with scarcely or no discernible siphunculi and a claviform cauda; they are an attractive yellow or green (in different shades) color mixed with black; microscopic examination (or even stereoscopic magnification) reveals numerous dorsal setae, mostly claviform, spatulate, flabelliform, or fungiform and often short or very short.

Saltusaphis scirpus Theobald, 1915

Studied Material: Maipú (Mendoza), yellow water trap, 28-II-2004, 1 alate viviparous female (J. Ortego *leg.*); *Cyperus rotundus* Linnaeus, 1753, 29-IV-2004, alate and apterous viviparous females and nymphs (J. Ortego *leg.*). Viviparous females greenish-black, relatively large (usually over 2 mm), somewhat depressed and oval-shaped, with a concave front and eyes fairly separated from the antennal insertion (very common characters in the subfamily). Antennae almost reaching body length, fore femur much more voluminous than the others, siphunculi subcylindrical, low, pigmented and very rough. Dorsum of apterae with small and abundant segmental sclerites (with pigmentation very similar to that of intersegmental sclerites) and numerous short and flabelliform setae, except the marginal ones on final segments, which are long (in particular on the two blunt tubercles of abdominal segment VIII) and claviform, blunt or pointed. Alatae with marginal and spinal (one or two) sclerites and small pleural plates on each abdominal segment, antennal segment III bearing 9 to 21 oval-transverse secondary sensoria, and wings with slightly bordered veins and spots on edge of pterostigma and tip of the veins.

Saltusaphis scirpus has been recorded in most of Europe (Bulgaria, Czech Republic, France, Germany, Greece, Hungary, Italy, Poland, Portugal, Rumania, Russia [with doubts], Slovakia, Spain, Sweden, and the Ukraine) and sub-Saharan Africa (Angola, Burundi, Kenya, Lesotho, Malawi, Mozambique, South Africa, Sudan, and Zimbabwe), several Asian countries (from the Mediterranean to India) and North America (U.S.A.), either under its valid name, or as *Hiberaphis iberica* Börner, 1949, *Saltusaphis africana* Eastop, 1953 and *Bacillaphis afghanica* Narzikulov & Umarov, 1970 (Nieto Nafría et al. 1998b). It is easily differentiated from *Thripsaphis unciniae*, so far the only species in the subfamily recorded from South America, by its dorsal sclerotization and pigmentation and shape of abdominal segment VIII (in apterae and alatae) and by the pigmentation of the wings. The presence of this species in Argentina could be due to (1) eggs in the soil of ornamental plants living in water or nearby, or in soil adhered to plant bulbs or tubercles, or (2) eggs or specimens living on ornamental Cyperaceae, for example *Cyperus*. The latter option is the most likely. The invasion could have originated from Europe, North America, or even South Africa.

New Data on the Subtribe Myzocallidina (Calaphidinae: Panaphidini):

The subfamily Calaphidinae Oestlund, 1918 is of holarctic origin, though some if its species now inhabit other parts of the world. Most of the genera and nearly all the species of *Myzocallis* Passerini, 1860 live on Fagaceae. This genus includes 42 taxa of species level, divided into 10 subgenera (Quednau 1999), two of which, *M.* (*Agrioaphis*) *castanicola* Baker, 1917 (specifically, the nominotypical subspecies) and *M.* (*M.*) *coryli* (Goeze, 1778), have been recorded in Argentina, Brazil, and Chile (Blackman & Eastop 1994; Quednau 1999). There are records (Fuentes-Contreras et al. 1997; Pérez Hidalgo et al. 1998; Bergmann et al. 2002; Ortego et al. 2004) of another four species of the subtribe in South America: *Hoplocallis picta* (Ferrari, 1872) in Argentina and Chile, *Tuberculatus* (*T.*) *querceus* (Kaltenbach, 1843) in Argentina, *Tuberculatus* (*Nippocallis*) *kuricola* (Matsumura, 1917) in Brazil, and *Tuberculatus* (*Tuberculoides*) *annulatus* (Hartig, 1841) in Argentina, Brazil, and Chile.

Myzocallis (*Myzocallis*) *boerneri* Stroyan, 1957

Studied Material: Junín (Mendoza), *Quercus suber* Linnaeus, 1753, 27-VIII-2004, alatae females and nymphs (J. Ortego *leg.*). *Myzocallis boerneri* was recorded (Nieto Nafría & Mier Durante 1998; Blackman & Eastop 1994; Quednau 1999) on various species of *Quercus* (*canariensis*, *castaneaefolia*, *cerris*, *ilex*, *infectoria*, *faginea*, *macedonica*, *persica*, *rotundifolia*, *suber*, and *variabilis*) in most of Europe, the Macaronesian Islands; Lebanon, Israel, Iran; South Africa; New Zealand, and U.S.A. (California). The species almost certainly entered Argentina via seedlings of European species of *Quercus* from Europe. It is differentiated from the other 6 species recorded for South America by the following key. Any doubts can be clarified by consulting the key by Quednau (1999).

1a. Abdomen with spinal tubercles 2

1b. Abdomen without spinal tubercles 3

2a. Abdominal segment III with one pair of large spinal tubercles joined at base. On Eurasian species of *Quercus*. When alive greyish-brown to brown with abundant powdered to filamentous wax . . . . *Tuberculatus* (*T.*) *querceus*

2b. Abdominal segments I and II with one pair of pale or slightly pigmented spinal tubercles and abdominal segment III with another bigger pigmented pair; all of them clearly separated at the base. On *Quercus* spp. When alive, pale green to yellowish–green or with shades of pink and even crimson coloring . . . . *Tuberculatus* (*Tuberculoides*) *annulatus*

3a. Setae of the antennal segment III at least 4.0 times the joint width of article. Siphunculi rising from marginal sclerites. On Eurasian species of *Castanea* and *Quercus*. When alive, pale green to red and with whitish wax. Alar veins (particularly the anterior ones) widely bordered. . . . . *Tuberculatus* (*Nippocallis*) *kuricola*

3b. Antennal setae much shorter (at most 1.0 times the joint width of article) 4

4a. Dorsum of the head and prothorax evenly dark and abdomen with a band of dark spinal plates (each with a small central depigmented area), marginal plates not close together and less pigmented than spinal ones. On Eurasian species of *Quercus*. When alive, yellow (cream to lemon) and black. . . . . *Hoplocallis picta*

4b. Abdomen without dark spinal band and head completely pale or with a dark spinal band 5

5a. Dorsum completely pale or with very faint marginal spots on prothorax and/or several abdominal segments. On *Corylus*. When alive, pale yellow or whitish-yellow . . . . *Myzocallis* (*M.*) *coryli*

5b. Head and prothorax with a dark spinal band and abdomen with pairs of spinal and marginal sclerites (spread out and well-pigmented in the nominotypical subspecies). On species of *Castanea* and *Quercus*. When alive, lemon to creamy yellow with black spots of varying size and intensity . . . . *Myzocallis* (*Agrioaphis*) *castanicola*

5c. Prothorax pale or with a pair of marginal spots at most and abdomen with pairs of marginal and spinal sclerites, less pigmented than the former. On Eurasian species of *Quercus*. When alive pale yellow to yellowish-green or even very pale greenish-yellow, with a cream-colored or dull brown thorax . . . . *Myzocallis* (*M.*) *boerneri*

New Data on the Tribe Macrosiphini (Aphidinae): Genus *Macrosiphoniella*:

The genus *Macrosiphoniella* Del Guercio, 1911 is one of the big genera in the very extensive tribe Macrosiphini, with approximately 140 species, 100 of which are classified in the nominotypical genus and some are little known. It belongs to the group of genera with long reticulate siphunculi, like *Macrosiphum* Passerini, 1860 and *Uroleucon* Mordvilko, 1914. Species of *Macrosiphoniella* have monoecious and basically holocyclic cycles (some are confirmed as being anholocyclic) and almost all of them live on species of Asteraceae. The distribution of each species varies greatly within the holarctic distribution of the genus as a whole. Some, such as *M. absinthii* (Linnaeus, 1758), are very widespread (possibly due to human activities spreading), while others have only been recorded in a few localities, for example, *M. aetnensis* Barbagallo, 1979 (Nieto Nafría et al. 2004). To date, four species from this genus had been recorded for South America (Smith & Cermeli 1979; Remaudière et al. 1991; Costa et al. 1993; Nieto Nafría et al. 1994): *Macrosiphoniella* (*M.*) *artemisiae* (Boyer de Fonscolombe, 1841) and in particular its nominotypical subspecies (known from Argentina only), *M.* (*M.*) *sanborni* (Gillette 1908) (recorded in Argentina, Brazil, Bolivia, Chile, Colombia and Venezuela), *M.* (*M.*) *tanacetaria* (Kaltenbach, 1843), and specifically its subspecies *bonariensis* E. E. Blanchard 1922, (described in Buenos Aires and former synonym of *italica* Hille Ris Lambers, 1966, and recorded from Argentina and Chile), and *M.* (*M.*) *yomogifoliae* (Shinji, 1924) (recorded in Brazil). Another four species: *M.* (*M.*) *abrotani* (Walker, 1852), *M.* (*M.*) *absinthii* (Linnaeus, 1758), *M.* (*M.*) *pseudoartemisiae* Shinji, 1933, and *M.* (*M.*) *tapuskae* (Hottes & Frison, 1931) are recorded for the first time.

Macrosiphoniella absinthii (Linnaeus, 1758)

Studied Material: Junín de Cuyo (Mendoza), *Artemisia absinthium* Linnaeus, 1753, 14-IX-2004, apterous and alate viviparous females (J. Ortego *leg.*). The species is normally found on *Artemisia absinthium*, but has been recorded (Heie 1995) on *Chrysamthemum zawadzkii*. It is widely distributed in Europe and there are records for North Africa and Canada (Nieto Nafría et al. 2004).

Macrosiphoniella abrotani (Walker, 1852)

Studied Material: Esperanza (Santa Fe), yellow water trap, 29-IX-1999, alate viviparous fe-

male (J. Ortego *leg.*); Malargüe: El Challao (Mendoza), *Artemisia* sp., 31-X-1999, apterous and alate viviparous females and nymphs (J. Ortego *leg.*); Maipú (Mendoza), *Artemisia* sp., 9-II-2002, apterous viviparous females (J. Ortego *leg.*); Rafaela (Santa Fe), *Artemisia annua*, 26-II-2001, apterous viviparous females and nymphs (I. Bertolaccini *leg.*). This species has been recorded on species of *Artemisia* and less frequently on some species of *Matricaria* and *Achillea*, in numerous European countries, Australia, and North America (Nieto Nafría et al. 2004).

Macrosiphoniella pseudoartemisiae Shinji, 1933

Studied Material: Malargüe (Mendoza), *Artemisia absinthium*, 5-IV-1994, apterous viviparous females (J. Ortego *leg.*); Trevelín (Chubut), *Artemisia absinthium*, 20-I-2000, apterous viviparous females (J. M. Nieto Nafría, J. Ortego and M. P. Mier Durante *leg.*). This aphid has been recorded (Lee et al. 2002) on *Artemisia annua*, *mongolica*, *princeps*, and *stolonifera* in North and South Korea, China, Russia (Far East), Japan and India. *Artemisia absinthium* is therefore a new host plant for this species. This is the most surprising of the four species now included in the South

American aphid fauna catalogue, given its origin and distribution; but *Macrosiphoniella yomogifoliae* has been recorded in Brazil.

Macrosiphoniella tapuskae (Hottes & Frison, 1931)

Studied Material: Barreal (San Juan), *Anthemis cotula* Linnaeus, 1753, 23-XI-2002, alate and apterous viviparous females and nymphs (M. P. Mier Durante, J. Ortego and J. M. Nieto Nafría *leg.*); San Rafael: El Sosneado (Mendoza), *Tanacetum vulgare* Linnaeus, 1753, 29-I-2000, apterous viviparous females (J. M. Nieto Nafría, J. Ortego and M. P. Mier Durante *leg.*). It has been recorded on different Asteraceae species, in particular *Achillea* and *Matricaria* in most European countries, and others in the Near East, North Africa, and North America (Nieto Nafría et al. 2004).

The following key can be used to differentiate the eight species, but carefully, due to the richness of species in the genus. Other characters (in square brackets) are given in order to corroborate the identification. Should any discrepancies arise regarding the described characters and those of the studied specimens another key for differentiating holarctic species must be used (for example, see Heie 1995).

1a. Siphunculi clearly longer than the cauda (Fig. 1g) and only 12-18% of their length reticulate. [Dark grey color, more or less yellowish or reddish, with ash-colored waxy powder, except for a spinal band and a strip in front of the insertion of the siphunculi. Without segmental sclerites on dorsum of abdomen (Fig. 1g). Antennal segment III with 6 to 26 secondary sensoria on proximal 2/3] . . . . *M. tapuskae*

1b. Siphunculi shorter (Figs. 1a-f, h) or slightly longer than cauda, and at least 40% of their length reticulate. 2

2a. Siphunculi pigmented, with pale basal area (Fig. 1a). [Green aphids, with waxy powder. Siphunculi 50-67% reticulate in length. Presiphuncular sclerites usually absent (Fig. 1a). Cauda with 11 to 25 setae. Antennal segment III with 2 to 13 secondary sensoria in proximal half] . . . . *M. abrotani*

2b. Siphunculi wholly pigmented, though not with the same intensity (Figs. 1b-f, h) 3

3a. Spinal sclerites fully-pigmented on abdominal segments II to VI (Fig. 1b). Antennal segment III with 29 to 55 secondary sensoria in proximal half. [Dark reddish-grey or purple aphids with waxy powder. Hind tibiae strongly pigmented (Fig. 1b). Siphunculi reticulate on 48-60% of its length. Cauda with 12 to 18 setae] . . . . *M. absinthii*

3b. Without spinal sclerites on abdominal segments II to VI (Figs. 1c-f, h). Secondary sensoria of antennal segment III varying in number and distribution 4

4a. Mid part of hind tibiae, at least, pale or scarcely pigmented (Figs. 1d, e) 5

4b. Hind tibiae strongly pigmented (Figs. 1c, f, h) 6

5a. Antennal segment III with 8 to 32 secondary sensoria, distributed along its entire length. Presiphuncular sclerites well-pigmented. On *Chrysanthemum*. [Dark grey or toffee-colored, without waxy powder. Siphunculi with 63-81% of its length reticulate. Cauda with 9 to 11 setae] . . . . *M. sanborni*

5b. Antennal segment III with 2 to 6 secondary sensoria, spread along proximal half. Presiphuncular sclerites tenuous (as tenous as setiferous sclerites on dorsum of abdomen) (Fig. 1d). On *Artemisia* [Green aphids with some waxy powder. Siphunculi with 62-71% of its length reticulate. Cauda with 9 to 13 setae. Dorsoabdominal setae blunt or with slightly expanded tip] . . . . *M. pseudoartemisiae*

6a. Processus terminalis 4.8 to 6.8 times ultimate rostral segment, which is 0.7 to 0.9 times second segment of hind tarsus. Cauda with 26 to 32 setae. Presiphuncular sclerites
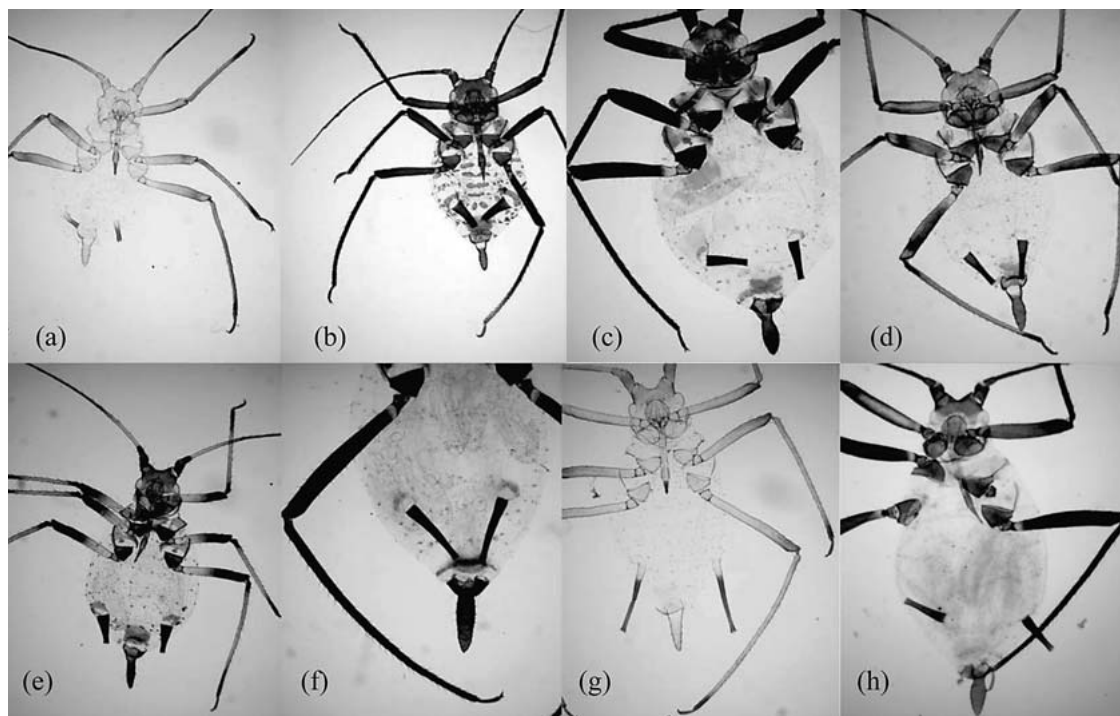
Fig. 1. (a) *Macrosiphoniella* (*M.*) *abrotani*, (b) *Macrosiphoniella* (*M.*) *absinthii*, (c) *Macrosiphoniella* (*M.*) *artemisiae*, (d) *Macrosiphoniella* (*M.*) *pseudoartemisiae*, (e) *Macrosiphoniella* (*M.*) *sanborni*, (f) *Macrosiphoniella* (*M.*) *tanacetaria bonariensis*, (g) *Macrosiphoniella* (*M.*) *tapuskae* and (h) *Macrosiphoniella* (*M.*) *yomogifoliae*.

    absent or present but scarcely pigmented (Fig. 1f). [Green to grey-brown aphids with waxy powder. Antennal segment III with 2 to 8 secondary sensoria in proximal half only] . . . . *M. tanacetaria bonariensis*

6b. Processus terminalis 2.4 to 5.0 times ultimate rostral segment, which is 0.8 to 1.3 times second segment of hind tarsus. Cauda with 10 to 36 setae (but with 27 setae at most if the processus terminalis is less than 1.0 times the cauda) 7

7a. Ultimate rostral segment 1.0 to 1.3 times second segment of hind tarsus. Processus terminalis 2.4 to 3.5 times ultimate rostral segment. Genital plate usually with 4-6 discal setae (exceptionally 2 or 8). On *Artemisia* and *Chrysanthemum*. [Green aphids covered with waxy powder. Presiphuncular sclerites absent or scarcely pigmented (Fig. 1h). Cauda with 17 to 24 setae. Antennal segment III with 2 to 8 secondary sensoria] . . . . *M. yomogifoliae*

7b. Ultimate rostral segment 0.8 to 1.1 times second segment of hind tarsus. Processus terminalis 3.6 to 5.0 times ultimate rostral segment. Genital plate with 2-3 discal setae. On *Artemisia vulgaris* and *A. absinthium*. [More or less greyish-green aphids with waxy powder. Presiphuncular sclerites present, though scarcely pigmented (Fig. 1c). Cauda with 19 to 27 setae. Antennal segment III with 3 to 14 secondary sensoria] . . . . *M. artemisiae*

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EVALUATION OF SEVERAL REDUCED-RISK INSECTICIDES IN COMBINATION WITH AN ACTION THRESHOLD FOR MANAGING LEPIDOPTERAN PESTS OF COLE CROPS IN ALABAMA

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ABSTRACT

Several reduced-risk insecticides were evaluated for management of three lepidopteran cole crop pests, *Plutella xylostella* (L.), *Pieris rapae* (L.), and *Trichoplusia ni* (Hübner) in central Alabama in 2004 (spring and fall plantings) and 2005 (spring only). The following formulated sprays were evaluated: Dipel® (*Bacillus thuringiensis* subspecies *kursatki*), XenTari® (*B. thuringiensis* subspecies *aizawai*), Dipel+XenTari (a premixed test formulation consisting of both subspecies of *B. thuringiensis*), Entrust® (a formulation of spinosad for use in organic crop production), and Novaluron (insect growth regulator). Variations in the populations of the three pest species were recorded from season to season with pest pressure being generally higher in both spring seasons than in the fall season. While moderate to high populations of *P. xylostella* and *P. rapae* were recorded in all three seasons, *T. ni* was detected only in spring 2005. An action threshold of 0.5 cabbage looper equivalents (CLE) per plant was used to determine the need for insecticide applications. Insecticide efficacy was determined by comparing densities of larvae and immatures (larvae + pupae) of each pest species, crop damage ratings, densities of key non-target arthropods, and number of insecticide applications in plots treated with each material versus untreated control plots. All five reduced-risk insecticide formulations were effective in reducing infestations of the three lepidopteran pests and in providing marketable cabbage and collards in Alabama. Among the treatments, Entrust® consistently produced the lowest mean damage ratings with the minimum number of applications per season. No significant effects of insecticide treatments were recorded in the numbers of spiders and lady beetles found per plant. The results also suggest that the 0.5 CLE action threshold can be used to produce marketable cabbage and collards in Alabama with only minimal applications of reduced-risk insecticides.

Key Words: Diamondback moth, *Plutella xylostella*, imported cabbageworm, *Pieris rapae*, cabbage looper, *Trichoplusia ni*, integrated pest management

RESUMEN

Varias insecticidas de riesgo reducido fueron evaluados para el manejo de tres plagas de lepidópteros del cultivo de col, *Plutella xylostella* (L.), *Pieris rapae* (L.) y *Trichoplusia ni* (Hübner) en el área central de Alabama en el 2004 (las siembras de la primavera y otoño) y el 2005 (solamente la primavera). Las siguientes formulaciones fumigadas fueron evaluadas: Dipel® (*Bacillus thuringiensis* subespecie *kursatki*), XenTari® (*B. thuringiensis* subespecie *aizawai*), Dipel+XenTari (una formulación de prueba pre-mezclada que consiste de ambas subespecies de *B. thuringiensis*), Entrust® (una formulación de "spinosad" para el uso en la producción de cultivos orgánicos) y Novaluron (un regulador del crecimiento de insectos). Las variaciones en la población de las tres especies de plagas fueron anotadas de una estación a la otra, con la presión de las plagas generalmente mas alta en ambas estaciones de primavera que en la estación de otoño. Mientras que poblaciones moderadas y altas de *P. xylostella* y *P. rapae* fueron registradas en las tres estaciones, *T. ni* (fue solamente detectada en la primavera del 2005. El umbral de acción de 0.5 equivalentes del gusano medidor de repollo (EGMR) por planta fue usado para determinar la necesidad para aplicar el insecticida. La eficacia del insecticida fue determinada comparando las densidades de las larvas e inmaduros (larvas y pupas) de cada especie de plaga, la clasificación del daño en el cultivo, las densidades de los artrópodos clave que no fueron objeto del tratamiento, y el número de aplicaciones de insecticida en parcelas tratadas con cada producto versus en las parcelas no tratadas (el control). Todas las formulaciones de insecticida de riesgo reducido fueron efectivas en reducir infestaciones de las tres plagas de lepidópteros y en proveer repollo y col de hoja para la venta en Alabama. Entre los tratamientos, el Entrust® de manera consistente produjó el menor promedio de clasificación de daño con el número mínimo de aplicaciones por estación. No efectos significativos de los tratamientos de insecticida fue registrado en el número de arañas y coccinellidos encontrados por planta. Estos resultados sugieron que el umbral de acción de 0.5 EGMR puede ser usado para producir repollo y col de hoja para la venta en el estado de Alabama con solamente un mínimo número de aplicaciones de insecticidas de riesgo reducido.

Cole crops, *Brassica oleracea* (L.), including cabbage, collards, broccoli, kale, brussels sprouts, and cauliflower, are an important component of diets in many parts of the world.

Cabbage and collards are the key cole crops grown in Alabama. Growers in the state utilize both spring and fall plantings for both crops, and often grow them in rotation with other vegetables (Kemble 1999). The key lepidopteran pests of cole crops in Alabama include the diamondback moth, *Plutella xylostella* (L.), imported cabbageworm, *Pieris rapae* (L.), and cabbage looper, *Trichoplusia ni* (Hübner) (Kemble 1999). *Plutella xylostella* and *P. rapae* are often the most abundant pests in many parts of Alabama, while infestations of *T. ni* are sporadic in nature (personal observation).

Caterpillars of the three lepidopteran species do direct damage to the marketable part of the plant by chewing holes in the foliage and producing frass (Harcourt et al. 1955; Shelton et al. 1982; Talekar & Shelton 1993; Tabashnik 1994), and are usually managed as a single caterpillar complex (Mahr et al. 1993). Tolerance of damage from these caterpillars is extremely low, basically zero to trace amounts of insect damage or frass in the final product (Morisak et al. 1984). In order to avoid significant economic loss, vegetable producers have typically managed these pests with an expensive therapeutic approach involving calendar-based applications of conventional insecticides, including various organophosphate, carbamate, and pyrethroid formulations. For instance, approximately 30,000 pounds of insecticide active ingredient are used annually for collard production in Alabama (Williams & Dangler 1992). Excessive and indiscriminate use of conventional insecticides has resulted in the development of pest resistance to insecticides (Hines & Hutchison 2001; Liu et al. 2002).

Globally, formulated sprays of microbial insecticides such as *Bacillus thuringiensis* and spinosad have been used widely as an alternative to chemical insecticides. However, development of pest resistance to microbial insecticides has been reported in several locations. For instance, resistance to *Bacillus thuringiensis* subspecies *kursatki* have been detected in field populations of *P. xylostella* in various locations in the mainland U.S. (Mahr et al. 1993; Shelton et al. 1993; Tang et al. 1997), and in several other locations throughout the world including Hawaii, Malaysia, the Philippines, Japan, Central America, and Thailand (Talekar & Shelton 1993; Rueda & Shelton, 1995; Tabashnik et al. 1997). Similarly, field populations of *P. xylostella* collected in Malaysia have been reported to show resistance to spinosad (Sayyed 2004). The problem of insecticide resistance is not limited to *P. xylostella*. Resistance to *B. thuringiensis* has been demonstrated in laboratory populations of *T. ni* (Estada & Ferre 1994)

and in greenhouse populations in British Columbia (Janmaat & Myers 2003).

Traditionally, more attention has been paid to insecticide-based control programs than biological control for management of lepidopteran pests of cole crops (Taleker & Shelton 1993; Biever et al. 1994; Xu et al. 2004). Although successful integrated pest management (IPM) programs have been developed and implemented in many parts of the world (Biever et al. 1994), it appears that insecticide-based control will remain the major tactic for managing caterpillar pests of cole crops for the foreseeable future (Xu et al. 2004).

Over the past several years, numerous biologically-based insecticides with novel modes of action have been developed and shown to have a high level of efficacy on lepidopteran pests of cole crops (Eger & Lindenberry 1998; Liu and Sparks 1999; Hill & Foster 2000; Hines & Hutchison 2001). These include microbial insecticides (e.g., several formulations of spinosad and *B. thuringiensis*) and insect growth regulators. These new materials are termed "reduced-risk insecticides" because of their narrow spectrum of activity and low toxicity to humans and non-target organisms, and are considered IPM-compatible. Although reduced-risk insecticides are increasingly being used by vegetable growers worldwide, little has been done to evaluate these materials in Alabama. The objective of this study was to evaluate the efficacy of several reduced-risk insecticides against lepidopteran pests of cole crops in Alabama. The materials evaluated included three formulations of *B. thuringiensis* (Dipel®, XenTari®, and Dipel+XenTari mixture) (Valent Biosciences Libertyville, IL), Entrust® (Dow AgroSciences, Indianapolis, IN), and Novaluron (Crompton (now Chemtura), Middlebury, CT). Dipel® is a formulation of *B. thuringiensis* subspecies *kursatki* and is the most commonly used microbial insecticide on Alabama vegetable crops (Joseph Kemble, personal communication). XenTari® is a formulated spray of *B. thuringiensis* subspecies *aizawai*, while Dipel+XenTari is a premixed test formulation consisting of both subspecies of *B. thuringiensis*. Entrust® is a natural insect control product formulated for the organic grower. The active ingredient, spinosad, is developed from a fermentation by-product of the soilborne actinomycete bacterium, *Saccharopolyspora spinosa* (Liu et al. 1999). Novaluron is an insect growth regulator (IGR) that works by inhibiting chitin synthesis. It is currently labeled in the U.S. as Diamond® for use on cotton and Rimon® for use on apples, potatoes, and sweet potato, and the registrant plans to label Novaluron for use on cole crops in the near future (K. Griffith, personal communication). These materials were evaluated over multiple field seasons (2004-2005) in central Alabama.

MATERIALS AND METHODS

General Methodology

This research was conducted over three growing seasons; spring 2004, fall 2004, and spring 2005 at the E.V. Smith Research center in Shorter, AL. Treatments were arranged in a randomized complete block design with three replicates in each spring season and four in the fall 2004 season. All seedlings were obtained from a nursery in western Georgia (Lewis Taylor Farms; Ty Ty, Georgia) and were planted bareground following a preseason fire ant (*Solenopis invicta*) treatment with Amdro® (active ingredient = hydramethylnon, BASF Corporation, Research Triangle Park, NC). Standard field preparation and crop production practices (i.e., irrigation) were used to establish cabbage or collard plants in all three field seasons.

In spring of 2004 'Bravo' cabbage was mechanically transplanted on 30-III-2004. Plots were 13.7 m by 9.1 m with plants spaced 45 cm apart within a row and 90 cm between rows for a total of 300 plants per plot. Plots were separated by a 15.2-m alley. The following four reduced-risk insecticides were compared: Dipel®, Xentari®, Dipel+Xentari combination, and Entrust®. In fall 2004, 'Top bunch' collards were mechanically transplanted 2-X-2004. Plots consisted of two 10-m rows, 100 cm apart with plants spaced 45 cm apart within a row and 90 cm between rows for a total of 40 plants per plot. Five reduced-risk insecticides were compared: Dipel®, Xentari®, Dipel+Xentari combination, Entrust®, and Novaluron. In spring 2005, 'Vates' collards were mechanically transplanted at the E.V. Smith Research Station on 22-IV-2005. The plot dimensions and treatments evaluated were as described for fall 2004.

Plots were evaluated weekly for pest infestation by sampling ten randomly selected plants per plot for larvae of *P. xylostella*, *P. rapae*, and *T. ni*. Eggs and pupae of the three species also were sampled. The number of immatures of each species was calculated by summing the number of larvae and pupae. Treatment applications were made only when larval counts exceeded a threshold of 0.5 cabbage looper equivalents (CLE) per

plant (Shelton et al. 1982, 1983). The CLE method accounts for the varying levels of feeding damage caused by the three species. In this method, 1 CLE = 20 *P. xylostella* larvae = 1.5 *P. rapae* larva = 1 *T. ni* larva (Shelton et al. 1982, 1983). In addition, plants also were sampled for aphids (number of plants with aphid infestation) and key non-target predatory insects in our fields, mainly spiders and lady beetle adults (Coccinelidae). Treatment applications were made with a CO_2 pressurized backpack sprayer using a 3-ft boom with 3 nozzles calibrated to deliver about 25 gpa at 40 psi. Insecticides were applied at the recommended rates. Dipel®, Xentari®, and Dipel+Xentari were applied at the rate of 1 pound per acre, Entrust® at 2 oz per acre, and Novaluron applied at the rate of 12 fluid ounces per acre. Based on the action threshold of 0.5 CLE, the average number of insecticide applications varied by treatment and season and ranged from 1.3 to 5 applications per season (Table 1).

At harvest, ten plants were randomly selected from each plot and rated for caterpillar feeding damage and marketability was quantified by the method of Greene et al. (1969). In this method cabbage plants grown in spring 2004 were rated based on insect feeding damage on a scale of 1 to 6 as follows: 1 = no apparent insect damage on head or inner wrapper leaves; 2 = no head damage, but minor feeding on wrapper leaves with 0-1% leaf area consumed; 3 = no damage on head, but moderate feeding damage on wrapper leaves with 2-5% leaf area consumed; 4 = minor feeding on head (but no feeding through outer head leaves), but moderate feeding on wrapper or outer leaves with 6-10% leaf area consumed; 5 = moderate to heavy feeding damage on wrapper and head leaves and a moderate number of feeding scars on head with 11-30% leaf area consumed; and 6 = severe feeding damage to head and wrapper leaves with heads having numerous feeding scars with ≥30% leaf area consumed (Greene et al. 1969). A similar method was used to assess marketability of collards in fall 2004 and spring 2005 with damage rating based solely on the percent of leaf area consumed (since collards is not a head-producing plant). A damage rating of ≤3 is considered marketable under normal conditions,

TABLE 1. MEAN (± SE) NUMBER OF APPLICATIONS OF EACH REDUCED-RISK INSECTICIDE TREATMENT PER PLOT DURING EACH SEASON. TREATMENT APPLICATIONS WERE MADE ONLY WHEN WEEKLY LARVAL COUNTS EXCEEDED A THRESHOLD OF 0.5 CABBAGE LOOPER EQUIVALENTS (CLE) PER PLANT.

Treatment/formulation	Spring 2004	Fall 2004	Spring 2005
Dipel DF	2.67 ± 0.19	1.50 ± 0.14	4.33 ± 0.19
Xentari DF	2.33 ± 0.19	1.25 ± 0.13	5.00 ± 0.00
Dipel+Xentari DF	2.67 ± 0.19	1.25 ± 0.13	4.67 ± 0.19
Entrust 80WP	2.33 ± 0.19	1.25 ± 0.13	3.67 ± 0.29
Novaluron	—	1.25 ± 0.13	4.00 ± 0.00

whereas a damage rating of ≤4 is marketable only under exceptional market conditions (Leibee et al., 1995).

Statistical Analysis. For each season, mean seasonal larval and immature counts of each lepidopteran species, number of plants with aphid infestation, numbers of key non-target beneficial arthropods (i.e., spiders and lady beetle adults), and mean damage rating at harvest were calculated for each treatment. Data were transformed by the square-root method $\sqrt{(x + 0.5)}$ and analyzed for significant treatment effects by analysis of variance (ANOVA) with the plots considered as blocks. Means were compared by the Tukey-Kramer HSD comparison for all pairs (JMPIN Version 4.0.2, SAS Institute Inc., 1998). Significant differences were established at the 95% confidence level ($P < 0.05$).

RESULTS

Infestation levels of the three lepidopteran pests varied with growing season. Moderate to high populations of *P. xylostella* and *P. rapae* were recorded during all three field seasons, while *T. ni* population was recorded only in spring 2005. In general, relatively higher populations of the lepidopteran pests were recorded in both spring seasons compared with the fall season. This was reflected also in the number of applications per insecticide treatment made during each season which averaged 2.5, 1.3, and 4.3 for spring 2004, fall 2004, and spring 2005, respectively (Table 1). In both spring seasons, caterpillar pest pressure as measured by CLE per plant per week in un-treated control plots began two weeks after planting and moderate caterpillar pressure was observed through harvest in spring 2004 (Fig. 1). Extremely high caterpillar pressure was recorded late in spring 2005 with CLEs greater than 3.5 per plant per week recorded in the last two weeks of the season (Fig. 1). In the lone fall season (fall 2004), however, caterpillar pest infestation did not begin until six weeks after planting, averaging less than 0.5 CLE per plant per week for the remainder of the season (Fig. 1). In general, no significant block (plot) effects were detected ($P > 0.05$) for any of the dependent variables in any of the seasons, suggesting that the plots were similar in pest abundance and treatment efficacy.

In spring 2004, all four reduced-risk insecticides resulted in reductions in the number of *P. xylostella* larvae ($F = 9.5$, $df = 4$, $P = <0.0001$) and immatures ($F = 8.9$, $df = 4$, $P = <0.0001$), and *P. rapae* larvae ($F = 3.3$, $df = 4$, $P = <0.0001$) and immatures ($F = 20.3$, $df = 4$, $P = <0.0001$) compared with the untreated control (Fig. 2A). However, significantly higher numbers of *P. rapae* immatures were recorded for Dipel® compared with the other insecticide treatments. Higher damage ratings were recorded in untreated control plots than in any of the treatments ($F = 65.3$, $df = 4$, $P = <0.0001$; Fig. 3A). Comparing the treatments, mean damage ratings were significantly lower in Entrust® than in Dipel+Xentari combination. No significant effects of insecticide treatments were recorded in the number of plants with aphids ($F = 0.3$, $df = 4$, $P = 0.89$; Table 2), and in the numbers of spiders ($F = 0.7$, $df = 4$, $P = 0.62$) or lady beetles ($F = 1.2$, $df = 4$, $P = 0.30$) found per plant (Fig. 4A).
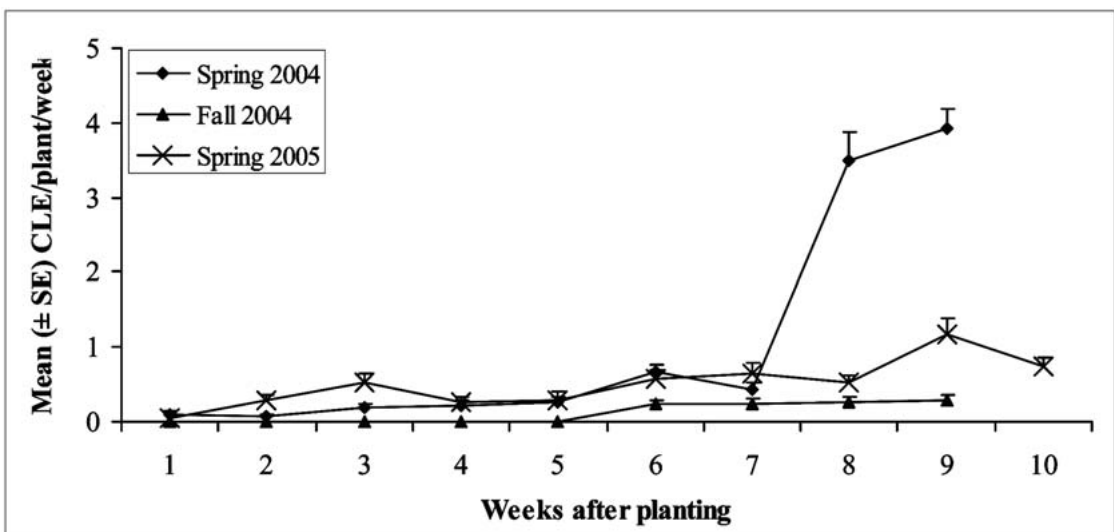

Fig. 1. Caterpillar pressure expressed as mean (± SE) number of cabbage looper equivalents (CLE) per plant recorded weekly after planting in untreated control plots during spring 2004, fall 2004, and spring 2005. Planting dates for spring 2004, fall 2004, and spring 2005 were March 30 2004, October 2 2004, and April 22 2005, respectively.
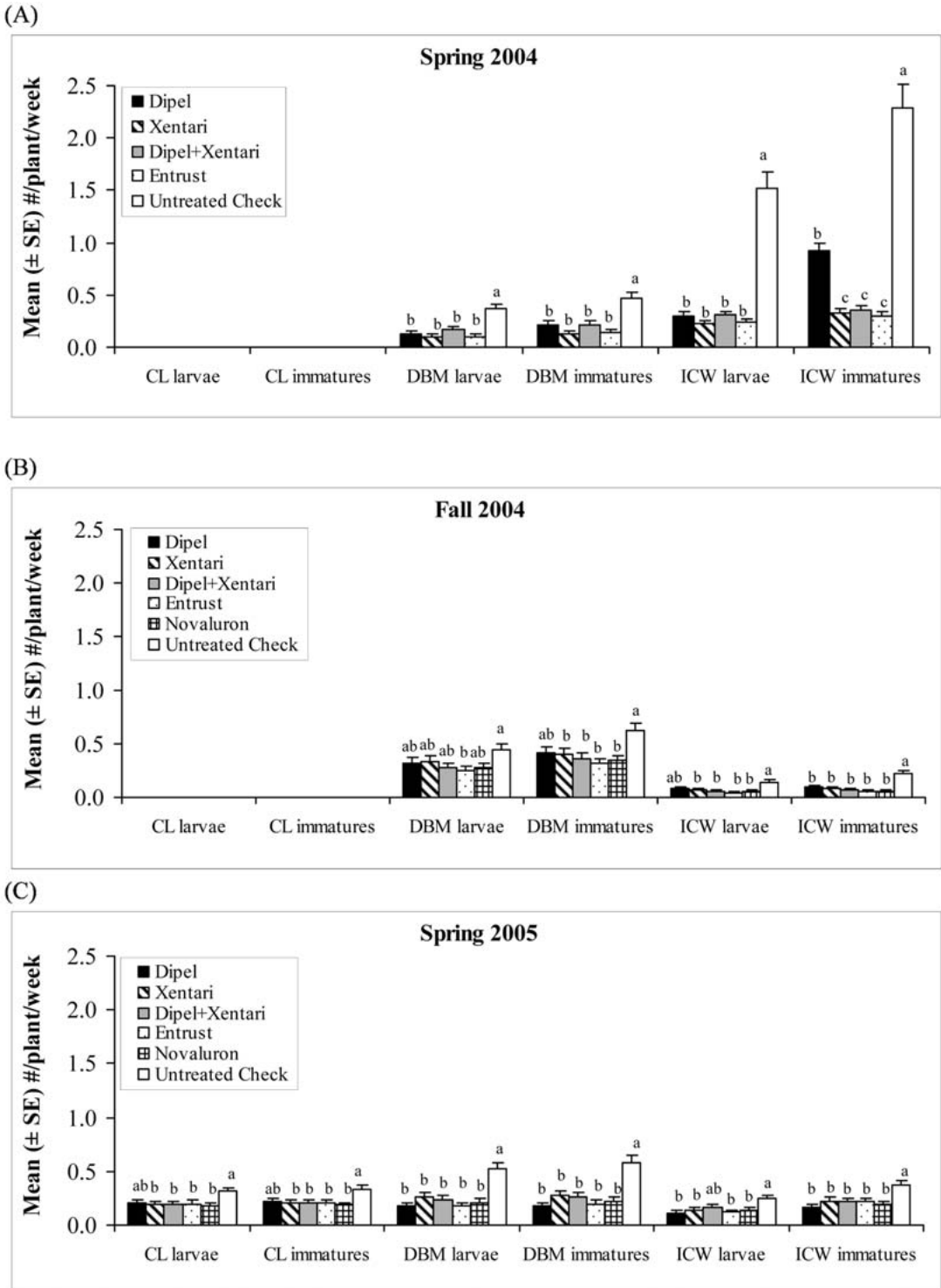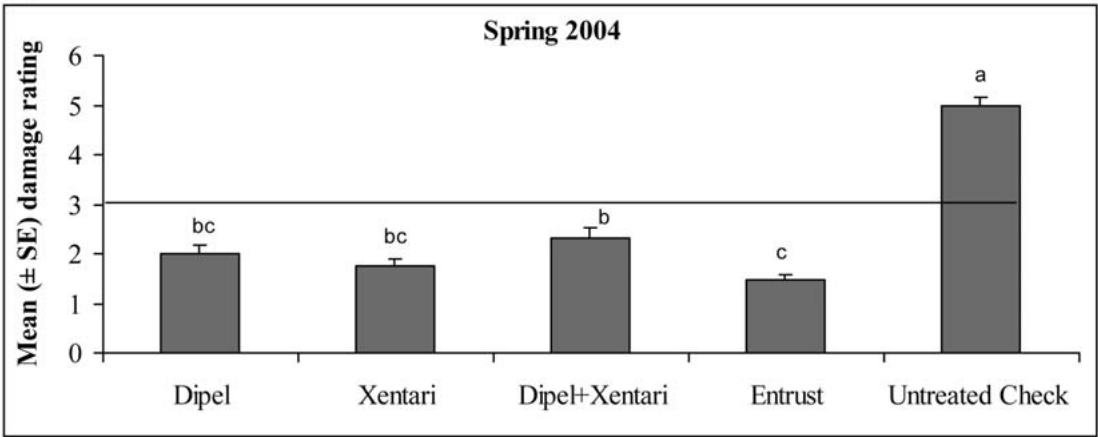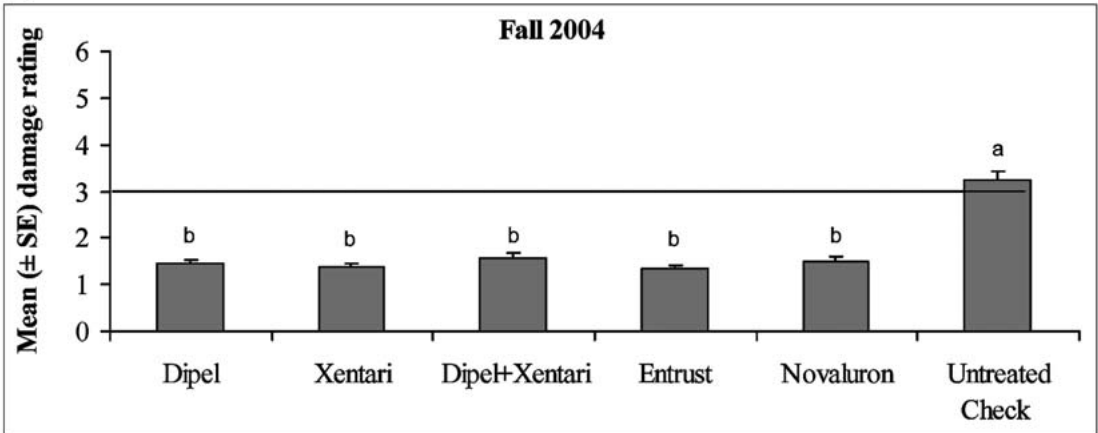
Fig. 2. Seasonal mean (± SE) number of larvae and immatures of lepidopteran species sampled per plant per week in plots treated with different reduced-risk insecticides during spring 2004 (A), fall 2004 (B), and spring 2005 (C). Key: CL = cabbage looper (*Trichoplusia ni*); DBM = diamondback moth (*Plutella xylostella*); ICW = imported cabbageworm (*Pieris rapae*). Means followed by the same letter are not significantly different ($P > 0.05$, Tukey-Kramer HSD).
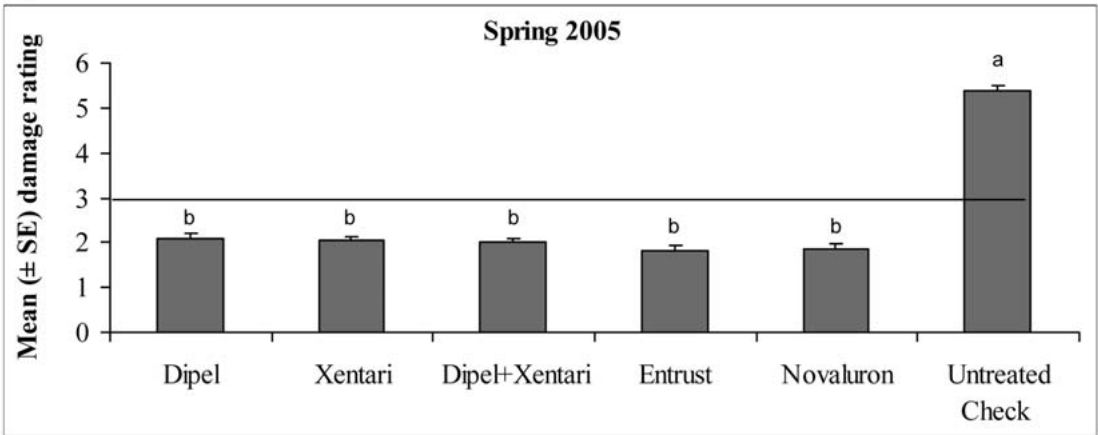
Fig. 3. Mean (± SE) damage ratings of plants harvested from plots treated with different reduced-risk insecticides during spring 2004 (A), fall 2004 (B), and spring 2005 (C). Line indicates marketability threshold of 3 above which produce is considered unmarketable. Means followed by the same letter are not significantly different ($P >$ 0.05, Tukey-Kramer HSD).

TABLE 2. SEASONAL MEAN (± SE) NUMBER OF PLANTS WITH APHID INFESTATION IN PLOTS TREATED WITH DIFFERENT REDUCED-RISK INSECTICIDES.

Treatment/formulation	Spring 2004	Fall 2004	Spring 2005
Dipel DF	0.019 ± 0.008	0.025 ± 0.008	0.033 ± 0.01
Xentari DF	0.019 ± 0.008	0.030 ± 0.009	0.043 ± 0.01
Dipel+Xentari DF	0.026 ± 0.01	0.028 ± 0.009	0.043 ± 0.01
Entrust 80WP	0.030 ± 0.01	0.030 ± 0.009	0.033 ± 0.01
Novaluron	—	0.019 ± 0.007	0.047 ± 0.01
Untreated Check	0.022 ± 0.009	0.056 ± 0.01	0.037 ± 0.01
P	0.89	0.10	0.93
No. plants sampled per treatment (n)	270	360	300

In fall 2004, a treatment effect was recorded for *P. xylostella* larvae ($F = 2.3$, $df = 5$, $P = 0.04$). However, only Entrust® resulted in significant reduction in *P. xylostella* larvae compared with the untreated control; no significant differences were recorded for the other treatments (Fig. 2B). With the exception of Dipel®, all treatments reduced *P. xylostella* immatures ($F = 4.4$, $df = 5$, $P = 0.0006$) and *P. rapae* larvae ($F = 5.3$, $df = 5$, $P < 0.0001$). Nonetheless, higher density of *P. rapae* immatures was recorded in the untreated control than in any of the treatments ($F = 11.3$, $df = 5$, $P < 0.0001$). All five treatments had lower mean damage ratings in comparison with the untreated control ($F = 38.7$, $df = 5$, $P < 0.0001$; Fig. 3B). No effects of treatments were recorded in the number of plants with aphids ($F = 1.8$, $df = 4$, $P = 0.10$; Table 2), and in the numbers of spiders ($F = 1.5$, $df = 4$, $P = 0.20$) or lady beetles ($F = 0.7$, $df = 4$, $P = 0.62$) found per plant (Fig. 4B).

In spring 2005, *T. ni* was collected in the field, whereas it was not present during the previous two seasons (Fig. 2C). In general, all treatments resulted in significant reductions in pest populations (Fig. 2C). All treatments except Dipel® reduced densities of *T. ni* larvae ($F = 3.3$, $df = 5$, $P = 0.006$) and immatures ($F = 3.7$, $df = 5$, $P = 0.003$) compared with the untreated control (Fig. 2C). For *P. xylostella*, lower numbers of larvae ($F = 8.1$, $df = 5$, $P < 0.0001$) and immatures ($F = 9.7$, $df = 5$, $P < 0.0001$) were recorded for all treatments compared with the untreated control. Similar treatment effects were recorded for *P. rapae* larvae ($F = 3.9$, $df = 5$, $P = 0.002$) and immatures ($F = 4.1$, $df = 5$, $P = 0.001$); however, *P. rapae* larval counts in plots treated with the Dipel+Xentari formulation were not significantly lower than larval counts in untreated control plots (Fig. 2C). A mean damage rating of 5.4 was recorded in the untreated control which was higher ($F = 101.4$, $df = 5$, $P < 0.0001$) than damage ratings in any of the five treatments (Fig. 3C). In all three seasons, mean damage ratings recorded in the treated plots were never above the marketability threshold of 3 (Green et al. 1969). No differences were recorded among the treatments in the number of

plants with aphids ($F = 0.26$, $df = 4$, $P = 0.93$; Table 2), numbers of spiders per plant ($F = 1.2$, $df = 4$, $P = 0.30$), and numbers of lady beetles per plant ($F = 0.8$, $df = 4$, $P = 0.55$) (Fig. 4C), suggesting little or no effects of insecticide treatments on the key non-target predators in our plots.

DISCUSSION

The goal of this study was to evaluate the efficacy of various reduced-risk insecticides in providing acceptable control of lepidopteran pests of cole crops in Alabama. In all three seasons, all materials tested resulted in the production of marketable produce with considerably lower pest pressure and crop damage ratings compared with untreated control plots which never yielded marketable produce. These results indicate that all five reduced-risk insecticides were effective in controlling lepidopteran pests of cole crops in Alabama. The results also suggest that the 0.5 CLE action threshold recommended by Shelton et al. (1982, 1983) can be used to produce marketable cabbage and collards in Alabama with only minimal applications of reduced-risk insecticides, particularly in locations with minor or no endemic populations of *T. ni*. Although resistance evaluation was not the primary goal of this study, our results confirming the high efficacy of the various microbial insecticides tested in this study may suggest that *P. xylostella* resistance to *B. thuringiensis* is currently not a major problem in central Alabama, considering that vegetable growers in this region have been applying Dipel® in their fields for years.

Although we did not always find significant differences among the reduced-risk insecticides tested in this study, Entrust® consistently produced the lowest mean damage ratings (although not always significant) with the least mean number of applications per season. The relatively higher efficacy of Entrust® recorded in this study may be due to its broad spectrum activity and multiple mode of entry. Entrust® differs from the other materials evaluated in this study in that it successfully kills insects from several orders, whereas the other treatments are selective to lep-
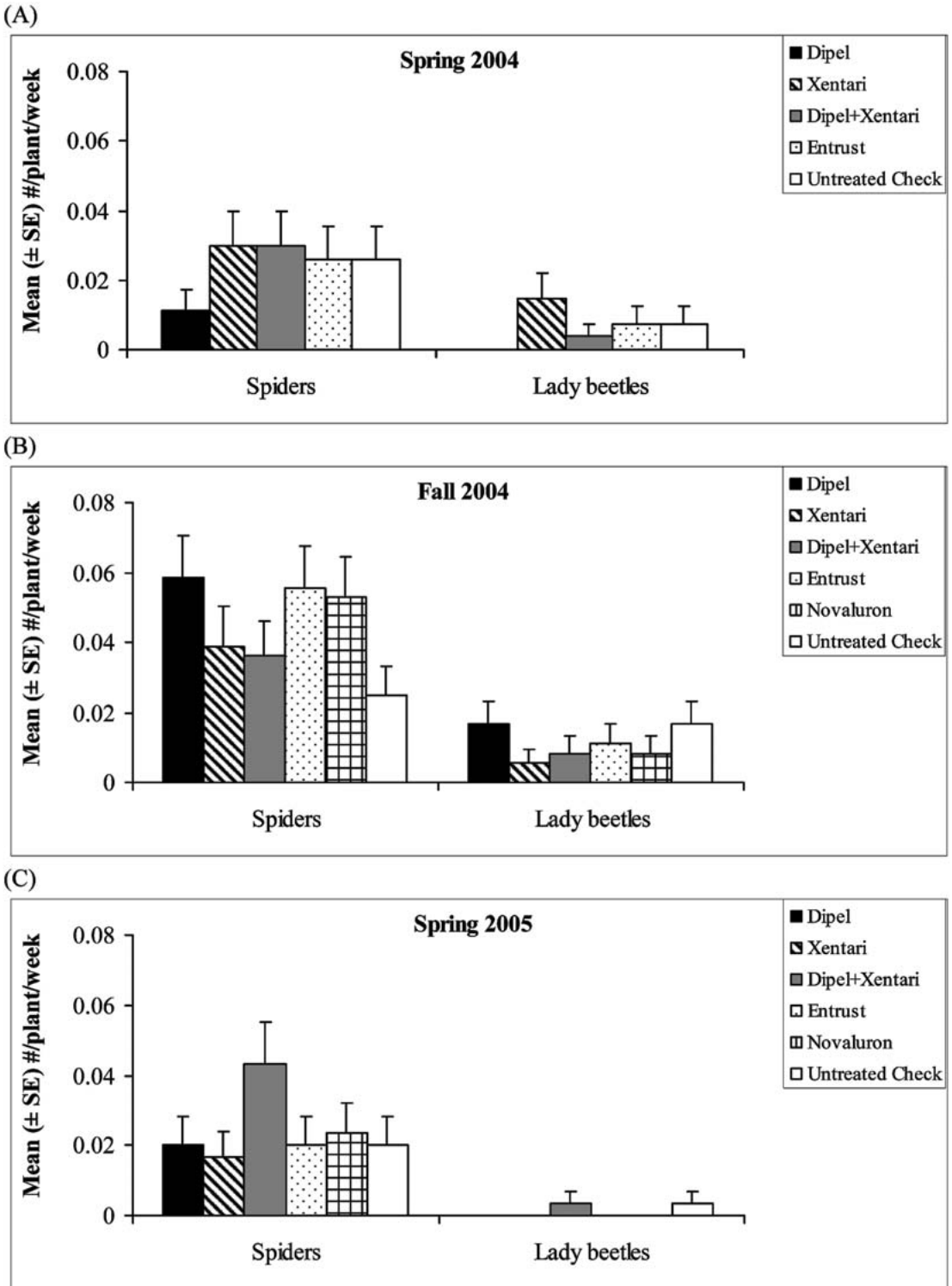
Fig. 4. Seasonal mean (± SE) number of non-target spiders and lady beetle adults found per plant per week in plots treated with different reduced-risk insecticides during spring 2004 (A), fall 2004 (B), and spring 2005 (C). Means are not significantly different (*P* > 0.05, Tukey-Kramer HSD).

idopterans only (Cisneros et al. 2002). In addition, spinosad, the active ingredient in Entrust® has both contact and ingestion activity (Eger & Lindenberry 1998; Liu et al. 1999), whereas the other reduced-risk insecticides must be eaten by the insects in order to be effective. It is thought that the broad spectrum activity of Entrust® will probably ensure some control of non-lepidopteran pests such as cruciferous flea beetles, harlequin bugs, aphids, and other minor pests that the other chemicals were not effective against. However, we did not observe in the current study a significant reduction in aphid-infested plants in Entrust®-treated plots compared to the other treatments or control. On the other hand, spinosad has been reported as toxic to beneficial insects such as *Diadegma insulare* (Cressons) (Hymenoptera: Ichneumonidae) (Xu et al. 2004), a very common and effective parasitoid of *P. xylostella* in North America (Mahr et al. 1993). Hill & Foster (2000) showed a 100% *D. insulare* mortality rate after 8 h of exposure to spinosad-treated brassica leaves, while Cisneros et al. (2002) recorded up to 98% mortality of predators exposed to high concentrations of this microbial insecticide. However, we did not record any significant effect of Entrust® or any of the other treatments on numbers of spiders and lady beetles, the two most important predators in our fields. Entrust® thus appears to be a promising tool for use in cole crop pest management and insecticide resistance management programs, considering that the active ingredient, spinosad has not been reported to share cross-resistance mechanisms with any other group of insecticides (Liu & Yue 2000; Wei et al. 2001). In general, Xentari® was second to Entrust® in producing acceptable damage ratings. However, the fact that this material had the highest average number of applications per season suggests that it may not provide economically acceptable control compared to the other treatments.

Significant variations in the populations of the three lepidopteran pests were recorded from season to season. In general, lepidopteran pest pressure was higher in both spring seasons than in the fall. Significant *P. xylostella* pressure was recorded in both spring seasons and in the fall, whereas *P. rapae* pressure was highest in spring 2004 followed by spring 2005. Furthermore, we recorded during spring 2004 about 60 flying *P. rapae* adults per plot in 5-min visual observations compared to about 8 flying adults in fall 2004, suggesting that this pest may be more severe in the spring than in the fall. The detection of *T. ni* in spring 2005 may have exacerbated total pest pressure during this season resulting in above threshold CLEs and the need to apply insecticides at a much higher frequency than in the first two seasons. This is especially likely since *T. ni* is the most voracious and damaging of the three pests (Shelton et al. 1982; Hines & Hutchison 2001). The reason

for the detection of *T. ni* only in spring 2005 may be due to later planting date for this season. In summary, our results confirmed the efficacy of the tested reduced-risk insecticides in managing direct pests of cole crops in Alabama in a threshold-based IPM program. These reduced-risk insecticides offer a wide range of pest management options available to vegetable growers and should be used wisely or in rotation with one another to minimize selection for resistance to any one given material. Obviously, the longevity of these new insecticides as effective IPM tools will depend on their judicious use, compatibility with natural enemies, and cost effectiveness, among other factors.

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HOST SELECTION BEHAVIOR OF *LEPTOPHOBIA ARIPA* (LEPIDOPTERA: PIERIDAE)

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Abstract

Host selection and egg laying behavior of wild populations of the mountain white butterfly, *Leptophobia aripa* (Boisduval), was observed in the presence of a group of host plants (*Brassica oleracea* L. var. *capitata*) of varying quality. Host variation was generated by manipulating three crop management variables: fertilization, water, and light. *Leptophobia aripa* was not indifferent to host quality variation, and showed great ability to evaluate and discern among a group of hosts. A sigmoidal relation was found between egg laying and host plant size. The latter was probably perceived through the host's diameter, or other physical and chemical characteristics related to this attribute. More detailed studies are necessary in order to understand which cues this insect uses to locate its host and which other attributes it evaluates upon deciding to lay eggs. This understanding could allow for the development of agro-ecological alternatives in controlling this insect, considered to be a crop pest in some regions of Mexico and Central America.

Key Words: mountain white butterfly, *Brassica oleracea*, host plant selection, host quality

Resumen

Se observó el comportamiento de selección y oviposición de poblaciones silvestres de *Leptophobia aripa* (Boisduval) ante un conjunto de plantas hospederas (*Brassica oleracea* L. var. *capitata*) de distintas calidades, generadas mediante cambios en tres condiciones de manejo del cultivo: fertilización, riego y luz. Su comportamiento no fue indistinto a las diferentes calidades de hospedera, sino que obedeció a una compleja selección. Mostrando una gran capacidad para evaluar y discriminar entre el conjunto de hospederas. Se encontró una relación altamente no lineal entre la oviposición y el tamaño de la planta, probablemente percibida a través del diámetro de la hospedera, o por otras características físicas y químicas relacionadas con este atributo. Son necesarios estudios más detallados que contribuyan a entender cuáles son las señales que este insecto usa para localizar su hospedera y que otros atributos evalúa al tomar la decisión de ovipositar. Esto permitiría desarrollar alternativas agroecológicas para su control, dado que en algunas regiones de México y Centroamérica se le considerar como plaga.

Translation provided by the authors.

All herbivorous insects show some degree of host selectivity. Most adult holometabolous species must select an appropriate host for larval growth and survival (Bernays & Chapman 1994). Under natural conditions, insects confront many external stimuli, their own internal physiological stimuli, and a series of environmental constraints (Visser 1986; Bernays & Chapman 1994; Badenes et al. 2004). This makes it very difficult to discern the relative importance to the insect of chemical, visual, and mechanical stimuli from host and non-host plants (Schoonhoven et al. 1998; Hooks & Johnson 2001). However, it is generally assumed that the host selection process in specialist insects is governed primarily by volatile chemical signals, later by visual stimuli, and finally by non-volatile chemical signals (Hern et al. 1996; Hooks & Johnson 2001).

Female butterflies reject many potential hosts when searching for egg laying sites. They demonstrate a hierarchy in host preferences, discriminating among plant species, among genotypes, among individuals with different phenological and physiological conditions, and even among plant parts, although not all discriminate at the finer scales (Thompson & Pellmyr 1991; Bernays & Chapman 1994). However, this knowledge is derived from studies of very few insect species (Bernays & Chapman 1994; Schoonhoven et al. 1998). Furthermore, there may be significant behavioral differences within a family, among species of the same genus, or even among different

populations of the same species (Jones 1977; Singer & Parmesan 1993; Reich & Downes 2003).

To this date, there are no studies on host selection behavior of the mountain white butterfly, *Leptophobia aripa* (Boisduval). This insect is a multivoltine species with overlapping generations. Females lay masses of 15 to 80 eggs (Bautista & Vejar 1999). The mountain white butterfly specializes in the family Brassicaceae, and it is an important pest of Brassica crops in Southeastern Mexico, Central America, and the Caribbean (CATIE/MIP 1990, Santiago et al. in press). However, it is not known which plant physiological stage is best suited for oviposition of *L. aripa*. In the case of cultivated plants, crop management choices may determine the quality of the plant as a host (Andow 1991).

The objective of the present study was to observe the egg laying behavior of *L. aripa* in host plant patches (*Brassica oleracea* L. var. *capitata*) of different qualities.

MATERIALS AND METHODS

The experiment was established in the Valley of San Cristóbal de Las Casas Chiapas, México (2,113 m.a.s.l.; C(w_2)(w); García 1973) within the cabbage production area of the Highlands of Chiapas. Cabbage plants of the variety Copenhagen Market were started in seed beds. Twenty five days after germination, each seedling was transplanted to a black plastic bag (20 cm high by 15 cm in diameter). The bag contained a 1:1 proportion of clay-loam forest soil and sand.

Sixty four plants were prepared. These were divided into eight groups of eight plants each, and placed in a greenhouse. In order to generate different host qualities, each group was submitted to one of eight treatments for 40 days. These treatments consisted of all possible combinations of two fertilization levels, two watering levels, and two photosynthetically active radiation (PAR) levels (Table 1). Nitrogen fertilization was equivalent to 100 kg Ha^{-1}, the most common dose ap-

plied to cabbage in the study zone (Santiago et al. in press). Treatments were irrigated with high or low water treatments every four and eight days, respectively, from August 1 to September 20, 2002. Accumulated irrigations (326 and 183 mm, respectively) were roughly equivalent to the high (320 mm) and low (195 mm) average cumulative rainfalls during the same period, to be found within the cabbage production zone where *L. aripa* was studied (Cervantes 1997).

Sixty five days after germination, the bagged plants were moved to an open field 200 m from a cabbage field to promote visits from wild populations of *L. aripa*. The 64 bags were randomly distributed in a square pattern without contiguous repetitions (Hurlbert 1984), with 50 cm between plants. Watering treatments were continued throughout the time of the plants' exposure to *L. aripa*.

For five days, *L. aripa*'s flights during host location and egg laying behavior were observed (for 1 h per day between 10 a.m. and 2 p.m.) and this information was recorded. A total of 28 individuals were observed from the time they entered until they left the group of host plants. The behavior of 8 females (that actually laid eggs during the five recorded hours) was classified into four types of acts: linear flight, turning flight, landing and egg laying. Each behavioral act was recorded on an experiment layout map.

The cabbage plants were reviewed daily in the afternoon (5 to 5:30 p.m.) for 11 days, and the number of eggs laid per plant during 9 h of exposure (8 a.m. to 5 p.m.) was recorded. After being counted, the eggs were carefully removed with a damp flannel cloth, in order to avoid hatching and to minimize visual or chemical stimuli from the eggs which could inhibit egg laying of conspecific females (Bernays & Chapman 1994). Hilker & Meiners (2002) reported for *Pieris brassicae* (L.) that egg removal might not completely eliminate such stimuli. However, in this study, *L. aripa* laid eggs repeatedly on most plants from which previously laid eggs were removed.

TABLE 1. DESCRIPTION OF FACTORS AND LEVELS FOR THE TREATMENTS. EACH LEVEL OF A FACTOR WAS COMBINED WITH BOTH LEVELS OF THE OTHER TWO FACTORS.

Factors	Level 1	Level 2
Nutrient (N)	N1: Without fertilizer.	N2: Foliar fertilizer (20% N - 30% P - 10% K - 1.6% micronutrients) at a dose of 12.5 g per plant. N dose equivalent to 100 kg Ha^{-1}. Applied 15 days after transplanting.
Water (W)	W1: Watered with a total of 3,240 ml over a period of 51 days. Equivalent to 183 mm of rainfall from August 1 to September 20.	W2: Watered with a total of 5,760 ml over a period of 51 days. Equivalent to 326 mm of rainfall from August 1 to September 20.
PAR(L)	L1: Mesh shade which eliminated 64% of the photosynthetically active radiation inside the greenhouse.	L2: 100% of the photosynthetically active radiation inside the greenhouse.

Each afternoon after sampling, the group of plants was enclosed with greenhouse plastic in order to prevent them from receiving rain water and additional butterfly visits.

Eighty two days after planting, the height and diameter of plants were measured, and above ground biomass was harvested to determine fresh weight per plant. Also, a 2-cm^2 leaf sample was taken from each plant for determining the foliar nitrogen and chlorophyll concentrations with standard methods (AOAC 1999).

The experiment was designed to relate oviposition to host plant management treatments, assuming that the latter produce variation in host plant parameters that are relevant for egg-laying behavior (Myers 1985; Hern et al. 1996; Hooks & Johnson 2001). To check this assumption, we also explored to what extent such variation was actually produced by treatments. Nutrient, water, and light treatment effects on plant height, diameter, aboveground fresh weight, leaf nitrogen concentration, and leaf chlorophyll concentration were analyzed with three-factor ANOVAs (Underwood 1997).

Because egg laying counts did not meet assumptions of normality due to numerous zero counts (Underwood 1997), statistical analysis was performed by logistical regression (Agresti 1996).

A step-wise multiple linear regression analysis was carried out between the number of eggs laid and the five parameters measured for each plant. A non-linear regression model was fitted between the number of eggs laid and that factor best explaining the egg-laying pattern observed in the linear model. Factors discarded in the linear model were proven to be non significant for the non-linear model as well. The non-linear regression model was fitted and selected with the program TableCurve™ 2D (AISN Software, Inc. 1994). The statistical software SPSS version 10.0.5 (1999) was used for the remaining analyses.

RESULTS

When a female *L. aripa* entered the host plant patches, on average 64% of behavioral acts were turning flights over the potential hosts, possibly for recognition and evaluating purposes. Landing on the host comprised 12% of behavioral acts. Egg laying was always preceded by a turning flight. Linear flights also were observed. The latter alternated with turning flights and landings. Sixty percent of linear flights were over lesser-quality hosts (e.g., non-fertilized plants). A typical search behavior in egg-laying *L. aripa* females is shown in Fig. 1, which shows that the butterfly flew over almost the entire group of plants and selectively laid eggs on up to four different highest-quality hosts.

The logistical regression model (maximum likelihood test: $\chi^2 = 14.001$, $df = 3$, $P = 0.003$) showed a greater probability of oviposition on fertilized plants (N2) than on non-fertilized plants

(N1) ($\chi^2_{Wald} = 4.163$, $df = 1$, $P = 0.041$). There was a marginally greater egg laying probability for plants which received more watering (W2) than on those which were watered less (W1) ($\chi^2_{Wald} = 3.212$, $df = 1$, $P = 0.073$). The probabilities of laying eggs on plants with a greater (L2) and lesser (L1) PAR availability were not different ($\chi^2_{Wald} = 0.965$, $df = 1$, $P = 0.326$) (Fig. 2).

None of the interactions among the three factors was significant: Nutrient × Watering ($\chi^2_{Wald} = 0.288$, $df = 1$, $P = 0.591$). Nutrient × PAR ($\chi^2_{Wald} = 0.039$, $df = 1$, $P = 0.843$). Watering × PAR ($\chi^2_{Wald} = 0.088$, $df = 1$, $P = 0.767$). Nutrient × Watering × PAR ($\chi^2_{Wald} = 0.021$, $df = 1$, $P = 0.885$).

Nutrient, watering, and PAR caused significant variation in physical and chemical plant parameters evaluated in this study (Tables 2 and 3). Fertilized plants (N2) were taller, had a greater diameter, greater fresh weight, greater nitrogen concentration, and greater chlorophyll concentration than non-fertilized plants (N1). Plants receiving more water (W2) had a greater diameter and greater fresh weight, but similar height, nitrogen concentration, and chlorophyll concentration as compared to less watered plants (W1). Plants exposed to greater PAR availability (L2) were the shortest, had a smaller diameter, less fresh weight, greater nitrogen concentration, and similar chlorophyll concentration as compared to plants with less available PAR (L1). (Some of these effects of PAR reduction were possibly caused by better soil humidity conservation in shaded bags).

Significant Nutrient × Watering interactions were found for plant weight and crown diameter. These plant parameters did not respond to nutrient addition at low watering levels, but responded strongly at high watering levels (Table 2). Significant Nutrient × PAR interactions were found for nitrogen concentration.

The step-wise multiple linear regression analysis determined that fresh weight is the parameter that best explains variation in the number of eggs laid per plant ($R^2 = 0.61$, $df = 59$, $F = 90.731$, $P < 0.0005$). The other four attributes evaluated proved to be non-significant (diameter, $P = 0.248$; height, $P = 0.245$; chlorophyll, $P = 0.615$; nitrogen, $P = 0.779$). When fresh weight was not included in the analysis, the only parameter selected as significant was diameter ($R^2 = 0.39$, $df = 59$, $F = 36.782$, $P < 0.0005$). Again, the other three parameters were not significant (height, $P = 0.905$; chlorophyll, $P = 0.718$; nitrogen, $P = 0.743$).

A non-linear regression model was fitted between fresh weight and number of eggs per plant. The best among biologically reasonable models was a sigmoidal function. This function shows an abrupt increase in the response variable when the fresh weight of the plant exceeds a threshold, estimated for this study to be between 30 and 40 g (Fig. 3).

ENTRANCE

Fig. 1. Schematized search behavior in an egg-laying female of *L. aripa* upon entering the group of mixed-quality hosts. Shaded cells indicate where eggs were laid, each cell contains a replicate of a determined treatment. For example N2W2L2 corresponds to fertilized plants, with more watering and with more PAR availability (see Table 1 for notation; only plants intersected by flight are labeled). Note that 11 out of 15 turning flight occurred over N2W2 plants, and all four cases of egg laying occurred on this same class of plants.

DISCUSSION

In this study, *L. aripa* was offered heterogeneous patches of hosts. Its egg laying behavior was not arbitrary or indifferent to options presented; rather the butterfly showed a capacity to evaluate and discriminate among the group of hosts. Selec-

tion behavior is common among Pieridae, but had not been previously documented for *L. aripa*.

Many studies have shown that Pieridae larvae survive and grow better on well fertilized and well watered Brassicaceae plants (e.g., Myers 1985; Chen et al. 2004). *Leptophobia aripa* preferred to lay eggs on plants that were fertilized and which
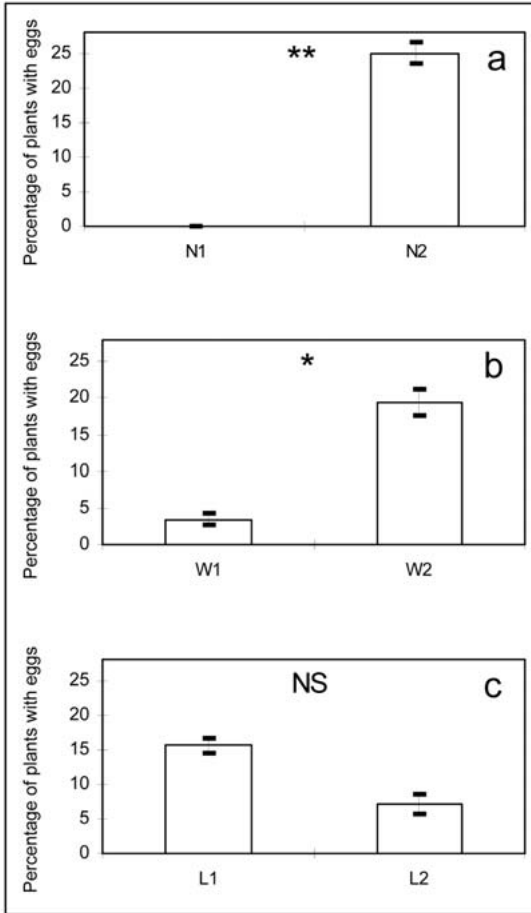
Fig. 2. Average percentage of cabbage plants on which *L. aripa* laid eggs (taken from 11 samples). a) N1: non fertilized plants, and N2: fertilized plants (**$P <$ 0.05). b) W1: plants with less watering, and W2: plants with more watering (*$P < 0.1$). c) L1: plants with lesser PAR availability, and L2: plants with greater PAR availability. Error bars: ± 1 SE.

grew under conditions of greater soil humidity. In this study, host size, probably perceived as foliar crown diameter, was the plant parameter factor associated to host preference by *L. aripa*. Host size increased significantly when both nutrient addition and high watering levels were present. Other plant parameters commonly modified by management (Chen et al. 2004), such as volatiles that act as cues and/or stimulate oviposition, were not studied and cannot be ruled out.

No single host management factor or host parameter has explained selection by Pieridae, and the importance of different factors varies and remains controversial. One of the species most closely related to *L. aripa* is *Pieris rapae* (L.), whose egg laying behavior has been widely stud-ied, but remains controversial. For instance, Root & Kareiva (1984) reported that *P. rapae* follows a random flight host search, and lays eggs without discriminating quality factors. Renwick & Radke (1983) found that *P. rapae* was not attracted by volatile host cues. They also found that host size and form were not important in egg laying behavior. Radcliffe & Chapman (1966) did not find a correlation between plant size and *P. rapae*'s egg laying preference. They concluded that color or chemical stimuli could be determining factors in host choice. In contrast, other authors have demonstrated that *P. rapae*'s flight and egg laying patterns are modified by factors such as plant size, phenology, species, humidity content, nutrients, leaf color and plant chemistry (Jones 1977; Latheef & Irwin 1979; Myers 1985; Andow et al. 1986; Jones et al. 1987; Hern et al. 1996; Hooks & Johnson 2001).

Another related species is *Pieris virginiensis* (Edwards). Flight and egg laying patterns of *P. virginiensis* are very similar to those of *P. rapae*. Their flight is markedly linear; they widely disperse their eggs, and leave behind apparently attractive hosts. Their egg laying behavior does not respond to host-plant size (Cappuccino & Kareiva 1985).

Egg laying behavior observed for *L. aripa*, unlike that reported for *P. rapae* and *P. virginiensis*, did respond to plant size. We found a sigmoidal relation, as would be expected with species that lay eggs in masses and confront host quality heterogeneity (Roitberg et al. 1999). Perhaps *L. aripa* perceived size through the host's foliar crown diameter, as this was the second most important plant parameter explaining host selection.

Host selection by *Leptophobia aripa* also could have occurred through other size-related physical and chemical characteristics not evaluated in this study. These signals could play an important role in other ecological interactions. For example, *Pieris napi* (L.) uses *Arabis gemmifera* (Mastum.) as a plant host. This plant species grows covered by neighboring vegetation, and for this reason is a host of inferior quality (in nutritional content and biomass), but it allows *P. napi* to avoid parasitism by the *Cotesia glomerata* (L.) wasp and the *Epicampocera succincta* (Meigen) fly (Ohsaki & Sato 1999).

Fertilization and watering treatments also could have modified the plant's chemical composition; in the case of members of Brassicaceae family, it could modify glucosinolate concentrations (Myers 1985; Mewis et al. 2002; Chen et al. 2004). These secondary metabolites are produced by the plants as a chemical defense (Renwick & Radke 1983; Lambdon et al. 2003; Müller et al. 2003). Specialized insects sometimes use these compounds as chemical cues, and even incorporate them into their body and use them to defend against predators and parasitoids (Messchendorp et al. 2000; Mewis et al. 2002). Several crucifer insects are known to have glucosinolate detoxification and sequestration mechanisms (Wadleigh &

TABLE 2. SUMMARY OF RESULTS FROM THREE-FACTOR ANOVAS TESTING THE EFFECTS OF NUTRIENT (N), WATER (W), AND PAR (L) ON PLANT HEIGHT, DIAMETER, ABOVE-GROUND FRESH WEIGHT, LEAF NITROGEN CONCENTRATION, AND LEAF CHLOROPHYLL CONCENTRATION. TEST OF SIGNIFICANT P VALUES < 0.05 ARE IN BOLD.

Source	df	Height		Diameter		Weight		Nitrogen		Chlorophyll	
		MS	F	MS	F	MS	F	MS	F	MS	F
N	1	1.6	14.9	619.1	38.0	30.7	41.2	0.2	11.9	2.3	13.5
W	1	0.4	3.7	377.5	23.2	10.1	13.6	0.0	0.1	0.2	1.2
L	1	1.6	14.8	691.5	42.5	24.9	33.4	0.2	9.0	0.3	1.8
NXW	1	0.1	0.6	258.6	15.9	5.2	7.0	0.1	2.8	0.0	0.1
NXL	1	0.4	4.0	23.0	1.4	1.6	2.1	0.2	9.0	0.0	0.0
WXL	1	0.3	2.7	30.9	1.9	2.5	3.4	0.0	0.6	0.3	1.5
NXWXL	1	0.3	3.1	55.8	3.4	2.9	3.9	0.0	0.4	0.0	0.2
Error	52	0.1		16.3		0.7		0.0		0.2	

TABLE 3. MEAN (± 1 SE) OF PLANT HEIGHT, DIAMETER, ABOVE-GROUND FRESH WEIGHT, LEAF NITROGEN CONCENTRATION, AND LEAF CHLOROPHYLL CONCENTRATION FOR EACH FACTOR LEVEL.

Factor	Height	Diameter	Weight	Nitrogen	Chlorophyll
N1	7.9 (0.5)	12.3 (0.9)	8.0 (1.2)	4.0 (0.1)	0.5 (0.1)
N2	11.2 (0.7)	19.6 (1.3)	37.7 (5.5)	4.6 (0.2)	0.9 (0.1)
W1	8.7 (0.6)	13.3 (1.0)	12.5 (2.0)	4.3 (0.2)	0.7 (0.1)
W2	10.2 (0.7)	17.9 (1.4)	30.6 (5.6)	4.3 (0.1)	0.6 (0.1)
L1	10.7 (0.5)	18.9 (1.0)	28.2 (4.8)	4.1 (0.1)	0.8 (0.1)
L2	8.1 (0.7)	12.0 (1.3)	14.5 (3.9)	4.5 (0.2)	0.6 (0.1)

Yu 1988). Müller et al. (2003) did not find glucosinolate sequestration in *P. rapae* and *P. brassicae*; the case for *L. aripa* still needs to be studied.

Another manner in which *L. aripa* could be attracted to larger plants is that observed in *P. brassicae*. This species, like *L. aripa*, tends to lay eggs
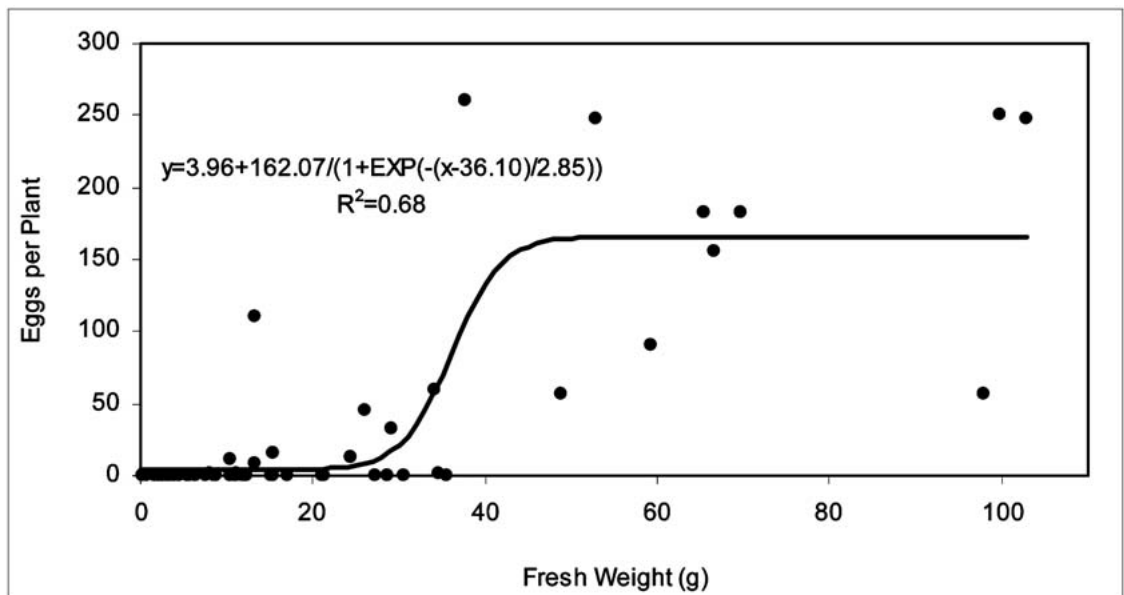

Fig. 3. Non-linear regression between fresh weight of cabbage plants and number of eggs laid by *L. aripa* per plant throughout 11 days of exposure. ($R^2 = 0.68$, $df = 59$, $F = 39.934$, $P < 0.001$).

in large masses when locating large-size hosts with abundant leaves (Stamp 1980; Le Masurier 1994). The aggregate lifestyle and conspicuous coloration of its larvae may provide a defense against predators and parasitoids (Stamp 1980; Le Masurier 1994).

In many cases, insect egg laying behavior results from balancing among factors which include minimizing parasitic and predatory risk, selecting the most nutritious host, avoiding intra-specific competition for food, and maximizing egg laying (Myers 1985; Ohsaki & Sato 1999). The insect internally weighs the various stimuli and inhibitors perceived through visual, chemical, and mechanical signals (Thompson & Pellmyr 1991; Hern et al. 1996).

Leptophobia aripa's searching and egg laying behavior observed in this study demonstrates its capacity to evaluate and discriminate among a group of hosts. Egg laying preference associated to host size has also been found for *P. brassicae* but not for *P. rapae*, *P. virginiensis* and *P. napi*. This confirms that related species may have significantly different behavior (Jones 1977; Singer & Parmesan 1993; Reich & Downes 2003).

Leptophobia aripa is a pest for Brassicaceae crops in some regions of Mexico and Central America. Producers in the region have adopted fertilizers and pesticides rather recently (Santiago et al. in press). Agroecological alternatives to heavy agrochemical use are desirable. Our findings suggest that nutrient addition to well-watered plants significantly increases plant weight (as expected) and, beyond a plant weight threshold, it also increases oviposition. It is important to study to what extent increased oviposition affects larval survival and growth, and cabbage head damage. Other plant parameters such as production of cue volatiles need to be investigated and their relation with plant size established. It is also important to study tradeoffs between plant size, cabbage head value, and crop damage caused by *L. aripa*, as well as the capacity of alternative management strategies (e.g., intercropping and moderate organic fertilization) to improve tradeoffs.

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FEEDING AND SIBLICIDAL CANNIBALISM IN
A MALE PARASITIC WASP (HYMENOPTERA: EULOPHIDAE)

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Abstract

Melittobia digitata Dahms is a small parasitic wasp known for its lethal male combat but subject to controversy regarding the occurrence of male feeding and cannibalistic feeding in particular. Here we report our observations supporting siblicidal cannibalism. To test the ability of a male's capability to feed we smeared sugary dye on the wasps' mouthparts and observed the dye passing through the digestive system to produce colored feces, confirming that males have a complete digestive tract. To document siblicidal feeding we injected other males with water-soluble dye, and paired them with undyed males. Undyed winners that appeared to feed on dyed losers were monitored; dye was evident in their feces. Finally, to determine if males benefit from feeding, we compared the longevity of artificially fed and unfed males; fed males lived significantly longer than non-fed males (Mann-Whitney U test = 81.5, N_1 = 26, N_2 = 26, $P < 0.001$). We discuss possible reasons for the comparative rarity of siblicidal cannibalism and its fitness implications.

Key Words: *Melittobia*, kin selection, uneven sex ratios, male combat

Resumen

Melittobia digitata Dahms es una avispa parasitoide conocida por sus combates letales entre machos pero que está sujeta a controversia respecto a la existencia de alimentación por estos in general, y canibalismo en lo particular. Se reportan aquí nuestros hallazgos en cuanto a canibalismo. Para probar la habilidad de un macho para comer se le untó una pasta azucarada coloreada en las partes bucales de la avispa. Se observó pasar a través del sistema digestivo para producir heces de color, confirmando así que los machos tienen un sistema digestivo completo. Para documentar canibalismo entre hermanos se inyectó a otros machos un colorante soluble en agua y se colocaron con machos normales. Se monitorearon los ganadores no coloreados parecieron alimentarse sobre los perdedores coloreados; el colorante era evidente en sus heces. Finalmente, se determinó el beneficio de alimentarse, comparando la longevidad de machos alimentados y no alimentados (artificialmente); los machos alimentados vivieron significativamente más tiempo que los no alimentados (Prueba U de Mann-Whitney = 81.5, N_1 = 26, N_2 = 26, $P < 0.001$). Discutimos razones posibles para la rareza del canibalismo entre hermanos y sus implicaciones adaptativas.

Melittobia (Hymenoptera: Eulophidae) are small, gregarious parasitoids of solitary wasps and bees, and assorted associates (Edwards & Pengelly 1966; Krombein 1967; Maeta & Yamane 1974). These parasitoids have intrigued biologists (e.g. Hamilton 1967) because of their unusual and highly inbred reproductive strategy. *Melittobia digitata* Dahms is also used in educational curricula under the name WOWBug® (Matthews et al. 1996, 1997).

Upon finding a suitable host, the female *Melittobia* stings it, feeds on hemolymph exuding from the sting wound, and then lays several hundred eggs, of which over 90% develop into females (Buckell 1928; Schmieder 1938; Dahms 1984). Females mate once with a brother, and then cooperate to chew an exit hole and disperse (Deyrup et al. 2005); their brothers remain behind to die within their natal host's cocoon (Dahms 1984).

While males' lives may be circumscribed within their natal cocoon, they are nonetheless action-filled. Males of most *Melittobia* species are highly pugnacious and frequently engage in fatal fights with their brothers (e.g., Graham-Smith 1919; Malyshev 1968; Matthews 1975; Hamilton 1979; Hartley & Matthews 2003; Abe et al. 2003); attacks on male pupae are also documented (Hermann 1971; Abe et al. 2005) and, because *Melittobia* are protandrous, male fighting can begin before the first females emerge. Occasionally, however, males also will attack females presented to them (Balfour-Browne 1922; Hermann 1971; Matthews 1975; Dahms 1984).

There has been speculation as to whether, in addition to the obvious advantage of dispatching potential rivals, such attacks might provide an opportunity for males to feed (Matthews 1975). Several biologists have gone on record as doubt-

ing that *Melittobia* males feed at all. For example, while Dahms (1984) observed attacks, he found no evidence of feeding and pointed out that a male's gaster grows increasingly thinner until he dies. Abe et al. (2005) categorically state that males of *M. australica* Girault do not feed. Balfour-Browne (1922) noted chewing attacks, but considered them to be an artifact of experimental conditions. Others disagree, reporting that males sometimes continue to chew on a defeated male sibling (Graham-Smith 1919; Matthews 1975) or on an attacked female (Hermann 1971) for relatively extended periods of time. If they were to ingest nutrients during this behavior, such cannibalism might provide a competitive advantage (Matthews 1975), enabling a male to live longer or produce more sperm.

Combat between males of *Melittobia digitata* is particularly intense. We noticed that *M. digitata* males in our laboratory cultures sometimes spent an extended period of time with their mandibles immersed in the tissues and hemolymph of a defeated male (Fig. 1). In one instance, a male killed an emerging male by biting through the emerging male's head capsule, and then inserted his mandibles deeper into the head capsule. The victor's palpi were highly active, with motions resembling those of feeding females. As we watched,
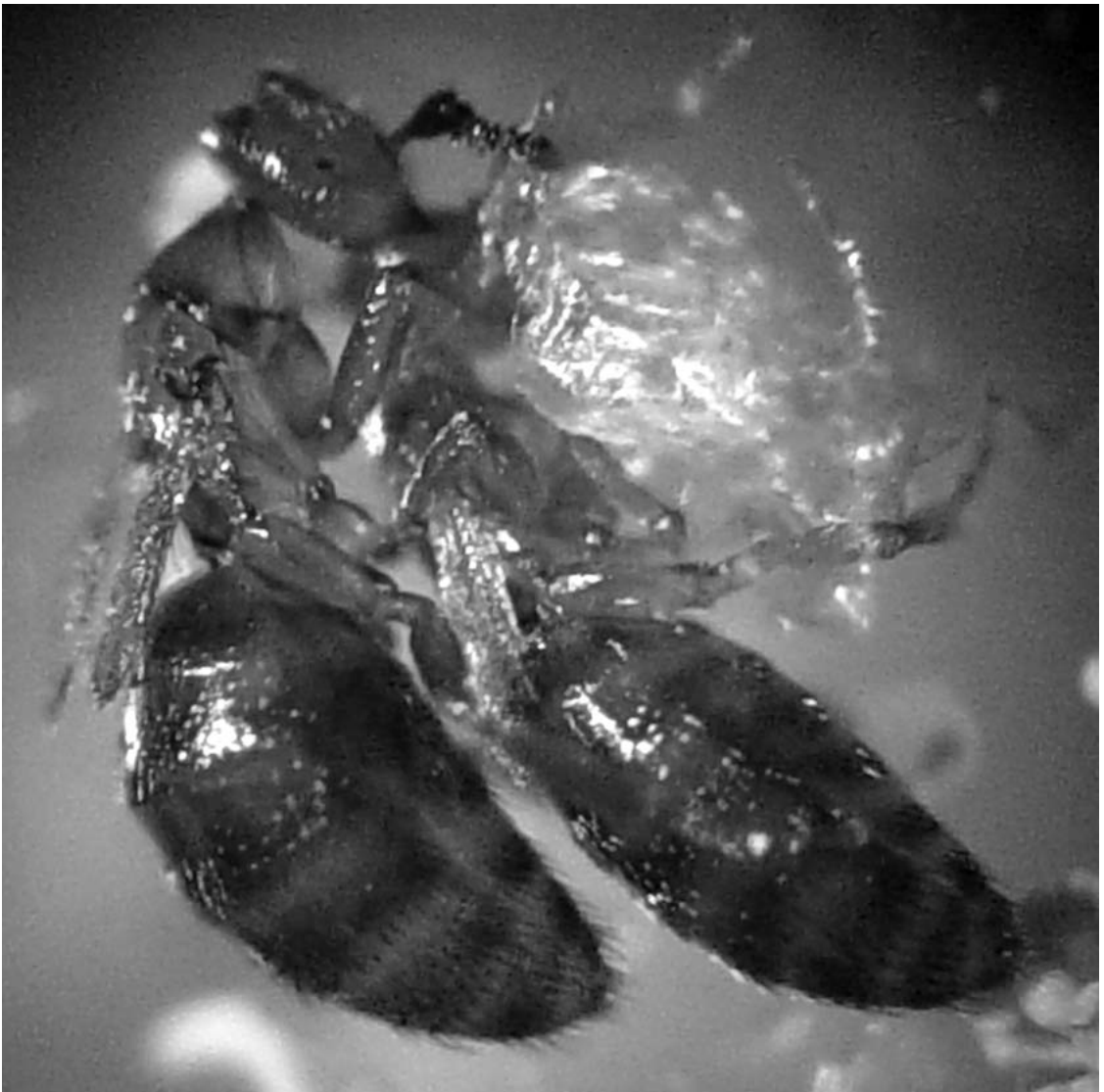

Fig. 1. A male of *Melittobia digitata* Dahms that appears to be feeding on a sibling male (Photo courtesy of Jorge M. González).

the abdomen of the male began to swell slightly, as if hemolymph were filling the crop. This observation lent support to a hypothesis that *M. digitata* males sometimes feed on a defeated male, and encouraged our experimental approach to male feeding with three objectives. The first was to determine whether male *M. digitata* have a functional digestive tract. The second was to resolve whether males ingest hemolymph from other males, and, if so, whether it passes through their digestive system. The last was to test whether individual males benefit from feeding.

MATERIALS AND METHODS

Experiment 1: Functional Digestive Tract

We collected 40 *M. digitata* male pupae (recognizable by the lack of compound eyes) developing in a single laboratory culture, isolated them individually in small tightly lidded plastic boxes ($50 \times 25 \times 18$ mm, Carolina Biological Supply Co., Cat. No. ER-14-4584), recorded eclosion dates, and randomly assigned the adults either to the control or experimental group. The controls were undisturbed. When wasps in the experimental group were 2 days old, we smeared their mouthparts with either "willow green", "cornflower blue", or "rose petal pink" cake icing dye (Wilton Enterprises).

After the passage of several hours to allow opportunity for treated males to groom, all males were transferred into clean boxes. We checked the boxes daily and recorded whether colored fecal droppings appeared. We also observed the males under a dissecting microscope to check for dye in their digestive system, and found that it was clearly visible through their translucent cuticle. A $?^2$ test in was used to determine whether individuals in the experimental and control groups differed significantly in passing colored fecal spots vs. undyed spots (Statistica 6.0).

Experiment 2: Feeding on Another Male

Because we reasoned that nutritionally stressed males would be more likely to feed, we stressed males by isolating individual late male pupae (±1 d until eclosion) and providing each with 10 newly eclosed virgin females. Males were allowed to mate *ad libitum* with these females for up to 5 days post-eclosion. After 3-5 days the males' gasters became thin and they appeared emaciated.

To produce weakened males with identifiable hemolymph as potential losers, we injected them in the abdomen with blue water-based dye (McCormick & Co., Inc.) using a glass pipette (Soda Lime Glass, 9", J. & H. Berge, Inc.) that had been stretched while heating it in an alcohol flame. Typically, the dyed male rapidly weakened, and was usually dead in 10 to 15 min.

An emaciated undyed male and a "weak" freshly dyed male were paired in a deep well projection slide arena (Carolina Biological Supply, Inc.). Because there was only a short window of opportunity for combat, we placed them next to each other to facilitate interaction; even then, most fighting was non-lethal. Even after lethal fights, most males did not attempt to feed on their defeated brother. However, we continued to dye, expose, and observe the males until we recorded 10 instances of undyed victors that killed their dyed brother and appeared to feed upon them. Each of these victors was placed into a separate observation box and observed for subsequent dye passage in its fecal droppings.

Experiment 3: Benefit from Feeding

To determine whether males benefit from feeding, we gathered 55 *M. digitata* male pupae from five cultures, isolated each pupa in a glass 1 dram vial, and inspected the vials daily, recording the date on which each male eclosed; 52 pupae eclosed as adults. Males that eclosed on the same day were assigned to an experimental (fed group, $n = 26$)) or a control (unfed, $n = 26$) group.

The experimental group was fed insect hemolymph from a *Trypoxylon* (*Trypargilum*) *politum* Say prepupa. Using an insect pin to puncture the host prepupal cuticle, we bled one drop of hemolymph onto a glass slide then gently transferred a male to the drop with a fine brush. Males immediately imbibed hemolymph from the drop. When a male did not drink voluntarily, we coaxed its head into the drop. The males invariably fed when their mouthparts touched the hemolymph, and we allowed males to feed to satiation. The control group of males was not fed. We did not give them water or insect saline solution; such resources do not occur in their natural habitat, because males seldom, if ever, leave the pupa case of their host.

All individuals in both groups were individually isolated in 1-dram glass vials and placed in an incubator at 30°C. We recorded how many days each male survived. The difference between the treatment and the control groups was analyzed using a Mann-Whitney U test and a survival analysis (Statistica 6.0).

RESULTS

In the first experiment, all colors of dye were immediately visible passing through the upper digestive system into the crop of all 20 treated males, and color appeared in their droppings when checked 24 h later. Whereas all males leave at least some fecal specs, no control males ever had droppings of a color similar to those of the fed males. This difference was very highly significant using a χ^2 test ($\chi^2 = 40.0$, $P < 0.001$) (Statistica 6.0).

In experiment 2, each of the males that we had suspected of feeding on his brother had blue color moving through the body and into the crop. This was confirmed when we checked 24 h later that dye was passed in droppings of all 10 individuals.

In experiment 3, individual male adult life spans varied, ranging from 12-16 d for unfed males, and from 13-18 d for fed males (Fig. 2). However, the lives of fed males were 1.5 d longer, on average, than those of unfed males (unfed ± SE = 13.2 ± 0.14, $\bar{x}$ = 13: fed ± SE = 14.7 ± 0.21, $\bar{x}$ = 15). Statistically, the difference was very highly significant (Mann-Whitney U test = 81.5, N_1 = 26, N_2 = 26, $P < 0.001$) (Statistica 6.0).

DISCUSSION

The results from experiment 1, demonstrating that the digestive tract of male *M. digitata* is complete and apparently functional, led to the second experiment, which established that males that defeat another male are capable of ingesting hemolyph from the defeated individual. The combination of these two experiments supports the assumption that the apparent feeding behavior that we had previously observed was correctly interpreted because males of *M. digitata* have a functional digestive tract and are capable and will imbibe hemolymph from another male.

We showed that *M. digitata* can feed, but our findings may not apply to all species in the genus. For example, *M. femorata* Dahms does not appear to have the same propensity for lethal male combat as *M. digitata* (R. W. M., unpublished data). While an *M. femorata* male conceivably could feed on a killed female, it would be unlikely to feed on a brother.

Records of feeding by adult male parasitoids are rare. Males of few species have access to
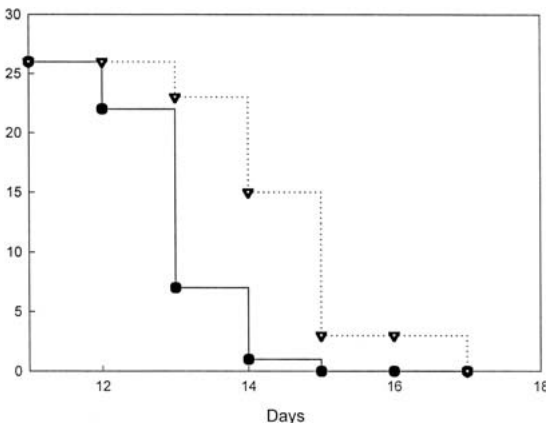

Fig. 2. Longevity of fed and unfed males of *M. digitata* at 30°C (dotted line and ▽ = males that were fed host hemolymph; solid line and ● = males that were unfed).

hemolymph, and *M. digitata* seems to take an advantage of an unusual situation. Nectar is a more usual food source for adult Hymenoptera, but nectar-feeding by parasitoids is also rare, and concentrated in a few families. At the Archbold Biological Station (Highlands Co., FL), where flower visitors have been studied for many years, there are few records of nectar feeding by male parasitoids. Among Ichneumonoidea, nectar feeding occurs in male *Agathis longipalpus* (Cresson) (Braconidae); among Chalcidoidea nectar feeding occurs in male *Leucospis affinis* Say, *L. robertsoni* Crawford and *L. slossonae* Weld (Leucospidae). In contrast, male aculeate Hymenoptera are frequent nectar feeders at the Archbold Biological Station, including numerous species representing 15 families (M.A.D., unpublished data).

Reports of adult male siblicidal cannibalism in insects are relatively rare. A situation somewhat similar to that of *Melittobia* occurs in ants of the genus *Cardiocondyla*; ergatoid males engage in lethal combat, usually won by an older male that attacks a recently eclosed sibling (Stuart 1987; Heinze et al. 1998). In this genus, however, workers remove the dead male from the nest or feed it to larvae (Stuart 1987). The situation confronting *Melittobia* males differs from that of ants in that *Melittobia* males exist in a closed system, without access to external resources.

In mites, female cannibalism has been reported (Schausberger & Croft 2000; Berndt et al. 2003), but its possible siblicidal nature seems to require further study. Schausberger & Croft (2000) reported that *Phtoseiulus persimilis* Athias-Henriot preferentially cannibalized non-siblings, but later Schausberger (2003) reported that if raised without contact with siblings, they preferentially cannibalized siblings. *Melittobia digitata* males have been reported to occasionally kill female siblings but whether they also cannibalize them is not clear (González & Matthews 2005).

Cannibalism for its own sake would seem to have several potential disadvantages. The three most applicable to *M. digitata* males are the risk of being injured or killed in attacking a similarly capable individual, the risk of contracting a disease from the consumed individual, and the evolutionary cost to fitness (Elgar & Crespi 1992). However, like *M. digitata* attacking male pupae, some species seem to avoid the problem of attacking a similar organism when early maturing individuals or individuals of a more advanced developmental stage kill a less capable immature individual (Elgar & Crespi 1992). However, this is not always the case; for example, cannibalism on peers has been recorded in intrauterine sharks (Wourms 1977; Hamlett & Hysell 1998). In *M. digitata* violent combat, presumably evolved in the context of local mate competition, usually quickly incapacitates the defeated male, thereby removing the risk of further injury. This would

leave victorious males free to consume the defeated male without further risk. Similarly, cannibalism among male *Melittobia digitata* seems unlikely to transmit disease, as the combatants are usually siblings, having fed off the same host, and lived their entire lives inside a sealed cocoon. The third potential disadvantage, loss of fitness in sibling competition, is a complex issue; kin selection models have endeavored to deal with this problem (Griffin & West 2002). However, cannibalism after combat adds yet another advantage in *M. digitata* male competition.

The third experiment showed that males who fed lived significantly longer than unfed controls. Lengthening one's adult life by the equivalent of 11% is no biologically trivial matter; presumably, those males that live longer secure more mates, dispatch more rivals, and have increased fitness relative to unfed males. Wiltz and Matthews (unpublished) found that males are more likely to die before exhausting their sperm, which makes longevity a better indicator of increased fitness than sperm production. We have observed males feeding on eclosing males and on pupae that are more vulnerable. Added longevity in males that emerge with the first generation of a few short wing females would benefit greatly in fitness by the extended overlap with the subsequently emerging group long wing females. Wiltz and Matthews (unpublished) study and our observations expose the possible benefits for males who can extend their lifespan by feeding.

We conclude that male cannibalism in *M. digitata* may not be rare when the advantages outweigh the disadvantages. The natural history of *M. digitata* appears to satisfy this criterion. The fact that a single male can potentially inseminate over 200 sisters and is likely to die before exhausting his sperm (B. Wiltz & R. Matthews, unpublished), as appears to occur routinely in some *Melittobia* species, provides a context in which male feeding and increasing life expectancy would be advantageous. Male *M. digitata* that defeat and then cannibalize brothers may also obtain nutrients needed to maintain sperm production and sex pheromone production (Consôli et al. 2002) for an extended life expectancy, as well as acquire the energy needed to successfully combat newly eclosing brothers (Abe et al. 2005) and repeatedly perform the relatively elaborate courtship displays that characterize the genus (Matthews & Matthews 2003, González & Matthews 2005).

ACKNOWLEDGMENTS

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SOLENOPSIS PHORETICA, A NEW SPECIES OF APPARENTLY PARASITIC ANT FROM FLORIDA (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

A new species of ant, *Solenopsis phoretica*, is described from a dealate queen found clinging to the petiole of a nest queen of *Pheidole dentata* Mayr in Gilchrist County, Florida. The position of the *Solenopsis* queen, as well as details of its morphology, strongly suggest that it represents a parasitic species. It is distinguished from other *Solenopsis* by its concave clypeal area and slender, elongate mandibles with an enlarged basal tooth. A single specimen is known.

Key Words: parasitic ant, parasitic *Solenopsis*, parasitic fire ant

RESUMEN

Se describe una nueva especie de hormiga, *Solenopsis phoretica* de una reina dealatada (que boto las alas) encontrada colgada al pecíolo de la reina hormiga de *Pheidole dentata* Mayr en el condado de Gilchrist en la Florida. La posición de la reina de *Solenopsis* y los detalles de su morfología, sugiere fuertemente que esta representa una especie parasítica. Se distingue esta especie de otras *Solenopsis* por tener la área del clipeo concavo y la mandíbula elongada y delgada con un diente basal engrandecido. Un solo specimen es conocido.

Solenopsis is a genus of over 180 described species (Bolton 1995). The genus shows variable habits. Many species are polyphagous, above-ground foragers, such as the notorious pest, *Solenopsis invicta* Buren. Other species, especially those species formerly placed in the subgenus *Diplorhoptrum*, are primarily subterranean foragers. Some of these subterranean species may issue from small galleries to carry off food and larvae from brood chambers of other ants (Hölldobler & Wilson 1990). A few species of *Solenopsis* are workerless parasites that were at one time placed in the genera *Labauchena* or *Paranamyrma* (Ettershank 1966). Here, we describe a new species of *Solenopsis* based on a single dealate queen. This species appears to be parasitic on other ants, but we do not know whether it is workerless, nor do we know whether it is closely related to any other parasitic species.

Character states defining *Solenopsis* are detailed by Ettershank (1966). In the North American fauna the genus can be recognized by the combination of a few character states: two-segmented petiole; two-segmented antennal club; propodeum lacking spines or angles; clypeus longitudinally bicarinate, with a median, apical marginal seta. The clypeal features are lacking on the species described below.

Solenopsis phoretica, Davis and Deyrup
new species

Diagnosis of dealate female (Fig. 1): The dealate female is distinguished from other *Sole-* *nopsis* by the following combination of character states: mandibles elongate, teeth lacking or vestigial, except for apical point and enlarged basal angle; clypeus concave, smooth.

Description of holotype dealate female: features visible in lateral view described from left side. Measurements in mm. Total length (length of head excluding mandibles + length of mesosoma + length of petiole + length of postpetiole + length of gaster): 3.03; head length: 0.55; head width at rear margins of eyes in frontal view: 0.55; length of mesosoma: 0.88; length of petiole: 0.30; length of postpetiole: 0.20; length of gaster 1.10. Color: yellowish brown, appendages yellow. Head: smooth, shining, sparsely covered with setigerous punctures separated by 2-8 times the width of a puncture, setae suberect, directed posteriorly in the frontal area, elsewhere directed anteriorly; ocelli not enlarged, each ocellus about the width of antennal scape at base; malar area long and narrow, slightly shorter than length of eye; mandibles elongate, over half the length of head at midline, apical tooth elongate, delimited proximally by a narrow notch apparently representing a vestigial tooth, inner profile of mandible strongly concave, concavity delimited proximally by strongly produced basal angle with a truncate apex; clypeus smooth, concave, without carinae, with four subapical elongate setae; antennae 10-segmented, scape reaching outer corners of head in frontal view, antennal club 2-segmented, club about as long as remainder of funiculus. Mesosoma: smooth and shining, with sparse setigerous
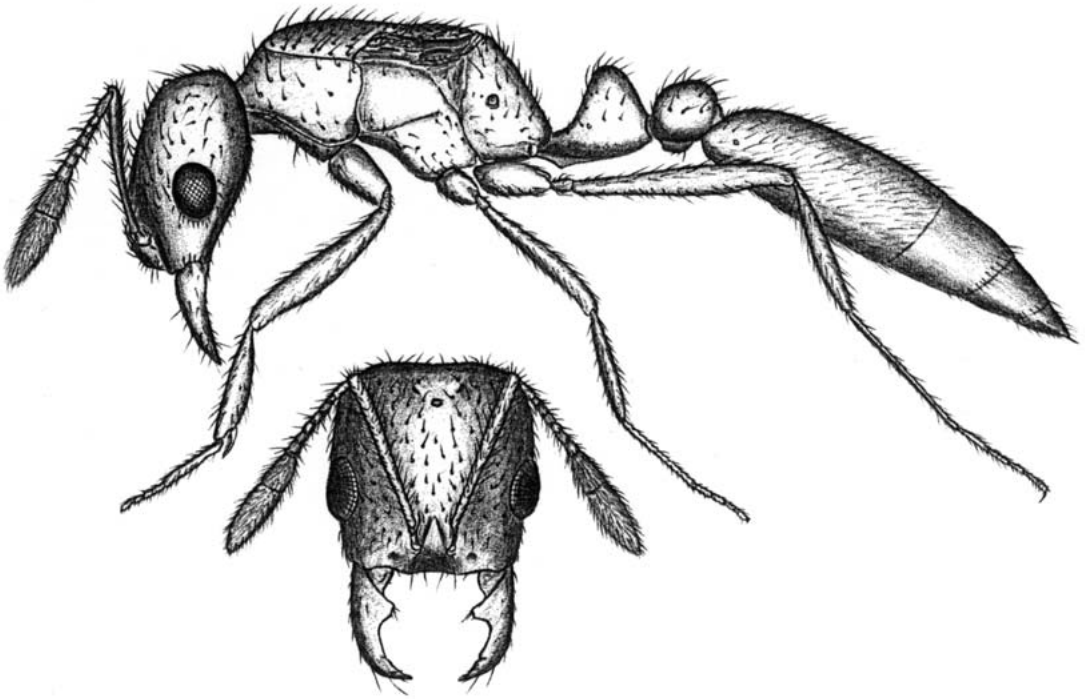
Figure 1. *Solenopsis phoretica*, **new species**, dealate queen, lateral view (above) and frontal view of head. Actual length of insect: 3.03 mm.

punctures on pronotum, near margins of mesonotum, mesopleura, sides of propodeum; disc of mesonotum and declivity of propodeum unpunctured; propodeum evenly declivitous in lateral view, only slightly convex; legs smooth, shining, with sparse, strong, semidecumbent, distally-directed hairs. Petiole: peduncle short, less than 0.25 length of base of petiole in lateral view; petiole in lateral view triangular, apex broadly and smoothly rounded; in posterior view apex strongly convex; ventral process narrowly expanded, with a small triangular tooth. Postpetiole: low and rounded above in lateral view, in posterior view about 1.5 times as wide as long, broadly convex. Gaster: in dorsal view with prominent, rounded anterior corners of first tergite; first tergite covered with sparse, long, posteriorly-directed hairs that are longer than the distance between them and emerging from inconspicuous punctures; tergites 2-4 smooth, with a subapical row of hairs.

Type locality and associated information: collecting data on label of holotype: FL: Gilchrist Co., Route 47, 2.5 miles north of junction with Route 232, 9 February 1992, Lloyd R. Davis. Mandibles locked around petiole of nest queen of *Pheidole dentata*.

We deposited the holotype specimen in the Museum of Comparative Zoology, Harvard University, Cambridge, MA.

Etymology: species epithet derived from *phoretos* (Greek), meaning "carried," referring to the phoretic relationship between the holotype and the nest queen of *Pheidole dentata*.

DISCUSSION

It is generally undesirable to describe a species of ant on the basis of a single queen. By convention and convenience, ant holotypes are generally workers. Workers, as well as males, may be very different from queens. In this case, no additional specimens have been found since the date of capture in 1992. Our intent is to alert the myrmecological community to this unusual species, in the hope that this exposure may lead to the discovery of more specimens and more natural history information.

Only a limited amount of speculation is justified, as only a single specimen is available. The generic placement of *S. phoretica* is based on its general resemblance to queens of such small *Solenopsis* species as *S. carolinensis* Forel and *S. abdita* Thompson. Resemblances include the two-segmented antennal club, smooth and shiny integument, the type and placement of setigerous punctures, and the shape of the petiole and postpetiole. If, however, the antennal club were three-segmented, rather than two-segmented, the spe-

cies could be plausibly placed in the genus *Monomorium*. We were also influenced by the precedent of parasitic species of *Solenopsis* with reduced or absent clypeal carinae, such as the South American *S. daguerrei* (Santschi). There is no evidence, however, that *S. phoretica* is closely related to *S. daguerrei* and its relatives. The latter species lacks a number of features found in *S. phoretica*: enlarged punctures bearing short setae on the head and mesosoma; angulate subpetiolar process; falcate mandibles with a strongly projecting basal angle. The petiole of *S. daguerrei* is sharply angulate above in lateral view, the postpetiole is narrow in posterior view, the anterior edge of the mesonotum is slightly protuberant, overhanging the pronotum, and the inner margins of the mandibles are oblique with four teeth (including the apical tooth).

We suspect that *S. phoretica* is parasitic because it was found clinging to the petiole of a nest queen of *Pheidole dentata* and because the mandibles and concave clypeal area fit exactly around the petiole. The radical nature of the clypeal and mandibular modifications suggest a relatively long phoretic association, although not necessarily with *P. dentata*. There are other local ants, such as *Pheidole crassicornis* Emery, *Solenopsis geminata* (Fabricius) and possibly *S. pergandei* Forel that have a petiole that might well accommodate the mandibles of *S. phoretica*. A phoretic relationship in which the parasite is attached to the petiole of the host queen is, to our knowledge, unique in ants. *Solenopsis daguerrei* queens cling to the neck of their host queen, immobilizing her, and greatly decreasing her reproductive ability (Silveira-Guido et al. 1973). There is also a highly specialized parasitic ant, *Teleutomyrmex schneideri* Kutter, whose queens ride about unattached on the host queen (Hölldobler & Wilson 1990).

It is impossible to define, on the basis of our single observation, the nature of the suspected parasitic relationship. *Solenopsis phoretica* seems equipped for a prolonged period of phoresy on its host, but it is still possible that *S. phoretica* dismounts after it is fully imbued with the odor of the host queen. It is tempting to suggest, by analogy with known parasitic *Solenopsis*, that *S. phoretica* is a workerless parasite, but there is no evidence of this, aside from the absence of worker *Solenopsis* in the host nest. Whatever relationship *S. phoretica* may have with its host, it is certain to be interesting and unusual. We hope that myrmecologists and other naturalists working in southeastern North America will be on the lookout for this species. It might not be necessary to find nest queens of the host, as at some point in the life cycle of *S. phoretica* there should be numerous alate *S. phoretica* in the host colony.

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ASSESSMENT OF FEMALE REPRODUCTIVE STATUS IN *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE)

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Abstract

Reliable methods are needed for assessing sexual maturity in field-captured tephritid fruit flies. To provide such a tool for female Caribbean fruit flies, *Anastrepha suspensa* (Loew), this study documented changes in ovarian development over a four-week period following adult eclosion. The ovarian maturation process was classified into six developmental stages. Stages 1-4 described sequential steps in the development of immature ovaries, stage 5 indicated presence of mature oocytes, and stage 6 was the ovipositional phase. For each stage, four morphometric characters were examined-length of ovary, width of ovary, an ovarian index (length of ovary multiplied by width of ovary), and length of terminal follicle. Ovarian characters were compared by stage and correlated with the number of mature oocytes per ovary (egg load). Ovarian index maximized the differences between sexually mature and immature ovaries, and ovary length provided the best separation of immature stages. All four characters were positively correlated with egg load, but ovarian index and ovary width were the two best indicators of mature oocytes. Use of these parameters to assess egg load would eliminate the need to tease apart ovaries and count mature oocytes, thereby providing an efficient method for processing large samples of flies. Classification of female sexual maturity based on an ovary staging system, in conjunction with assessment of egg load in mature stages, would facilitate evaluation of the physiological age structure of a fly population captured in field deployed traps.

Key Words: Caribbean fruit fly, ovary development, sexual maturation, oocyte, egg load

Resumen

Se necesitan métodos confiables para apreciar la madurez sexual de las moscas de la familia Tephritidae que han sido capturadas en el campo. Para proveer una medida para las hembras de la mosca de fruta del Caribe, *Anastrepha suspensa* (Loew), éste estudio documenta cambios en el desarrollo del ovario sobre un periódo de cuatro semanas despues de la eclosión del adulto. La madurez del ovario fué clasificada en seis etapas de desarrollo. Etapas 1-4 describieron los pasos en secuencia en el desarrollo del ovario inmaduro, etapa 5 indicó la presencia de oocitos maduros, y la etapa 6 fué la fase oviposicional. Por cada etapa, cuatro carácteres morfométricos fueron examinados—longitud del ovario, anchura del ovario, un índice del ovario (longitud del ovario multiplicado por la anchura del ovario), y longitud del folículo terminal. Los carácteres del ovario fueron comparados por etapa y correlacionados con el número de oocitos maduros por cada ovario (carga de huevos). El índice del ovario aumentó las diferencias entre los ovarios sexualmente maduros e inmaduros, y la longitud del ovario proveyó la mejor separación de los estados inmaduros. Todos los carácteres fueron correlacionados positivamente con la carga de huevos, pero el índice y la anchura del ovario fueron los indicadores mejores de los oocitos maduros. El uso de estos parámetros para apreciar la carga de huevos eliminaría la necesidad de separar los ovarios y contar los oocitos maduros, proveyendo un método eficiente para procesar una muestra grande de moscas. Clasificación de la madurez sexual de las hembras basada en un sistema de etapa de ovario, en conjunción con la apreciación de la carga de huevos en etapa de madurez, facilitaría la evaluación de la estructura de la edad fisiológica de una población de moscas capturada en mosqueros.

Translation provided by the authors.

Tephritid fruit flies in the genus *Anastrepha* are serious economic pests of fruit crops throughout tropical and subtropical regions of the Americas (Aluja 1994). The Caribbean fruit fly, *A. suspensa* (Loew), is a quarantine pest for the citrus industry and a production pest of guava and other fruits in Florida (Greany & Riherd 1993). The Mexican fruit fly, *A. ludens* (Loew) and West Indian fruit fly, *A. obliqua* (Macquart), though not established in Florida, pose additional invasive threats due to proximity of populations in Mexico and the Caribbean (White & Elson-Harris 1992). Traditionally, monitoring programs for tropical fruit flies have relied on McPhail traps containing liquid protein baits, typically hydrolyzed yeast (Steyskal 1977; Heath et al. 1993). Ammonia was

recognized as the primary fruit fly attractant emitted from liquid protein baits (Bateman & Morton 1981), and ammonia-based synthetic lures have been developed for *Anastrepha* spp. including ammonium acetate and putrescine (Heath et al. 1995; Thomas et al. 2001) and ammonium bicarbonate and putrescine (Robacker 1999).

Relative capture of *Anastrepha* fruit flies among traps baited with liquid protein bait formulations and synthetic lures has been highly variable (Epsky et al. 2004). Field trials of *A. suspensa* found that at sites with a high percentage of mated females, flies made choices among the liquid protein bait formulations tested while at sites with lower percentages, flies were less discriminating (Epsky et al. 1993). In laboratory trials, sexually immature females consumed more protein than sexually mature females (Landolt & Davis-Hernandez 1993). Using a combination of electroantennography (EAG) and behavioral bioassays, Kendra et al. (2005a, 2005b) evaluated dose-response of *A. suspensa* to ammonia. EAG recordings from females 1-14-d old showed that antennal response to ammonia was not constant, but varied depending upon the age/sexual maturity of the flies. The antennal response of sexually mature and immature females correlated with differences in behavioral response to ammonia in flight tunnel bioassays (Kendra et al. 2005b). These laboratory results support the hypothesis that the variability seen in field captures may be due, in part, to the physiological age structure of the fly population during the monitoring period.

Female tephritid fruit flies are sexually immature at eclosion (anautogenous) and the ovarian maturation process is dependent upon multiple factors, including temperature, photoperiod, diet (especially protein availability), and chemical cues (Fletcher 1989; Wheeler 1996; Papaj 2000; Aluja et al. 2001). Therefore chronological age is not equivalent to physiological age. Dodson (1982) found that wild *A. suspensa* require at least 14 d to reach sexual maturity, whereas laboratory-reared strains can mature within 7-8 d (Mazomenos et al. 1977; Kendra et al. 2005b). In addition to genetic strain differences, the presence of males has been shown to affect the rate of ovarian development in *A. suspensa* (Pereira et al. 2006). With such variability in maturation rate, reliable methods are needed to ascertain sexual maturity and mating status in female fruit flies, particularly field-collected specimens. The most accurate methods to determine mating status entail examination of the sperm storage organs (spermathecae and ventral receptacle) for presence of spermatozoa (Dodson 1982; Fritz & Turner 2002; Twig & Yuval 2005), and field cage tests found that 100% of sexually mature *A. suspensa* females were inseminated within a 72-h period (Dodson 1982). To differentiate between sexually mature and immature females, studies on *A. suspensa* have used measurements of ovary length (Nation 1972; Dodson 1982), ovary length and width (Dodson 1978) or ovarian index (ovary length multiplied by width, Landolt & Davis-Hernandez 1993). Nation (1972) also confirmed sexual maturity by the presence of mature terminal oocytes that are ~1 mm long and opaque. However, there are decreases in both the percent of sexually mature females with mature oocytes once oviposition starts (Dodson 1982) and in the number of eggs oviposited over the fairly long life span of *A. suspensa* females (Sivinski 1993), making reliance on single factor determinations unreliable for flies trapped in the field. In this report, we critically examine several ovarian morphometric characters, document changes in these characters for 28 d following adult eclosion, and assess how reliably each character serves as an indicator of sexual maturity in *A. suspensa*.

MATERIALS AND METHODS

Insects

Anastrepha suspensa were obtained from a laboratory colony maintained at the USDA-ARS, Subtropical Horticulture Research Station, Miami, FL. Rearing conditions consisted of a photoperiod of 12:12 (L:D), 70% RH, and ambient room temperature ($25 \pm 2°$C). In preparation for this laboratory study, pupae (12 d old) were removed from the colony, placed on weighing trays, and held in screen cages ($30 \times 30 \times 30$ cm). Once adult flies began to emerge, pupal trays were transferred to new cages every 24 h until emergence ceased, typically 4 d. Since females tended to emerge earlier than males, the first cage often contained only females and therefore was discarded. The remaining cages were mixed-sex (~1:1 sex ratio) and contained flies of known age, staged at 1-d intervals. Adult flies were provisioned with water (agar blocks) and food (refined cane sugar and yeast hydrolysate, 4:1 mixture) *ad libitum*. No oviposition medium was provided since the females readily laid eggs on the mesh sleeves of the rearing cages. Known-aged females were collected and stored in 70% ethanol until dissection.

Morphological Studies

Flies were dissected under a stereomicroscope (at 25× magnification), their ovaries were removed, and ovarian development was classified according to the system described for *Bactrocera cacuminata* (Hering) (Raghu et al. 2003). This system identifies six stages in the ovarian maturation process (Fig. 1): previtellogenic phases (stages 1 and 2), vitellogenic phases (stages 3 and 4), appearance of mature oocytes (stage 5), and an ovipositional phase (stage 6). After determining the developmental stage, measurements were
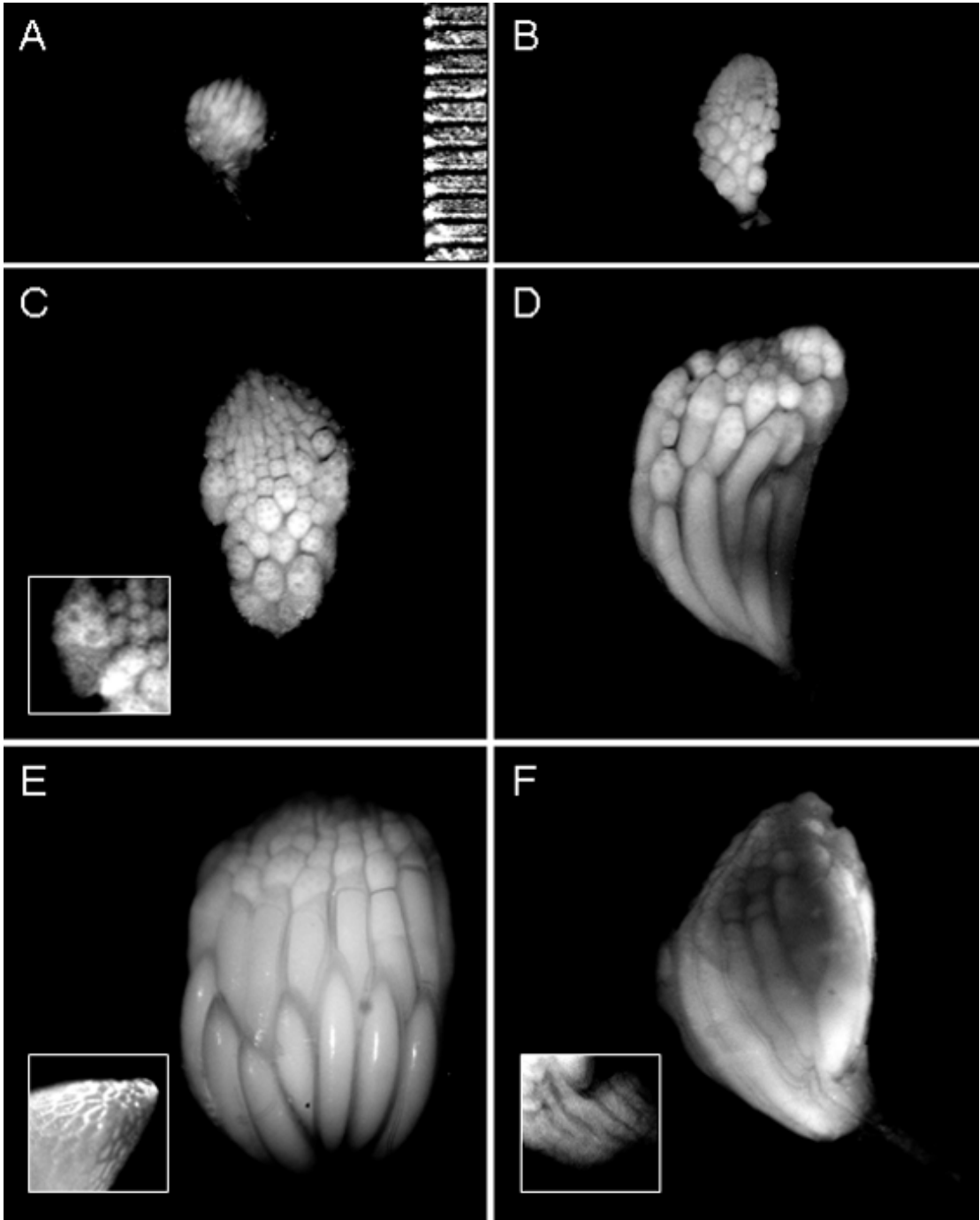
Fig. 1. Stages of ovarian development in adult *Anastrepha suspensa*, adapted from classification system of Raghu et al. (2003). Stages 1 (A) and 2 (B) represent follicles in early and late previtellogenic development, respectively. Stage 3 (C) marks initiation of vitellogenesis, accumulation of yolk in terminal follicles; Inset shows enlarged follicle containing a yolk-filled oocyte (dark lower portion) capped with trophocytes (nurse cells). Stage 4 (D) indicates late vitellogenesis, at which point yolk occupies more than half the follicle. Stage 5 (E) denotes ovaries with mature oocytes, characterized by an intact chorion (eggshell) with a reflective surface and a reticulated pattern (pronounced near the micropyle) visible at high magnification (inset). Stage 6 (F) indicates onset of oviposition, confirmed by presence of residual follicular bodies (corpora lutea) at base of the ovary (enlarged in inset). All ovary images at same magnification, scale unit = 0.1 mm.

taken of the ovary length, ovary width, and length of terminal follicle (i.e., the largest, most advanced follicle). All measurements were made with a hand-held micro-scale (to 0.1 mm; Mini-tool, Inc., Los Gatos, CA) placed beneath the ovary. Additionally, ovary length was multiplied by ovary width to obtain ovarian index, a standard method for assessing sexual maturation (Landolt & Davis-Hernandez 1993; Kendra et al. 2005b). After the ovaries were measured, they were teased apart carefully with fine insect pins (size 00, Elefant brand, Austria) and the number of mature oocytes (egg load) was counted. To be considered mature, oocytes had to lack accompanying trophocytes (nurse cells, Fig. 1C) and possess a fully developed chorion (eggshell, Fig. 1E), confirmed by the presence of a characteristic reticulated pattern in surface architecture visible at 100X magnification. Finally, the dorsal length of thorax (from anterior edge of mesonotum to posterior end of mesoscutellum) (Sivinski 1993) and length of forewing (from base of costal vein to wing apex where vein R_{4+5} terminates at the margin) were measured as independent indicators of overall female size. Measurements were recorded from females that were 1-28 d post-eclosion, and ten females were dissected for each age class.

Statistical Analysis

Regression analysis was used to describe the relationship between chronological age and ovarian developmental stage using SigmaPlot 8.0 (SPSS Inc., Chicago, IL). Several regression models were tested including polynomial, hyperbolic, logarithmic, and sigmoidal. Differences in response variables (ovarian characters) among the developmental stages were analyzed by one-way analysis of variance (ANOVA) with PROC GLM (SAS Institute 1985) followed by Tukey's test ($P = 0.05$) for mean separation. The Box-Cox procedure, which is a power transformation that regresses log-transformed standard deviations ($y + 1$) against log-transformed means ($x + 1$), was used to determine the type of transformation necessary to stabilize the variance before analysis (Box et al. 1978). Correlations among ovary length, ovary width, ovarian index, follicle length, and number of mature oocytes (egg load) within each developmental stage were determined with two-at-a-time comparisons by PROC CORR. Additional comparisons determined correlation between egg load and the four ovarian characters over the entire 28-d period (all developmental stages combined). Finally, analysis of covariance (ANCOVA) with PROC GLM was used to evaluate effect of differences in size among the sampled females on comparisons among morphometric characters.

RESULTS

Fig. 1 depicts the six stages of ovarian development in adult *A. suspensa*, and comparisons of morphometric characters and egg load among the different stages are given in Table 1. The relationship between ovarian developmental stage and female chronological age was best fit by a sigmoidal model, and this is presented in Fig. 2A.

All ovaries from 1-2 d old adults were classified as stage 1 (Fig. 1A). Stage 1 ovaries were very small and consisted of parallel, previtellogenic ovarioles. Ovary length and width were approximately equal, and these two measurements were positively correlated ($r = 0.64238$, $P = 0.0023$). In addition, ovarian index was positively correlated with both ovary length ($r = 0.90382$, $P < 0.0001$) and ovary width ($r = 0.90204$, $P < 0.0001$) in stage 1 and all subsequent stages; this was not unexpected since ovarian index is a compound character derived from ovary length and width. All 3-d-old and some 4-5-d-old adults had ovaries classified as stage 2 (Fig. 1B). During this stage, separate follicles were first discernible within the ovarioles, but they were still previtellogenic. The ter-

TABLE 1. OVARIAN CHARACTERS (MEAN ± SD) AT EACH DEVELOPMENTAL STAGE IN ADULT *A. SUSPENSA*.

Stage	n	Age (d)	Ovary length (mm)	Ovary width[1] (mm)	Ovarian index[1] (mm²)	Follicle length (mm)	Egg load[2]
1	20	1-2	0.29 ± 0.06 a	0.27 ± 0.05 a	0.08 ± 0.03 a	0.10 ± 0.00 a	0.0 ± 0.00 a
2	20	3-5	0.58 ± 0.15 b	0.36 ± 0.09 ab	0.21 ± 0.08 ab	0.11 ± 0.03 a	0.0 ± 0.00 a
3	14	4-6	0.90 ± 0.15 c	0.49 ± 0.08 b	0.45 ± 0.12 b	0.26 ± 0.07 b	0.0 ± 0.00 a
4	9	6-7	1.38 ± 0.34 d	0.71 ± 0.12 c	0.97 ± 0.28 c	0.52 ± 0.19 c	0.0 ± 0.00 a
5	21	7-9	1.88 ± 0.22 e	1.29 ± 0.26 e	2.45 ± 0.71 e	1.06 ± 0.12 e	18.2 ± 13.49 c
6	196	9-28	1.56 ± 0.20 d	1.05 ± 0.18 d	1.66 ± 0.43 d	0.96 ± 0.12 d	4.2 ± 3.80 b
		F	279.95	205.08	204.32	479.28	64.01
		df	5, 274	5, 274	5, 274	5, 274	5, 274
		P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Means within a column followed by the same letter are not significantly different (Tukey's mean separation test [$P = 0.05$]).
[1]Data were square-root ($x + 0.5$) transformed prior to analysis; non-transformed means are shown.
[2]Data were log ($x + 1$) transformed prior to analysis; non-transformed means are shown.
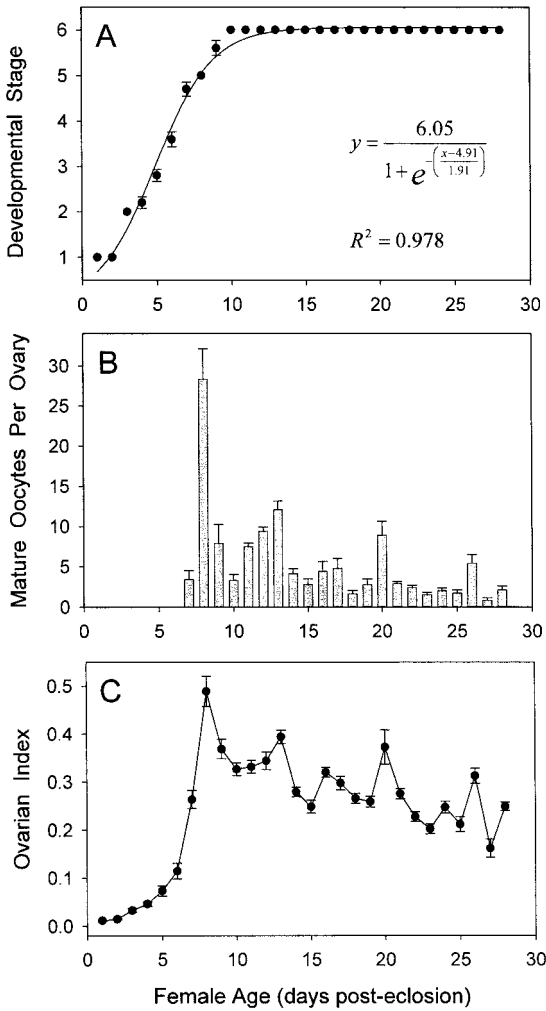
Fig. 2. Three methods for assessing reproductive status in female *Anastrepha suspensa*. (A) Ovarian maturation depicted by developmental stages according to the system of Raghu et al. (2003). (B) Number of mature oocytes per ovary (egg load). (C) Ovarian index (length of ovary multiplied by width of ovary), standardized relative to length of forewing. All three graphs present mean values (±SE) recorded from ovaries dissected 1-28 d after adult emergence, n = 10 females per day.

minal follicles ranged in length from 0.1-0.2 mm. Stage 2 ovaries were longer but not wider than stage 1 ovaries, and there were no correlations among ovary length, width or follicle length during stage 2. Stage 3 (Fig. 1C) was characterized by the onset of vitellogenesis, the accumulation of yolk in the terminal follicles, which occurred in females 4-6 d old. During stage 3 both the ovaries and the terminal follicles were longer than in stage 2, and ovary width and ovarian index were greater than in stage 1. There were positive correlations between ovary length and follicle length (r

= 0.54852, P = 0.0422) and between ovarian index and follicle length (r = 0.66225, P = 0.0099), but no correlations between other paired measurements. Observations of asynchronous gonadotrophic cycles were first noted during stage 3. By stage 4 (Fig. 1D), the yolk content exceeded 50% of the terminal follicle, and this stage included adults that were 6-7-d-old. All morphometric characters were greater in stage 4 ovaries than in previous stages, and as was observed for stage 3, there were positive correlations between ovary length and follicle length (r = 0.92467, P = 0.0004) and between ovarian index and follicle length (r = 0.91403, P = 0.0006). Stages 1-4 comprised the classes of sexually immature females, during which egg load remained at zero (Table 1). At the first appearance of mature oocytes, found in flies 7-9-d-old, ovaries were classified as stage 5 (Fig. 1E). The largest egg loads were recorded during stage 5, and accordingly the largest values for all ovary measurements were obtained from females in this stage. There were positive correlations between ovary length and ovary width (r = 0.65385, P = 0.0013), between ovary length and egg load (r = 0.67036, P = 0.0009), between ovary width and egg load (r = 0.91356, P < 0.0001), and between ovarian index and egg load (r = 0.90050, P < 0.0001) during stage 5. Initiation of oviposition marked the transition to stage 6 (Fig. 1F), confirmed by the presence of at least one residual follicular body (corpus luteum) formed after a terminal follicle releases its oocyte. Based on this criterion, ovaries from the majority of sexually mature females (9-28-d-old) were classified as stage 6; however, considerable variation was observed within this age range. Developmental asynchrony increased with age, and was pronounced by late stage 6, giving the ovaries of older females an irregular morphology compared with those of younger mature females. In addition, stage 6 was characterized by an overall decline in egg load (Fig. 2B) and ovary size (Fig. 2C) with increasing age, and mean values of all morphometric characters decreased in stage 6 compared to stage 5 ovaries. Despite this decrease, all measurements except for ovary length were significantly greater in stage 6 than in stage 4. All stage 6 characters were highly and positively correlated when paired with the other characters measured (P < 0.0001).

Mature oocytes were first detected in females 7 d old, and were present in some females sampled each day thereafter up to day 28 (Fig 2B). Mean egg load fluctuated over this period, with maximum number of mature eggs on day 8, and secondary peaks on days 13, 20, and 26. All four morphometric characters were positively correlated with egg load, with the highest correlations obtained with ovarian index (r = 0.78319, P < 0.0001) (Fig. 2C) and ovary width (r = 0.74641, P < 0.0001) from females 1-14-d-old. Correlations decreased with increasing female age throughout weeks 3 and 4.

Forewing length and thorax length were evaluated as characters indicative of overall insect size. As expected, there was no relationship between female age and either measurement, nor were there differences in either measurement among females from the different developmental stages. Wing length (mean ± standard deviation) was 5.92 ± 0.196 mm and measurements ranged from 5.0 - 6.4 mm. Thorax length was 2.44 ± 0.105 mm and ranged from 2.0 - 2.7 mm. The two measurements were positively correlated (r = 0.50896, $P < 0.0001$). However, since the wing is a longer structure, a greater range of length differences could be measured, giving better resolution to size differences among female flies. Therefore, wing length was used to adjust for female size in ANCOVA. The adjustment was not significant for any of the morphometric characters; therefore, a measurement of overall female size did not improve the classification of the females among the stages. The greatest effect was observed in the analysis of ovary length (F = 2.06; df = 5, 268; P = 0.0714), indicating that in tests of flies that are more variable in size, accounting for individual size may improve use of ovary length measurements as an indicator of sexual maturity.

DISCUSSION

The objective of this study was to identify a reliable method by which sexual maturity of female Caribbean fruit flies can be assessed based on morphological evidence. The photographic documentation and morphometric analysis presented in this report indicate that this can be accomplished by classifying ovarian development into six distinct stages, adapting the system proposed by Raghu et al. (2003). As has been reported in *B. cacuminata* (Raghu et al. 2003) and other tephritid species (Fletcher et al. 1978), the gonadotrophic cycles in *A. suspensa* ovarioles were not synchronous. Throughout the early stages of oogenesis, most terminal follicles were observed to be developing in phase; but during the later stages, some follicles were noticeably delayed. Due to this asynchrony, consistent assignment of ovaries to a particular developmental stage was achieved by evaluating the state of the most advanced ovarioles.

The ovaries of *A. suspensa* initially increased in length and then in width during a maturation phase which spanned the first 8-d post-eclosion in our laboratory population. Of the four characters examined, ovary length provided the best separation of immature stages during this maturation phase, but ovary length alone did not discriminate between stage 4 (immature) and stage 6 (mature) ovaries. Distinguishing between these two stages required inspection for residual follicular bodies and assessment of gross ovary morphology. Ovarian index, which combined the contributions of length and width, effectively maximized the differences between immature and mature ovaries. Ovarian index has been used previously for assessment of sexual maturity in this same strain of *A. suspensa*, and Kendra et al. (2005b) concluded that peak EAG response to ammonia occurred in immature flies (4-6-d-old) and peak response to carbon dioxide occurred in sexually mature flies (10-12-d-old). Classification by developmental stage now provides further interpretation of those results. Maximal antennal response to ammonia was measured from females with stage 3 ovaries actively undergoing vitellogenesis (deposition of yolk proteins), and this coincides with the age of peak protein consumption reported by Landolt & Davis-Hernandez (1993). Maximal response to carbon dioxide was found in stage 6 females during the ovipositional phase, which is consistent with the theory proposed by Stange (1999) that carbon dioxide serves as a close-range oviposition attractant for tephritid fruit flies.

The presence of mature oocytes in an ovary is regarded as the definitive character for female sexual maturity (Nation 1972; Aluja et al. 2001). Some 7-d-old females had mature oocytes, but by 8 d of age all females had mature oocytes under laboratory conditions. In a previous study with laboratory reared *A. suspensa*, indicator variable analysis also identified day 8 as the breakpoint between sexually immature and mature females (Kendra et al. 2005b). The transition from maturation phase to oviposition phase is marked by substantial changes both physiologically and behaviorally. The 8-d-old females (stage 5) had the maximum average egg load, and this was followed by secondary peaks at 5-7-d intervals. Approximately 10% of the 9-28-d old females (stage 6) had no mature eggs present in the ovaries. This included 30% of the 18-d-old females and 50% of the 28-d-old females. Fluctuations in egg load versus age suggest that eggs are laid in batches initially, when ovarioles are most synchronous. Over time, the cyclic pattern diminished apparently due to increasing asynchrony in oogenesis. Once a female is sexually mature, with fully developed eggs, she may switch from food-seeking behaviors, which allow her to obtain protein for egg development, to oviposition-site seeking behaviors, which enable her to locate suitable host fruit. Predominance of these two activities may alternate throughout stage 6 as females undergo successive cycles of oviposition. Although food-seeking behavior was thought to be primarily an activity of sexually immature females, the cyclic fluctuation of egg load indicates that, despite being sexually mature, a female might not engage in host-seeking/oviposition behaviors until she possessed an appropriate egg load. Thus, determination of egg load of sexually mature females (especially in stage 6) may provide further discrimination among females captured in field trials.

The six-stage system is a useful means of evaluating female sexual maturity, but its accuracy

for stages 4-6 depends upon assessment of mature oocytes within the ovaries. Without inspection for the presence of a chorion, it is possible to misidentify a full-sized terminal follicle as a mature oocyte, as supported by the lack of correlation between follicle length and egg load in stage 5. Also, stage 6 females may have oviposited all mature eggs at time of capture or mature oocytes may be concealed within the ovary (PK, personal observation). Therefore, the most reliable method for determination of egg load consists of ovary removal, careful separation of ovarioles and counting of mature oocytes, which is very time-consuming. The ideal screening method for field-captured flies would consist of a quick dissection followed by one or two simple measurements. Based on comparisons of the morphological characters examined in this study, ovarian index and ovary width are reliable indicators correlated with egg load. Use of these parameters to assess egg load would facilitate efficient processing of large samples of flies.

Classification of female sexual maturity by ovarian developmental stage, in conjunction with assessment of egg load in the mature stages, would facilitate evaluation of the age structure of a fly population responding to specific lures in field trapping studies. Although a laboratory strain of *A. suspensa* was used for this study, the proposed classification system should have broad applications since it is based on several ovarian characters and reflects female physiological age. In addition, standardization for insect size may improve resolution of ovary measurements as parameters for assessing maturity status in more variable field populations. The utility of this method for wild populations of *A. suspensa* and other tephritid species will need to be addressed in complementary studies.

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CUTEREBRA BOT FLIES (DIPTERA: OESTRIDAE) AND THEIR INDIGENOUS HOSTS AND POTENTIAL HOSTS IN FLORIDA

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ABSTRACT

Typical mammal hosts (indigenous rodents and lagomorphs), geographic distributions and phenologies of the five species of *Cuterebra* bot flies occurring in Florida are described. This coverage includes a reevaluation of some previously reported host records and presentation of unpublished data on larval infestations and captures of adult *Cuterebra* in Florida. In addition, indigenous species of Florida rodents for which there appear to be no in-state reports of larval infestation are listed (both native species of lagomorphs in Florida are hosts of *Cuterebra* within the state). Many gaps in our knowledge of the biology of these flies in Florida are identified, but based on available information, it appears that Florida is not exceptional when compared with certain other areas of North America in *Cuterebra* species diversity or the species of native rodents that apparently are not used as larval hosts. The geographic affinities of the Florida *Cuterebra* are Nearctic. Four of the species (*C. americana* (Fabricius), *C. buccata* (Fabricius), *C. emasculator* Fitch and *C. fontinella* Clark) have broad ranges in North America, whereas *C. cuniculi* (Clark) appears to be restricted to southern Georgia and Florida.

Key Words: lagomorph, parasite, phenology, rodent, species diversity

RESUMEN

Se describen los hospederos mamíferos típicos (roedores y lagomorfos indígenas), la distribución geográfica y la fenología para cinco especies de tórsalo (moscas del género *Cuterebra*) que ocurren en la Florida. Algunos de los pasados informes de los hospederos son re-evaluados y se presentan datos no publicados sobre las infestaciones de larvas y adultos de *Cuterebra* recolectados en Florida. Se presenta una lista de las especies de roedores indígenas de Florida las cuales aparentemente no tienen un registro de infestación de estas larvas dentro del estado (ambas de las especies nativas de lagomorfos en Florida son hospederos de *Cuterebra*). Muchas incognites en nuestro conocimiento de la biología de estas mosca en Florida estan identificadas, pero basadas sobre la información disponible, parece que Florida no es excepcional cuando se compara con ciertas otras áreas de America del Norte en cuanto de la diversidad de especies de *Cuterebra* o las especies de roedores nativos que aparentemente no son usados como hospederos de las larvas. Las afinidades geográficas de las moscas *Cuterebra* de Florida son Nearcticas. Las especies *C. americana* (Fabricius), *C. buccata* (Fabricius), *C. emasculator* Fitch y *C. fontinella* Clark tienen un rango geográfico amplio en America del Norte mientras que *C. cuniculi* (Clark) aparentemente es restringida en el sur de Georgia y Florida.

Cuterebra bot flies (Diptera: Oestridae; often listed as Cuterebridae) (e.g., Sabrosky 1986; Alcock & Kemp 2004; Burns et al. 2005) are obligate parasites of many native (indigenous) rodents (mice, rats, tree squirrels, etc.) and lagomorphs (rabbits, hares, etc.) in the Americas (Sabrosky 1986). Larvae (bots) of these dipterans are subcutaneous parasites that live in encapsulated pockets known as warbles. Depending on the species of *Cuterebra* and its host, the larvae develop for four to six weeks, ingesting body fluid and excreting and respiring through a hole (the warble pore) they create in the host's skin (Catts 1982; Slansky & Kenyon 2003). In addition to species they typically parasitize, these insects occasionally infest 'atypical' hosts, especially non-native (= non-indigenous or adventive; Frank & McCoy 1995) rodents and lagomorphs and non-rodent/ non-lagomorph mammals (including humans) (Sabrosky 1986; Baird et al. 1989; Glass et al. 1998; Harris et al. 2000; Suedmeyer et al. 2000; Safdar et al. 2003; F. S., unpublished data).

Most of the 30+ species of *Cuterebra* are temperate zone species, with flies in other cuterebrine genera (*Dermatobia*, *Metacuterebra*, *Alouattamyia*, *Rogenhofera* and *Pseudogametes*) occurring in subtropical and tropical climates (Catts 1982; Sabrosky 1986; Guimaraes 1989; Colwell & Milton 1998; Bergallo et al. 2000; note, however, that Pape (2001) suggested that the latter three genera likely should be included in *Cuterebra*). Based on morphological features of the adults and on larval hosts, Sabrosky (1986) divided *Cuterebra* into four 'groups', defined by a species within

the group: the rodent-infesting *'americana'* and *'fontinella'* groups, and the lagomorph-infesting *'buccata'* and *'cuniculi'* groups.

Diverse biogeographic patterns are exhibited by various taxa of Florida's indigenous entomofauna and other biota; these may include precinctive species, either depauperate or high species diversity, declining diversity from north to south (e.g., peninsula effect), and affinities to different geographic regions (e.g., Frank 1986; Peck 1989; Choate 1990; Deyrup 1990; Frank & McCoy 1995). In this paper I address various components of the biogeography of *Cuterebra* in Florida, a topic that has previously not been investigated. I review literature relevant to the presence in the state of flies in this genus and of their indigenous mammal hosts. This coverage includes a reevaluation of some previously reported host records as well as presentation of unpublished data on larval infestations and captures of adult *Cuterebra* in Florida. In addition, I list the indigenous rodents occurring in the state for which there appear to be no in-state reports of *Cuterebra* larval infestation (both native lagomorph species are hosts for larvae of these flies in Florida). Finally, I discuss the diversity and geographic affinities of these flies in Florida and address the question of whether there are an exceptional number of vacant niches (potential host species) for *Cuterebra* species in the state.

MATERIALS AND METHODS

Published literature was reviewed to determine which species of *Cuterebra* and other cuterebrines occur in Florida, as well as their typical hosts, ranges, and phenologies. Unless indicated otherwise, information before 1986 was obtained from Sabrosky (1986), who not only compiled and synthesized most of the published information available at that time on *Cuterebra* but also reported numerous unpublished records resulting from his examination of specimens from many private and museum collections. Information on mammal species in Florida was obtained from the American Society of Mammalogists (undated), the Florida Fish and Wildlife Conservation Commission (2004a,b) and Brown (1997a,b), unless cited otherwise. Nomenclature follows that of the International Taxonomic Information Service (ITIS 2004).

RESULTS

Cuterebra in Florida

Five species of *Cuterebra* occur in Florida: *C. americana* (Fabricius), *C. buccata* (Fabricius), *C. cuniculi* (Clark), *C. emasculator* Fitch, and *C. fontinella* Clark. There apparently are no verified published records for flies of other *Cuterebra* species or in other cuterebrine genera occurring

naturally in Florida. Worth (1950a) listed "*Dermatobia*-like" larvae removed from roof (or black) rats, *Rattus rattus* (L.) (a non-indigenous, atypical host species), captured in Hillsborough Co., but this appears to be a misidentification of second instar *Cuterebra* larvae, as done previously (Townsend 1892). In subsequent reports (Worth 1950b,c) in which he thanked a *Cuterebra* taxonomist, C. W. Sabrosky, for identifying the larvae, Worth no longer mentioned *Dermatobia*. Below I discuss the typical hosts, ranges and phenologies in Florida for these five species.

C. americana

Typical Hosts. There apparently is only one main typical host species for larvae of *C. americana,* the eastern wood rat *Neotoma floridana* (Ord), which ranges throughout the northern two thirds of peninsular Florida and the Panhandle (there is also an isolated population on Key Largo). There appear to be only two published infestation reports for this host in Florida. Without any additional information, Johnson (1930) stated that he "obtained *Cuterebra* larvae from the large wood rat" (presumably *N. floridana*) in the state, and Worth (1950b) reported capturing *Cuterebra*-infested individuals of this species in Hillsborough county.

Distribution. County records for captures of adult *C. americana* in Florida include Alachua, Citrus, Duval, Hillsborough, Lake, Orange, Pasco, and Sarasota. If Worth's (1950a,b) reports of infested *R. rattus* captured in Dade Co. involved *C. americana*, as suspected by Sabrosky (1986), then this species would appear to occur throughout peninsular Florida. However, in Worth's papers the larvae were not described and no mention was made of obtaining adults for definitive species identification even though Sabrosky (1986) stated that Worth "reared" these specimens (in fact, Worth thanks Sabrosky for identifying the larvae only to the level of *Cuterebra* sp.). In addition, the typical host (*N. floridana*) of this species apparently does not occur in Dade Co. Finally, larvae of at least one other Florida *Cuterebra* species (*C. buccata*) have been recorded infesting *Rattus* species as atypical hosts. Taken together, these caveats would appear to call into question the presence of *C. americana* in Dade Co. Because this species has been reported from Georgia and Louisiana (as well as from several other states from eastern Colorado to Virginia and southward), it likely also occurs throughout the Florida Panhandle.

Phenology. Sabrosky (1986) provided no dates for adult captures or host infestations. An adult female *C. americana* was collected in Alachua Co. on 7-X-1992 (P. M. Choate, Dept. Entomology & Nematology, University of Florida, personal communication). Worth (1950b) captured *R. rattus* in-

fested with *Cuterebra* (possibly *C. americana*; but see above) in Dade Co. in January and *Cuterebra*-infested *R. rattus* and *N. floridana* in Hillsborough Co. in late February through early March (these were the only times that trapping was done; see also Worth 1950c). Because the data are so limited, the phenology of this species in Florida is uncertain, but it appears to be univoltine outside the state (Goertz 1966).

C. buccata

Typical Hosts. Larvae of this species typically infest eastern cottontails, *Sylvilagus floridanus* (J. A. Allen), and probably also individuals of other *Sylvilagus* species. Both *S. floridanus* and the marsh rabbit *S. palustris* (Bachman) are widespread in Florida, but the presence in the state of the swamp rabbit *S. aquaticus* (Bachman), which might occur in the extreme western Panhandle, is uncertain. There appear to be no definitive records of infestation of rabbits of either of these species by larvae of *C. buccata* in Florida. However, Worth (1950a,b) reported that individuals of *S. palustris* were commonly infested with larvae of *Cuterebra* (Sabrosky (1986) does not mention these records). Although these larvae were not identified to species, they probably were either *C. buccata* or *C. cuniculi* (see below), the only *Cuterebra* species in Florida known to use rabbits as their typical hosts.

Distribution. This is a very widespread species, reported from all states east of the western mountain states except Maine, Vermont and Rhode Island. According to Sabrosky (1986), supposed records of this species from St. Johns and Collier counties (Johnson 1895, 1913) in Florida presumably involved another species (*C. fontinella*; see below). *Cuterebra*-infested *S. palustris* collected in Hillsborough Co. (Worth 1950a,b) may have involved this species, and/or possibly *C. cuniculi* (see below). Sabrosky (1986) considered as valid the claim of Knipling & Bruce (1937) that a larva of this species was removed from a cow in September in Sumter Co. However, the involvement of *C. buccata* (or indeed any species of *Cuterebra*) in this infestation is questionable for a variety of reasons: (1) the larva was a second instar, and no species identification key for this stage of the *Cuterebra* lifecycle was then (nor is now) available; (2) the authors provide no information on the characteristics used to identify this larva either as a species of *Cuterebra* or as *C. buccata* in particular; (3) a cow is a very unusual atypical host for *Cuterebra* larvae, and I am aware of no other reports documenting cattle as hosts; and (4) cattle are subject to parasitization by larvae of cattle warble flies (two species of *Hypoderma*), both of which occur in Florida (Glick 1976). Larvae of these insects typically form warbles on the backs of these animals, which was the site of the supposed *Cuterebra* larva. Thus, although a *Cuterebra* larva may have infested a cow, as stated by Knipling & Bruce (1937), I consider this conclusion highly unlikely.

Phenology. There apparently are no definitive phenological records for this species in Florida, although *C. buccata* larvae may have infested the *S. palustris* trapped by Worth (1950a,b) in late February to early March (the only time that trapping was done). Thus, the phenology of this species in the state cannot presently be determined, but elsewhere it appears to be at least bivoltine.

C. cuniculi

Typical Hosts. The typical hosts for *C. cuniculi* are *S. floridanus* and *S. palustris,* with infestation records for both hosts in the state.

Distribution and Phenology. *Cuterebra cuniculi* is very restricted in distribution, apparently occurring only in Florida and southern Georgia. County and date records for this species in Florida (adults, unless indicated otherwise) include Alachua (May and December), Broward (August), Collier (April), Dade (May), Hamilton (October), Highlands (May and December), Indian River (a larva from *S. palustris* in June; the adult emerged in October), Orange (May), Palm Beach (May and December; also, a larva from an unspecified host in October with the adult emerging in November; and another adult in November from a larva (no date) infesting *S. palustris*), Polk (March), St. Johns (April) and St. Lucie (a larva from *S. floridanus* in December; the adult emerged in February). Worth's (1950a,b) records of *Cuterebra*-infested *S. palustris* trapped in Hillsborough Co. during late February through early March (the only time that trapping was done) likely would have involved this species and/or *C. buccata*. Apparently, there are no records for this species from counties in the Panhandle. From the records listed above, it is likely that this species occurs at least throughout the peninsular part of the state and that it has two or more generations during the year. Based on very limited data, it appears to be bivoltine in Georgia.

C. emasculator

Typical Hosts. The typical hosts for this species include tree squirrels (*Sciurus* sp.), and eastern chipmunks, *Tamias striatus* (L.). There are Florida infestation records for eastern gray squirrels, *S. carolinensis* Gmelin, and fox squirrels, *S. niger* L., both of which are widespread throughout the state. In contrast, *T. striatus* is restricted to the northern portions of a few counties in the Panhandle (Escambia, Holmes, Okaloosa, Santa Rosa, and Walton) (Gore, 1990), and there appear to be no published *Cuterebra*-infestation records for individuals of this species in Florida. Southern flying squirrels, *Glaucomys volans* (L.), which are

widely distributed in Florida, have rarely been reported to be parasitized by *Cuterebra* larvae (presumably *C. emasculator)* in the state or elsewhere, suggesting that *G. volans* is an atypical host species for *Cuterebra* larvae.

Distribution. *Cuterebra emasculator* is widely distributed throughout eastern North America from just west of the Mississippi River to the Atlantic coast. Published records for Florida include Alachua (Sabrosky 1986; Forrester 1992; Slansky & Kenyon 2000; 2002) and Columbia (Coyner 1994; Coyner et al. 1996) counties, although the latter record may not have involved *C. emasculator*. A recent study has extended the known range of this species to over 40 additional counties throughout the northern and central regions of the state (including the Panhandle) (F. S., unpublished data). Apparently, *C. emasculator* is rare in or absent from the southern counties despite the presence of potential host squirrels.

Phenology. Sabrosky (1986) does not provide phenological data for this species in Florida, but infested squirrels typically are observed in the state from July through October (Slansky & Kenyon 2000; 2002; 2003; F. S., unpublished data). Coyner's (1994; Coyner et al. 1996) report of finding one individual of *S. niger* (out of 123 examined fox squirrels) with a larva presumed to be *C. emasculator* on 21-II-1991 is exceptional. Because no information was given that the larva was definitively identified to species, the possibility exists that it was of a different species such as *C. cuniculi*, which, unlike *C. emasculator*, appears to have a winter generation. *Cuterebra emasculator* appears to be univoltine in Florida and throughout its geographic range (Bennett 1972a,b; F. S., unpublished data).

C. fontinella

Typical Hosts. The main typical hosts for *C. fontinella* apparently are the white-footed mouse *Peromyscus leucopus* (Rafinesque) and the cotton mouse *Peromyscus gossypinus* (LeConte) (records in Sabrosky (1986) and Durden (1995)). However, adults of this species have been reared from a variety of other indigenous rodents, including the deer mouse *Peromyscus maniculatus* (Wagner) (mice of this species apparently are the main typical hosts for a closely related species, *Cuterebra grisea* Coquillett), the golden mouse *Ochrotomys nuttalli* (Harlan), the northern grasshopper mouse *Onychomys leucogaster* (Wied-Neuwied), the Mexican spiny pocket mouse *Liomys irroratus* (Gray), the woodland jumping mouse *Napaeozapus insignis* (Miller), the meadow vole *Microtus pennsylvanicus* (Ord), and the yellow-pine chipmunk *Tamias amoenus* J. A. Allen (records in Sabrosky (1986); also, Clark & Durden (2002) for *O. nuttalli*). Of these, only *P. gossypinus*, *O. nuttalli,* and a subspecies of *M. pennsylvanicus* occur in Florida. *Cuterebra*-infested indi-

viduals of *P. gossypinus*, which occurs statewide, and *O. nuttalli*, which is found in the northern half of peninsular Florida and the Panhandle, have been captured in the state (Pearson 1954; Layne 1963; Bigler & Jenkins 1975). In addition, Layne (1963) trapped *Cuterebra*-infested Florida mice, *Peromyscus* (= *Podomys*) *floridana* (Chapman), which occur only in Florida (the central portion of the peninsula). It is likely that the mice in the latter three studies were parasitized by *C. fontinella*. If so, then *O. nuttalli*, *P. gossypinus,* and *P. floridana* would apparently constitute the typical hosts for this *Cuterebra* species in the state.

Distribution. *Cuterebra fontinella* is a very widespread species, occurring throughout most of the continental US (except Alaska), southern Canada, and northeastern Mexico. Sabrosky (1986) provides a distribution map for this species, including several records for Florida. Because of the small size of this map and the large symbols used to mark collection locations, identification of the counties involved is somewhat tenuous, but these appear to be Alachua, Broward, Citrus, Collier, Columbia, Dade, Hillsborough, Lee, Manatee, Monroe, Orange, Pinellas, Sarasota, St. Lucie, Union, and Volusia. Pearson's (1954) infestation records are for Levy Co., and Bigler & Jenkins (1975) performed their study in Monroe Co. Layne (1963) did extensive trapping throughout the northern half of the state (Alachua, Clay, Gilchrist, Levy, Putnam and St. Johns counties) and some in Highlands Co. Individual county records were not presented in the latter study but apparently *Cuterebra*-infested mice were found in each of these counties. According to Sabrosky (1986), Johnson (1895) originally thought a fly captured in St. Johns Co. was *C. buccata* but he later correctly identified it as *C. fontinella* (Johnson 1913). However, in the latter publication he provided a separate record for *C. buccata* from Collier Co., but Sabrosky (1986) indicated that Johnson more likely was again dealing with *C. fontinella*. Apparently, there are no published records for this species from the Panhandle.

Phenology. Sabrosky (1986) provided no phenological data for *C. fontinella* in Florida. An adult female *C. fontinella* was captured in Alachua Co. on 19-IV-2003 (P. M. Choate, Dept. Entomology & Nematology, University of Florida, personal communication). Pearson (1954) reported trapping *Cuterebra*-infested *P. gossypinus* in all months of the year except February and March, with almost half of these records in June; he did not report capture dates for the *Cuterebra*-infested *P. nuttalli* he trapped. Bigler & Jenkins (1975) also captured *Cuterebra*-infested *P. gossypinus* during most months of the year; no trapping was done in December, but parasitized mice were caught in every other month except October, with peaks in the prevalence of infestation in January and June. Layne (1963) found *Cuterebra*-

infested *P. floridana* in all quarters of the year. If these latter three studies involved *C. fontinella* (as is likely), then this species probably has two or more generations per year in Florida. It appears to be at least bivoltine in other southeastern states (Durden 1995, Georgia; Clark & Durden 2002, Mississippi) and elsewhere (e.g., Goertz 1966; Wolf & Batzli 2001, Illinois).

Indigenous Rodents not Known to be Parasitized by *Cuterebra* Larvae in Florida

Several species of indigenous rodents occur in Florida for which no published records of parasitization by *Cuterebra* larvae in this state apparently exist. These are listed below, along with published reports and a few unpublished records of *Cuterebra* infestation (or indication of the apparent lack thereof) from elsewhere in the ranges of these, and in some cases closely related, taxa.

Castoridae and Aplodontidae. American beavers, *Castor canadensis* Kuhl, occur in the Panhandle and northern third of peninsular Florida. Apparently, there are no published *Cuterebra*-infestation records for this species in any part of its range in North America. Sabrosky (1986) listed only two records of mountain beavers, *Aplodontia rufa* (Rafinesque) (note that this species belongs to a different family (Aplodontidae) than *C. canadensis*), parasitized by *Cuterebra* larvae (Oregon and Washington). These limited records suggest that no *Cuterebra* species uses beavers of either of these two species as typical hosts.

Geomyidae. The southeastern pocket gopher *Geomys pinetis* Rafinesque is the only member of this family in Florida. It is found in the Panhandle and the northern half to two thirds of the Florida peninsula. One individual of this species captured by Worth (1950a; probably in Hillsborough Co.) was not parasitized by *Cuterebra* larvae. Sampling of *G. pinetis* in Alachua Co. for an entire year and in Alabama, Florida and Georgia primarily from December through February (totaling over 150 individuals trapped) yielded no specimens obviously infested with *Cuterebra* larvae (P. E. Skelley, FDACS/DPI, Gainesville, FL, personal communication). In the western US, the northern pocket gopher *Thomomys talpoides* (Richardson) is the typical host of *Cuterebra polita* Coquillett (a member of the *'americana'* group). There appear to be no *Cuterebra*-infestation records for the several other species of *Geomys* and *Thomomys* in North America.

Muridae. A number of indigenous murid rodents occur in Florida for which no published *Cuterebra*-infestation records in the state appear to be available. The marsh rice rat *Oryzomys palustris palustris* (Harlan) has a statewide distribution in Florida. There is also a subspecies, the silver rice rat *O. p. natator* Chapman (sometimes listed as the invalid *O. argentatus* Spitzer and Lazell), which is apparently limited to some

of the Lower Keys. There appear to be no *Cuterebra*-infestation records for any members of this genus in North America; none are listed in Sabrosky (1986) and no infested individuals were captured by Worth (1950a), Pearson (1954), Durden (1995), or Clark & Durden (2002). However, parasitization of another member of this genus, *O. russatus* (Wagner), by *Metacuterebra apicalis* (Guerin-Meneville) in South America has been well documented (Bergallo et al. 2000; Bossi et al. 2002; both Brazil). The hispid cotton rat *Sigmodon hispidus* Say and Ord is distributed statewide in Florida. Goertz (1966) reported that individuals of this species were very rarely parasitized by an unknown species of *Cuterebra* (possibly *C. americana*) in Oklahoma, whereas no such infestations were found in Florida (Worth 1950a; Pearson 1954; Bigler & Jenkins 1975) or elsewhere in North America (Clark & Kaufman 1990, Kansas; Boggs et al. 1991, Oklahoma; Clark & Durden 2002). Disney (1968) reported infestation of *Sigmodon* sp. cotton rats in Honduras by larvae of *Cuterebra* (= *Metacuterebra*) *flaviventris* (Bau).

Two species of *Peromyscus* mice occur in Florida, and *Cuterebra*-infested individuals of one of these, *P. gossypinus*, have been captured in the state. However, the other species, *P. polionotus* (Wagner), which is comprised of several subspecies (beach mouse, oldfield mouse, etc.) variously distributed in Florida, is apparently lacking in *Cuterebra*-infestation records. Another indigenous mouse species in Florida, the eastern harvest mouse *Reithrodontomys humulis* (Audubon and Bachman), occurs throughout the northern two thirds of the peninsula and in the Panhandle. Little or no parasitization of *Reithrodontomys* mice has been reported from elsewhere in North America (Goertz 1966; Hensley 1976, Virginia; Sabrosky 1986; Clark & Kaufman 1990; Boggs et al. 1991; Clark & Durden 2002), which suggests that members of this genus may serve only occasionally as atypical hosts for *Cuterebra* larvae.

Two species of *Microtus* voles occur in Florida: the pine (or woodland) vole *M. pinetorum* (LeConte), found in the central part of the northern one third of the peninsula, and a rare subspecies of the meadow vole *M. pennsylvanicus*, namely the Florida saltmarsh vole *M. p. dukecampbelli* Woods, Post & Kilpatrick, which inhabits saltmarshes in the Cedar Key area (Levy Co.). There are several records from outside Florida of individuals of *M. pennsylvanicus* and other *Microtus* voles parasitized by larvae of various *Cuterebra* species (Clough 1965, Wisconsin; Maurer & Skaley 1968, New York, North Dakota and Pennsylvania; Getz 1970, Wisconsin; Hensley 1976, *M. pennsylvanicus* but not *M. pinetorum*; Boonstra et al. 1980, British Columbia, Canada), as well as reports of *Cuterebra*-infested *Clethrionomys* voles (Sabrosky 1986, Manitoba and Quebec, Canada; Bowman 2000, New Brunswick, Can-

ada), which do not occur in Florida. However, none of the *Microtus* voles captured by Sillman (1955, Ontario, Canada), Goertz (1966), Shoemaker & Joy (1967, West Virginia), Hensley (1976, *M. pinetorum*), Clark & Kaufman (1990), Boggs et al. (1991), Bowman (2000), or Clark & Durden (2002), nor any of the *Clethrionomys* individuals trapped by Maurer & Skaley (1968) or Hensley (1976), were infested with *Cuterebra* larvae.

Round-tailed muskrats, *Neofiber alleni* True, are distributed throughout much of peninsular Florida, with some isolated populations in the Panhandle. Sabrosky (1986) provided records of infestation of an individual of this species (location not given) and of the muskrat *Ondatra zibethicus* (L.) (Michigan). These limited records suggest that no *Cuterebra* species uses these muskrat species as typical hosts.

Sciuridae. There are few reports of flying squirrels (*Glaucomys* species) parasitized by *Cuterebra* larvae. Apparently, the only published North American record is for an individual of *G. volans* in Alachua Co., Florida (Forrester 1992), and I am aware of a few such cases from other eastern states (F. S., unpublished data). Because of the rarity of these records, it is likely that *Glaucomys* species are atypical hosts of *Cuterebra* (presumably *C. emasculator*). *Tamias striatus*, which is restricted in Florida to the northern portions of certain counties in the Panhandle, is a frequent host of *C. emasculator* outside the state, especially in the northern portion of its range.

DISCUSSION

From the above coverage, it is evident that there are many gaps in our knowledge, specific to Florida, of the biology of the *Cuterebra* species occurring in the state. The most complete data on host species, county distribution, and phenology within Florida are available for *C. cuniculi* and *C. emasculator*. However, if the studies of Pearson (1954), Layne (1963), and Bigler & Jenkins 1975) involved *C. fontinella* (as is likely), then aspects of the biology of this species in Florida also are reasonably well understood. The least amount of information is available for *C. americana* and *C. buccata*.

Obviously, more studies are required to provide the information needed to better understand the biology of these five *Cuterebra* species in Florida. The mammals that serve as typical and atypical hosts for these species within the state need to be determined, or in some cases better documented. In addition, the distributions and phenologies of these species within the state need to be established for some of the species or better delineated for the others. A key limitation in the research required to achieve these goals involves the difficulty of determining *Cuterebra* species when only larval specimens are available. Generally, the larvae of these flies cannot be identified to species based on their external features; instead, they usually need to be reared to the adult stage, for which definitive morphologically-based descriptions are available (Sabrosky 1986). However, obtaining adults from larvae can be problematic; second and early third instars removed from their hosts are unable to pupate, and although more mature third stadium larvae can pupate, they may enter pupal diapause, which can delay obtaining adults by several months (e.g., Bennett 1972a;b). In addition, there can be substantial mortality of diapausing pupae (F. S., unpublished data). The problem of species identification of the larvae will be overcome as comparative DNA sequences become available for more species of *Cuterebra* (Otranto et al. 2003; Noel et al. 2004; F. S., unpublished data). At a broader level, third stadium *Cuterebra* larvae can be separated into species that typically parasitize rodents and those that infest lagomorphs, based on certain features of their cuticular ornamentation (Knipling & Brody 1940; Baird & Graham 1973).

Limitations in our knowledge prevent a meaningful biogeographic analysis of the in-state distribution of the Florida species of *Cuterebra* (Deyrup 1990). However, it is possible to address some broader patterns for these flies in Florida. Although the biogeography of the genus has not been studied quantitatively (e.g., species/area relationships), the number of *Cuterebra* species (five) occurring in Florida appears comparable to that in certain other states of similar area (Illinois and Washington; species distributions from Sabrosky (1986)). In addition, Florida is inhabited by members of all four of the *Cuterebra* groups. The state contains each of the species chosen by Sabrosky (1986) to name these groups, as well as *C. emasculator*, which is in the '*fontinella*' group. Thus, Florida does not appear to be either depauperate or unusually rich in its total number of *Cuterebra* species or in representatives of Sabrosky's (1986) four *Cuterebra* groups. However, before definitive conclusions can be reached regarding *Cuterebra* species diversity within Florida, the effects of habitat heterogeneity, host species diversity, historical influences, and other relevant biogeographic factors must be investigated for the entire genus.

Regional affinities of the indigenous entomofauna of Florida are diverse. In many cases these reflect relationships to taxa in other areas of the southeastern US, but for some groups there are affinities to taxa in southwestern North America or in the Caribbean region (Frank 1986; Peck 1989; Choate 1990; Deyrup 1990). The five species of *Cuterebra* occurring in Florida are all Nearctic temperate zone species with eastern distributions, but three (*C. americana*, *C. buccata,* and *C. fontinella*) range very broadly into western North America. In contrast, *C. emasculator* is found from just west of the Mississippi River eastward to the Atlantic Ocean, and *C. cuniculi* is the

most narrowly distributed, apparently occurring only in southern Georgia and in Florida (Sabrosky 1986). Only one other species, *Cuterebra abdominalis* Swenk, a member of the '*cuniculi*' group, is present in the southeastern US. Although ranging broadly from the Midwest to the Atlantic coast, this species apparently does not occur in Florida. Thus, there are no precinctive species of *Cuterebra* in Florida (although *C. cuniculi* comes close to being in this category) and there appear to be no Caribbean ties for the Florida species of this genus. In addition, Neotropical species in other cuterebrine genera are absent from Florida, despite the subtropical climate in the southern part of the state (Henry et al. 1994).

There appear to be several vacant niches for *Cuterebra* species in Florida, in terms of the presence of indigenous rodent species that apparently seldom if ever serve as hosts for flies in this genus. It appears that 11 of the 17 (65%) native rodent species within Florida fall into this 'vacant niche' category (note that for these numbers, the various subspecies are not considered separately): *C. canadensis, G. pinetis, G. volans, M. pennsylvanicus, M. pinetorum, N. alleni, O. palustris, P. polionotus, R. humulis, S. hispidus,* and *T. striatus.* None of these species are restricted to Florida (although some of the subspecies are), and most of them appear to show little or no infestation by *Cuterebra* larvae outside the state as well. Of these species, apparently only *M. pennsylvanicus* and *T. striatus* are typical hosts of *Cuterebra* larvae outside Florida. It is likely that further study will demonstrate that individuals of both of these species serve as hosts for *Cuterebra* larvae within Florida because species that typically parasitize these rodents elsewhere (*C. fontinella* and *C. emasculator,* respectively) are present in the state. Thus, although additional research on host use within Florida, as well as comparative studies of other areas of North America, are required before a definitive conclusion can be reached, Florida does not appear to be exceptional in its apparently unutilized, potential host species among its indigenous rodents. Indeed, apparently the only unique aspect of the association between *Cuterebra* species and their typical host species in Florida is the parasitization of individuals of the Florida mouse (*P. floridana*), which apparently occurs only in the state, by larvae of an unidentified species of *Cuterebra* (Layne 1963; probably *C. fontinella*).

In conclusion, there are many unanswered questions about *Cuterebra*/ host species associations in Florida and elsewhere. In addition to the need to better understand these flies' biology, such as their typical and atypical host species, geographic ranges, and phenologies. Questions such as what factors determine the suitability of rodents and lagomorphs to serve as hosts, both between and within these orders, as well as in comparison with mammals in other orders, and what

are the effects of larval infestation on the performance of individual hosts and host species population dynamics, remain to be answered. For example, in Florida there are several 'at-risk' (endangered, threatened, or of special concern) species and subspecies of rodents and a lagomorph (Florida Fish and Wildlife Conservation Commission 2004b) that might be affected by *Cuterebra* larval infestation, but even the most basic data on prevalence and intensity of parasitization within these populations are apparently lacking; similar situations occur in certain other states as well (Slansky & Kenyon 2003). Throughout North America, domestic felines with outdoor access can become infested with *Cuterebra* larvae (F. S., unpublished manuscript). Unlike with most other hosts, such occurrences can be fatal to the cats (Glass et al. 1998), and yet information as basic as which species of *Cuterebra* are involved in these cases is not available. Thus, it would seem that additional research on *Cuterebra* and host associations both within and outside Florida is well justified.

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TWO SPECIES OF CECIDOMYIIDAE PREDACIOUS ON CITRUS RUST MITE, *PHYLLOCOPTRUTA OLEIVORA*, ON FLORIDA CITRUS

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ABSTRACT

Larvae of two undescribed species of Cecidomyiidae (Diptera) were found preying upon *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) on Florida citrus. Identifications to genus were made from adults reared in the laboratory. The two species had distinctive larval coloration. One larval type was completely yellow and was identified as *Feltiella* n. sp., while the second larval type had an orange color with a transverse white band close to the mouthparts. The latter cecidomyiid was identified as belonging to a genus near *Lestodiplosis* in the broad sense. *Feltiella* n. sp. (n = 17) and the species near the genus *Lestodiplosis* (n = 12) consumed 33.8 ± 4.6 (mean ± SEM) and 43.0 ± 6.4 citrus rust mite eggs; 14.2 ± 1.4 and 15.0 ± 2.0 citrus rust mite nymphs, and 3.0 ± 0.4 and 5.6 ± 0.9 citrus rust mite adults/10 min., respectively. There were no significant differences (P > 0.05) in the consumption rates of either predator on any rust mite life stage. These data indicate that *Feltiella* n. sp. and the species near the genus *Lestodiplosis* are both efficient predators of *P. oleivora* eggs, larvae, and nymphs.

Key Words: Acari, citrus, Diptera, Eriophyidae, *Feltiella, Lestodiplosis,* predation

RESUMEN

Dos especies de larvas no descritas de Cecidomyiidae (Díptera) fueron encontradas depredando *Phyllocoptruta oleivora* (Ashmead) (Acari: Eriophyidae) en cítricos de Florida. Las identificaciones fueron hechas en adultos criados en el laboratorio. Los dos cecidomidos tienen distintivas coloraciones larvales. Un tipo es completamente amarillo y es identificado como *Feltiella* n. sp., mientras que la otra tiene un collar blanco cerca de las partes bucales. Este cecidomido fue identificado como una especie cercano a *Lestodiplosis* en un amplio rango. *Feltiella* n. sp. (n = 17) y la especie cercana al genero *Lestodiplosis* (n = 12) consumieron 33.8 ± 4.6 (AVG ± SEM) y 43.0 ± 6.4 huevos del acaro de tostado, 14.2 ± 1.4 y 15.0 ± 2.0 ninfas del acaro del tostado, y 3.0 ± 0.4 y 5.6 ± 0.9 adultos del acaro del tostado /10 min., respectivamente. No hubo diferencias significances entre las dos especies en los rangos de consumo de los diferentes estadios del acaro del tostado. Con los datos presentados aquí es evidente que *Feltiella* n. sp. y la especie cercana al genero *Lestodiplosis* son depredadores eficientes de huevos, larvas y ninfas de *P. oleivora*.

Translation provided by the authors.

The citrus rust mite (CRM), *Phyllocoptruta oleivora* (Ashmead), and the pink citrus rust mite *Aculops pelekassi* (Keifer) are pests on Florida citrus (Acari: Eriophyidae) (Denmark 1963; Childers & Achor 1999). Both species cause rind blemish injuries to developing and mature fruit. Other types of damage include reduced bonding force of fruit, premature fruit drop, reduced yields, and lower juice quality (Allen et al. 1994). The pink citrus rust mite also can cause leaf distortion, crinkling, and stunting of new shoot growth (C. C. Childers, unpublished). A third species, the citrus bud mite, *Aceria sheldoni* (Ewing), is frequently found on citrus but is not considered an economic pest in Florida (Childers & Achor 1999). Natural enemies of citrus rust mites include predatory phytoseiid and stigmaeid mites (Muma 1961; Muma & Selhime 1971; Peña 1992; Childers 1994). Hubbard (1883) found "a little coral-red maggot and a yellow midge larva" (Diptera: Cecidomyiidae, formerly Itonididae) feeding on CRM in Florida. Later, Muma et al. (1961) reported *Itonidini* species feeding on *P. oleivora* and the six-spotted mite, *Eotetranychus sexmaculatus* (Riley), on citrus in Lake Alfred, Florida.

The larvae of the cosmopolitan genus *Feltiella* (Diptera: Cecidomyiidae) and all described species form a group associated exclusively with tetranychid species (Gagné 1989), and this genus belongs in the tribe Lestodiplosini that is composed

entirely of predators or parasitoids of insects and mites (Gagné 1989, 1994, 1995). The identification of these mite-eating cecidomyiids is difficult; for example, in Europe many authors are using *Feltiella acarisuga* (Vallot) and *Therodiplosis persicae* Kieffer as synonyms (i.e., Colombo et al. 1993; Piatkowski 2000; Putte 2002). The larval stage of *F. acarisuga* (Vallot) is a well-known predator of the two-spotted spider mite, *Tetranychus urticae* Koch (Gillespie et al. 1998).

Feltiella acarisuga completes its life cycle in 8 to 10 d in Italy (Roberti 1954) and 29 d on average in Israel (Sharaf 1984). These differences in developmental times are likely dependent on temperature and/or humidity differences. The crops included in studies with *F. acarisuga* were apple (Roberti 1954), eggplant (Sharaf 1984), cucumber (Gillespie et al. 1998, 2000), strawberry (Easterbrook 1998), and plants in greenhouses (Opit et al. 1997; Enkegaard et al. 2000). *Feltiella occidentalis* (Felt) occurs on strawberry in California (Oatman et al. 1985) and *F. minuta* has been found on eggplant (Ho & Chen 1998).

There are few examples of eriophyid predation by larval cecidomyiids. Nijveldt (1969) compiled a list of cecidomyiid species and their respective eriophyid prey. The larva of a *Medetera* species (Diptera: Dolichopodidae) was reported preying on *Aculus schlechtendali* Nalepa on apple in Washington (Rathman et al. 1988). The eriophyid *Aceria litchii* Keifer is a serious pest of lychee (*Litchii chinensis* Sonnerat) in Australia and China, and the larva of *Arthrocnodax* sp. (Cecidomyiidae) was observed preying upon *A. litchii* (Waite & Gerson 1994).

The objectives of this study were to identify two cecidomyiids observed preying upon citrus rust mites in different citrus orchards in Florida, as well as to compare and quantify their consumption of different CRM stages.

MATERIALS AND METHODS

Cecidomyiid Collection

Sampling for cecidomyiid larvae was conducted between June and August and again from October to the first week of December 2001 in a 'Hamlin' orange orchard in Lake Alfred, Florida. Larvae were collected from citrus leaves and fruits and transferred individually to Petri dishes with a 5-0 sable brush. Individual fruits with high numbers of CRM (>100 cm²) were collected to prepare individual rearing arenas with an adequate food source for maintaining the two dipteran species. Some of the cecidomyiid larvae were allowed to complete their development so that adults could be obtained for identification while others were used for feeding experiments and behavioral observations. Data obtained on individual prey experiments were recorded separately.

Rearing of Cecidomyiid Larvae

Individual rectangular, transparent plastic containers (Pioneer Plastics Inc #295C, Eagan, MN) with semi-tight lids (31 cm long × 24 cm wide × 11 cm deep) served as rearing chambers for the midge larvae. A lightly moistened piece of paper towel was placed on the bottom of each container to provide increased humidity. Each CRM-infested orange arena was carefully examined and all other arthropods removed. Between six to eight oranges were then placed individually on PVC rings (3.5 cm diam. × 1 cm high) inside each plastic rearing container. Two or three cecidomyiid larvae of the same type were added to each fruit in the same container and then covered with a lid (*n* ≥ 20 for yellow and orange, respectively). The containers were held in an environmental chamber at 25 ± 1°C, 60 ± 10% RH under fluorescent lights set to a photoperiod of 14:10 (L:D) h. The cecidomyiid larvae were observed daily and the oranges infested with CRM were replaced as required. As each cecidomyiid pupa formed, it was removed and isolated in a 5-cm-diam. Petri dish held under the same environmental conditions. However, pupae were difficult to find and often escaped detection so that many adults emerged in the plastic container. Pupae were found attached to fruit, on or under the paper toweling, or attached to the plastic walls of the rearing units.

Cecidomyiid Predation on Citrus Rust Mites

The predatory behavior of cecidomyiids was observed on individual Hamlin oranges heavily infested with all stages of CRM. Individual oranges were placed on PVC rings (3.5 cm diam. × 1 cm high) as described earlier. The presence of high densities of CRM was confirmed on each fruit by using a dissecting stereomicroscope (>100 cm²). Since it was not possible to estimate the age of the larvae used in the experiments, larvae of similar lengths (1.6-1.8 mm) were selected for each assay. A single cecidomyiid larva was placed on a fruit within the center field of view by using a stereomicroscope and monitored for 10 min. rotating the fruit to maintain a constant focus on the maggot's movements. The number of eggs, combined larvae and nymphs, and adult CRM stages consumed per larva during each 10-min. interval were tallied separately for each cecidomyiid species. In total, 17 yellow and 12 orange cecidomyiid larvae were observed. Data were analyzed with a single factor analysis of variance (Zar 1984). Sub-samples of eriophyid mite populations were collected from fruits into 80% ethanol and then later slide-mounted in a modified Hoyer's medium and identified to species (Baker et al. 1996). Multiple slides were prepared with each containing 10 or more rust mite motile stages.

RESULTS

Cecidomyiid Collection and Rearing

Two distinct types of larvae were observed. One was yellow in color (Fig. 1a) and the other was orange with a white collar behind the mouthparts (Fig. 1b).

Success in rearing cecidomyiids to the adult stage was obtained with larvae collected during November and December, but those collected during July and August did not complete development (Fig. 2). Adult males and females were reared from yellow larvae whereas only two females were reared from orange larvae. The two distinct types of larvae were identified as belonging to two different genera. The yellow larvae were an undescribed species of *Feltiella* and the females obtained from the orange larvae were identified as a species near the genus *Lestodiplosis* (in the broad sense). However, they do not fit well in the genus *Lestodiplosis*, suggesting that this species may represent a new genus.

Predation by Cecidomyiids on Citrus Rust Mites

All eriophyids sub-sampled from each 10-min observation interval were identified as the citrus rust mite, *Phyllocoptruta oleivora*. The number of eggs, combined larvae and nymphs, and adult citrus rust mites consumed during 10-min observations by both species are shown in Fig. 3. There were no significant differences ($P > 0.05$) between the rust mite stages consumed by either *Feltiella* n. sp. or the second dipteran species. *Feltiella* n. sp. and the second species consumed 33.8 ± 4.6 (mean ± SEM) and 43.0 ± 6.4 ($F = 1.99$; 1,27 *df*; $P = 0.16$) CRM eggs; 14.2 ± 1.4 and 15.0 ± 2.0 CRM larvae and nymphs ($F = 1.41$; 1,27 *df*; $P = 0.24$), and 3.0 ± 0.4 and 5.6 ± 0.9 CRM adults ($F = 0.26$; 1,27 *df*; $P = 0.60$), respectively.

DISCUSSION

Previous reports of cecidomyiid species feeding on CRM were based largely on anecdotal information. To date, there is no reliable description of CRM feeding by cecidomyiid predators in Florida or other citrus growing areas of the world. McMurtry (1977) and Perring & McMurtry (1996) cited Muma et al. (1961), who only mentioned a predatory midge he recovered from *P. oleivora* colonies. There was no empirical quantification or scientific description of cecidomyiid feeding behavior. Furthermore, all citations refer to the original descriptions by Hubbard (1883) who apparently described the two types of larvae (one yellowish and the other orange with a white collar) reported here. Adults of *Feltiella* n. sp. (yellow larva) and a species near the genus *Lestodiplosis* (orange larva with a white collar) were success-

fully collected, reared, and identified to genus in this study. Voucher specimens are deposited with the USDA, Systematic Entomology Laboratory, Beltsville, MD.

Yothers & Mason (1930) reported that these midges appeared on occasion but only when high numbers of CRM were present. This was observed with *Feltiella minuta* (Felt) when it increased on *Tetranychus kanzawai* Kishida (Ho & Chen 1998). Both species examined in this study fed on *P. oleivora* eggs, larvae, and nymphs of CRM and completed their development to adults on this diet. If we extrapolate the average number of all *P. oleivora* stages consumed in 10 min to 4 min, then 1.2 adults, 5.7 nymphs and larvae, and 13.5 eggs could be consumed in 4 min by a single midge larva. This rate of consumption surpasses the predatory capacity of *Iphiseiodes quadripilis* (Banks), one of the most abundant phytoseiids on Florida citrus (Villanueva & Childers 2005), feeding on *A. pelekassi*, (Villanueva 2002) in which a female starved for 24 h fed on 1.8 ± 0.5 *A. pelekassi* in 4 min. A similar comparison was shown between *F. minuta* and *Amblyseius womersleyi* Schicha on eggplant (Ho & Chen 1998).

Laboratory observations of the two cecidomyiid predators revealed that they search for CRM eggs by continuously moving the anterior part of their bodies to the left and to the right while moving forward and changing direction. This appeared very similar to the 'questing' behavior described for syrphid larvae seeking aphids (Bargen et al. 1998). Once an egg is detected during this sweeping movement, it is rapidly consumed while the larva continues onward. Consumption of CRM eggs by either cecidomyiid species was difficult to observe due to the small size and transparent color of the egg (about 30-40 μm). The presence of *P. oleivora* eggs is essential for larval development of both cecidomyiids for two reasons. First, larvae of both species were frequently found on the bottom of the plastic containers completely separated from the fruit. However, the arenas the cecidomyiid larvae had abandoned were still infested with motile stages of CRM but lacked CRM eggs that were previously consumed. When a new fruit with abundant CRM eggs was provided, the cecidomyiid larvae would immediately begin feeding and remained on the fruit. Second, on many occasions, both species of larvae encountered adult CRM but usually they were not consumed. Other times, the larva would raise the adult CRM off the substrate with their mouthparts and appear to cast the adult aside. On occasion, when the attacks on CRM adults were successful, the larval mouthparts were directed to the ventrolateral part of the mite's body, just behind the second pair of legs. This was not observed when the attacks were directed to the immature rust mite stages. Larval and nymphal CRM stages appeared to be more vulnerable and
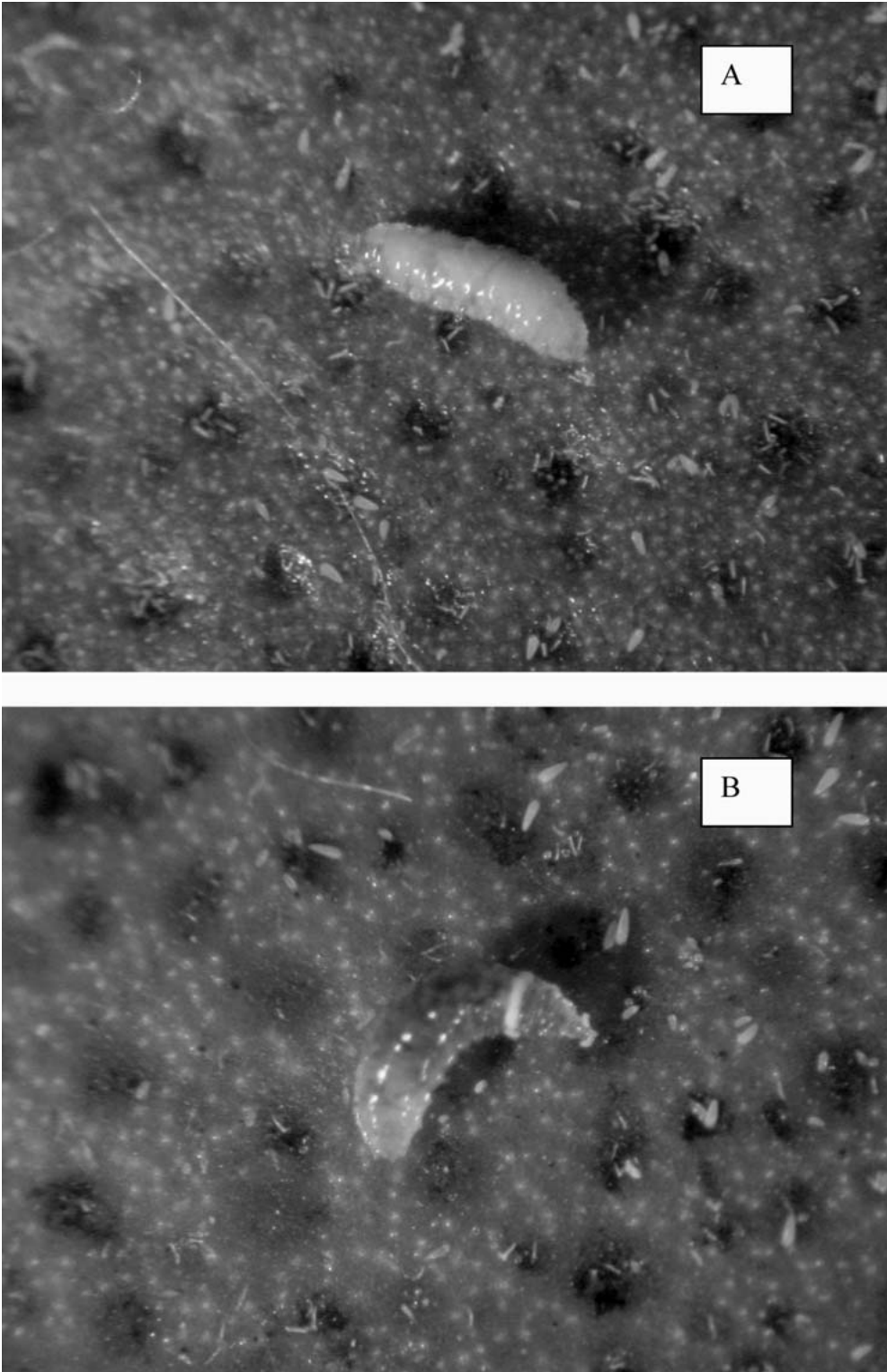
Fig. 1. Two morphologically distinct larval types of two new species of cecidomyiids surrounded by their citrus rust mite *Phyllocoptruta oleivora* prey. (A) *Feltiella* n. sp. and (B) a species near the genus *Lestodiplosis*. Note the color and ring around the anterior end of the larva.

Fig. 2. Adult *Feltiella* n. sp., a predator of the citrus rust mite *Phyllocoptruta oleivora* reared in the laboratory. The vertical bars beneath the insect are in millimeters.

were usually eaten when grasped on any part of their bodies.

The relative abundance of these cecidomyiids was not recorded in this study because the priority was to identify the species involved and quantify their predation on CRM stages. Most larvae collected in the field were more abundant on fruit than on leaves during October and November. The predominance of midge larvae on fruit surfaces was consistent with the higher densities of CRM present on fruits than on leaves.

Observations in both laboratory and field showed that larvae of both cecidomyiid species were capable of jumping or springing off a plant substrate when disturbed. Larvae were observed to raise the middle part of their bodies into an inverted U-shape while keeping both the head and terminal ends attached to the substrate. The larva would then rapidly release its hold on the substrate and leap or spring from the plant surface using tension on the sternal spatula or breastbone to provide the springing action. Cecidomyiid larvae falling to the ground would likely desiccate and die. From observations in the labo-

ratory rearing arenas, it appears that this movement is directional. When the fruit in the rearing containers had a few CRM eggs, the larva would
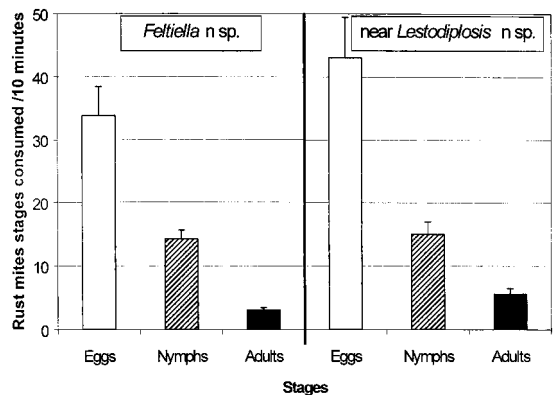

Fig. 3. Mean numbers ± SEM of different citrus rust mite (*Phyllocoptruta oleivora*) stages consumed by larvae of *Feltiella* n. sp. and a species near the genus *Lestodiplosis*.

abandon the fruit and be found on an adjoining new fruit that had abundant rust mite eggs.

The dispersal behavior of these midges and their unnoticed predation on CRM eggs likely contributed to earlier failed attempts to rear these species. Similar situations were encountered by Yothers & Mason (1930) who wrote: "These larvae are very small and extremely delicate, and all attempts to rear them to maturity have failed". Few accurate studies on the biology of predacious cecidomyiids and their effects on prey mite populations are available. This is the case for cecidomyiids preying on Eriophyidae in general, and also on different species of Tetranychidae (Chazeau 1985).

In this study, we demonstrated that these two undescribed cecidomyiids are capable of feeding and reproducing on an exclusive diet of CRM. Both species appeared to have highly specialized abilities eating CRM eggs and immature stages.

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THE INTRODUCTION OF THE EXOTIC Q BIOTYPE OF *BEMISIA TABACI* FROM THE MEDITERRANEAN REGION INTO CHINA ON ORNAMENTAL CROPS

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ABSTRACT

The Q biotype of *Bemisia tabaci* (Gennadius), which has been described from the Mediterranean/North African region, was identified for the first time infesting ornamental crop species in several locations in China. Identification and partial distributions of the exotic B biotype and the recently introduced Q biotype in China were established by using the mitochondrial cytochrome oxidase I gene (mtCOI) as a molecular marker. Collections of *B. tabaci* were made from representative geographical locations and plant hosts in different provinces of China. MtCOI sequence analysis revealed that collections from Beijing [AY582872, AY589499], Yunnan [AY518189, AY587516], and Henan [AY587514] shared >99.6% sequence identity with the Q biotype from Spain [AY587513, AY562216, AY596950]. The Q type from China shared 98.9-99.4% nucleotide sequence identity with Q-like relatives of *B. tabaci* described from Israel [AY518191, AY582869]. Phylogenetic analyses indicated that certain *B. tabaci* populations that are present in China are the Q biotype, and that the Q biotype now in China may have originated from Spain or other nearby locations where the Q biotype has been identified. This is the first report of the introduction of the Q biotype from the Mediterranean region into China. The specific outcomes of the Q biotype as an invasive species in Asia are presently unknown. Certain Q biotype populations from Spain have been reported to exhibit resistant to neonicotinoid insecticides, which are commonly used for controlling this pest and virus vector in ornamental and field crops. Thus, the close monitoring of the Q biotype in China and elsewhere, particularly where commercial plants are grown for export or received for importation, respectively, is essential to avoid the further geographical expansion of the habitat of the Q biotype.

Key Words: *Bemisia tabaci* biotype Q, geographical origin, introduced species, mitochondrial COI gene, phylogenetic analysis

RESUMEN

El biotipo Q de *Bemisia tabaci* (Genn.), el cual fue descrito de la región Mediterráneo/Norte de Africa, fue identificado por primera vez infestando especies de cultivos ornamentales en varios lugares de China. La identificación y la distribución parcial del exótico biotipo B y el recién introducido biotipo Q en China fueron determinados usando el gen citocromo oxidase I mitocondrial (mtCOI) como un marcador molecular. Se realizaron colecciones de *Bemisia tabaci* de lugares geográficos representativos y hospederos de plantas en diferentes provincias de China. El análisis de la secuencia de mtCOI reveló que las colecciones de Beijing [AY582872, AY589499], Yunnan [AY518189, AY587516] y Henan [AY587514] compartieron >99.6% de la identidad de la secuencia con el biotipo Q de España. [AY587513, AY562216, AY596950]. El tipo Q de China compartieron 98.9-99.4% de la identidad de la secuencia de nucleótidos con los relacionados de clase como del tipo Q de *B. tabaci* descritos de Israel [AY518191, AY582869]. El análisis filogenético indica que ciertas poblaciones de *B. tabaci* que estan presentes en China son de biotipo Q biotype, y el biotipo Q que ahora esta presente en China puede haberse originado en España u otros lugares cercanos donde el biotipo Q ha sido identificado. Este es el primer informe de la introducción de biotipo Q de la región Mediterránea a China. Los resultados específicos de biotipo Q como una especie invasora en Asia son en estos momentos desconocidos. Ciertas poblaciones del biotipo Q de España han sido reportadas que muestran resistencia a los insecticidas neonicotinoides que se usa regularmente para controlar esta plaga y vector de virus en cultivos ornamentales y de campo. Por esto, es esencial realizar un monitoreo extensivo del biotipo Q en China y en otros lugares, particularmente donde se siembra plantas comerciales para exportación o recibidas para importación para evitar una mayor expansión geográfica del habitat del biotipo Q.

The *Bemisia tabaci* (Genn.) complex (Brown et al. 1995b) is a hemipteran (Aleyrodidae) pest that feeds on plant phloem. It also is the most important vector worldwide of several genera of plant virus (Brown 2000, 2001; Brown & Bird 1992).

Bemesia tabaci is best described as a species complex that comprises an unexpectedly large number of genetically variable populations, some of which are discernible owing to distinct phenotypes (Brown et al. 1995a,b). Well-studied *B. tabaci* populations that have been differentiated are referred to as races (Brown & Bird 1992) or biotypes (Brown et al. 1995a; Costa & Brown 1991). The B biotype (Costa & Brown 1991) is a particularly aggressive *B. tabaci* variant. It has an extremely broad host range, is highly fecund, and disperses relatively long distances (Brown 2000; Brown et al. 1995b), and has become established in many locations beginning approximately in 1988-present (Costa & Brown 1991; Costa et al. 1993). Since that time, it has been of considerable concern as a pest and virus vector in subtropical and temperate, mild climate zones where the majority of the world's vegetable and fiber crops are produced (Brown et al. 1995a,b; Brown 2000). Population genetics studies have shown that the B biotype probably originated from the Middle Eastern/North African region (Frohlich et al. 1999).

In China *B. tabaci* has become an important agricultural pest in the late 1990s (Chu et al. 2004; Zhang 2000), and the introduction of the B biotype into China was first reported in 2002 (Luo et al. 2002). The B biotype is now known to be widespread in a number of provinces in China where vegetables, cotton, and ornamentals are produced (Chu et al. 2004; Wu et al. 2003; this report).

In China and elsewhere, the understanding that *B. tabaci* is a polymorphic, cryptic species that can upsurge without warning and cause great damage to crop and ornamental species is lacking. This has often resulted in the delayed recognition of upsurges in local whitefly populations and/or of exotic introductions (Reitz & Trumble 2002), such as the B biotype. This realization has prompted an accelerated interest in practicing routine monitoring of *B. tabaci* populations to detect early the potentially invasive *B. tabaci*, or otherwise upsurgent haplotypes, with particular emphasis on those that are associated with the global commercial plant industry.

The purpose of this study was to determine if the Q biotype (Costa et al. 1993; Guirao et al. 1997) was present in ornamentals and/or annual flowering or bedding plants in China. Such knowledge is important because the Q biotype has only recently been recognized as a potentially invasive pest species in the vegetable and ornamentals industries in the Mediterranean/Middle Eastern region (Guirao et al. 1997; Horowitz et al. 2003). The introduction and establishment of the Q biotype is

anticipated, or was possibly expected to already have occurred, in at least certain locations, and is expected to have important and far-reaching economic relevance. The potential for damage will further be exacerbated if Q biotype populations exhibit resistance to a well-known neonicotinoid (Nauen et al. 2002; Rauche & Nauen 2003), upon which the industry presently relies to control *B. tabaci*. Resistance to this compound has already been reported in Spain and Israel (Ebert & Nauen 2000; Rauche & Nauen 2003).

Recent studies have demonstrated that the mitochondrial cytochrome oxidase I (mtCOI) gene (Brown 2001; Brown et al. 1995b; Frohlich et al. 1999) is a highly informative coding sequence for differentiating populations and haplotypes/biotypes in the *B. tabaci* complex. In this study, the mtCOI was used as a population genetics marker to detect the presence of and identify the Q biotype, and subsequently to determine its partial distribution in a number of provinces in China, which routinely produce ornamental and bedding plants. Phylogenetic analysis of the mitochondrial COI sequence for *B. tabaci* collections from China revealed for the first time that the Q biotype is distributed in multiple locations throughout the country.

MATERIALS AND METHODS

Whitefly Collections

Adult whiteflies (*B. tabaci*) were collected live and placed into tubes containing 95% ethanol. Populations were collected from representative locations and plant species throughout select provinces of China (Table 1).

Whiteflies DNA Extraction, the Polymerase Chain Reaction, and Sequencing

Individual whiteflies were subjected to lysis and DNA extraction following the procedure of Frohlich et al. (1999). Polymerase chain reaction (PCR) (Saiki et al. 1988) primers were employed to amplify a fragment of the *B. tabaci* mitochondrial COI gene (800-820 bp), using parameters and PCR primers, as described by Frohlich et al. (1999).

PCR assays were conducted with 2 μL of each template DNA in a total reaction volume of 25 μL. The PCR reaction mix and PCR conditions followed Frohlich et al. (1999) with a little modification, and 1 unit of Taq DNA polymerase was contained in the PCR reaction mix. PCR products were separated on 1.0% agarose gels, and bands were visualized by ethidium bromide staining and viewed with a UV light source. PCR products were purified with a kit (EZ Spin Column DNA Gel Extraction Kit purchased from Sangon Technology Company, Shanghai) according to the man-

TABLE 1. BIOTYPE, GEOGRAPHICAL SOURCE, HOST PLANT, AND GENBANK ACCESSION NUMBER FOR *B.TABACI* MITO-
CHONDRIAL CYTOCHROME OXIDASE DNA (MTDNA COI) SEQUENCES.

Geographical location and year	Host plant	GenBank Accession number	Acronym	Whitefly haplotype or biotype
Zhengzhou 2003	*Brassica oleracea* L.	AY582870 AY518186	HeNanBoleAY582870	B
Zhengzhou 2003	*Gossypium hirsutum* L.	AY587515	Zhengzhou	B
Zhengzhou 2003	*Cucurbita moschata* L.	AY596949	Zhengzhou	B
Zhengzhou 2003	*Brassica oleracea* L. *var. capitata* L.	AY582873	Zhengzhou	B
Zhengzhou 2003	*Ipomoea batatas* L.	AY589497	Zhengzhou	B
Zhengzhou 2 003	*Solanun melongena* L.	AY587514	HeNanSmelAY587514	Q
Beijing 2003	*Cucumis sativus* L.	AY587519	BJCsatAY587519	B
Beijing 2003	Unknown	AY582867	BJAY582867	B
Beijing 2003	*Capsicum annuum* L.	AY596953 AY582871	BJ	B
Beijing 2003	*Cucumis sativus* L.	AY589498	BJ	B
Beijing 2003	*Heliantus annuus* L.	AY582872 AY589499	BJInilAY582872	Q
Zaozhuang, Shandong 2003	*Cucumis sativus* L.	AY587518	SDZZAY587518	B
Taian, Shandong 2003	Unknown	AY587517	SDTAAY587517	B
Nanjing, Jiangsu 2003	*Gossypium hirsutum* L.	AY518185	JSNJAY518185	B
Zhejiang 20003	Unknown	AY566182	ZJAY566182	B
Zhejiang 2003	Unknown	AY596952	ZJNBAY596952	(non-B/Q) Asian clade
Shanghai 2003	*Euphorbia pulcherrima* Willd.	AY550274	ShHaiEpulAY550274	B
Shanghai 2003	*Brassica albo-glabra* Bail.	AY550273	Shanghai	B
Kunming, Yunnan 2003	*Euphorbia pulcherrima* Willd.	AY587516 AY518189	YN2AY587516 YNAY518189	Q
Haikou, Hainan 2003	*Solanun melongena* L.	AY518187	HaiNAY518187	B
Tulufan, Xinjiang 2003	*Euphorbia pulcherrima* Willd.	AY582868	XJAY582868	B
Israel 2003	*Gossypium hirsutum* L.	AY518190	IBAY518190	B
Israel 2003	*Gossypium hirsutum* L.	AY518191 AY582869	IQAY518191	Q-like
Spain 2003	*Solanum lycopersicum* L.	AY596951	SB AY596951	B
Spain 2003	*Solanum lycopersicum* L.	AY596950 AY587513 AY562216	SQWAY596950 SQ1AY587513 SQ2	Q
Arizona, USA 2003	*Hibiscus rosa-sinensis* L.	AY518194	AZAY518194	B

TABLE 1. (CONTINUED) BIOTYPE, GEOGRAPHICAL SOURCE, HOST PLANT, AND GENBANK ACCESSION NUMBER FOR *B.TABACI* MITOCHONDRIAL CYTOCHROME OXIDASE DNA (MtDNA COI) SEQUENCES.

Geographical location and year	Host plant	GenBank Accession number	Acronym	Whitefly haplotype or biotype
California, USA 2003	*Solanun melongena* L.	AY589496	California	B
California, USA 2003	*Euphorbia pulcherrima* Willd.	AY550272	CLAY550272	B
Texas, USA 2003	*Brassica oleracea* L. var. *capitata* L.	AY518192	TXAY518192	B

ufacturer's instructions. The DNA sequence for each PCR product was determined from the 5'end at the Sangon Technology Company, Shanghai.

Phylogenetic Analyses and Identification of *B. tabaci* Haplotypes in China

The mtCOI sequences for select, representative whiteflies (geographical and host or origin) were determined, and reference mtCOI sequences were obtained from the GenBank database. The DNA sequence was obtained for one to three individuals from each of 30 whitefly collections (Table 1). The mtCOI sequences were aligned with the CLUSTAL W algorithm (Thompson et al. 1994). Distances were calculated with the Kimura 2-parameter model of MEGA2.1 (Kumar et al. 2001). The NJ (Neighbour-Joining) and UPGMA (Unweighted Pair Group Method with Arithmatic Mean) algorithms available in MEGA2.1 (Kumar et al. 2001) were used to infer phylogenetic relationships, respectively. Two thousand bootstrap replicates were performed for each analysis.

RESULTS

Phylogenetic Analysis of the *B. tabaci* MtCOI

The mtCOI sequence was edited to remove PCR primer sequences, which yielded a ~470-bp fragment for each *B. tabaci* mtCOI sequence. The mtCOI sequences have been deposited in GenBank and the Accession Number for each is shown parenthetically (Table 1). Because the same haplotype was typically observed in field populations, only one mtCOI sequence was included for each representative haplotype per field collection.

The mtCOI sequence was used to identify biotypes and haplotypes, based on phylogenetic relationships. The mtCOI NJ (Fig. 1) and UPGMA (not shown) trees revealed similar results and four main clades were supported, each by robust bootstrap value. Three distinct clades were revealed with two major nodes strongly supported by 100% bootstrap values.

One major clade (I) contained sequences for the B biotype, and this identification was based on a high degree of shared nucleotide identity with reference sequences for the B biotype (Costa & Brown 1991). These collections were from Zhengzhou in Henan Province [AY582870, AY518186, AY587515, AY596949, AY582873, AY589497], Beijing [AY587519, AY582867, AY596953, AY582871, AY589498], Shandong [AY587518, AY587517], Jiangsu [AY518185], Zhejiang [AY566182], Shanghai [AY550274, AY550273], Hainan [AY518187], and Xinjiang [AY582868]. The latter haplotypes grouped closely with the reference sequences for the B biotype previously identified in Israel [AY518190], Spain [AY596951], and the U.S.A., including Arizona [AY518194], California [AY589496], and Texas [AY518192].

A second major clade (II) contained *B. tabaci* identified as the Q biotype (Guirao et al. 1997; Moya et al. 2001) or variants of the Q haplotype sequence. The Q-like haplotype was identified in collections from Yunnan [AY587516,AY518189], from Zhengzhou in Henan [AY587514], and from Beijing [AY582872, AY589499]. The Q haplotype has been identified previously in Spain [SQ1AY587513, SQWAY596950], where it was subsequently characterized in biological terms as the Q biotype (Guirao et al. 1997; Moya et al. 2001), and in Israel [IQAY518191] (Horowitz et al. 2003). The latter population/haplotype, which is a very close relative of the Spanish Q biotype, is probably indigenous to Israel, because *B. tabaci* is composed of several genetically distinct groups with a strong geographical association between more closely related biotypes (Frohlich et al. 1999; De Barro et al. 2000).

The third clade (III) was represented by a haplotype for a population collected from Zhejiang, China [ZJNBAY596952], which appears to be of Asian origin.

Nucleotide Divergence Estimates

Within and between nucleotide sequence divergence were calculated for field collections from China and reference population sequences for the
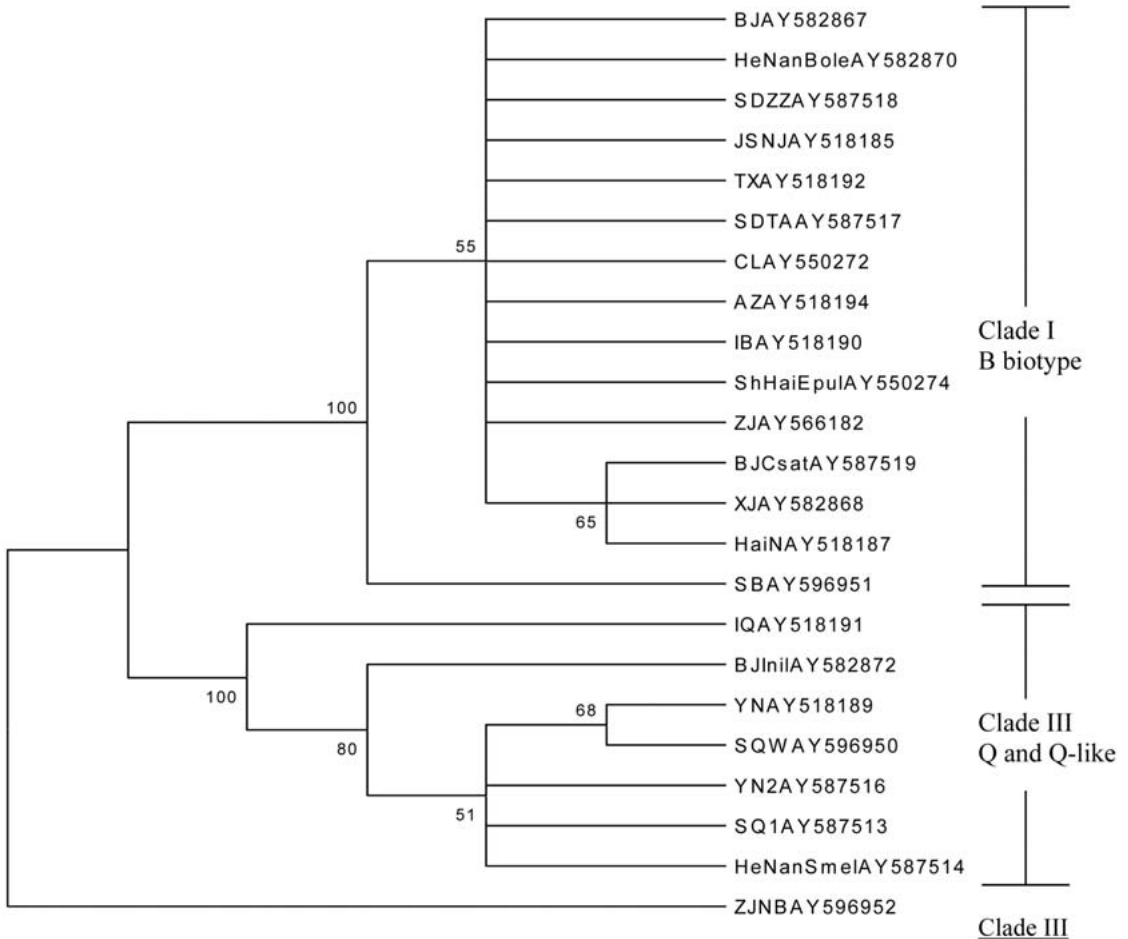
Figure 1. Phylogenetic tree for *B. tabaci* based on a fragment (~450 bases) of the mitochondrial cytochrome oxidase I gene. The tree was inferred by using the UPGMA method and 2000 bootstrap replicates. Abbreviations for whitefly collections are shown in Table 1.

B and Q biotypes of *B. tabaci*. Populations from Beijing [BJAY582867, BJCAY587519], Xinjiang [XJAY582868], Shandong [SDZZAY587518, SDTAAY587517], Henan [HeNanBoleAY582870], Jiangsu [JSNJAY518185], Shanghai [ShHaiEpulAY550274], Zhejiang [ZJAY566182], Hainan [HaiNAY518187] from China shared more than 99.6% nucleotide sequence identity with *B. tabaci* B biotype identified from Spain [SBAY596951], Israel [IBAY518190], Arizona [AZAY518194], California [CLAY550272], and Texas [TXAY518192]. These mtCOI sequences were 100% identical with B biotype sequences for populations collected from various other locations, worldwide.

The nucleotide divergence estimates indicated that accessions from Beijing [BJlnilAY582872], Yunnan [YN1YNAY518189, YN2AY587516] and Henan [HeNanSmelAY587514] shared >99.6%

nucleotide sequence identity with the Q biotype from Spain [SQ1AY587513, SQWAY596950]. The mtCOI sequences from the latter collections from China likewise shared 98.9%-99.4% nucleotide sequence identity with one population of *B. tabaci* from Israel [IQAY518191].

Sequence comparisons collectively suggest certain collections of *B. tabaci* from China are the Q biotype, and the *B. tabaci* population from Israel [IQAY518191] also is Q-like. The populations from Israel and China were slightly more divergent from one another than collections from China were from sequences obtained from *B. tabaci* Q biotype from Spain. The mtCOI DNA sequence (~470 bp) for whiteflies identified as the Q biotype from China was highly invariant (99-100% nucleotide identity) (data not shown), suggesting that they originated recently from a single or a very few introductions and/or original source(s).

The collection from Zhejiang [ZJNBAY596952] shared only 82.3%-83.5% nucleotide sequence identity with reference *B. tabaci* sequences included here, indicating that the Zhejiang haplotype was neither B nor Q biotype, and likely represents a divergent *B. tabaci* population that originated 'locally' and is indigenous to Asia.

DISCUSSION

Recently, the Q biotype of *B. tabaci*, which had been a relatively benign pest in the Mediterranean region (Simón et al. 1999), has been recognized as a serious pest and virus vector, owing to its ability to reach high population densities (Moya et al. 2001; Simón et al. 1999) and to develop resistance to at least one neonicotinoid (Rauch & Nauen 2003). These characteristics have been noted together with an increase in Q biotype infestations in southern Spain, where the B biotype is now almost absent, despite its introduction there in the mid-1990s (Simón et al. 1999). What is now recognized as the Q biotype has been identified in the Iberian Peninsula, in Sardinia and Sicily, and in Morocco (Brown 2000; Moya et al. 2001), the general region (Mediterranean/Middle East/North Africa) to which Q-like haplotypes are thought to be indigenous (Brown 2000).

Significant differences in host suitability (Muniz 2000) and developmental parameters (Muniz & Nombela 2001) for the B and Q biotypes, with respect to four weed species that occur in the winter months, were determined in no-choice assays. Except for *Lactuca serriola* L., the mean reproductive parameters for the Q biotype were significantly greater than those for the B biotype (Muniz 2000). The Q biotype showed higher daily infestation rates than the B biotype on most tomato varieties tested (Nombela et al. 2001). On sweet pepper, the generation time for the Q biotype was found to be shorter than that of B biotype at 33 and 17 d, respectively. The number of cumulative generations of Q biotype also was somewhat greater than for the B biotype (Muniz & Nombela 2001). Furthermore, the resistance of the Q biotype to pesticides has been shown to be more resilient than observed for the B biotype (Anthony et al. 1995; Costa et al. 1993; Rauch & Nauen 2003). These collective results are likely linked to the increased pest status of the Q biotype in the Mediterranean region during the past several years.

Prior to this study, the Q biotype had not been reported in China. The high intra-population homogeneity suggests that a recent introduction of this biotype has occurred in China. Knowledge of insecticide resistant-Q haplotype populations in Spain (Ebert & Nauen 2000) and Israel (Nauen et al. 2002), and also that Spain and the Canary Islands are important producers of ornamentals crops that could have made their way to China in commercial trade, suggests a link between the movement of ornamentals species from the Mediterranean region and the recent presence of the Q type in China. Importation records have revealed that poinsettia and other ornamental plants were imported to China from Spain for the International Horticultural Exposition held in 1999 in Kunming, Yunnan Province. Such plants could have provided one possible route of entry into China for the Q biotype.

Herein, we report that the Q-biotype of *B. tabaci* was identified for the first time on infested ornamentals plants in several different regions of China. It is now important to closely monitor the potential establishment and spread of the Q biotype in the country to avoid its further dissemination, which could be devastating to vegetable and ornamental production. We have identified the Q biotype in distant locations in China, suggesting that multiple introductions may have occurred, or that plants from a single introduction were moved long distances from the original sources. Additional studies will be required to test this hypothesis and to determine if the Q biotype is sufficiently adapted to conditions in China to establish as an invasive pest and vector of plant viruses in ornamental, vegetable, and fiber crops.

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POPULATION DYNAMICS OF THE FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) AND ITS PARASITOIDS IN NORTHWESTERN ARGENTINA

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ABSTRACT

In order to know the population dynamics of the fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), and its parasitoids in northwestern Argentina, larvae were weekly collected at two different agrological regions (Tafí Viejo, and Vipos) over four years. The relationship between larval and parasitoid populations, climatologic factors, percent of infested plants, parasitoid relative importance index, abundance of the parasitoids, and percent parasitism were estimated. FAW attacked cornfields when the plants achieved V1 and V2 stages. Temperature and rainfall were the climatologic factors that significantly affected pest density, and temperature affected the parasitoid abundance as well. The FAW parasitoids collected were *Campoletis grioti* (Blanchard), *Chelonus insularis* (Cresson), *Ophion* sp. and *Archytas* spp. (possibly *marmoratus* and/or *incertus*). The average parasitism percentage was 39.4% and 15% in T. Viejo and Vipos, respectively. Parasitoid abundance in both regions was similar, but diversity was different possibly relating to the native surrounding vegetation in Vipos. This is the first report of population dynamics of the fall armyworm and its parasitoids in northwestern Argentina.

Key Words: *Spodoptera frugiperda*, parasitoid complex, population fluctuation, performance, corn

RESUMEN

Para conocer la dinámica poblacional del gusano cogollero del maíz (GC), *Spodoptera frugiperda* (J. E. Smith) y la de sus parasitoides en el Noroeste argentino, se colectaron larvas semanalmente en dos regiones agrológicas (Tafí Viejo y Vipos) durante cuatro años. Se determinó la relación entre la fluctuación poblacional de la plaga y la de sus parasitoides con los factores climáticos. Se estimó el porcentaje de plantas dañadas, el índice de importancia relativa, la abundancia de los parasitoides, y el porcentaje de parasitismo. El GC ataca los cultivos de maíz ni bien son implantados, cuando las plantas alcanzan las etapas fenológicas V1 y V2. La temperatura y la precipitación fueron los factores climatológicos que afectaron significativamente la abundancia de la plaga. La abundancia de los parasitoides también fue afectada por la temperatura. Los parasitoides colectados fueron *Campoletis grioti* (Blanchard), *Chelonus insularis* (Cresson), *Ophion* sp., and *Archytas* spp. (posiblemente *marmoratus* y/o *incertus*). Los porcentajes de parasitismo fueron 39.4% y 15% en T. Viejo y Vipos, respectivamente. La abundancia de los parasitoides en las dos zonas estudiadas fue similar, pero la diversidad fue diferente posiblemente relacionada a la vegetación circundante en Vipos. Este es el primer estudio sobre la dinámica poblacional del GC y la de sus parasitoides en el Noroeste argentino.

Translation provided by the authors.

INTRODUCTION

Insects are a dominant component of agricultural ecosystems, and they impact crop yields in many ways. Several species are pests of row and horticultural crops, reducing yields by the transmission of diseases or by direct damage. Other insects are natural enemies of pest species, and can be used as biological control agents for reducing pest organisms. Insects are considered indicators of biodiversity, providing a means of determining the effects of agricultural practices on whole communities or on abundance and dynamics of individual species (LaSalle 1993).

Understanding the factors that influence the distribution and abundance of an insect is a fundamental issue of insect ecology and is a practical concern with insects that cause economic damage (Baskauf 2003). Insect population dynamics have fundamentally different characteristics depending on the strength and form of exogenous (density-independent) vs. endogenous (density-dependent) forces. Many factors affect population abundance such as competition, natural enemies, and resources, but the relative contribution of exogenous and endogenous effects remains an open question for nearly all biological populations (Ylioja et al. 1999).

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) was recognized as a destructive pest of many agricultural crops more than 200 years ago (Luginbill 1928). It is an important economic pest of corn, rice, sorghum, peanut, alfalfa, cotton, Sudan grass, soybean, tobacco, oat, wheat, sugarbeet, and diverse pasture grasses such as Bermuda grass, Johnson grass, and others (Sparks 1979; Andrews 1980; Capinera 1999), and it is widely distributed in America. Its distribution extends eastward into the Caribbean, southward to northern Argentina and northern Chile, and northward through Central America, Mexico, the United States, and southern Canada (Andrews 1980).

Because the FAW has a wide distribution, it is subjected to much climatic diversity, namely, temperature, moisture, and soil type. The environmental factors influencing development and survival, as well as genotype, agricultural practices, crop phenology, and plant maturity may contribute to the dynamics of the system in a given locale (Harrison 1984a; Pair et al. 1986; Barfield & Ashley 1987; Simmons 1992; Riggin et al. 1993).

The FAW is the most important corn pest, causing yield losses fluctuating from 17% to 72% in northeastern Argentina (Perdiguero et al. 1967). However, studies related with the population dynamics of FAW in Argentina, and how environmental factors affect this phenomenon were not previously reported in Argentina. Reports related to the relationship between date and damage by FAW in commercial corn in northwestern Argentina have been published by Willink et al. (1993a, b), and Sosa (2002a, b).

FAW has a diverse complex of natural enemies in the Americas and the Caribbean basin (Ashley 1979; Ashley et al. 1982; Molina-Ochoa et al. 2003). In Argentina at least thirteen species of hymenopteran parasitoids and eight dipteran parasitoids are known to attack FAW (Vera et al. 1995; Virla et al. 1999; Murúa et al. 2003; Murúa & Virla, 2004). However, there is a lack of information on the natural distribution of FAW and its parasitoids in northwestern Argentina, as well as the influence of environmental factors on their dynamics. We report the population dynamics of FAW, its parasitoids, and the influence of environmental factors on the dynamics in the northwestern region of Argentina.

MATERIALS AND METHODS

Sampling Sites

Two agrological regions (Zuccardi & Fadda 1985) of northwestern Argentina were systematically sampled for FAW larvae during the crop-growing part of the year over a period of four years. The two regions were located in the province of Tucumán. The first region was Tafí Viejo (Department of Tafí Viejo), located between the coordinates 26° 44' S, 65° 14' W, and 609 m altitude. This region is part of the Chaco Pampeana Plain, and it is characterized by good availability of soil moisture during the year. The cornfield used for monitoring was seven ha in size and planted with the regional corn variety Leales 23.

The second region was Vipos (Department of Trancas) located at 26° 28' S, 65° 18' W, and 786 m altitude, in the Intermontana of Tapia-Trancas basin. This region is characterized by soil moisture availability only during the rainy part of the year (December-January), and irrigation is usually required. The cornfield sampled at Vipos was ca. 40 ha, and was also planted with the corn variety Leales 23. Other commercial crops grown in the area included corn, soybean, pumpkins, and vegetables. All commercial fields routinely applied insecticides.

Both regions exhibit agrological differences in their hydrological conditions such as rainfall and evapotranspiration. These differences determine the planting date for each region. Corn can be planted during late October at Tafí Viejo, while the planting date at Vipos occurs from late December to early January.

Sampling for FAW larvae was conducted weekly at Tafí Viejo from October 1999 to January 2000 (Year 1), from October to December in 2000 (Year 2), from October to December 2001 (Year 3), and November 2002 to January 2003 (Year 4). In Vipos, the larvae were sampled from January to March in 2000 (Year 1), from October to December in 2000 (Year 2), from October to December 2001 (Year 3), and from January to March in 2003 (Year 4). Insecticides were not applied in any of the fields sampled in this study.

Larval Sampling

FAW larvae were sampled beginning approximately 10-12 d after the date corn plants exhibited two ligulate leaves, and continued until the beginning of the reproductive stage (R1) (Ritchie et al. 1992). The sampling period lasted about five to seven weeks. Fifty corn plants were randomly sampled at each sampling date, and divided in

five groups of ten plants. The plants were checked for the presence of FAW eggs, larvae, and/or adults following the methodologies used by Willink et al. (1993a,b), García Roa et al. (1999), and Fernández (2002). The number of corn infested plants was recorded in order to determine the percentage of infested plants (Harrison 1984b).

FAW larvae collected from cornfields were placed in glass vials (12 cm long × 1.5 cm diameter) containing a piece of fresh corn leaf, and were kept in a chamber under controlled conditions at 25 ± 2°C, 70-75% RH, and 14L:10D photoperiod. FAW larvae were then transferred to similar tubes containing 1 cm³ of artificial diet (Osores et al. 1982). The diet vials containing FAW larvae were maintained in the laboratory until the parasitoids had emerged, or until FAW adult emergence (Riggin et al. 1993).

Parasitoid Identification

Parasitoids were recorded and identified by several specialists. Tachinids were identified by Lic. Susana Avalos (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina), and *Campoletis grioti* (Blanchard) by Dra. Carolina Berta (Fundación Miguel Lillo, Departamento de Zoología, Entomología, San Miguel de Tucumán, Argentina). The remaining parasitoids were compared to specimens previously identified by Dr. Luis De Santis (Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, La Plata, Argentina).

Population Variables

The Percent of infested plants (% IP) (Harrison 1984b, Urbaneja García 2000, Diez 2001) was calculated by the following equation:

$$\% \text{ IP} = \frac{\text{Infested plants}}{\text{total plants}} \times 100$$

The relative importance index (RII) of the parasitoid species allows for an estimation of the species not only considering its abundance but also its occurrence or frequency. In this way, species poorly represented in individual numbers but frequently recovered over a long period can be balanced with numerous species with sporadic occurrence (Remes-Lenicov & Virla 1993; Rueda 1999; Diez 2001). It was calculated by the following formula:

$$\text{PRII} = \frac{\text{No. of individuals of the species "i"}}{\text{Total No. different species}} \times \frac{\text{No. of samples species "i" occurred}}{\text{Total No. of samples}} \times 100$$

Frequency (F) is the percent of individuals of certain species in relation to total individuals of all species (Canal Daza 1993; Molina-Ochoa et al. 2001; Molina-Ochoa et al. 2004), and was calculated by using the following formula:

$$F = \frac{\text{No. individuals of species "i"}}{\text{No. total collected individuals}} \times 100$$

Percent of parasitism (%P) was calculated according to Van Driesche (1983); Pair et al. (1986), and Crisóstomo-Legaspi et al. (2001), as follows:.

$$\%P = \frac{\text{No. total parasitized individuals}}{\text{No. total individual observed}} \times 100$$

Statistical Analysis

Percent data were angularly transformed and subjected to analysis with the software Statistics © 5.5 (2000). In order to determine differences between and among FAW and parasitoid collections from the same region and those from different regions, student t tests were performed.

Regression analyses also were performed to determine the relationship between FAW populations and parasitoid abundance with temperature and rainfall (Diez 2001; Schliserman 2001) by a stepwise approach. For the analyses, the mean of low and high temperatures and mean rainfall during the sampling week, and the mean of low and high temperatures and rainfall recorded in the two weeks previous to the sampling date, were used. From these data it was possible to estimate the week in which the environmental factors most affected the FAW populations, and the abundance of FAW parasitoids.

RESULTS AND DISCUSIÓN

The percent of infested plants (%IP) by FAW larvae was higher at Vipos (≅20%) than at Tafí Viejo (5.5%) ($t = 0.0001$, $P < 0.001$, $df = 75$). The annual percentage of infested plants at Tafí Viejo was highest during 2001 (9%), while at Vipos the highest record was during 2000 (71.3%). The %IP during the four year study in Tafí Viejo were 5.8%, 0.1%, 8.9%, and 8.9%. However in Vipos the %IP were 71.3%, 19.7%, 21.3%, and 18.4%. The total percent of infested plants (%TIP) in Tafí Viejo, and Vipos were 5.5, and 30%, respectively. ANOVA revealed significant differences in the number of infested plants among years during the 4-year study in both areas (T. Viejo: $F = 106.38$; $P < 0.001$; $df = 72$, and Vipos: $F = 91.46$; $P < 0.001$, $df = 74$). The results obtained at Tafí Viejo (early planting) and Vipos (late planting) agreed with those previously reported by Willink et al. (1991) for the Tucumán region, and Sosa (2002a) for the North of Santa Fé province. Earlier plantings had lower levels of FAW infestation and damage, a response similar to that reported by Mitchell (1978), and Harrison (1984b) on corn infested by corn earworm and fall armyworm, respectively.

FAW Collection

About 2400 corn plants were examined in Tafí Viejo, and 132 FAW larvae were collected and 52 parasitoids were recovered. The mean number of FAW larvae per 10 plants was 0.58, 0.013, 0.89, and 0.88 during years 1, 2, 3, and 4, respectively. Larvae were collected as early as late September because of the early planting date. FAW larvae were not found in the first phenological stages (one to three ligulate leaves, V1-V3). One larva was recorded in year 2 during the late crop-growing season, when the plants had seven to eight leaves (V7-V8). Overall, years 1 and 3 produced higher densities of FAW larvae during the vegetative stages V3 to V6, similar to what was found by Hernández-Mendoza (1989) in Colima, México. In contrast, higher larval densities were recorded in years 2 and 4 at the end of the vegetative period.

In Vipos, 2750 plants were examined, 540 larvae were collected, and 82 parasitoids were obtained. The mean number of FAW larvae per 10 plants was 2.59, 2.17, 1.25, and 1.83 during years 1, 2, 3, and 4, respectively. During years 1 and 2, higher larval numbers were recorded in the V1-V3 stages. Larval populations were consistent throughout the vegetative plant phase for the other years. Overall, larval densities diminished with the age of the cornfield, achieving the lowest numbers during the beginning of the corn's reproductive stages. Comparing FAW population fluctuations in both locations during this 4-year study, FAW at Vipos exhibited significantly higher larval numbers ($t = 1.99$, $P < 0.001$, $df = 72$).

FAW and Corn Phenology

FAW infestations displayed a plant age-dependent response at both localities during the 4-year study. Reduced mean larval numbers were related to plant age and development. Mitchell et al. (1974), and Beserra et al. (2002) found that the distribution of FAW larvae and eggs varied according to the phenological stage of the corn. During the early plant stages (V1-V3), first and second instars were predominant, and about one to six larvae per plant were found. During V4 and V6, only one larva was usually recovered per plant. Carvalho & Silveira (1971) found that small and medium larvae would coexist, but the number of larvae per plant decreased as larval size increased. Larval cannibalism, larval mortality from disease or predators, and larval age are possible factors that influence distribution.

FAW and Climatic Factors

In Tafí Viejo a temperature-dependent response was obtained with respect to FAW population abundance [Y = -1.34 + 1.29 log Max 2T° (mean high temperatures recorded in the week previous to the sampling plus mean high temperatures recorded in the second week previous to the sampling) - 0.29 log rainfall 2 (mean rainfall recorded in the week previous to the sampling plus mean rainfall recorded in the second week previous to the sampling)]; $n = 30$; $P < 0.001$; $R^2 = 0.99$). Barfield & Ashley (1987) reported that corn phenology and temperature affected larval development, food consumption, and adult female longevity and fecundity, and that developmental times were temperature-dependent and were modified by the stage of corn consumed. However, at Vipos an associated response was observed with rainfall [Y = 3.89 + 0.5 log rainfall 0 (mean rainfall during the sampling week) –0.18 log Max T°0 (means of high temperatures during the sampling week)]; $n = 30$; $P < 0.01$; $R^2 = 0.219$). Silvain & Ti-A-Hing (1985) found that the highest populations of FAW moths and larvae were observed during the rainy seasons, and lowest during the dry seasons.

Insect phenology, density, and number of FAW generations are influenced by the climatic conditions in a given region. Climatologic differences between and among localities could explain the phenological differences, and climatologic conditions among years also could explain fluctuations in pest abundance in each area (Dent 1991; Diez 2001).

Parasitoid Species

Considering the diversity of parasitoids reported from the Tucumán area (Vera et al. 1995; Virla et al. 1999; Berta et al. 2000; Murúa et al. 2002; Murúa et al. 2003; Murúa & Virla 2003; Murúa & Virla 2004) few species were recovered in our study. Only two ichneumonids, *Campoletis grioti* (Blanchard) and *Ophion* sp., one braconid, *Chelonus insularis* (Cresson), and possibly one or two species of tachinids, *Archytas marmoratus* (Town.) and/or *A. incertus* (Macquart) were found. All species were collected at Vipos (Table 1), and only *C. grioti* was recovered at Tafí Viejo. Of all parasitoids collected at Vipos, *Archytas* spp. comprised 38.3%, *C. grioti* 35.8%, *Ch. insularis* 22.2%, and *Ophion* sp. 3.7%. Seasonally, *Ophion* sp. was collected when corn plants were V4, whereas *Archytas* spp. and *C. grioti* were collected at the end of the crop cycle. These results are in agreement with those by Virla et al. (1999) and Vera et al. (1995), who reported *C. grioti* and *Ophion* sp., respectively, attacking FAW larvae collected from corn in Argentina. Our results are also in agreement with Molinari & Avalos (1997), who showed that the dipteran parasites attacked the last instars of FAW in Argentina. No differences were determined in the abundance of parasitoids in both locations during the 4-year study ($t = 1.36$, $P = 0.19$, $df = 38$). We speculate that differences in early or late corn planting would not affect the abundance of FAW parasitoids.

TABLE 1. FREQUENCY AND RELATIVE IMPORTANCE OF THE FAW PARASITOID COMPLEX IN VIPOS DURING THE FOUR-YEAR STUDY.

Species	Frequency during year (%)				Relative Importance during year (%)				
	1	2	3	4	1	2	3	4	Total
C. grioti	52.2	44.4	33.3	5.5	0.26	0.19	0.15	0.004	0.13
Ch. insularis	5.6	33.3	33.3	38.9	0.01	0.007	0.11	0.07	0.056
Ophion sp.	34.8	5.6	4.2	0.0	0.02	0.007	0.146	0.0	0.005
Dipteran spp.	25.0	44.4	29.2	57.9	0.1	0.2	0.1	0.2	0.14

Percent Parasitism

Campoletis grioti was the single parasitoid responsible for 39.4% parasitism at Tafí Viejo. In Vipos, overall percent parasitism during the 4-year study was 15%. The tachinid species, *C. grioti*, *Ch. insularis*, and *Ophion* sp. caused 5.7%, 5.4%, 3.3%, and 0.6% of total FAW parasitism, respectively. The highest record of annual parasitism was recorded during year 2 with 10.5% for *C. grioti* and the dipteran species, but during year 3, *C. grioti*, and *Ch. insularis* each caused 8.5% of parasitism (Table 2).

Kogan et al. (1999) mentioned that cultural practices developed in a plot can affect in a positive or negative way the natural enemy populations, increasing or inhibiting the parasitoid colonization in cultivated fields. These practices could also have direct or indirect effects, directly through environment alterations and indirectly affecting the host plant architecture, lack of food, or refuge.

The lack of vegetation surrounding the sampling area in the cornfields at Tafí Viejo could be a reason for the low diversity found. This area was surrounded by lemon groves where insecticide applications are commonly applied. It is known that the presence of spontaneous vegetation associated with the crop results in higher numbers and diversity of natural enemies related to this vegetation (Altieri & Whitcomb 1980; Hoballah et al. 2004).

It is important to consider that *C. grioti* is a oligophagous parasitoid that attacks different hosts of several genera in the family Noctuidae. Another possible cause for low diversity is early planting in Tafí Viejo that reduces FAW infestation, and damage to cornfields (Willink et al. 1991). Conversely, in Vipos corn is planted later and the fields were surrounded by native vegetation without significant anthropogenic disturbances affecting potential parasitoid refuges. Higher diversity of parasitoids and higher rates of parasitism in Vipos also may be related to a

TABLE 2. ABUNDANCE AND PERCENT PARSITISM OF FAW PARASITOIDS OBTAINED IN TAFÍ VIEJO AND VIPOS DURING THE FOUR-YEAR STUDY.

Location	Abundance and percent parasitism (%)							
	Tafí Viejo				Vipos			
Year	1	2	3	4	1	2	3	4
NPS*	500	750	450	700	900	350	750	750
FAWCL	29	1	40	62	233	76	94	137
Species								
C. grioti	4	1	18	29	12	8	8	1
	(13.8)	(100)	(45.0)	(46.8)	(5.2)	(10.5)	(8.5)	(0.7)
Ch. Insularis	—	—	—	—	2	1	8	7
					(0.8)	(1.3)	(8.5)	(5.1)
Ophion sp.	—	—	—	—	1	1	1	—
					(0.8)	(1.3)	(1.1)	
Dipteran spp.	—	—	—	—	5	8	7	11
					(2.1)	(10.5)	(7.5)	(8.0)
Total parasitoids collected (%)	4	1	18	29	20	18	24	19
	(13.8)	(100)	(45.0)	(46.8)	(8.6)	(23.7)	(25.5)	(13.9)

(*) Number of plants sampled, FAWCL = FAW collected larvae.

more diverse habitat with more forest, orchards, groves, and pastures near to cornfields (Molina-Ochoa et al. 2001; Hoballah et al. 2004). Overall, the percent parasitism measured in this study was similar to other studies. Berta et al. (2000) reported parasitism ranging between 5.26% and 50% in cornfields with and without insecticide application in the province of Tucumán, respectively. Luchini & Almeida (1980) listed FAW parasitoids occurring in Brazil and considered *C. grioti* the most important parasitoid causing about 95% parasitism.

Ashley (1986) and Andrews (1988) listed *Ch. insularis* occurring throughout North America highlighting its role as parasitoid of FAW by showing parasitism of 63% in southern Florida; however, Pantoja & Fuxa (1992), Molina-Ochoa et al. (2001), and Molina-Ochoa et al. (2004) reported lower levels of parasitism by *Ch. insularis* of about 5% in Puerto Rico and Mexico, respectively. This braconid has the broadest distribution in Latin America and South America (Molina-Ochoa et al. 2003). Lewis & Nordlund (1980) consider this parasitoid an excellent candidate for augmentative release because it can be introduced throughout its overwintering zone. It is capable of early-season colonization, and can be used in direct therapeutic releases on target crops.

The ichneumonid *Ophion* sp. caused the lowest level of FAW parasitism in Vipos, ranging between 0.8 and 1.3% during the 4-year study. Similar results have been reported by Molina-Ochoa et al. (2001). Gross & Pair (1991) state that *Ophion flavidus* (Brullé) parasitized 4th, 5th, and 6th instar FAW with equal success, but were minimally successful in completing development on late 6th instars. This parasitoid caused 19.5% of parasitism in June in southern Georgia. *Ophion* sp. has been reported previously in Argentina (Vera et al. 1995).

Dipteran parasites played an important role in Vipos in the 4-year study, providing high parasitization levels. *Archytas marmoratus* and /or *A. incertus* caused levels of parasitization between 2.1%, and 10.5%. The importance of these species in Argentina and other South American countries was emphasized by Molina-Ochoa et al. (2004) based on reports by Molinari & Avalos (1997) and Virla et al. (1999).

Parasitoids and Climatic Factors

Temperature was the most important climatic factor influencing parasitoid populations in both locations. Similar responses of other parasitoids have been reported by Diez (2001), and Schliserman (2001) for the Tucumán region, such as those attacking the fruit flies, *Ceratitis capitata* (Weid.), *Anastrepha fraterculus* (Weid.), and citrus leafminer, *Phyllocnistis citrella* (Stainton).

The maximum temperature in Tafí Viejo was the most important factor [$Y = -1.55 + 1.3 \log Max 2T°$ (mean high temperatures recorded in the week previous to the sampling plus mean high temperatures recorded in the second week previous to the sampling) $- 0.3 \log$ rainfall 0 (mean rainfall during the sampling week)]; $n = 38$; $P < 0.001$, $R^2 = 0.99$) affecting the parasitoid population fluctuation.

In Vipos minimum temperature was important factor but no climatic factor was a significant variable describing parasitoid populations [$Y = 2.62 + 0.387 \log T° Min 0$ (mean low temperatures during the sampling web) $- 0.18 \log$ rainfall 1(mean rainfall recorded in the week previous to the sampling)]; $n = 30$; $P = 0.11$; $R^2 = 0.085$). Factors, such as insecticides, farming and cultural practices, and other natural enemies, may be influencing parasitoid populations.

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GENETIC EVIDENCE FOR TWO INTRODUCTIONS OF THE FORMOSAN SUBTERRANEAN TERMITE, *COPTOTERMES FORMOSANUS* (ISOPTERA: RHINOTERMITIDAE), TO THE UNITED STATES

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ABSTRACT

Exotic introductions of Formosan Subterranean Termite (FST) to the United States from Asia have had significant economic consequences. Multiple introductions through marine transport have been proposed, but identification of these routes has yet to reveal more than one lineage in the continental U.S. DNA sequencing of a 640-bp cytochrome oxidase II (COII) mitochondrial DNA (mtDNA) marker to 60 disjunct populations, revealed two independent lineages spanning the continental U.S., Hawaii, Japan, and China. Limited genetic variation was observed with this marker. Group I constitutes a largely Asian clade, while Group II is comprised of both Asian and southern U.S. populations. This is the first study which has documented 2 distinct lineages to continental United States and Hawaii.

Key Words: invasive species**,** DNA sequence, genetic variation, molecular diagnostics, termite

RESUMEN

Las introducciones exóticas de la termita subterránea de Formosa (TSF) de Asia a los Estados Unidos han tenido consecuencias económicas significativas. Introducciones multiples por medio del transporte marino han sido propuestas, pero la identificación de estas rutas todavia no ha revelada mas que un linaje en los Estados Unidos continental. La secuenciación de un marcador de 640-bp del citocromo-c-oxidasa II de ADN mitocondrial (mtADN) a 60 poblaciones separadas, revelo dos linajes independientes atravesando los Estados Unidos continental, Hawaii, Japan y China. El marcador mostró una variación genética limitada. El grupo I constituye un clado principalmente asiático, mientras el grupo II consiste de poblaciones asiáticas y del sur de los Estados Unidos. Este es el primer estudio que documenta los dos linajes distintas en los Estados Unidos y Hawaii.

Formosan subterranean termite (FST) *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), has long been suspected to have originated from Formosa (the Island of Taiwan), but endemic to mainland China due to the identification of a termitophile from there (Kistner 1985). FST has been reported from 14 southern provinces in China with a northern limit of 33°28' N and a western limit of 104°35'E (Gao et al. 1982; He & Chen 1981; Lin 1986) (Fig. 1). Introductions of this exotic pest have been documented around the world following closely with trade routes extending to the United States and beyond (Chhotani 1985). Historical shipping trade between the east and west over the past 450 years (Welsh 1996; Lim 1997), and the likely introduction(s) of FST to the continental U.S. after World War II (La

Fage 1987), have made tracking introduction points difficult. Trading centers in Guangdong Province (e.g., Macau, Guangzhou, Shenzhen, and Hong Kong), Fujian Province (e.g., Puyuan) and Shanghai Province, China, and Taiwan have provided likely ports of origin for FST (See Province Map, Fig 1). Gay (1967) suggests that introductions of FST into Guam, Midway Island, the Marshall Islands, and the Hawaiian islands are most likely due to shipping trade.

FST is believed to have been introduced to Japan almost 300 years ago (Mori 1987; Su & Tamashiro 1987; Wang & Grace 1999; Vargo et al. 2003), and has been hypothesized to have been introduced to Hawaii almost 100 years ago (Su & Tamishiro 1987). The history of FST introductions to the continental United States is more am-

Fig. 1. Provincial Map of China based on Wang et al. (2002). Shaded provinces reflect areas with known *Coptotermes formosanus* infestations.

biguous because of likely misidentifications. For example, early samples of *Coptotermes* in Houston, Texas, during the 1950s were identified as *C. crassus* Snyder, but were later positively identified as *C. formosanus*.

Presently, FST is distributed across the southeast United States (Spink 1967; Howell et al. 1987; La Fage 1987; Su & Tamashiro 1987; Appel & Sponsler 1989; Chambers et al. 1998; Su & Scheffrahn 1998a; Cabrera et al. 2000; Hawthorne et al. 2000; Howell et al. 2000; Su & Scheffrahn 2000; Hu et al. 2001; Scheffrahn et al. 2001; Jenkins et al. 2002), and disjunct populations in southern California (Atkinson et al. 1993; Haagsma et al. 1995) are thought to have originated from Hawaii. Without doubt, their continued presence and growing distribution(s) have been exacerbated by commerce and trade practices within the United States (Cabrera 2000; Jenkins et al. 2002; Glenn et al. 2003), and by the general lack of education and research funding directed towards this problem until recently (Oper-

ation Full Stop, a FST interdiction research unit located in New Orleans, Louisiana was initiated by the United States Department of Agriculture, Agricultural Research Service in 1998).

Several studies applying genetic or biochemical interpretations of FST populations have attempted to identify introduction routes of FST. However, while multiple entry points appear likely, the lack of genetic variation in this invasive species has made identification of these routes difficult to achieve. Studies applying cuticular hydrocarbons (Haverty et al. 1990), allozymes (Korman & Pashley 1991; Strong & Grace 1993; Broughton & Grace 1994; Wang & Grace 2000), mitochondrial DNA (mtDNA) (Jenkins et al. 2002), and microsatellite DNA (Vargo & Henderson 2000; Husseneder & Grace 2000; 2001a, b; Husseneder et al. 2002) have been reported, but current literature has not conclusively established the origins of alternative routes to the United States. These studies have implicated that more than one introduction route existed, but

they have not corroborated their suppositions with the inclusion of additional FST populations which might elucidate this observation.

Presumably, this could be attributed to the overall lack of genetic diversity of FST globally. In introduced populations, the lack of clear colony boundaries and the potential for considerable mixing of individuals among colonies may lead to the formation of colonies which could extend over large areas making colonial identity difficult, an observation observed in unicolonial ant species (Argentine ant *Linepithema humile*) (Tsutsui et al. 2000, 2001). Alternatively, it may be that the natural dispersal of FST alates is more significant than previous recorded distances (Messenger & Mullins 2005), an explanation proposed for the low mitochondrial DNA (mtDNA) divergence among sites spanning across states such as Georgia (Jenkins et al. 2002). However, human-aided dispersal of FST would be equally plausible as a contribution to low mtDNA divergence. Some argue that the lack of genetic diversity in FST could be due to genetic bottlenecks (Strong & Grace 1993; Broughton & Grace 1994) with limited founder effect. Others suggest the possibility of significant inbreeding due to neotenic involvement (Wang & Grace 1995). For this to be acceptable, one must assume that there would be some inbreeding depression or fixation.

Herein, we report that while multiple introductions of FST (to the United States) are presumed, limited genetic variation in this species restricts the clarification of exactly where these exotic introductions originated from when using some molecular markers. We provide evidence of 2 distinct lineages, occurring in the continental United States and in the Hawaiian Islands, with identical lineages from China.

MATERIALS AND METHODS

Coptotermes formosanus were collected from all known continental United States where FST has been reported, the Hawaiian Islands, Japan, Hong Kong, and China (Table 1). Morphological identification of specimens used in this study were performed by applying the keys of Scheffrahn et al. (1994), and verified with a FST molecular diagnostic method (Szalanski et al. 2004). Voucher specimens, preserved in 100% ethanol, are maintained at the Arthropod Museum, Department of Entomology, University of Arkansas, Fayetteville, AR, the University of Florida-Ft. Lauderdale Research and Education Center, Ft. Lauderdale, FL, and the Center for Urban and Structural Entomology, Department of Entomology, Texas A&M University, College Station, TX.

Alcohol preserved specimens were allowed to dry on filter paper, and DNA was extracted from individual worker, or soldier heads by using the Puregene DNA isolation kit D-5000A (Gentra,

Minneapolis, MN). Extracted DNA was resuspended in 50 µL of Tris:EDTA and stored at -20°C. Polymerase chain reaction (PCR) was conducted with the primers TL2-J-3037 (5-ATGGCAGATTAGTGCAATGG-3) designed by Liu and Beckenbach (1992) and described by Simon et al. (1994) and Miura et al. (1998), and primer TK-N-3785 (5-GTTTAAGAGACCAGTACTTG-3) from Simon et al. (1994). These primers amplify a 3' portion of the mtDNA COI gene, tRNA-Leu, and a 5' section of the COII gene. PCR reactions were conducted with 1 µL of the extracted DNA (Szalanski et al. 2000), with a profile consisting of 35 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 60 s. Amplified DNA from individual termites was purified and concentrated by using Microcon-PCR Filter Units (Millipore, Bedford, MA).

Samples were sent to The University of Arkansas Medical School DNA Sequencing Facility (Little Rock, AR) for direct sequencing in both directions with an ABI Prism 377 DNA sequencer (Foster City, CA). To facilitate genetic comparison with existing GenBank DNA sequences, 113 bp from the 5' end of the sequence was removed, and the remaining 667 bp was used. GenBank accession numbers for the FST haplotypes found in this study are AY453588 and DQ386170. DNA sequences were aligned with BioEdit version 5.09 (Hall 1999) and Clustal W (Thompson et al. 1994). The distance matrix option of PAUP* 4.0b10 (Swofford 2001) was used to calculate genetic distances according to the Kimura 2-parameter model (Kimura 1980) of sequence evolution.

RESULTS AND DISCUSSION

Introduction of exotic termites to the United States is an ongoing problem that is invariably sustained by modern trade and limited or non-existent quarantine regulations.

Native populations (in China) of FST should possess greater genetic diversity. For this reason, focusing on the nature of genetic variation in populations from China and neighboring Asian countries (Vargo et al. 2003) is a logical starting point when evaluating the nature of introduced populations to the United States (Husseneder et al. 2002) and its territories. In the present study we evaluated native populations of FST from Guangdong, Shanghai, and Fujian provinces (Hong Kong, Puyuan, Guangzhou, and Xhinhui) in China. However, only two distinct COII haplotypes were observed.

Applying *C. acinaciformis* (Froggatt), *C. lacteus* (Froggatt), and *Heterotermes cardini* (Snyder) as outgroups, Haplotype group I contains locations from Hong Kong, Japan AB109529, Hsin-Hui (presently known as Xhinhui), China (from Jenkins et al. 2002), Puyuan and Guangzhou, China, Oahu, HI, Nagasaki, Japan, and Ft. Worth, TX [presumably this sample was collected from

TABLE 1. *COPTOTERMES FORMOSANUS* COLLECTION DATA.

Location	Country	N	Hap	Source
Hong Kong	China	3	1	This study
	Asia	1	1	AB109529
Ft. Worth, TX	USA	1	E(1)	Jenkins et al. 2002
Oahu, Hawaii	USA	1	1	This study
Hong Kong	China	2	2	This study
HI	USA	1	2	AY536406
Maui, HI	USA	2	2	This study
GA	USA	2	2	AY536405, AY027489
Cairo, GA	USA	2	2	AY683220
Lawrenceville, GA	USA	1	2	AY683213
Tucker, GA	USA	1	2	AY683214
Dallas, GA	USA	1	2	AY683214-15
Savannah, GA	USA	2	2	AY683217-219
GA	USA	1	2	This study
Spindale, NC	USA	1	2	This study
Forest City, NC	USA	1	2	This study
Rutherfordton, NC	USA	1	2	This study
Marco Island, FL	USA	1	2	This study
Trinity, FL	USA	1	2	This study
Niceville, FL	USA	1	2	This study
Florida City, FL	USA	1	2	This study
Temple Terrace, FL	USA	1	2	This study
Palm Beach, FL	USA	1	2	This study
Pompano Beach, FL	USA	1	2	This study
Galveston, TX	USA	2	2	This study
San Antonio, TX	USA	1	2	This study
Garland, TX	USA	1	2	This study
Rockwall, TX	USA	1	2	This study
Stennis Sp Ctr, MS	USA	4	2	This study
New Orleans, LA	USA	2	2	AY536407,AY683217
Lake Charles, LA	USA	3	2	This study
New Orleans, LA	USA	3	2	This study
St. Rose, LA	USA	1	2	This study
New Orleans, LA	USA	1	B(2)	Jenkins et al. 2002
SC	USA	1	C(2)	Jenkins et al. 2002
Nagasaki	Japan	2	2	This study
Puyuan	China	1	3	AY536403
Guangzhou	China	1	4	AY536404
Mobile, AL	USA	1	D	Jenkins et al. 2002
GA	USA	1	A	Jenkins et al. 2002
Hsin-hui (Xhinhui)	China	1	G	Jenkins et al. 2002
Hsin-hui (Xhinhui)	China	1	H	Jenkins et al. 2002
Oahu, HI	USA	1	F	Jenkins et al. 2002

Grapevine, TX, because the only known occurrences of FST in Tarrant County, TX, occur in the Northeast portion of this county (pers. Comm. Mike Merchant)]. Group II contains several FST populations from disjunct locations: Hong Kong, North Carolina, South Carolina (Jenkins et al. 2002), Georgia, Florida, Alabama (Jenkins et al. 2002), Mississippi, Louisiana, Texas, Oahu and Maui, HI (Figs. 2 and 3). Representative taxa from group I were slightly more divergent based on Maximum likelihood analysis (Fig. 3). Inclusion of FST sequence data from Jenkins et al. (2002), des-

ignated by their respective haplotype descriptions (A through H), also fall within the two groups presented herein (Table 2, Figs. 2 and 4).

Fei and Henderson (2003) noted that incipient colony establishment was somewhat more restrictive for outbred primary reproductives, owing discrepancies to environmental adaptive resource differences from two disjunct populations from Louisiana. Furthermore, Coaton & Sheasby (1976), and Lenz & Barrett (1982) suggest that dominant use of neotenics for colony growth in *C. formosanus* may be a successful strategy to in-
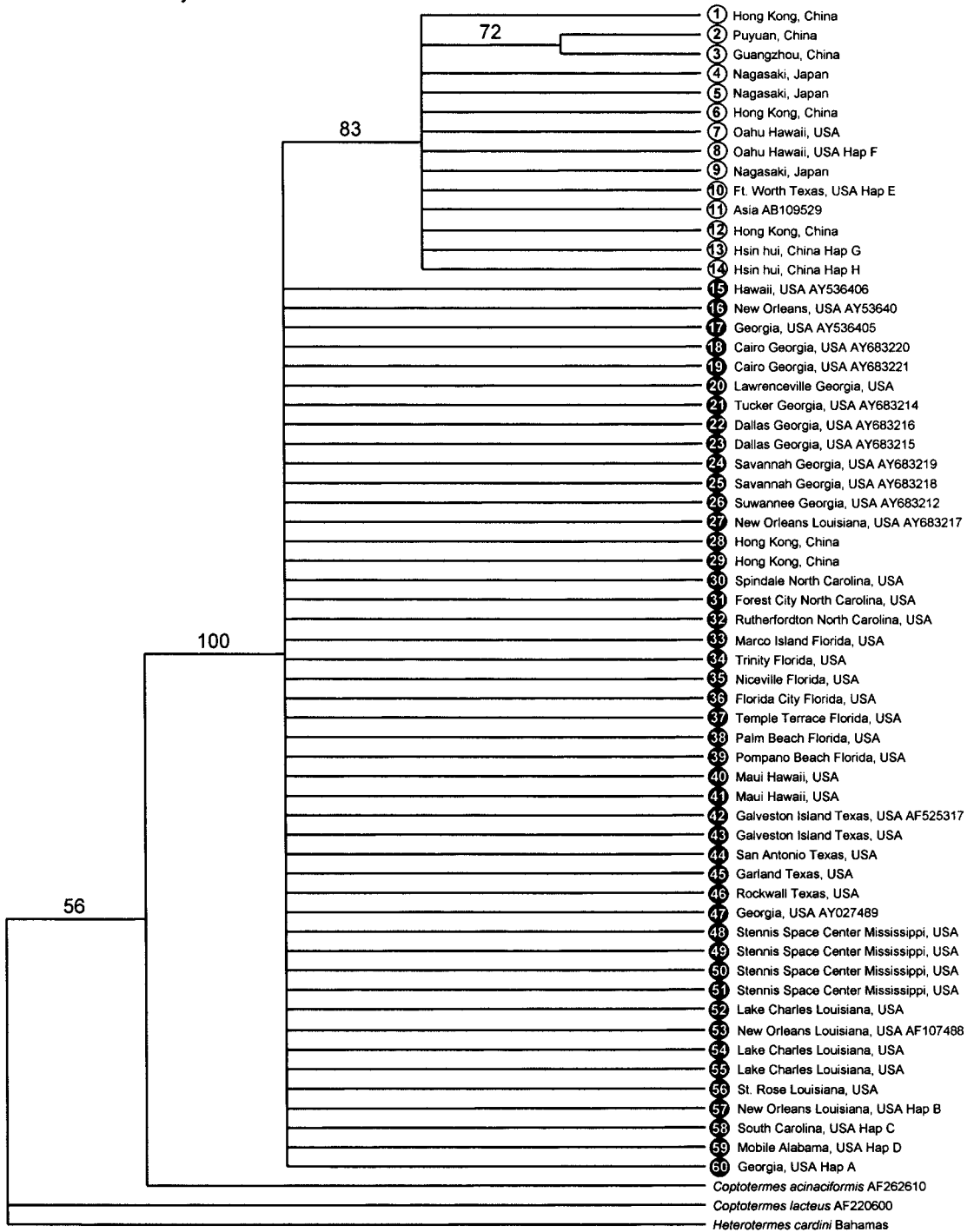
Maximum Parsimony

Fig. 2. Maximum Parsimony Analysis of *Coptotermes formosanus* lineages in North America. For consistency, open and closed circles reflect the different mtDNA COII lineages of *C. formosanus*, while the numbers are used for comparison and clarification of geographic location in Figures 3 and 4.
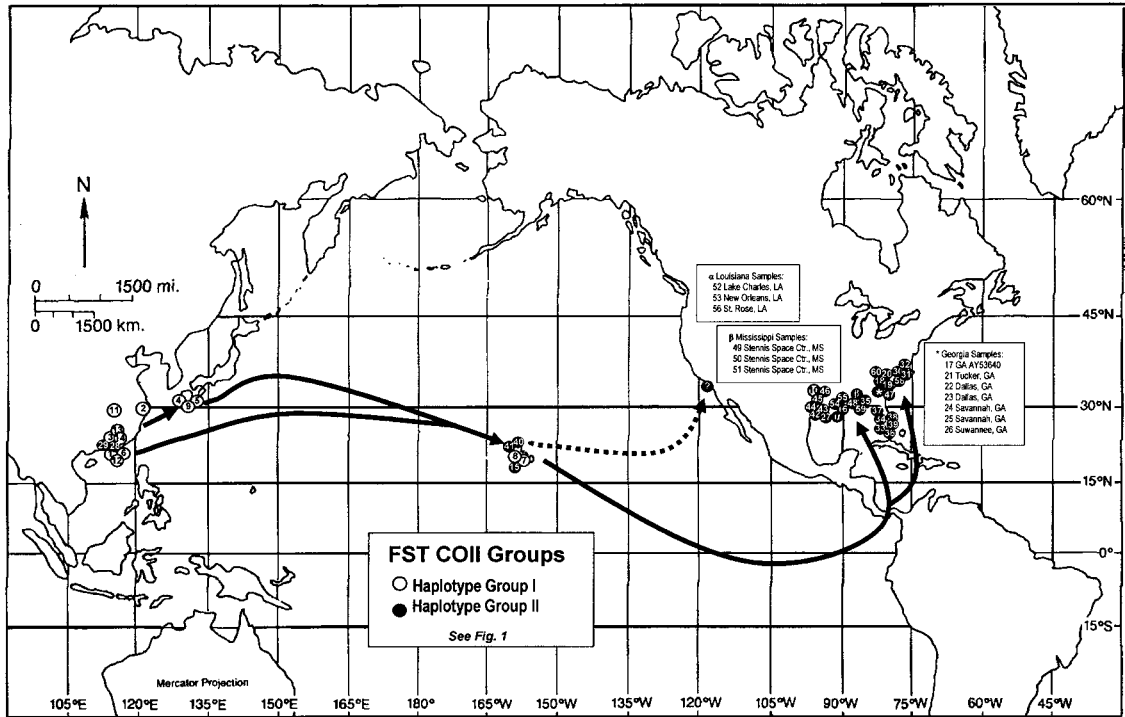
Fig. 3. Introduction routes of *Coptotermes formosanus* from Asia to North America. Dashed arrow pointing towards Southern California suggests the introduction from Hawaii based on anecdotal information that has not been corroborated in genetic studies to date.

vade new environments. If this adaptive strategy is true for *C. formosanus*, reduced genetic variation may be the result and would account for some of the limited population viscosity observed to date. Habitat fragmentation and anthropogenic disturbances significantly reduce population viscosity. More comprehensive studies of FST may not reveal significant genetic diversity. For FST, reduced genetic variation does not necessarily mean reduced fitness or vigor, but may simply imply that there is greater reproductive plasticity. For example, Hyashi et al. (2004) demonstrated that *Reticulitermes speratus* (in Japan) can utilize facultative parthenogenic reproduction. This would be a significant establishment capability for termites like FST when introduced to non-endemic locations such as the United States.

There have been numerous emigrations of people to Hong Kong throughout history. Major migrations of Chinese settlers from mainland China to Hong Kong have been recorded as early as the Song Dynasty (960-1279) (Welsh 1996). After the end of World War II and the communist takeover of mainland China in 1949, hundreds of thousands of people emigrated from China to Hong Kong (Welsh 1996). In fact, locations such as Xhinhui, a treaty port in 1904, was an important outlet for Chinese emigrants to the United States (Anonymous 2004).

The introduction of FST to the U.S. likely occurred several times, perhaps more than ten different occasions (RHS, personal communication). Given this fact, it is remarkable that the established link between the U.S. and China has never been substantiated for more than one FST lineage.

Populations of FST from Japan appear only in one of the presented clades (Group I, Fig. 2), and further sampling from more locations (in Japan) may provide additional information on whether Japan could have contributed more significantly to FST introductions to Hawaii or the continental United States. Group I (Fig. 2) is largely comprised of samples from Asian/Pacific locations but has one sample (Ft. Worth, TX) that was collected in the continental U.S. (Fig. 3). This is significant because it implicates a second introduction route to the continental U.S. that has never been identified in previous studies. Group II, is comprised of FST samples from nearly all known southeastern states (Alabama, Florida, Georgia, Louisiana, Mississippi, North Carolina, South Carolina), Texas, Hawaii, and several FST from China. Both clades are well-supported by strong bootstrap support (>80%) by both parsimony and Liklihood analyses (Figs. 1 and 3).

Although FST distributions have been more recently updated (Wang et al. 2002), the lack of a

TABLE 2. HAPLOTYPE VARIATION AT 15 NUCLEOTIDE SITES FOR NINE *COPTOTERMES FORMOSANUS* HAPLOTYPES.

Hap	8	11	19	32	33	46	176	211	222	297	333	427	643[a]	653[a]	662[a]
1	C	G	A	A	T	A	A	A	A	T	A	G	A	T	A
2	.	.	.	.	.	.	.	.	G	A	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.
4	.	.	.	.	.	.	.	.	.	.	.	A	G	.	.
A[a]	.	.	G	.	.	.	.	.	G	A	.	.	.	.	C
D[a]	.	.	.	.	.	.	.	.	G	A	C	.	.	.	.
F[a]	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
G[a]	G	T	.	.	.	.	T	.	.	.	.	.	.	A	.
H[a]	.	.	.	T	A	T	T	T	.	.	.	.	.	.	.

[a]Jenkins et al. (2002).

geographic explanation for a second lineage introduced to the United States remains unclear (Wang & Grace 2000). Sequence data obtained from GenBank, from Jenkins et al. (2002), provides a second haplotype match in the continental United States (haplotype E from Ft. Worth, TX) that represents the first documented case corroborating multiple lineages from presumably multiple introductions (at least two in the present study). These two distinct haplotypes share one commonality—both groups have representatives with identical haplotypes (lineages) from Hong Kong, Japan, Hawaii, and the continental United States (Fig 3).

There were numerous FST samples where repeated attempts to amplify sufficient DNA for sequencing of the mtDNA COII gene were not successful (e.g., FST from San Diego, California and Tai Chuong, Taiwan). These results were not surprising, as we have routinely observed ~60% efficiency when using the COII marker with FST. However, amplification of the 16S rRNA for these samples was successful. We routinely observe >90% efficiency for this marker with FST. While the utility of the 16S marker is excellent for phylogenetic studies of the genus *Coptotermes* (JWA, unpublished), for molecular diagnostic methods (Szalanski et al. 2004), or other rhinotermitids (Szalanski et al. 2004; Austin 2004a; 2004b), it does not provide the degree of genetic variation suitable to discern the two distinct FST haplotypes observed in this study. The slightly larger COII amplicon (640 bp versus 428 bp of 16S rRNA) provides only a small increase in resolution between FST populations, even though it works well for other Rhinotermitidae (Austin et al. 2002, 2004c). Our laboratory experience with FST suggests that in general, it is more difficult to extract high quality DNA from Coptotermes for genetic studies when compared to other rhinotermitids, a problem that may be more common than reported. Additional problems may include the presence of unknown inhibitors, method of sample preservation (some preservation methods are

known to provide poorer quality DNA for genetic studies (Post et al. 1993; Reiss et al. 1995; Dillon et al. 1996) or the age of samples provided.

While the idea that multiple introductions to the United States have been proposed, alternate introduction routes have never been substantiated in literature. This study provides a glimpse of some of the difficulties encountered working with FST. Most notably, it would appear that the low genetic variation detected with our COII marker in this species does not equate to reduced fitness or establishment capability.

Populations of nearly all species, social or otherwise, exhibit at least some degree of genetic differentiation among geographic locales (Ehrlich & Raven 1969). Herein, we present two distinct COII haplotypes of FST in the continental United States (one based on our own samples evaluated, and a second from Jenkins et al. (2002)). However, our results appear to contradict the degree of variation described by Jenkins et al. (2002). They describe 8 different COII haplotypes (maternal lineages) from 14 geographic locations across the southeast United States, Hawaii, and China. Applying the COII marker to 60 geographic locations (Table 1) we only identified 2 haplotypes—one in Japan, two in Hawaii, the continental United States, and China, respectively. Noting that many of the variable sites in Jenkins et al. (2002) occur at positions 651 through 685 of their slightly larger COII amplicon (total size of the amplicon was 685), it is unclear where the discrepancies occurred. One possibility may be due to sequence error that could only be detected by comparison with greater taxon sampling. Other possibilities may be due from improper sequence alignment or mispriming of template DNA during PCR. We elected to include all taxa from Jenkins et al. (2002) into our sequence dataset (COII lineages A through H), which may have provided an advantage due to our larger number of locations sampled. As with animal populations, additional genetic structure normally is to be expected over increasing spatial scales, where populations can show additional differenti-
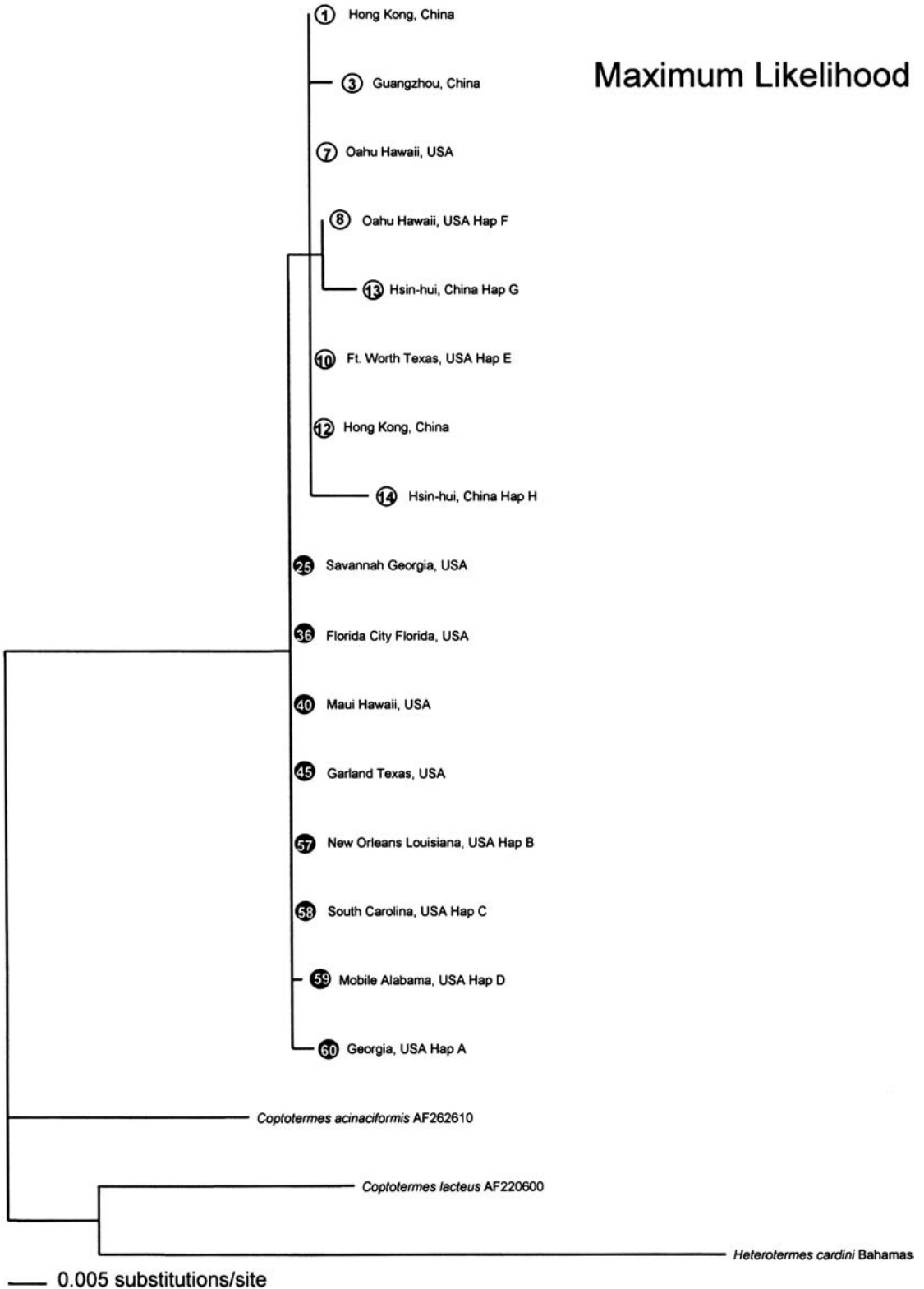
Fig. 4. Maximum Likelihood analysis *Coptotermes formosanus* lineages in North America.

ation due to spatial habitat structure and isolation by distance (Avise 2004). However, our results seem to refute this generalization for FST, a fact probably attributed to its establishment ability in fragmented urban ecosystems and their indirect interactions with humans.

The preponderance of FST research appears to support our findings. Haverty et al. (1990) found no differences in qualitative cuticular hydrocarbon profiles among four FST populations in the U.S. Korman & Pashley (1991) concluded that populations from Florida and New Orleans are in the same group and are very closely related to each other, a finding also corroborated within the present study (Fig. 3). Strong & Grace (1993) concluded that low genetic and phenotypic variability in introduced FST populations to Hawaii could have been from a single event. Broughton & Grace (1994) observed that only 9 of 16 different restriction enzymes cut mtDNA zero or once. Vargo et al. (2003) was unable to detect significant isolation by distance among colonies at the spatial scale studied (0.7-70 km) from 2 disjunct populations of FST in Japan, nor from populations in New Orleans, LA and Oahu, HI. This suggests a general lack of strong population viscosity in introduced populations of FST. The finding also seems to be contrary to Jenkins et al. (2002), whose FST samples ranged in distance from 6-37 km in Atlanta, GA. Wang & Grace (2000), applying enzymatic polymorphisms, concluded that at least two introductions to the United States have occurred, but the second clade in their study lacked sufficient samples from China to determine the origin of a second route.

More recently, the utility of mtDNA markers for identifying where exotically introduced *Heterotermes* (Szalanski et al. 2004), *Nasutitermes* (Scheffrahn et al. 2004) and *Cryptotermes/Procryptotermes* (RHS, unpublished) to the United States is being investigated. The principal caveat with studies of this nature is that significant representation of taxa is essential, particularly when dealing with species of limited genetic variation like FST. A secondary caveat is that tremendous skill in identifying termites morphologically is essential to ensure the validity of a genetic study based on known, identified samples. Because FST was likely misidentified when it was first observed in the continental United States, little attention was given, and subsequent populations have developed over the years. This was one of the reasons behind developing molecular diagnostics for this species (Szalanski et al. 2004), and a need to genetically review some species to corroborate their original identifications (Scheffrahn et al. 2004). As population-level studies for FST from various locations across the world continue to accumulate (see Vargo et al. 2003), perhaps a better understanding of local factors which contribute to the low genetic diversity observed in FST will become more apparent. Given the 300 years of known occurrence in Japan (Vargo 2003) and the lack of genetic variation in China, it is unlikely we will observe significant variation in this species within the U.S. Random genetic drift is unlikely to occur at a rate that we will detect anytime soon. Perhaps more intuitively, we should not assert our scientific prejudices about the nature of reduced genetic variation in FST (causing some reduction in fitness), or Isoptera in general, until we more exhaustively investigate their basic biology and reproductive systems.

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SIZE, FECUNDITY, AND GONADIC MATURATION
OF *TOXOTRYPANA CURVICAUDA* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, is an important pest of papaya. It is distributed from Florida, USA, to northern South America. We studied aspects of its biology on papaya, *Carica papaya*. Females and males emerged within a 3-d period with similar numbers emerging daily. Females are heavier than males but had similar longevity. Puparial length, puparial weight, and adult weight did not correlate with adult longevity. First chorionated eggs were recorded 4 d after emergence. Females 6 d old had an average of 44 ± 2.2 (sem) chorionated eggs. Heavier females have a reproductive advantage as they have more chorionated eggs than light females. More than 85% of females lived at least 6 d.

Key Words: egg maturation, longevity, emergence rhythm, *Carica papaya*, Mexico

RESUMEN

La mosca de la fruta de la papaya, *Toxotrypana curvicauda* Gerstaecker, es un insecto plaga de la papaya, *Carica papaya,* que se distribuye desde la Florida en los EUA hasta la parte norte de Sudamérica. En esta ocasión, estudiamos aspectos de su biología en *Carica papaya*. Ambos sexos emergen en un lapso de 3 días con similar número de hembras y machos emergiendo diariamente. Las hembras son más pesadas que los machos pero presentan similar longevidad. No existe relación entre longevidad del adulto y el largo o ancho de la pupa o por el peso del adulto. Los primeros huevos corionados se observaron en hembras de 4 d de edad. En promedio cada hembra madura sexualmente (6 d de edad) presentó 44 ± 2.2 huevos corionados. Las hembras pesadas tienen una ventaja reproductiva ya que presentan más huevos corionados que hembras ligeras. La mayoría de las hembras (> 85%) vivieron al menos 6 días.

Translation provided by the authors.

The papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker (Diptera: Tephritidae), is one of seven species of the genus *Toxotrypana* (White & Elson-Harris 1992). *Toxotrypana curvicauda* has been reported from south Florida and south Texas, through much of Central America, to northern South America including some Caribbean islands (Knab & Yothers 1914; Eskafi & Cunninghan 1987; O'Doherty & Link 1993).

Several authors have reported on the biology of this species. Knap & Yothers (1914) and Mason (1922) produced the first reports on the life cycle. Landolt (1984) studied ovary and egg development and reported that males are ready to mate on the day they emerge while females have a 6-d premating period. Aluja et al. (1994) evaluated preference for papaya varieties and Landolt & Hendrichs (1983) and Aluja et al. (1997a, b) reported on spatial and temporal distribution of this fly. The sex pheromone of *T. curvicauda* was identified by Chuman et al. (1987). Improvements in trapping techniques with sex pheromone were done by Landolt et al. (1988), Landolt & Heath (1990), and Heath et al. (1996). Castrejón-Gómez et al. (2004) reported the use of food attractants for capturing the papaya fruit fly.

Body size and/or weight have been traditionally considered key determinants of an organism's ecological and physiological properties (Thornill & Alcock 1983; Honěk 1993), because it is strongly correlated with many physiological and fitness characteristics (Reiss 1989; Roff 1992). Female weight is generally accepted as an index of potential fecundity, assuming a positive relationship between the number of oocytes in the ovarioles and the weight of the female. Thus, heavy females have more eggs available for fertilization (Tzanakakis 1989; Fay 1989; Sivinski 1993). Similarly, large size or weight has been associated with greater longevity (Bloem et al. 1994). In mass rearing facilities, weight is an important parameter when evaluating the quality of laboratory populations (Chambers & Ashley 1984).

There is no published information available on puparial morphometry and adult emergence rhythm of the papaya fruit fly. Also, the relationships among puparial weight and number of mature eggs, and gonadic maturation and female age have not been explored. This information is important for understanding the biology of *T. curvicauda* on *C. papaya* and for planning research on the mating system as a tool for management of this papaya pest.

MATERIALS AND METHODS

Insects

Insects were collected as larvae from infested papaya fruits obtained from an experimental papaya plantation at the CEPROBI (Centro de Desarrollo de Productos Bióticos) grounds at Yautepec, Morelos, Mexico. Detailed information on localization, native vegetation, and climatic information of the CEPROBI grounds can be found in Aluja et al. (1997a). Mature larvae (100-200) were placed for pupation into a plastic cylindrical container (11 cm high and 8.5 cm diameter) with sterile soil (6 cm deep) and covered with mesh secured with a rubber band. Containers were watered as necessary to keep soil moist. One week after pupation, puparia were recovered and washed under running water and dried on paper toweling. An Ohaus balance (Explorer, 0.0001 g accuracy, made in Switzerland) was used to weigh the puparia. Each puparium was numbered and kept individually in a plastic container (9.5 cm high and 3 cm diameter) with sterile soil until adult emergence. Each puparium was photographed under a Nikon SMZ 1500 stereoscope with a Nikon Coolpix 4500 digital camera. SigmaScan v5 (Systat Software Corporation) was used to measure puparial length and width. Adults were weighed on their emergence day with the same balance noted above. Adults were maintained individually in plastic containers, and fed water and sugar (Sharp & Landolt 1984). Puparia and adults were kept under natural humidity (50-60%), temperature (27 ± 2°C) and light regime (12L:12D). This experiment was carried out from August to September, 2004. For each insect, we recorded puparial weight, length, width; and adult emergence, weight, longevity and sex.

Relationships among Puparial and Adult Weight and Number of Chorionated Eggs

To determine whether puparial and adult weight correlated with the number of chorionated eggs, 40 females (6-8-d-old) were killed by freezing (8 min in a domestic freezer, -20°C). The abdomen was removed from the body and dissected under the stereoscope (described above). Both ovaries were removed, immersed in 1% acetocarmin for 60 s and transferred to clean saline solution. Non-chorionated eggs retain the stain but the presence of the chorion prevents stain absorption (e.g., Fernando & Walter 1999). Unstained eggs were classified as mature and were presumed to be available for oviposition, while stained eggs were classified as immature eggs.

Gonadic Maturation

To determine if there was a relationship between adult age and the number of chorionated eggs, we compared the number of mature eggs contained in one ovary of females at 0, 2, 4, 6, 8, 10, and 12-d of age. We dissected 20 females for each age. Dissection, egg staining, and egg counting followed the methodology described above.

Statistical Analysis

Differences in puparial weight, length and width, as well as adult weight and longevity between males and females were determined with a t test. Regression analysis was used to determine the influence of puparial and adult weight on adult longevity and on the number of mature eggs of 6- to 8-d-old females. Analysis of covariance (ANCOVA) followed by a least squared means (LSM) test was used to determine if the number of chorionated eggs was affected by female age. Puparial weight was used as a concomitant variable. All statistical analyses were carried out with SAS (SAS Institute 1996). All data are reported as mean ± standard error.

RESULTS

Female puparia were significantly heavier and longer than male puparia (Table 1). Similarly adult females were significantly heavier than adult males (Table 1). In contrast, puparial width, puparial period and adult longevity were similar for both sexes (Table 1). More than 85% of females

TABLE 1. AVERAGE (± STANDARD ERROR) PUPAL AND ADULT CHARACTERISTICS OF *TOXOTRYPANA CURVICAUDA* ON *C. PAPAYA*.

Variable	Females (*n* = 226)	Males (*n* = 175)	*t*	*P*
Pupal weight (mg)	75.0 ± 0.90	70.2 ± 1.00	3.58	<0.001
Pupal length (mm)	10.3 ± 0.04	9.9 ± 0.05	6.165	<0.001
Pupal width (mm)	2.4 ± 0.05	2.3 ± 0.03	1.75	>0.05
Pupal period (d)	21.6 ± 0.06	21.6 ± 0.07	0.01	>0.05
Adult weight (mg)	48.8 ± 0.60	42.7 ± 0.70	6.639	<0.001
Adult longevity (d)	19.9 ± 1.14	20.0 ± 0.93	0.05	>0.05

lived at least 6 d. However, female and male minimum and maximum longevity were 2-59 and 2–55 d, respectively (Fig. 1).

Puparial length increased as puparial weight increased, for both sexes (males: F = 369, df = 1,172, P > 0.0001; females: R^2 = 0.68 and F = 423, df = 1,223, P > 0.0001, R^2 = 0.65). There were no relationships between puparial weight and longevity or for adult weight and longevity for either females (F = 0.007, df = 1,92, P > 0.05; F = 0.25, df = 1,92, P > 0.05) or males (F = 1.1, df = 1,141, P > 0.05 and adult F = 2.57, df = 1,141, P > 0.05), respectively.

Sex Ratio and Emergence Rhythm

Most insects emerged during the first two days at the start of adult emergence (Fig. 2). Similar numbers of males and females emerged in the first (t = 0.452, P = 0.66), second (t = 0.31, P = 0.76) and third day (t = 1.27, P = 0.23). However, significantly more females emerged overall (t = 2.96, P = 0.003), resulting in a 1:1.26 male:female sex ratio.

Relationship between Puparial and Adult Weight, and Number of Chorionated Eggs

Heavier females have a reproductive advantage over light females as heavier females had more chorionated eggs than light ones (Fig. 3A, B).

Gonadic Maturation

No chorionated eggs were obtained from 0- and 2-d-old females. Thus, these data were removed from the statistical analysis. The number of chorionated eggs significantly increased up to 100 eggs as female age increased (Fig. 4). Females at 10 d had more than double the number of chorionated

Fig. 1. Cumulative mortality of male and female *T. curvicauda* at 27 ± 2°C, 50-60 RH and, 12L:12D at Yautepec, Morelos, Mexico.
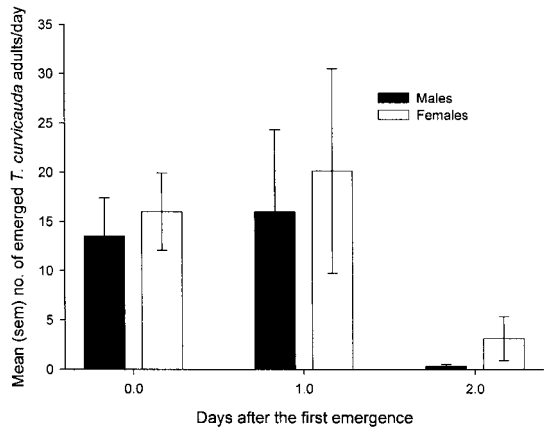
Fig. 2. Mean (± standard error) number of *T. curvicauda* adults that emerged per day starting with first adult emergence; n = 226 and 175 for females and males, respectively.

eggs of 4-d-old females (Fig. 4). There was no difference in number of chorionated eggs produced by 10- and 12-d-old females.

DISCUSSION

Toxotrypana curvicauda females had a 6-d precopulatory period. During the female's precopulatory period, ovaries and eggs increase in length and width (Landolt 1984). Our results show that the first chorionated eggs appeared later than 2 d after emergence, indicating that several days are required to produce mature eggs, as in many tephritids (Williamson 1989). Females that were 6-d-old had more than 45 chorionated eggs per ovary, and 10-d-old females had more than 70 chorionated eggs per ovary. In contrast, Knab & Yothers (1914) indicated that females had around 100 eggs and Rojas (1992) reported 67.8 ovarioles per females. However, none of the preceding authors related the number of eggs or ovarioles with female puparial or female adult weight. Our results indicate that research addressing female fecundity also should consider the positive and linear relationships between puparial and adult weight as well as age and the number of chorionated eggs.

According to Mason (1922), *T. curvicauda* females may lay 2 to 30 eggs during each oviposition. However, Rojas (1992) and Landolt & Reed (1990) reported that on average each female laid 5.4 and 29 eggs, respectively. This indicates that for females to lay their full egg-load, gravid 6-d-old females need to oviposit more than three times a day. According to Landolt and Hendrichs (1983), females may oviposite from 1 to 13 times each. Under laboratory conditions, gravid females may oviposit more than three times in a day

Fig. 4. Mean (± standard error) number of chorionated eggs per ovary of *T. curvicauda* females of different ages. Bars topped with the same letter are not significantly different (LSM; *P* = 0.05).

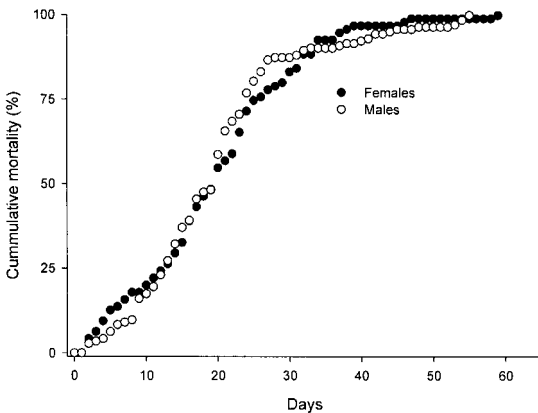Fig. 3. Relationship between (A) pupal weight and number of chorionated eggs and (B) female adult weight and number of chorionated eggs in *T. curvicauda*.

(A. Jiménez-Pérez, personal observation). This suggests that females may find at least three suitable oviposition substrates daily indicating its importance as a pest.

Knab & Yothers (1914) failed to find immature eggs in mature females and indicated that the oviposition period should be short. However, our observations indicate that mature and immature eggs coexist within the ovary, as indicated by Williamson (1989) for most tephritid species.

A precopulatory period has important implications for populations. It increases pre-reproductive mortality and decreases population growth. A female biased sex ratio may be a mechanism to diminish the effects of pre-reproductive female mortality. Puparial weight can be an additional factor used to assess the number of chorionated eggs. Our results will aid us in planning future research programs on *T. curvicauda*.

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BIOLOGY AND MATING BEHAVIOR OF THE COCONUT MOTH *ATHELOCA SUBRUFELLA* (LEPIDOPTERA: PHYCITIDAE)

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ABSTRACT

The coconut moth, *Atheloca subrufella*, is responsible for most of the flower and fruit shedding in coconut cropping systems. Despite this, little is known with regard to its biology and behavior. In order to understand its biology, the duration and viability of the egg, larval, and pupal stages, number of instars, pupal weight of males and females, sex ratio, adult longevity, and fecundity were determined. In the mating behavior study, observations included mating time and duration. Duration and viability of the egg, larval, and pupal stages were 3.0 and 93.0, 14.3 and 85.0, and 11.2 d and 91.0%, respectively, totaling 28.5 d (egg-adult) with 72.0% viability. There were four instars, with head capsule means of 0.27, 0.45, 0.80, and 1.33 mm for the 1st, 2nd, 3rd, and 4th instars, respectively. The sex ratio was 0.55, and the mean pupal weight was 22.2 mg for males and 25.2 mg for females. The pre-oviposition, oviposition, and post-oviposition periods averaged 2.4, 7.5, and 5.5 d, respectively. The longevity of males and females was 17.5 and 15.2 d, with a mean fecundity of 216 eggs. With regard to mating behavior, 91.0 and 9.0% of the tested pairs mated on the first and second day of adult life, respectively. Mating always began between 1900 and 2300 h, corresponding to an interval between 45 and 285 min after dusk, with a mean mating duration of 95 min.

Key Words: Insect, Aracaceae, palms, coconut palm pest

RESUMO

A traça-do-coqueiro *Atheloca subrufella* é responsável por boa parte da queda de flores e frutos na cultura do coqueiro. Apesar disso, ainda pouco se conhece sobre sua biologia e comportamento. Para o estudo da biologia deste inseto, determinaram-se a duração e viabilidade das fases de ovo, larva e pupa, o número de ínstares, peso de pupas de machos e fêmeas, razão sexual, longevidade dos adultos e fecundidade. Para o estudo do comportamento sexual observou-se o horário e a duração do acasalamento. A duração e a viabilidade das fases de ovo, lagarta e pupa foram de 3,0 e 93,0; 14,3 e 85,0; e 11,2 dias e 91,0%, respectivamente, totalizando 28,5 dias (ovo-adulto) e 72% de viabilidade. O número de ínstares foi 4, com médias de cápsula cefálica de 0,27; 0,45; 0,80 e 1,33mm, para o 1ª, 2ª, 3ª e 4ª ínstares, respectivamente. A razão sexual foi 0,55 e o peso médio de pupas 22,2 mg para machos e 25,2 mg para fêmeas. As durações dos períodos de pré-oviposição, oviposição e pós-oviposição foram de 2,4; 7,5 e 5,5 dias, respectivamente. A longevidade de machos e fêmeas foi de 17,5 e 15,2 dias, com fecundidade média de 216 ovos. Em relação ao comportamento sexual, 91,0 e 9,0% dos casais, copularam no primeiro e segundo dia de vida, respectivamente. O início da cópula ocorreu sempre entre as 19 e 23 horas, correspondendo ao intervalo de 45 a 285 min, após o entardecer, com uma duração média da cópula de 95 min.

The coconut moth *Atheloca subrufella* (Hulst, 1887) [= *Hyalospila ptychis* (Dyar, 1919)] (Lepidoptera: Phycitidae) is one of the most important coconut pests (Ferreira et al. 2002). In Brazil, the first reports on the occurrence of this insect are dated to the beginning of the 20th century in Bahia and Pernambuco (Bondar 1940; Costa Lima 1949). It has been reported in the states of Amazonas (Sefer 1963), Sergipe, and Rio de Janeiro (Silva et al. 1968). More recently, with the expansion of coconut production in several regions of the country, its now occurs in all of the Brazilian states where coconuts are grown (Ferreira et al. 2002). The coconut moth also occurs in the south of the USA (Georgia and Florida), the north of Mexico (Habeck & Nickerson 1982; Hodges et al. 1983; Adams 2004), Cuba, and the Virgin Islands (Bondar 1940; Heinrich 1956; Kimbal 1965; Moore 2001). Palm trees in the family Arecaceae are the most important hosts, with reports mainly in the genera *Cocos*, *Attalea*, *Syagrus*, *Sabal*, and *Serenoa* (Bondar 1940; Costa Lima 1949; Kimbal 1965; Silva et al. 1968; Moura & Vilela 1998; Moore 2001; Ferreira et al. 2002).

Although significant damage occurs in different regions, the bioecology of *A. subrufella* is still unclear and little known. The adult is a microlepidopteran with a wingspan from 14 to 18 mm; the moth is brownish and lives protected by open coconut spathes during the day (Bondar 1940). As soon as the inflorescence opens, the moth lays its eggs on female flowers (Moura & Vilela 1998). The newly-hatched caterpillars feed on the carpels of still-tender flowers or, if the flower has already been fertilized, they penetrate the developing coconut through the lower part of the bracts (Bondar 1940; Ferreira et al. 2002). Attacked flowers are aborted and fall off; the presence of the pest can be recognized by the feces at the site and by a change in color in female flowers, which become dark brown (Bondar 1940; Moura & Vilela 1998). In young coconuts, the caterpillar feeds on the mesocarp, opening a series of galleries and causing premature shedding of fruits. Feces and small fecal pellets bound by silk strands can be visualized on the external surface of young coconuts, facilitating recognition of the pest. Attacked coconuts that do not fall off become deformed and have reduced commercial value (Bondar 1940; Ferreira et al. 2002).

Controlling *A. subrufella* is particularly difficult because of the continuous development of inflorescences in the coconut palm, which makes agrochemical spraying not viable. In addition, spraying insecticides may affect a number of beneficial and pollinating insects, such as bees (Moura & Vilela 1998); moreover, the effectiveness of insecticides is low, because the caterpillars are protected between the bracts of young coconuts.

Thus, the purpose of this work was to study in detail the biology and mating behavior of *A. subrufella*, aimed at future studies for the integrated management of this pest, with emphasis on the production of sex pheromone.

MATERIALS AND METHODS

Starting a Stock Rearing

The insects used in the experiments were collected from infested young coconuts in commercial dwarf coconut plantations in the municipal district of Touros-RN, Brazil. Infested coconuts were taken to Emparn's (Empresa de Pesquisa Agropecuária do Rio Grande do Norte) Laboratory of Entomology, where they were arranged on plastic trays containing sterilized sand for pupation. The pupae obtained were separated and sent to the Insect Biology Laboratory of Departamento de Entomologia, Fitopatologia e Zoologia Agrícola of Escola Superior de Agricultura "Luiz de Queiroz" (ESALQ), Universidade de São Paulo (USP), in Piracicaba, SP, Brazil, where a stock rearing in young coconuts was initiated to support the biology and behavioral studies. The biology experiments were carried out with laboratory-reared second-generation insects, while the behavioral studies were conducted with third-generation insects.

Adults were maintained in cages made from PVC tubes 10 cm in height × 10 cm in diameter and lined with paper towel to provide an egg-laying substrate. The ends of the tube were covered with a 12-cm diameter Petri dish to prevent the insects from escaping. Adults were fed a 10% honey solution; the food and paper towel containing eggs were replaced every two days.

Coconut Moth Biology

Upon hatching, 150 larvae were placed in clear plastic cups 7 cm height and 6 and 5 cm diameter at the base and top, respectively, to determine the duration and viability of the egg, larval, and pupal stages, pupal weight of males and females, sex ratio, longevity of males and females, and fecundity, as recommended by Parra (2001). Young coconut fruits (2.5 cm 0.5) were placed inside the cups, maintained on filter paper (same diameter as the cup), in order to absorb the excess moisture released by the fruit and to avoid or reduce contamination by saprophytic microorganisms. After ten days of larval development, each fruit was replaced with a fresh one. Pupae were later individually placed in plastic cups 3.0 cm height × 1.5 cm diameter, and arranged on a perforated metal tray and containing filter paper inside, which was moistened daily in order to maintain adequate humidity during that stage of development. Sexes were separation during the pupal stage, according to the methodology by Butt & Cantu (1962).

The number of instars was determined by daily measuring the head capsule width of 15 caterpillars with an ocular micrometer attached to a stereoscopic microscope, according to Parra & Haddad (1989).

Longevity and fecundity determinations were made on each of 25 pairs in PVC cages, as previously described. Food was replaced daily, and the number of eggs and mortality of males and females were recorded. Rearing was conducted in an air-conditioned room at a temperature of 25 ± 1°C, relative humidity of 70 ± 10%, and 14h photophase.

The χ^2 test was used to determine the possible occurrence of protogyny, considering a daily sex ratio of 0.5 as the expected values. Regression analysis was used to determine a mathematical model that would best explain the daily emergence rhythm of males and females.

Mating Behavior of Coconut Moth

Preliminary visual observation studies with *A. subrufella* pairs demonstrated that mating in this species always occurred after dusk. Therefore,

5 groups of 9 pairs in their first day of life were formed, in clear plastic cups 13.0 cm in height and 8.5 6.0 cm in diameter at the base and top, respectively, inverted on a Petri dish 9.0 cm in diameter × 0.8 cm in height, containing filter paper moistened with distilled water at the base. These pairs were arranged in a greenhouse under natural light, temperature of 25 ± 3°C, and relative humidity of 70 ± 10%. Visual observations were made every 10 min, from the beginning of dusk until the mating activities ceased; the age, time at the start and end of copulation, duration, and a description for the courtship and copulation activity were recorded. Observations were made with a hand flashlight (Maglite® with a red filter). The light source was maintained at a ca. 60 cm from the arena so as to prevent possible interference with insect behavior. This experiment was conducted until the fifth day of life of those adults, and only pairs that performed at least one copulation during that period were considered for the analysis.

The sunset time for Piracicaba-SP was obtained from Observatório Nacional in Rio de Janeiro-RJ, Brasil, and occurred on average at 1830 h in March 2004 during the bioassays (Moreira 2004).

RESULTS

Biology

The means (± SEM) for duration of the egg, caterpillar, and pupal stages was 3.0 ± 0.01; 14.3 ± 0.09, and 11.2 ± 0.09 d, and viability was 93.0 ± 0.04; 85.0 ± 0.30, and 91.0 ± 0.29%, respectively. The complete cycle (egg-adult) lasted 28.5 ± 0.96 d and total viability was 72.0 ± 0.34%.

Four instars were determined for *A. subrufella*, with mean head capsule width of larvae of 0.27, 0.45, 0.80, and 1.33 mm, for the 1st, 2nd, 3rd, and 4th instars, respectively. The mean pupal weight was 22.2 ± 5.0 mg for males and 25.2 ± 4.1 mg for females. The sex ratio was 0.55 and the longevity of males and females was 17.5 ± 1.19 and 15.2 ± 0.95 d, respectively.

The durations of the pre-oviposition, oviposition, and post-oviposition periods were 2.4 ± 0.20, 7.5 ± 0.68, and 5.5 ± 0.84 d, respectively. In average terms, the oviposition rate was 29 eggs per d, and fecundity was 216.4 ± 20.86 eggs per female. Egg-laying decreased through the oviposition period, but oviposition until the 14th day was observed for some females (Fig. 1).

Females emerged in higher numbers than males during the first two d of emergence, indicating that this species presents the protogyny phenomenon (Fig. 2). Significant values were determined by the χ^2 test for the first two d (higher number of emerged females) and for the last two d (higher number of emerged males). There were no significant differences for the third and fourth d.
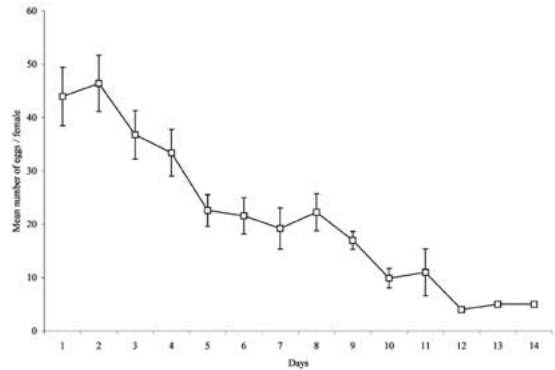
Figure. 1. Daily oviposition of *A. subrufella* reared during the larval stage on young coconut fruits. Temp.: 25 ± 1°C; RH: 70 ± 10%, photophase: 14h. Bars indicate the standard error of the mean (SEM).

The regression analysis factor for the emergence rhythm of males factor was significant ($y^{0.5}$ = -4.55 + 4.92x − 0.59x^2; R^2 = 0.86; F = 10.02; P = 0.01; df = 2), where the coefficients were different from zero by Student's "*t*" test (P ≤ 0.05). The emergence rhythm of females was significant (y = - 36.99 + 262.05/x − 214.64x^2; R^2 = 0.97; F = 20.99; P = 0.01; df = 2), indicating that the equations fit the data well.

Mating Behavior

From 45 *A. subrufella* pairs observed, 29 mated (64.4%). Of these mating pairs, 91.0 and 9.0% copulated on the first and second days of life, respectively.

The beginning of copulation always occurred between 1900 and 2300 h, corresponding to the interval from 45 to 285 min after dusk. The mating frequency from 2100 to 2200 h was statistically different from 1900 to 2000, but it was not different from 2000 to 2100 h, and from 2200 to 2300 h (Fig. 3). The mean copulation duration was 95 min (43-149 min).

DISCUSSION

There have been few reports on the biology of *A. subrufella*. Data presented in this paper suggest high biotic potential, although in the field climatic factors and the action of natural enemies must contribute to reducing this potential. According to Bondar (1940), the life cycle of this moth is approximately 25 to 30 d, while Moura & Vilela (1998) mentioned 40 d; these authors did not present durations for the different stages. In the present work, the life cycle lasted 28.5 d, on average, for a temperature of 25°C. The females normally begin laying eggs about 2 d after mating, laying a higher number of eggs in the very
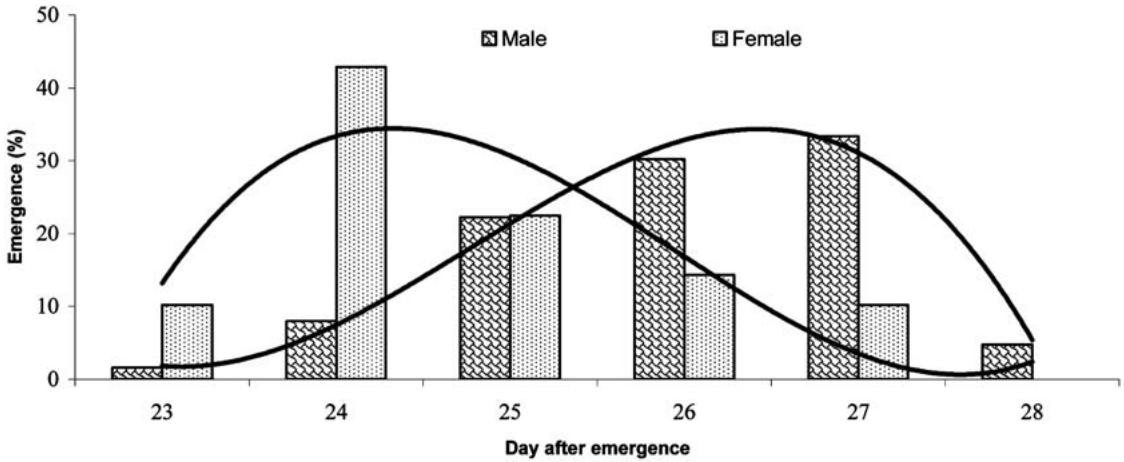
Figure. 2. Emergence of *A. subrufella* females and males. The solid and dashed lines indicate the emergence trend for males and females, respectively. Temp.: 25 ± 1°C; RH: 70 ± 10%, photophase: 14h.

first days. Even though the females have a longevity of about 2 weeks, the oviposition period is short, around 7 d, with a mean fecundity of 216 eggs per female. The post-oviposition period was 5.5 d. Eggs were laid individually, initially showing a pale-yellow color, becoming slightly reddish later, and acquiring a dark hue on the last day of embryonic development. The caterpillar stage lasted 2 weeks on average, and caterpillars may reach 15 mm in length. The pupal stage lasted 11
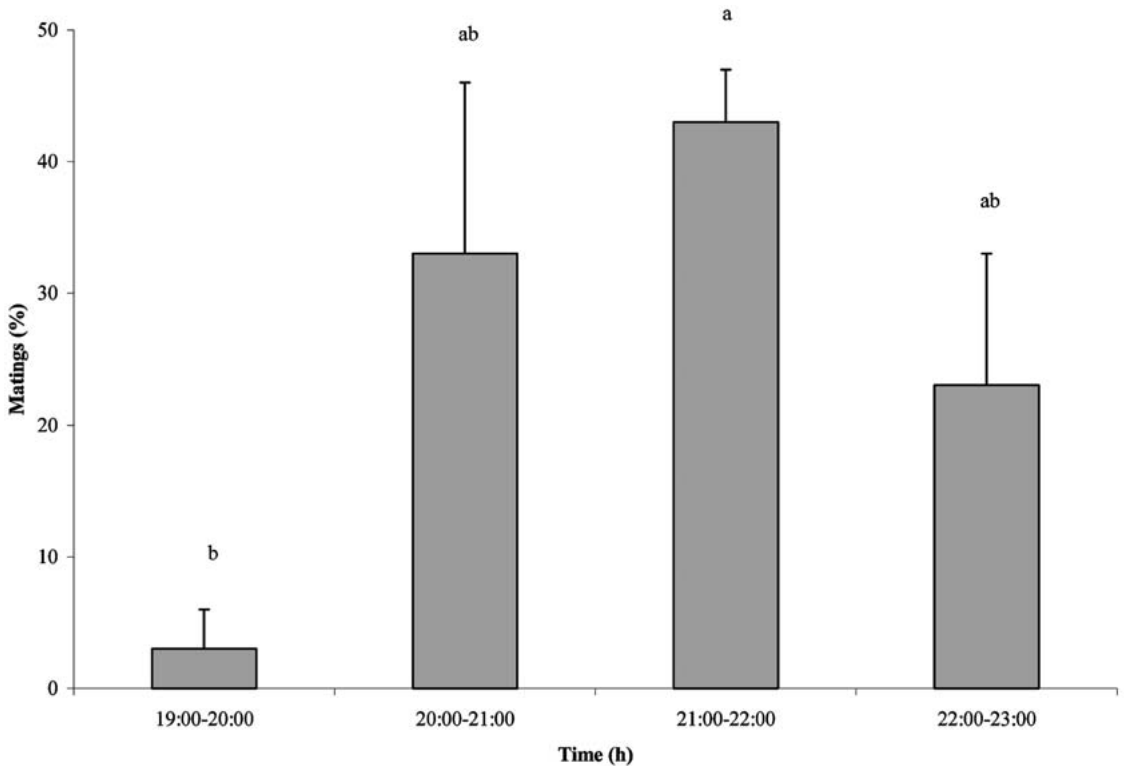
Figure. 3. Mating time of *A. subrufella*. Means followed by the same letter are not statistically different by Tukey test at the 5% probability level. Sunset time for Piracicaba-SP, Brazil, at 1830 h (March 2004).

d, differing from findings by Bondar (1940), who found a duration from 6 to 8 d. These differences may be associated with abiotic factors, not mentioned by Bondar (1940).

The emergence rhythm of *A. subrufella* adults indicates that females begin emerging before males (protogyny). The percentage of emerged females was significantly higher than that of males in the first two d (Fig. 2). The biological reason for this emergence pattern is still little understood in insects (Thornhill & Alcock 1983), but it has been demonstrated in other lepidopteran species and could represent an evolutionary strategy to promote mating between individuals from distinct populations (Uematsu & Morikawa 1997).

Mating always started during the scotophase, and were mainly concentrated in a period of two to four h after dusk. After the beginning of dusk, females and males became very agitated, especially the latter. With time, the females remained almost motionless, possibly in a "calling" position, even though no exposure of any exogenous gland or abdomen movements could be observed. A male near a female showed frenetic antennal and wing movements, walking in a semi-circular fashion around the female's body. Once the female was receptive, the male assumed a (contrary position) in relation to her, at a 180° angle, then walked backwards, with light wing and abdomen movements until copulation was achieved; this could last 1 h and 30 min on average ($P \leq 0.05$). If for some reason the female rejected the male's lunge before copulation was accomplished, the male restarted the whole procedure. In general, more than 90% of females mated on the first day of life.

More than 12 annual generations with high biotic potential may develop, because, in addition to laying more than 200 eggs, the insect shows high viability in all developmental stages. Development of an artificial diet for *A. subrufella*, which is available for other insects in the same family (Singh 1983), would facilitate the development of future management strategies for this pest.

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EVALUATION OF *FRANKLINIELLA BISPINOSA* (THYSANOPTERA: THRIPIDAE) AS A VECTOR OF THE *TOMATO SPOTTED WILT VIRUS* IN PEPPER

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ABSTRACT

Frankliniella occidentalis is the key vector responsible for the emergence of *Tomato spotted wilt virus* as a global threat to agriculture. *Frankliniella bispinosa* is a common thrips in Florida, the Bahamas, and Bermuda, but the role of *F. bispinosa* in the epidemiology of the virus is not known. The purpose of this study was to determine the ability of *F. bispinosa* to acquire and transmit *Tomato spotted wilt virus* in pepper. In laboratory experiments, the number of larvae produced per *F. bispinosa* female was less than the number of larvae produced per *F. occidentalis* female. The larvae of *F. bispinosa* successfully acquired *Tomato spotted wilt virus*, although at a lower percentage than *F. occidentalis*. Viruliferous adults of both species transmitted the virus to pepper. Our results confirm the competence of *F. bispinosa* as a vector of *Tomato spotted wilt virus*.

Key Words: *Frankliniella occidentalis*, Tospovirus, vector competence, viral acquisition, viral transmission, *Capsicum annuum*

RESUMEN

El trips, *Frankliniella occidentales*, es un vector clave y responsable para la emergencia del virus de la marchitez manchada del tomate como una amenaza global para la agricultura. Un otro especie de trips común en Florida, Bahamas y Bermuda es *Frankliniella bispinosa*, pero su papel en la epidemiología del virus de la marchitez manchada del tomate no es conocido. El propósito de este estudio fue para evaluar la habilidad de *F. bispinosa* para adquirir y transmitir el virus de la marchitez manchada del tomate al chile. Las larvas de *F. bispinosa* adquirieron con buen éxito el virus de la marchitez manchada del tomate, aunque a un porcentaje menor que en *F. occidentalis*. Adultos viruliferos de las dos especies transmitieron el virus a chile. En experimentos del laboratorio, el número de larvas producidas por hembra de *F. bispinosa* fue menor que el número de larvas producidas por la hembra de *F. occidentalis*. Nuestros resultados confirman la capacidad de *F. bispinosa* como un vector del virus de la marchitez manchada del tomate.

In addition to damaging plant tissues while feeding, some species of thrips vector *Tomato spotted wilt virus* (TSWV), a tospovirus transmitted through the saliva of thrips during feeding (Hunter & Ullman 1992; Ullman et al. 1997). Tomato spotted wilt was first observed in 1915 in Australia and described as the "spotted wilt" of tomatoes (Brittlebank 1919) that later was associated with transmission by thrips (Pittman 1927). The viral etiology was reported by Samuel et al. (1930).

TSWV causes economic loss in many agricultural crops. The virus has a broad host range, infecting over 1000 plant species, and causing an estimated crop loss of one billion US dollars per year throughout its host range (Prins & Goldbach 1998). Tomato, tobacco, lettuce, pepper, papaya, eggplant, green beans, artichokes, broad beans, celery, some ornamental plants, and other plants experience severe losses due to the virus (Rosella et al. 1996).

Ullman et al. (1997) reviewed the relevant scientific literature involving the relationship between TSWV and its thrips vectors. Only first and second instars of vector thrips species acquire TSWV during feeding upon an infected host, and the virus survives molting, pupation, and the replacement of tissues during the prepupal and pupal stages of thrips development. Adults are unable to acquire TSWV. Infected adults are responsible for transmission and spread.

Outbreaks of tomato spotted wilt are difficult to manage. Growing seedlings under cover, avoiding sequential plantings, removing acquisition hosts for the larvae, rotating with non-susceptible crops, and use of UV-reflective mulch are sometimes useful as management tactics (Cho et al. 1998; Kucharek 1990; Momol et al. 2004; Rosella et al. 1996). Tomato hybrids were developed with a single-gene dominant resistance trait, but this resistance was overcome by strains of the virus

(Rosella et al. 1996). Attempts to regulate vector populations with insecticides have not been successful, and populations of thrips developed resistance to broad-spectrum insecticides (Brodsgaard 1994; Immaraju et al. 1992). Further, primary spread of TSWV is not prevented by insecticides because insecticide-exposed viruliferous adults successfully transmitted the virus before death (Momol et al. 2004).

Thrips species known to transmit TSWV are *Thrips tabaci* (Lindeman), *Thrips setosus* (Moulton), *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Trybom), *Frankliniella fusca* (Hinds), and *Frankliniella intonsa* (Trybom) (Sherwood et al. 2001). *Frankliniella occidentalis* is the primary vector of TSWV due to its increasingly global distribution (Wijkamp et al. 1995). *Frankliniella bispinosa* (Morgan), which is distributed in parts of the southeastern US, Bermuda, and the Bahamas (Nakahara 1997), has been suspected, but not proven, as a vector of TSWV (Tsai et al. 1996; Webb et al. 1998).

The plants on which adult thrips can be collected have been cited in the literature as host plants (Mound and Teulon 1995), but adults frequently inhabit flowers that are not reproductive hosts. Adults of *F. bispinosa* are abundant in the flowers of bell pepper, *Capsicum annuum* L., in Florida along with adults of other species, including *F. occidentalis* (Funderburk et al. 2000; Hansen et al. 2003; Reitz et al. 2003). The suitability of pepper as a reproductive host of *F. bispinosa* has not been determined, and the possible role of *F. bispinosa* in TSWV epidemics is unknown. The purpose of our research was to determine the competence of *F. bispinosa* as a vector of TSWV in pepper. An experiment was conducted to determine the ability of *F. bispinosa* to reproduce and acquire the virus on pepper compared to the key vector, *F. occidentalis*. Another experiment was conducted to verify that *F. bispinosa* adults are able to transmit the TSWV to uninfected pepper.

Materials and Methods

Pepper Establishment and Maintenance

Individual 'Camelot X3R' bell peppers were transplanted into a 16 x 16 cm pot containing soil mixture (Fafard 3B Mix, Agawam, MA) and about 100 were maintained under greenhouse conditions. Plants were fertilized with Peat-Lite special 15-16-17 fertilizer (Scotts-Sierra Horticultural Products Company, Marysville, OH) and Miracle-Gro Bloom Booster 10-52-10 fertilizer (Miracle-Gro, Marysville, OH). Virus acquisition and transmission experiments were conducted in growth rooms at 23 to 25°C under a photoperiod of 14 h light, 10 h darkness.

Virus Acquisition Experiment

Plants for use in TSWV acquisition trials were mechanically inoculated with TSWV isolates collected from naturally infected pepper plants at the North Florida Research and Education Center. Using a mortar and pestle, we homogenized TSWV infected leaf tissue in 5% sodium sulfite solution containing diatomaceous earth in order to prepare an inoculum. Cheesecloth was used to apply inoculum to 3 or 4 leaves per experimental plant. Seven to 10 days after mechanical inoculum, experimental plants were tested for TSWV infection by a commercially available double antibody sandwich enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Elkhart, IN). Glass tubes and polystyrene balls (Precision Plastic Ball Co., Chicago, IL) were used in place of a microplate. Samples were scored for the presence of a colorimetric reaction indicating TSWV infection.

Individual TSWV infected pepper plants between 8 and 10 weeks of age were covered by a polyethylene cylindrical cage (35 cm × 15 cm) ($n = 23$ and 25 for *F. bispinosa* and *F. occidentalis*, respectively). The opening at the top was covered with fine mesh to prevent thrips escape, and there were two side openings (2.5 cm × 2.5 cm) covered with mesh. Ten females of *F. occidentalis* or *F. bispinosa* were introduced into the cage containing one infected plant. The adults were removed after 6 d. The plants were visually inspected for larvae at 6, 8, and 10 d after initial infestation with adult thrips. Each larva when found was transferred to green bean pods. After developing into adult, each was tested with an indirect ELISA to detect for the presence of the NSs protein encoded by TSWV RNA (Bandla et al. 1994). The NSs protein is present in thrips cells as a result of TSWV replication, demonstrating that the thrips is a host for TSWV.

The mean numbers of *F. occidentalis* larvae and *F. bispinosa* larvae recovered per cage after 10 days were compared with a two sample *t*-test [PROC TTEST in SAS System Software (SAS Institute 1999)]. The percent virus acquisition of *F. occidentalis* and *F. bispinosa* larvae also was compared by a two-sample *t*-test.

Virus Transmission Experiment

Transmission trials were conducted to confirm that the adults of *F. bispinosa* and *F. occidentalis* adults transmit TSWV to pepper. Cohorts of about 50 *F. bispinosa* and *F. occidentalis* larvae were allowed to feed on infected tomato fruit until developing into pupae. After developing into an adult, 5 to 10 of each species from each cohort were tested to verify TSWV acquisition with the indirect ELISA method described previously (Bandla et al. 1994). Twenty putatively viruliferous *F. occidentalis* or *F. bispinosa* adults from co-

horts that tested positive were introduced into a polyethylene cage (55-cm-long ×x 30-cm-wide × 48-cm-high) containing 4 healthy pepper plants of 8 weeks old. There were 19 and 8 cages established for *F. occidentalis* and *F. bispinosa*, respectively. The peppers were tested after 21 days for TSWV by ELISA as described above. Transmission by *F. occidentalis* and *F. bispinosa* was compared by a chi-square test.

RESULTS

Pepper was a suitable reproductive host for *F. occidentalis* and *F. bispinosa* in our study. The mean total number (+ SEM) of larvae recovered over 10 d when introducing 10 *F. occidentalis* or *F. bispinosa* adult females on individually caged pepper plants infected with TSWV was 47.7 (±7.2) and 15.3 (±2.5), respectively. The difference was significant ($t = -4.08, df = 58, P < 0.0001$).

A higher percentage of *F. occidentalis* acquired the virus versus *F. bispinosa* ($t = -2.07, df = 53, P < 0.05$).The mean percent acquisition (±SEM) of TSWV by *F. occidentalis* and *F. bispinosa* larvae feeding on infected pepper plants, as determined by an indirect ELISA to detect for the presence of the NSs protein encoded by the virus RNA, was 21.9 (±3.1) and 14.6 (±2.9), respectively.

The adults of *F. bispinosa* and *F. occidentalis* successfully transmitted TSWV to pepper. In the virus transmission experiments, pepper plants exposed to TSWV-infected adults of *F. bispinosa* were ELISA positive in 4 out of 8 replicates. Pepper plants exposed to viruliferous adults of *F. occidentalis* were ELISA positive in 6 out of 19 replicates. The difference in transmission between the two species was not significant ($\chi^2 = 0.8; df = 3$).

DISCUSSION

The results from the acquisition experiment indicate that under laboratory conditions *F. occidentalis* is more likely to acquire the virus, and thus may be a more effective vector in pepper than *F. bispinosa*. The number of larvae of *F. occidentalis* produced per female was 3.1-fold greater, indicating that *F. occidentalis* has a greater intrinsic capacity than *F. bispinosa* to increase on pepper. The greater the intrinsic capacity of increase of a vector species on a host plant the greater the potential for acquisition and spread of TSWV (Peters et al. 1996). Differences in feeding preferences and host suitability between *F. occidentalis* and *F. bispinosa* may result in varying abilities of each species to acquire TSWV depending on the host plant. Webb et al. (1998) observed in laboratory studies higher rates of acquisition of TSWV for *F. bispinosa* than for *F. occidentalis* when the larvae fed on *Datura stramonium* L.

Van de Wetering et al. (1999) analyzed 14 populations of *F. occidentalis*. Each population ac-

quired and transmitted TSWV, but there were marked differences in their efficiency, expressed as the percentage of transmitting adults. Laboratory experiments also do not account for ecological factors that influence thrips populations under field conditions, such as parasitism or predation that may reduce thrips populations, nor does it account for differences in mobility and other behaviors between the two thrips species that might affect the spread of TSWV in field peppers. Ramachandran et al. (2001) showed that the adults of *F. bispinosa* moved more rapidly in field peppers than the adults of *F. occidentalis*. This behavior allowed the adults of *F. bispinosa* to more frequently escape predation of *Orius insidiosus*.

The abundance of *F. bispinosa* and *F. occidentalis* in certain geographical regions also should be considered when assessing these species as a potential threat as a vector of TSWV in field pepper. Hansen et al. (2003) found *F. bispinosa* in much greater abundance than *F. occidentalis* in central Florida, while both species were abundant in northern Florida.

In this study, we have shown that under laboratory conditions *F. bispinosa* is a competent vector of TSWV in pepper. The species reproduced and acquired TSWV from infected pepper plants. Viruliferous adults of *F. bispinosa* transmitted TSWV to pepper. Reproduction of *F. occidentalis* on pepper and virus acquisition by the larvae feeding on pepper was greater than that by *F. bispinosa*; however, species-specific attributes may play a role in the ability of both vectors to vector TSWV in field conditions. The adults of *F. bispinosa* are more mobile within pepper fields than the adults of *F. occidentalis* (Ramachandran et al. 2001). TSWV epidemics occur on field pepper in our region (Gataitis et al. 1998), but the role of each species in disease epidemiology under field conditions is not understood.

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METAMASIUS CALLIZONA (COLEOPTERA: DRYOPHTHORIDAE): LONGEVITY AND FECUNDITY IN THE LABORATORY

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ABSTRACT

Metamasius callizona (Chevrolat) is native to southern Mexico and Guatemala. It was detected in Broward County, Florida, in 1989 and has spread to 20 counties in southern Florida, where it devastates populations of native epiphytic bromeliads and also attacks cultivated bromeliads. Larvae mine into stems of larger bromeliads, killing them. New data were obtained at ≈25°C and a photoperiod of 14:10 L:D to optimize cultures of this insect to serve as hosts for the production of biological control agents. After pairing with males, it took an average of 28.9 d (±17.8, range 8-89 d) for females to begin laying eggs; thereafter, each female laid eggs for the remainder of her life, or within just a few days of her death. The total duration of life of 75 ovipositing females averaged 156.4 d (±96.7, range 26-387 d); their lifetime egg production averaged 39.6 eggs (±40.0, range 2-188 eggs).

Key Words: Mexican bromeliad weevil, biological control, Florida, invasive species, adventive insects

RESUMEN

Metamasius callizona (Chevrolat) es nativo del sur de México y Guatemala. Fue detectado en el condado Broward de Florida en 1989. Ahora, ocupa 20 condados del sur de Florida donde destruye poblaciones nativas de bromeliáceas epífitas y ataca a bromeliáceas cultivadas. Las larvas minan los tallos de las bromeliáceas grandes causandoles la muerte. La nueva información presentada aquí es para mejorar las colonias de este insecto como huésped para agentes de control biológico. Después de aparearse, las 75 hembras bajo investigación mantenidas bajo 25°C y un fotoperíodo 14:10 luz:obscura iniciaron la oviposición en 28,9 días (±17,8 con un rango de 8-89) y continuaron oviposición durante toda la vida. La duración de vida de las 75 hembras fue de 156,4 días (±96.7 con un rango de 26-387); y la producción total de huevos por cada una fue de 39,6 (±40,0 con un rango de 2-188).

Translation provided by the authors.

Two Neotropical species of *Metamasius* arrived and became established in Florida in the 1980s. The first was *M. hemipterus* (L.), which is a secondary pest of sugarcane and some ornamental palms (Weissling & Giblin-Davis 1998). The second, with which we are concerned here, is *M. callizona* (Chevrolat), a pest of bromeliads (Larson & Frank 2004).

Metamasius callizona is one of many invasive insects in Florida (Frank & Thomas 2004). It was first detected in Florida in 1989, and is a pest of cultivated bromeliads such as *Ananas comosus* (L.) (pineapple) and numerous genera, species, and hybrids of ornamental bromeliads (Frank & Thomas 1994). It can be managed in plantings of cultivated bromeliads by applications of chemical insecticides. However, it is also a devastating pest of Florida's native bromeliad populations, and has spread to 20 counties in southern and central Florida. These 20 counties contain habitats for virtually all of the range of 11 of the 12 at-risk

bromeliad species, and part of the range of the 12th species. Insecticides are impracticable for the control of *M. callizona* because of the epiphytic growth of all native Florida bromeliads, their occurrence in nearly all of south Florida including Federal, state, and county parks, and the potential environmental damage to non-target organisms on land and in water bodies from widespread spraying (Frank 2002). The weevil is destroying 'naïve' populations of 'protected' endangered and threatened native bromeliad species (Frank & Cave 2005).

Metamasius callizona arrived in Florida as a contaminant of ornamental bromeliads imported from Mexico (Frank & Thomas 1994). We believe that its large distribution within Florida occurred by movement of weevil-contaminated ornamental bromeliads. Thus, there is great risk in places that import ornamental bromeliads from Mexico and, now, from Florida. These include Hawaii, with its pineapple industry, and Puerto Rico, with

not only a pineapple industry but also a rich native bromeliad flora. Constant vigilance is needed to guard against this.

As part of a biological control program aimed at *M. callizona*, the development of eggs, larvae, and pupae was investigated in the laboratory (Salas & Frank 2001). To complement those studies, we report here on laboratory longevity and fecundity of adult females.

The weevil genus *Metamasius* was traditionally placed in the family Curculionidae (e.g., Anderson 2002). However, Anderson (2003) and others reassigned it and related genera to a family named Dryophthoridae, which previously was the subfamily Dryophthorinae (= Rhynchophorinae) of Curculionidae. Because of this and other changes in classification of Curculionoidea, the name 'weevil' seems to apply to insects of several families.

MATERIALS AND METHODS

A greenhouse culture of *M. callizona* had been maintained since the early 1990s at the Entomology and Nematology Department, University of Florida. The original stock was collected in various Broward County parks in southern Florida. It was augmented from time to time with freshly-collected specimens to promote genetic diversity. The greenhouse was heated in winter and cooled in summer to eliminate temperature extremes. By 1995, pineapple crowns, discarded by grocery stores, were adopted as the sole food for adults, ovipositional substrate, and site for development of the immature stages. By 2001, the rearing was concentrated within cages of various sizes in the greenhouse to reduce escape from the greenhouse by adults, and predation by frogs (*Hyla* sp.) and lizards (*Anolis* sp.). Provided that air humidity was high (natural air humidity supplemented by watering from a garden hose with sprinkler head once every 2 d), this system was adequate for maintenance of the weevil culture. It eliminated need for culture of potted bromeliads and it minimized labor. The most laborious aspect was to extract weevil pupae from cocoons, and these as well as adults and larvae, from pineapple crowns, once development of most of each cohort within a cage had reached the pupal stage.

Beginning in August 2004, weevil pupae, extracted from cocoons in the greenhouse culture, were brought indoors to a rearing room and housed individually in plastic vials. The rearing room was maintained at ± 25°C (high 25.4 ± 0.3, low 24.3 ± 0.3, n = 449 d). Air humidity was supplemented by two electrical humidifiers (RH high 48.1 ± 5.8%, low 40.2 ± 5.8%, *n* = 449 d) although it was perhaps of little consequence to the weevils within the closed vials with moist pineapple leaves. A photoperiod of L:D 14:10 was maintained with overhead fluorescent lighting (495 lux) in the windowless room. This allowed the exact date of emergence of the resultant adult weevils to be recorded. Within 3-5 d of its emergence, each female was assigned a code number and paired with a coded male of similar age and placed in a transparent plastic vial (7 cm h, 3.8 cm internal diam.) with snap cap. Immediately, four lengths of pineapple leaf (≈ 5 cm) were added as food and ovipositional substrate. Those leaves had been kept chilled since their collection from grocery stores, and within each vial they provided moisture. Pineapple leaf lengths were replaced in each vial (with a living weevil) once every 2 d.

We examined each vial daily for survival of adult weevils and, using a dissecting microscope, for presence of eggs. As soon as the first egg was detected within each vial, the male weevil was removed and placed in a separate vial. Most eggs were oviposited singly in pockets cut by females in pineapple leaf lengths, but some were detected being held against the floor or the walls of the vials by moisture. Every egg observed was recorded and removed. Removal often resulted in destruction of the egg; therefore, fertility was not recorded. Female weevils were initiated to this regime until 75 of them had begun to oviposit. Data were recorded daily until all 75 females had died, and then were analyzed statistically.

The question of whether oviposition declines within the lifetime of a female was addressed by comparison of the number of eggs laid during the initial and final halves of the reproductive period. To achieve this, we recorded on spreadsheets an absolute scale (day of emergence to day of death) **a** (first egg laid) and **b** (death). We subtracted **a** from **b** to calculate midpoint (**x**) for each female that oviposited. Thus, we defined the initial and final halves of the reproductive period, then noted the number of eggs laid in each of those two periods, to present descriptive statistics. For this analysis, the two periods had to be of equal duration in whole days; to equalize them when there was a midpoint day, we ignored any data for that midpoint day; thus the total number of eggs recorded in this analysis is very slightly less than the actual total number recorded. For convenience, we considered these to be the two halves of the reproductive period although we acknowledge that the reproductive period could be deemed to end on the day the last egg was laid (which varied from 2 to several days earlier).

RESULTS AND DISCUSSION

The total duration of life of the 75 ovipositing females studied averaged 156.4 d (± 96.7 [SD], range 26-387 d); their lifetime egg production averaged 39.6 eggs (± 40.0, range 2-188 eggs). After pairing females with males, it took an average of 28.9 d (± 17.8, range 8-89 d) for females to begin laying eggs.

The daily oviposition by the 75 females that oviposited (after pairing) is shown in Fig. 1A. In the first 14 d after pairing, only one egg had been laid because almost all females were still in the preoviposition period. Not until d 89 were all surviving females in the group contributing eggs. However, by then, considerable mortality had occurred (Fig 1B). A graph of ovipositing females that survived 100 d (plotted but not shown) indicates a gradual build-up in daily oviposition until about d 89 cf. a rapid build-up after ≈ d 14.

Oviposition does not decline within the life of a female. We compared the number of eggs that females laid during the initial and final halves of the reproductive period. Thirty females laid more eggs in the initial half of their reproductive period, 39 in the final half, and six equally. Seven of eleven females that survived > 300 d laid more eggs in the final half of their reproductive period than in the initial half. Despite halving of the number of ovipositing females by d 140 (Fig. 1) there was no evidence of a decline in numbers of eggs laid daily. There was thus no evidence that fecundity declined as females aged. Rather, the evidence suggests that each female continued ovipositing until shortly before death.

The median interval between last oviposition and death was 5 d (mean 8.3 ± 8.8 d). If we accept that the oviposition rate was either 0.32 eggs/female/day (from first egg laid until death), or one egg every 3 d (see below), then there is virtually no room for an explanation other than senescence for cessation of oviposition.

No regular periodicity in oviposition by any female was detected, so the irregularities of the data presented in Fig. 1A are the result of random variation. Variation in numbers of eggs laid daily by any female was 0-4, with 0 (76.95%) followed by 1 (20.47%), 2 (2.34%), 3 (0.22%), and 4 (0.02%) calculated from day of pairing. The total fecundity of each female was highly correlated (n = 75, r = 0.7693, P < 0.001) with longevity, but yet 23% (1.00-0.77) of the variation was not explained by longevity. The longevity of males, held separately, appeared to match that of females.

The 75 females laid a total of 2,973 eggs. The sum of oviposition days, if calculated (**a**) from pairing to death of each female, was 11,505, but if calculated (**b**) from first egg to death was 9,392. An oviposition rate (eggs/female/day) might be calculated as (**a**) 2973/11505 = 0.26 or (**b**) 2973/9392 = 0.32. On the above evidence, we might expect ≈ 3,194 eggs from every 100 females treated similarly, assuming that 80.6% of them oviposit. In attempts to mass-produce weevils as hosts for a laboratory-reared biological control agent, it should be remembered that older females continue to oviposit at an unreduced rate.

The proportion of female *M. callizona* that laid eggs (80.6%) was very similar to that of *M. hemipterus* (76%) as was their preovipositional period (28.9 d) compared with that of *M. hemipterus* (27.0
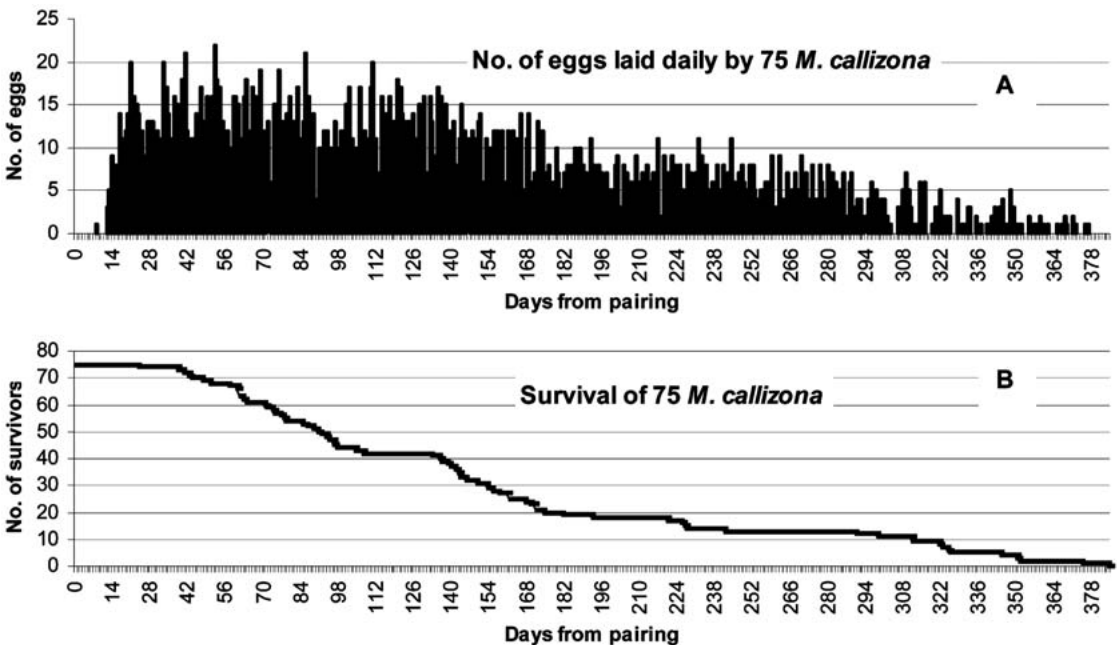
Fig. 1. A. Number of eggs laid daily by 75 *Metamasius callizona* females from day of pairing (3-5 d after emergence from the pupal stage) until the last died. B. Survival of those same 75 females over the same time period.

d) as reported by Weissling et al. (2003). The lifetime egg production (fecundity) per female *M. callizona* was 20% less (39.6 vs 51.6 eggs) in a slightly longer life (156.4 vs 142.3 d), and with maximal recorded lifespan longer (387 d vs 204 d). However, because of necessarily different substrates used and different procedures and temperature regimes, the reported differences are not statistically valid. We may only conclude that these characteristics of the two weevil species are similar. Weissling et al. (2003) calculated mean egg production during the oviposition period of *M. hemipterus* as 1.1 eggs/female/d ± 0.02, but did not explain the calculation method; we, however, calculate 0.32 eggs/female/d (see above) for *M. callizona* for which we cannot give an SD because of our method of calculation; the two values differ widely. One reviewer argued that the range of fecundity in our study was so enormous as to be unlikely unless some females had not mated successfully. However, only the 75 females that began to oviposit (80.6% of the total) were included in the study, variation in longevity was enormous, and 77% of variation in fecundity was explained by longevity.

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CLASSICAL BIOLOGICAL CONTROL OF THE PAPAYA MEALYBUG, *PARACOCCUS MARGINATUS* (HEMIPTERA: PSEUDOCOCCIDAE) IN THE REPUBLIC OF PALAU

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Abstract

The papaya mealybug (PM), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae), a pest in Central America and the Caribbean, was noted to have established on Palau in March 2003 and was causing serious damage to papaya, plumeria, hibiscus, and other plants. The parasitoids *Anagyrus loecki* Noyes, *Pseudleptomastix mexicana* Noyes and Schauff, and *Acerophagus papayae* Noyes and Schauff (Hymenoptera: Encyrtidae) totaling 24,586 were imported from Puerto Rico and field released in Palau from August 2003 to June 2004. *Anagyrus loecki* and *A. papayae* appear to be promising biological control agents of PM in Palau. No field recovery of *P. mexicana* was made in spite of several field releases. The reduction of the papaya mealybug population density levels below detectable levels was observed in a six-month period following the introduction of these exotic parasitoids. Following the successful implementation of a classical biological control program, the risk of this mealybug spreading to other islands in the Republic of Palau and to neighboring Micronesian Islands has been considerably reduced.

Key Words: Papaya mealybug, *Paracoccus marginatus*, Hemiptera, Pseudococcidae, *Anagyrus loecki*, *Pseudleptomastix mexicana*, *Acerophagus papayae*, Hymenoptera, Encyrtidae, biological control, Palau

Resumen

El estabecimiento en Palau de la cochinilla de papaya (PC), *Paracoccus marginatus* Williams y Granara de Willink (Hemiptera: Pseudococcidae), una plaga del Centroamerica y el Caribe, fue anotada en marzo del 2003. Esta plaga causa daño severo en papaya, hibiscus y otras plantas. Un total de 24,586 de los parasitoides, *Anagyrus loecki* Noyes, *Pseudleptomastix mexicana* Noyes & Schauff, y *Acerophagus papayae* Noyes & Schauff (Hymenoptera: Encyrtidae), fueron importados de Puerto Rico y liberados en Palau de agosto de 2003 hasta junio de 2004. Las especies *Anagyrus loecki* y *A. papayae* parecen ser agentes de control biológico prometadores de PC en Palau. El parasitoide, *P. mexicana* no fue recuperado en el campo a pesar de varias liberaciones de esta especie en el campo. La reducción en el nivel de la densidad de la población de la cochinilla de papaya a un nivel no detectable fue observada por un período de seis meses después de la introducción de estos parasitoides exóticos. La implementación exitosa de este programa de control biológico clásico ha reducida el riesgo que esta cochinilla se disperse a las otras islas en la Republica de Palau y las Islas de Micronesiano cercanas.

The papaya mealybug (PM), *Paracoccus marginatus* Williams and Granara de Willink (Hemiptera: Pseudococcidae) is native to Mexico and/or Central America (Miller et al. 1999). It was first described in 1992 (Williams & Granara de Willink 1992) and re-described by Miller & Miller (2002). In April 2003, G.W. Watson, Natural History Museum, London, England (Currently, California Department of Food and Agriculture, Sacramento) confirmed the identity of PM following a March 2003 report of heavy infestations of mealybugs on papaya *Carica papaya* L. (Caricaceae) on the island of Koror and in the southern state of Airai on the island of Babeldaob of the Republic of Palau (Anonymous 2003). Papaya mealybug has a wide host range of over 60 species of plants (Mey-

erdirk & Kauffman 2001). Its distribution and damage symptoms have been reviewed by Meyerdirk et al. (2004). The establishment of PM in Guam in 2002 and Palau in 2003 was flagged as a serious concern for the neighboring islands in the Pacific (Meyerdirk et al. 2004). This concern has been justified by its recent establishment on Maui in the Hawaiian Islands (Heu & Fukada 2004).

Since the establishment of PM in Palau, home gardeners have been washing mealybugs from papaya trees with water using hoses and farmers have been using insecticides to control PM without much success. In response to the pressure from PM some homeowners have elected to cut their papaya trees and some commercial growers have abandoned papaya cultivation.

Successful classical biological control programs on hemipterans in recent years include cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in Africa (Neuenschwander 2001), mango mealybug, *Rastrococcus invadens* (Williams) (Hemiptera: Pseudococcidae) in West Africa (Bokonon-Ganta & Neuenschwander 1995; Pitan et al. 2000), red coconut scale, *Furcaspis oceanica* Lindinger (Hemiptera: Diaspididae) in Saipan and Guam (Muniappan et al. 2003), pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae) in the Caribbean (Kairo et al. 2000), and most recently *P. marginatus* in Guam (Meyerdirk et al. 2004).

In an attempt to develop a classical biological control program for the papaya mealybug, the parasitoids *Anagyrus loecki* Noyes, *Acerophagus papayae* Noyes and Schauff, and *Pseudleptomastix mexicana* Noyes and Schauff (Hymenoptera: Encyrtidae) were collected originally in Mexico. They were later cultured and mass produced in a cooperative effort with the Puerto Rico Department of Agriculture at San Juan, Puerto Rico and USDA, Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ). These parasitoids have been shipped to the Dominican Republic, Florida, and Guam (Meyerdirk et al. 2004).

This paper documents the introduction and establishment of the parasitoids and suppression of PM in the Republic of Palau.

Materials and Methods

Plumeria (*Plumeria* spp.) (Apocynaceae) was selected as the study plant to monitor population densities of PM and the newly released exotic parasitoids as described in Meyerdirk et al. (2004). Even though papaya is one of the main hosts of PM, it was not chosen for sampling because the plants are fragile and heavy infestation of PM kills the plants in a short period. On the other hand, plumeria trees are hardy and are distributed throughout Palau. Samples taken on plumeria included four mature leaves removed from two terminal shoots per quadrant selected at random,

totaling 16 leaves per tree for mealybug density counts. The length and width of each leaf were measured. Only the lower surface of the leaves was examined under a dissecting microscope because 99% of all developmental stages of PM are located on the lower surface of the leaves. Mechanical counters were used to tally the total number of mealybugs per stage of development. Stages counted included egg masses as single individual units with eggs alone, egg masses with eggs and crawlers, second and third instars of males and females, adult male and female mealybugs, and mummies with and without exit holes. Second and third instars and adult male and female stages were totaled per leaf. All 16 leaves were used to average the number of stages per leaf per study site. One tree represented one study site. A total of nine study sites, each with one plumeria tree, served as the source of counts for this study.

Additional plumeria leaves were collected from each study site showing signs of PM infestation in order to isolate a total of 100 individual mealybugs. Late second and third instars and adult females were individually collected for percent parasitization records. These counts were conducted on a monthly schedule with mealybugs removed from the leaf samples and individually encapsulated in clear gelatin capsules (size 0). These capsules were labeled and placed in Ziploc plastic bags for 30 days in the laboratory in an air conditioned room (25°C). After the 30-day period, each capsule was examined to determine if the mealybug was parasitized. Emerged parasitoids were counted and identified to species. The parasitoids *A. loecki*, *A. papayae*, and *P. mexicana* were shipped from Puerto Rico to Palau from August 5, 2003, to June 25, 2004, with an interruption from October 2, 2003, to May 6, 2004, because of low parasitoid culture production.

The survey for population density estimation of PM before the release of the parasitoids was carried out on August 5, 2003. Release of *A. loecki*, *A. papayae*, and *P. mexicana* was carried out from August 6, 2003, to June, 2004. In total, 24,586 parasitoids were released at 13 sites over a 10 month period (Table 1).

Cryptolaemus montrouzieri Mulsant (Coleoptera: Coccinellidae), a pre-existing mealybug predator established in 1939 in Palau (Esaki 1940), was sampled during papaya mealybug density counts by using a beat sheet (53 × 53 cm). A total of four strokes with a beating stick (60 cm long) against plumeria terminals were used to knock off *C. montrouzieri* adults and larvae, which were then counted in each quadrant of each plumeria tree at random. Notes were taken on other predators observed at each study site.

Data collected on the number of egg masses, 2nd and 3rd instars, and adult males and females per whole plumeria leaf sample were converted to total number of mealybugs per 100 sq cm of leaf.

TABLE 1. PAPAYA MEALYBUG PARASITOIDS RELEASED AT DIFFERENT LOCATIONS IN PALAU DURING 2003-2004.

Release sites	Parasitoids[1]	Dates of release							
		08/06/03	08/26/03	09/23/03	10/02/03	05/06/04	05/11/04	05/21/04	06/25/04
PITI Compound, Malakal	AP	200	—	200	100	—	20	—	350
	AL	—	200	—	—	—	15	—	125
	PM	—	—	200	—	—	—	—	185
Old Sea Plane Port, Meyuns	AP	200	—	200	100	—	—	—	320
	AL	—	—	200	—	—	17	—	130
	PM	—	200	—	100	—	180	—	145
Palau High School, Medalaii	AP	200	—	200	—	—	—	250	550
	AL	200	—	200	—	—	—	100	120
	PM	200	—	200	—	—	—	130	150
Catholic Church, Ngerbeched	AP	200	200	200	—	—	—	450	—
	AL	200	—	200	—	—	—	135	—
	PM	200	—	200	—	—	—	150	—
Asahi Baseball Field, Ngerbeched	AP	200	—	200	100	—	370	—	580
	AL	—	—	—	—	—	87	—	150
	PM	—	200	—	100	—	—	—	165
Palau Community College, Medalaii	AP	200	—	200	—	—	50	325	—
	AL	—	—	—	—	—	10	150	—
	PM	—	200	—	—	—	—	175	—
Behind Palasia Hotel, Dngeronger	AP	200	—	200	—	—	—	125	—
	AL	—	—	—	—	—	—	150	—
	PM	—	200	—	—	—	—	175	—
City Cab, Iyebukel	AP	400	—	200	—	—	—	125	—
	AL	—	—	—	—	—	—	150	—
	PM	—	200	—	—	—	—	100	—
Airai View Hotel, Airai	AP	200	200	200	—	—	115	150	—
	AL	200	—	200	—	—	92	175	—
	PM	200	—	200	—	—	—	130	—
Chengina Masang, Malakal	AP	—	—	200	—	—	—	950	1565
	AL	—	—	—	—	—	—	825	340
	PM	—	—	—	—	—	—	850	515
Gov. Laura Ierago residence, Meyuns	AP	—	—	200	—	—	—	—	—
	AL	—	—	—	—	—	—	—	—
	PM	—	—	—	—	—	—	—	—
Pasqual Tiakl, Ngerbeched	AP	—	—	200	—	—	—	—	—
	AL	—	—	—	—	—	—	—	—
	PM	—	—	—	—	—	—	—	—
Klou Klubev. Peleliu	AP	—	—	—	—	2000	—	—	—
	AL	—	—	—	—	220	—	—	—
	PM	—	—	—	—	1400	—	—	—
Total	—	3200	1600	4000	500	3620	956	5320	5390

[1]AL: *Anagyrus loecki*; AP: *Acerophagus papayae*; PM: *Pseudleptomastix mexicana*.

RESULTS

The sampling sites, numbering one through nine, in Palau are shown in Fig. 1. The locations and number of parasitoids released are given in Table 1. In total, 13, 270 *A. papayae*, 4,441 *A. loecki*, and 6, 875 *P. mexicana* were released. No parasitoids were observed in a pre-release survey conducted on Aug. 5, 2003. Monthly sampling to determine the establishment of the introduced parasitoids and percentage of parasitism is presented in Fig. 2.

Papaya mealybug population densities on plumeria for the nine sampling sites, the reduction in population density of various stages of PM, and parasitoid mummies over a period of one year from the time of release of the parasitoids are shown in Fig. 3. Very few *C. montrouzieri* larvae and adults were encountered in the surveys. It varied from zero to a maximum of nine per survey from nine sites. The increase in leaf length and width of plumeria indicating healthy growth at the sampling sites was the apparent result of the PM pop-
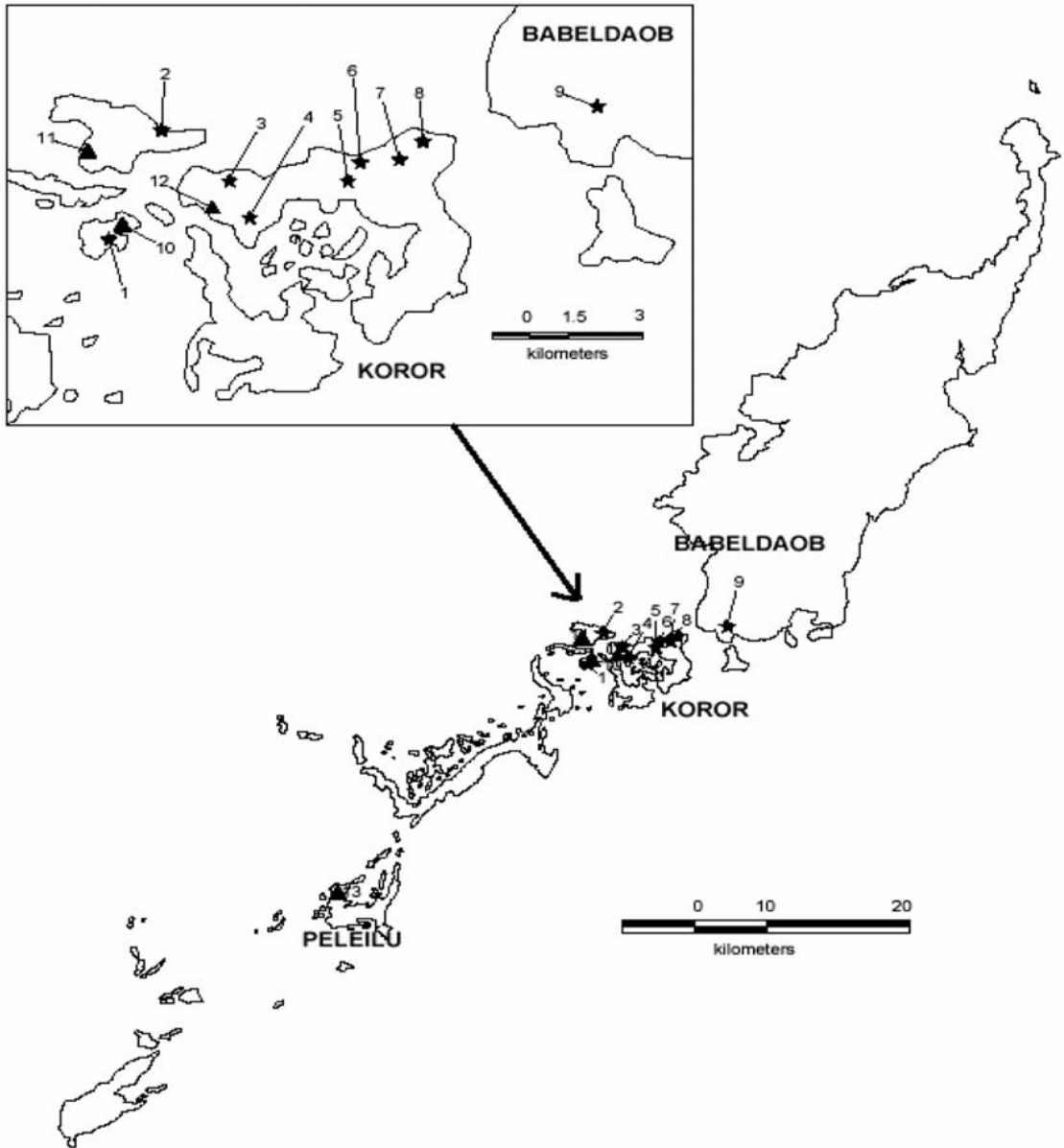
Fig. 1. Parasitoid release and sampling sites in the republic of Palau. ★—Parasitoid release and sampling sites (1 Piti compound; 2 Old sea plane port; 3 Palua high school; 4 Catholic church; 5 Asahi baseball field; 6 Palau community college; 7 Palasia hotel; 8 City cab; 9 Airai View hotel). ▲—Parasitoid release sites only (10 Chengina masang; 11 Governor Laura Ierago's residence; 12 Pasqual Tiaki; 13 Peleliu).

ulation suppression as shown in Fig. 4. In March 2004, PM was observed in the southern island Peleliu; however, the parasitoids also had moved with it and kept the population of PM localized and at a very low level. Sampling for parasitoids in the island of Peleliu in June 2004 indicated fortuitous introduction of the parasitoid *A. papayae*. Parasitism ranged from 8 to 49%. Subsequently all three parasitoids were released on this island.

DISCUSSION

The use of exotic natural enemies to suppress pest population has long been an integral part of biological control, which has continually proved very valuable in eliminating pest problems (Van Driesche & Bellows 1996). This tactic has been applied to pests in a wide variety of natural, agricultural, and urban settings (Bellows & Fisher
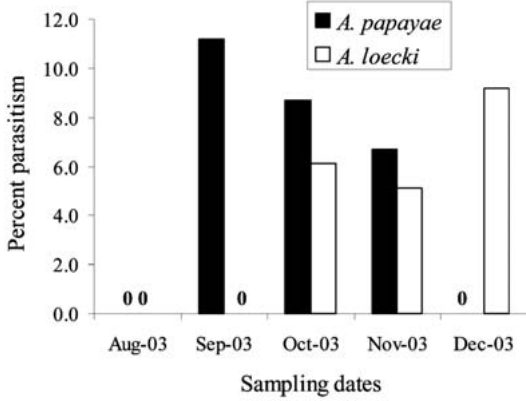
Fig. 2. Percent parasitism on *Paracoccus marginatus* by the parasitoids *Acerophagus papayae* and *Anagyrus loecki*.

1999; Mackauer et al. 1990; Wellings 1998). According to DeBach & Rosen (1991), 164 species of insect pests are being permanently controlled by classical biological control. For example, for 75 species control was "complete", for 74 species "substantial," and for 15 species "partial" control. Meyerdirk et al. (2004) reported successful biological control of PM in Guam, and this study presents yet another successful biological control effort in the western Pacific.

Survey of PM on Palau before the release of the parasitoids proved that there were no local parasitoids that shifted to this mealybug. A few adults and larvae of *C. montrouzieri* were collected in the beat-net samples. Parasitoids of PM imported from Puerto Rico were released within four months of confirmation of the establishment of PM on Palau. Even though the establishment of the parasitoids was confirmed within a month of their release at the sampling sites, releases were

Fig. 3. Population (Mean ± SEM) fluctuations of papaya mealybug and its parasitoids in Palau

Fig. 4. A measurement (Mean ± SEM) of the *Plumeria* spp. leaves from the time of release of the parasitoids.

continued until June 2004 in an effort to suppress the PM population and to prevent further spread to different parts of Palau. Four months after introduction of the parasitoids there was a reduction in PM population density below a detectable level in the surveys. In February 2004, one out of nine sites had 106 egg masses, 212 second and third instars and adults, and 12 mummies per 100 sq. cm. Other sites had none, possibly due to local extinction of the parasitoids in those areas. In May 2004, a few PM were observed in two out of nine sites; however, in these sites 17 and 2047 mummies also were recorded. In July 2004, no detectable incidence of PM or the mummies was observed.

Among the three parasitoids released, only *A. papayae* and *A. loecki* were recovered from the field; *P. mexicana* was not found. Recovery of the parasitoids from the field indicated that *A. papayae* was the first to become established. *A. loecki* was also established, but was recovered a month later. When the releases of parasitoids were discontinued in early October, *A. papayae* was recovered from the field in November 2003, but not in December. On the other hand *A. loecki* was recovered until December 2003. Of the three parasitoids released in Koror and Airai, *A. papayae* was the only parasitoid that established fortuitously in Peleliu.

A low incidence of the hyperparasitoids, *Eunotus* sp. (Hymenoptera: Pteromalidae) 0.4% and *Procheiloneurus dactylopii* (Hymenoptera: Encyrtidae) 0.8%, also was observed. The introduction of the parasitoids and the suppression of the PM have resulted in steady increase in width and length of the plumeria leaves. Almost all papaya, plumeria, and hibiscus plants recovered and no symptoms of damage were noted a year after introduction of the parasitoids. Suppression of PM in Guam (Meyerdirk et al. 2004) and in Palau has considerably reduced the possibility of this mealybug spreading to other Micronesian islands.
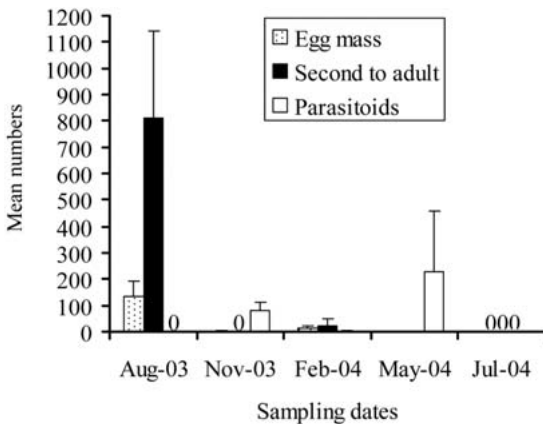
Similar results have been noted in the Dominican Republic, Puerto Rico, and Guam with about 97% reduction in PM populations a year after the introduction of the parasitoids (Kauffman et al. 2001; Meyerdirk & Kauffman 2001; Meyerdirk et al. 2004). The programs in Guam and Palau are classic examples of technology transfer of a classical biological control program from the Caribbean to the Pacific.

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PREY PREFERENCE BY *DELPHASTUS CATALINAE* (COLEOPTERA: COCCINELLIDAE) ON *BEMISIA ARGENTIFOLII* (HOMOPTERA: ALEYRODIDAE): EFFECTS OF PLANT SPECIES AND PREY STAGES

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ABSTRACT

Plant species and insect stages were studied for their effects on feeding by predator *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) on the silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) (Homoptera: Aleyrodidae). To study the influence of plant species, immature whitefly prey were presented simultaneously to starved predator adults on leaf cuttings of five different plant species: cotton (*Gossypium hirsutum* L.), tomato (*Lycopersicon esculentum* Miller), hibiscus (*Hibiscus rosa-sinensis* L.), cowpea (*Vigna unguiculata* [L.], Walpers ssp. *unguiculata*), and collard (*Brassica oleracea* var. *acephala* DC). Percentage predation over 24 h was significantly highest on cotton, followed in rank order by collards, cowpea, tomato, and hibiscus. Different predation rates may have been caused by differential response to volatile secondary compounds released by the leaf cuttings. Host stage preference was studied by presenting individual adult predators with equal numbers of prey (200 per replicate) in three aggregate life stages: eggs, small nymphs (1st to 3rd instars) and large nymphs (4th instar to pupae). Adults consumed significantly higher numbers of eggs in a 24-h predation period compared with small or large nymphs. These findings suggest that among the plant species tested, *Delphastus catalinae* may be most effective on early-season cotton or immediately after whitefly infestation when eggs are predominant.

Key Words: silverleaf whitefly, predation, tomato, vegetables, hibiscus, cotton

RESUMEN

Las especies de plantas y las estadias de insectos fueron estudiados para sus efectos en la alimentación del depredador *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) sobre la mosca blanca (*Bemisia argentifolii* Bellows y Perring) (Homoptera: Aleyrodidae). Para estudiar la influencia de las especies de plantas, las inmaduras de la mosca blanca presas fueron presentadas a los adultos depredadores habrientos sobre esquejes de hojas de cinco especies diferentes de plantas: algodón (*Gossypium hirsutum* L.), tomate (*Lycopersicon esculentum* Miller), hibiscus (*Hibiscus rosa-sinensis* L.), caupí o garbanzo (*Vigna unguiculata* [L.], Walpers ssp. *unguiculata*), y hoja de col (*Brassica oleracea* var. *acephala* DC). El porcentaje de depredación por un período de 24 h fue significativamente el mas alto en algodón, seguido en el orden del mas alto hasta el mas bajo por la hoja de col, caupí, tomate y *Hibiscus*. Las diferencias en las tasas de depredación pueden ser causadas por sus respuestas diferenciales a los compuestos de volátiles secundarios liberados de los esquejes de hojas. La preferencia de la estadia de hospedero fue estudiada al presentar a los individuos de adultos depredadores un número igual de presas (200 por replica) en tres estadias de vida agregadas: huevos, ninfas pequeñas (1º a 3º estadia) y ninfas grandes (4º estadia a pupario). Los adultos consumieron significativamente mas huevos en un periodo de depredación de 24 h, comparados con las ninfas pequeñas o grandes. Estos hallazgos sugieron que entre las especies de plantas probadas, *Delphastus catalinae* puede ser lo mas efectivo en algodón en el principio de la estación o inmediatamente después de la infestación de mosca blanca cuando los huevos son predominantes.

Delphastus catalinae (Horn) (Coleoptera: Coccinellidae) is a promising predator for biological control against whiteflies (Simmons & Legaspi 2004), especially under greenhouse conditions (Liu 2005). Development, survivorship and fecundity were studied in the laboratory against the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae) (= *B. tabaci* Gennadius Biotype B) (Liu 2005). In that study, it was reported that mean adult longevity was

146.6 d, net reproductive rate (R_0) was 276.8, gross reproductive rate (*GRR*) was 325.1, generation time (*T*) was 35.6 d, doubling time (*DT*) was 4.8 d and intrinsic rate of increase (r_m) was 0.158. These parameters suggest that the beetle is capable of regulating *B. argentifolii* and other whiteflies under greenhouse conditions (Liu 2005). In a previous study, Hoelmer et al. (1993) reported adult longevity of 60.5 d for females and 44.8 d for males of *D. pusillus* (LeConte) (= D. catalinae) at 28°C. Number of prey *B. argentifolii* consumed by adult predators decreased with prey age: 167.1 eggs or 11.6 early 4th instars per day. In an applied biological control program, *Delphastus* may persist without augmentation efforts only in large populations of *Bemisia* because of the need for large numbers of eggs in its diet (Hoelmer et al. 1993).

In laboratory studies with ornamental plants, predation efficacy of *D. catalinae* was compared against *Coleomegilla maculata lengi* Timberlake (Coccinellidae) feeding on the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Aleyrodidae) (Lucas et al. 2004). Adult and 4th instar *C. maculata* were more efficient predators of whitefly nymphs and eggs on glabrous fuchsia (*Fuchsia hybrida* Voss cv "Lena Corolla"). However, *D. catalinae* adults were more efficient on pubescent poinsettia plants (*Euphorbia pulcherrima* Willd ex Klotzch cv "Dark Red Annette Hegg"). In comparison with *Nephaspis oculatus* (Blatchley) (Coccinellidae), *D. catalinae* displayed faster rates of movement, but smaller searching areas in their larval stages when feeding on *B. argentifolii* on hibiscus (Liu & Stansly 1999). However, the adults searched in similar patterns and *D. catalinae* did so more quickly as compared with the other predators. *Nephaspis oculatus* also consumed prey more slowly. Furthermore, lower and higher temperature extremes for 24-h survival of both adult and immature *D. catalinae* were found to be about 0 and 40°C, respectively (Simmons & Legaspi 2004). At 25°C, adult *D. catalinae* can survive almost 6 mo at 25°C (50% survived 3.4 mo.); this has implications for commercial shipment and survival of mild winters (Simmons & Legaspi 2004). In exclusion cage experiments, *D. catalinae* caused 55% and a 67% decreases in densities of *B. argentifolii* in two field seasons (Heinz et al. 1999). However, open field evaluations on cotton showed no significant effects of releases of 3.5 or 5.5 *D. catalinae* beetles per plant.

In this study, we investigated the effects of cotton, vegetable and ornamental plant species on predation by *D. catalinae* on immature stages of *B. argentifolii*. We also determined preferences for different lifestages of whitefly prey.

MATERIALS AND METHODS

Performance on Plant Species

Adult *Delphastus catalinae* of undetermined age and sex were starved before they were individually confined for 24 h in a 150-mm plastic Petri dish lined with filter paper. As a source of water, a damp cotton ball was placed in a small dish in the test arena. Dishes used for the predation study contained a circular leaf section of each plant species that was previously infested with *B. argentifolii*. When necessary, additional cuttings were added to present ≈50 whitefly nymphs and eggs per plant species. Leaf cuttings were glued on to a filter paper lining a dish (150×25 mm) and placed within the same dish. Five plant species were used: cotton (*Gossypium hirsutum* L. var "DP 458 B/R"®), tomato (*Lycopersicon esculentum* Miller cv "Burbank") (Pennington Seed Inc., Madison, GA), hibiscus (*Hibiscus rosa-sinensis* L.) (Espositos Nurseries, Tallahassee, FL), cowpea (*Vigna unguiculata* [L.] Walpers, cv "Mississippi Silver") (Cross Seed Company, Charleston, SC), and collard (*Brassica oleracea* ssp. *acephala* DC, cv "Georgian") (Cross Seed Company, Charleston, SC). Each Petri dish comprised one replicate; each treatment was replicated 10 times (treatments × replicates = 5 × 10). One adult predator was released in the center of each Petri dish and allowed to feed for 24 h. After the 24-h exposure, the predator was removed from each dish and the number of remaining eggs and nymphs were recorded. This experiment was conducted in a ThermoForma growth chamber (ThermoForma, Marietta, OH) maintained at 26°C, 60% RH and 14L: 10D photoperiod.

Host Stage Preference

Collard greens (cv "Georgian") were grown in pots containing MetroMix 200, (Scotts-Sierra Horticulture Products Co. Marysville, OH). A maximum of 4 leaves per potted plant were covered with an organza bag (16 cm × 20 cm) to prevent whitefly infestation. All other leaves were removed. Oviposition sites on the plant were restricted by securing only one leaf between two cardboard sheets (15 cm × 15 cm) and foam (1.27 cm). Ten circular holes were previously bored in the underside cardboard with a No. 10 cork borer. The cardboard was held in place with a 9-gauge wire frame and binder clips. The plant stem was covered with aluminum foil. The plants were placed in screened cages (61 × 61 × 61 cm). Approximately 200-300 adult whiteflies were released into each cage and allowed a 24-h oviposition period. Infestations were staggered to assure adequate numbers of each lifestage: eggs, small (1st-3rd instars) and large nymphs (4th-pupae) at the time of the experiment. Leaf cuttings containing ≈200 prey items of each stage were made with the cork borer and they were placed in a feeding arena (150 × 25 mm dish lined with filter paper). Each leaf cutting was glued to the filter paper to reduce wilting time. A single starved (8-h starvation period) adult *D. catalinae* was placed in the

center of each arena and allowed 24 h to feed. Each dish was secured with a rubber band. After the 24-h exposure, the predators were removed and the numbers of remaining prey were recorded by lifestage (eggs, small and large nymphs). The experiment was replicated 10 times. These tests were conducted in a ThermoForma growth chamber under conditions described as above.

Data Analysis

Predation on different plant species was analyzed as a One-Way ANOVA on percentage of whitefly hosts eaten. Data were transformed by the arcsine transformation prior to analysis but are presented as untransformed means (Sokal & Rohlf 2003). Predation on different host stages was analyzed as a One-Way ANOVA on numbers of prey eaten. When treatment effects were significant, means were separated by Tukey's HSD (P = 0.05). All analyses were performed with Systat 10.2 (Systat Software Inc., Point Richmond, CA).

RESULTS AND DISCUSSION

Performance on Plant Species

The proportions of whitefly hosts consumed were significantly different among plant species (F = 25.22; df = 4, 45; P < 0.01; R^2 = 0.69). Predation was significantly highest on cotton, followed in rank order by collard, cowpea, tomato, and lowest on hibiscus and ranged from 98.6% to 46.1% (Fig. 1). Previous studies have shown that different plant species can have profound effects on predation rates. In *Serangium parcesetosum* Sicard (Coccinellidae) feeding on *B. argentifolii* (Legaspi et al. 1996), predation was highest on cucumbers, followed by tomato and cantaloupe, and lowest on hibiscus, which are similar to the results presented here. In the predatory stinkbug *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) feeding on 4th instar *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), attack rates were higher and handling times shorter on sweet pepper and eggplant, compared with tomato (De Clercq et al. 2000). Lower predator performance on tomato was attributed to the presence of glandular trichomes and allelochemicals (De Clercq et al. 2000). The architecture of host plant leaves may affect predation rates, such as that in the lady beetle *Propylea quatuordecimpunctata* (L.) (Coccinellidae) feeding on the Russian wheat aphid, *Diuraphis noxia* (Mordvilko) (Aphididae) (Messina & Hanks 1998). Lower predation rates on broad-leaved crested wheatgrass, *Agropyron desertorum* (Fisher ex Link) Schultes was attributed to the presence of refuges such as rolled leaves, compared with the slender-leaved Indian ricegrass, *Oryzopsis hymenoides* (Roemer & Schultes) Ricker (Messina et al. 1997). Moreover, predation rates may be af-
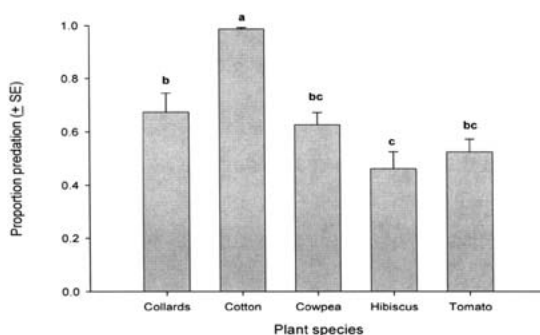
Fig. 1 Proportion of predation in 24 h (mean ± SE) by *Delphastus catalinae* on *Bemisia argentifolii* on different host plants. Bars with common letters are not significantly different (Tukey HSD; P = 0.05).

fected by interactions between different biotic and abiotic factors. In *Macrolophus pygmaeus* Rambur (Hemiptera: Miridae) feeding on green peach aphids, *Myzus persicae* (Sulzer) (Homoptera: Aphididae), predation rates were higher in darkness than in a light environment, and differences were more marked on pepper plants rather than eggplant (Perdikis et al. 2004).

Host Stage Preference

Total numbers of each host stage were not significantly different at the start of the experiment; about 200 prey items of eggs, small and large nymphs were presented to individual predators at the start of the feeding period (F = 0.029; df = 2, 27; P = 0.972; R^2 = 0.002), thereby permitting analysis on numbers of prey eaten (rather than percentages). Higher numbers of eggs were consumed in the 24-h predation period, compared with small or large nymphs (F = 84.933; df = 2, 27; P < 0.01; R^2 = 0.863) (Fig. 2). Predators are known to display different prey-preference responses when presented with various life stages of a prey. In some studies, adult prey was preferred over the egg stage. When eggs of Colorado potato beetle *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and aphids (*M. persicae*) were presented to *C. maculata* in equal numbers, females did not prefer either prey at low densities, but preferred aphids at high densities (Hazzard & Ferro 1991). Other studies have shown preferences for the egg stage of prey. For example, the phoretic mite, *Macrocheles peregrinus* Krantz (Acari: Macrochelidae) feeding on the horn fly, *Haematobia irritans exigua* De Meijere (Diptera: Muscidae), showed a strong preference for eggs of other dipterans (Roth et al. 1988). *Coleomegilla maculata lengi* neonates displayed strong preferences for conspecific eggs, even in the presence of essential prey and benefited from such cannibalistic behavior (Gagne et al. 2002). When presented
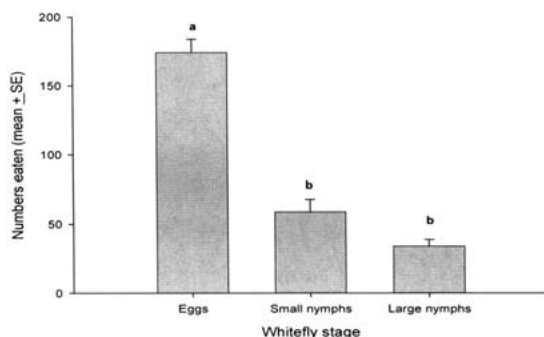
Fig. 2. Host stage preference by adult *Delphastus catalinae*. Mean numbers of whitefly prey eaten in 24 h. Bars with common letters are not significantly different (Tukey HSD; $P = 0.05$).

with a choice between younger or older eggs, *C. maculata lengi* larvae displayed preferences for younger eggs of *Trichoplusia ni* (Hübner) (Noctuidae) (Roger et al. 2001). Finally, lifestage preferences may change with predator specificity. Blackwood et al. (2001) tested 13 species of phytoseiid mites for preferences between eggs and larvae of the two-spotted spider mite, *Tetranychus urticae* Koch (Arachnida: Acari: Tetranychidae), and found that oligophagous, specialized spider mite predators generally displayed a preference for eggs, whereas more polyphagous, generalist predators showed no prey-stage preferences.

In this study, we found that the proportions of whitefly eggs and nymphs consumed were highest on the cotton disks, followed by collards, cowpea, and tomato, and lowest on hibiscus. The collard and cowpea had glabrous leaves while the leaves from the other plant species in this study had trichomes. Because this study was not performed with whole plants, the effects of leaf structure (e.g., trichomes, refuges) were not likely to be strong factors affecting predation rates. More likely is the possibility that *D. catalinae* responded differentially to volatile secondary compounds released by the plant leaf cuttings. *Delphastus catalinae* adults displayed a strong preference for *B. argentifolii* eggs followed by small, then large nymphs, despite their presence in equal numbers in the feeding arena. The data suggest that in an applied biological control context, *D. catalinae* adults are most likely to be effective against *B. argentifolii* in cotton and early in the season for other crops, or when the egg stage is most abundant.

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A NEW MEMBER OF THE GROWTH-PROMOTING GLYCOPROTEINS FROM DIAPREPES ROOT WEEVIL (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

A new member belonging to the family of growth-promoting glycoproteins referred to as imaginal disc growth factors, IDGF, was identified from the root weevil *Diaprepes abbreviatus* (L.), (Coleoptera: Curculionidae). The imaginal disc growth factor full length cDNA transcript, designated as *idgf-DRW*, was cloned and identified from tissue of adult, teneral DRW females. Sequencing and subsequent homology comparisons of the nucleotide sequence (GenBank accession no. AY821658) indicated that the open reading frame (ORF) consisted of 1329 bases and encoded a putative protein of 442 amino acid residues with a calculated molecular weight of 49.5 kDa and a pI value of 6.68. BLASTX comparisons of the *idgf*-DRW cDNA sequence showed that the deduced amino acid sequence designated as IDGF-DRW (AAV68692.1) had 43% to 51% similarity with IDGF1-5 and DS47 from *Drosophila melanogaster*, *D. simulans*, and *D. yakuba*, 51% similarity with IDGF in *Pieris rapae*, and 51% similarity with an IDGF-like protein in *Bombyx mori*. Signal P analysis revealed that the predicted IDGF-DRW contained a signal peptide of 23 amino acid residues located at the N-terminus, similar to other known IDGF proteins. The structure of IDGF-DRW was predicted based on the characterized IDGF2 from *D. melanogaster* as the model. The deduced amino acid sequence for the IDGF-DRW protein had 48% similarity with *Drosophila melanogaster* IDGF2. The predicted IDGF-DRW displays the characteristics found in *Drosophila* IDGFs, the fold of family 18 glycosyl hydrolases, with an insertion (Gly304 to Phe392) in the beta barrel between strand β7 and helix alpha-7 that forms an additional α+β domain similar to that of *Serratia marcescens* chitinases A and B. An identified nucleotide change which results in an amino acid change within the active binding site in IDGF-DRW also was observed. The significant similarities of IDGF-DRW to other members within the family of IDGFs support its classification as a new member of the invertebrate growth factors and the first IDGF to be identified from a coleopteran.

Key Words: AAV68692, AY821658, cDNA, *Diaprepes abbreviatus*, gene expression, IDGF

RESUMEN

Identificamos a un nuevo miembro de la familia de glicoproteínas que promueven el crecimiento en invertebrados conocidas como factores de crecimiento de discos imaginales, IDGF (siglas en ingles), del gorgojo *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). El transcripto complete derivado del ADN del IDGF, identificado como idgf-DRW, fue clonado e identificado apartir del tejido de hembras adultas tenerales de *D. abbreviatus*. El secuenciamiento y subsecuente comparación de la homología a nivel de secuencia nucleotidíca (accesión AY821658 de GenBank) señalaron que el marco abierto de la lectura (ORF) consiste de 1329 bases y codifica para una proteína de 442 aminoácidos con un peso molecular calculado de 49.5 kDa y un valor pl de 6.68. Las comparaciones de BLASTP de la secuencia de cADN del idgf-DRW mostraron que la secuencia deducida de la proteina identificada como IDGF-DRW (AAV68692.1) tenía un 43% -51% de identidad con IDGF1-5 y DS47 de *Drosophila melanogaster, D. simulans* y *D. yakuba*, 51% de identidad con IDGF de *Pieris rapae*, y 51% de identidad con una proteina de *Bombyx mori* similar a IDGF. El análisis SignalP reveló que

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el IDGF-DRW predicho contiene un peptido de señal de 23 aminoácidos situados en el N-término, similar a otras IDGF previamente caracterizadas. La estructura de IDGF-DRW fue predicha usando el IDGF2 de *D. melanogaster* como modelo. El IDGF-DRW predicho presenta el doblez característico de las glycosyl hydrolasas de la familia 18, con una inserción (Gly304 a Phe392) en el beta barrel entre la banda β7 y la hélice alpha-7 que forma un dominio adicional α+β similar al de las chitinasas A y B de *Serratia marcescens*. También fue validado al cambio de un nucleótido que da lugar a un cambio de un aminoácido dentro del sitio activo de ligamiento de IDGF-DRW. Las significativas semejanzas de IDGF-DRW con otros miembros dentro de la familia IDGF apoyan su clasificación como un nuevo miembro de los factores de crecimiento de invertebrados. Este es el primer IDGF identificado en coleópteros.

Translation provided by the authors.

Information about the action of insect growth-regulating hormones is of interest for two reasons. First, the functions and cellular actions of invertebrate and vertebrate hormones have been shown to be remarkably conserved; thus, what we learn about insects may also enhance our understanding of hormonal regulatory processes in vertebrates. Second, by understanding how insect hormones function and interact in the regulation of insect development, we may be able to devise safe and specific agents to disrupt the insect life cycle, thus increasing the efficiency of efforts to manage agricultural pests and disease vectors. The pest system herein is the combination of *Diaprepes abbreviatus* (L.), *Diaprepes* root weevil, DRW, and *Phytophthora* which can cause severe tree decline and destroy groves within a few years (Graham et al. 1996, 2003). *Diaprepes* continues to be a major concern of citrus growers in Florida due to the difficulty of timely detection of larval infestations in the soil, and the paucity of effective management options (Lapointe 2000). To identify genes which regulate DRW development we constructed a gene expression cDNA library from DRW.

Little information on insect growth factors was available until 1996 when an insect growth factor was purified from the conditioned medium of an embryonic cell line, NIH-Sape-4, of *Sarcophaga peregrine* (flesh fly) (Homma et al. 1996). In 1999, a new developmental gene family of growth-promoting glycoproteins, referred to as the imaginal disc growth factors (*idgf*), was identified in *Drosophila melanogaster* (Kawamura et al. 1999). The IDGF proteins promote cell proliferation in the imaginal discs and were the first polypeptide growth factors to be reported from invertebrates. Throughout all developmental stages in variable patterns, IDGF proteins are expressed suggesting that *idgf* is an important gene having multiple functions during the entire life cycle (Kawamura et al. 1999). Though little is known about their precise mode of action, *idgf* genes appear to cooperate with insulin to stimulate the growth of imaginal disc cells. IDGF proteins are structurally related to chitinases from which they may have evolved, rather than to known growth factors, but have no known catalytic activity. Several nonenzymatic proteins with sequence homology to chitinases have been described in vertebrates indicating that the typical chitinase-like fold may be present in proteins with a wider range of biological functions other than chitin degradation (Hakala et al. 1993; Morrison & Leder 1994; Shackelton et al. 1995; Hu et al. 1996; Owhashi et al. 2000; Chang et al. 2001; Varela et al. 2002). The current *idgf* gene family in *Drosophila* is comprised of six members (*idgf1-5* and *ds47*) which encode proteins sharing 40-50% similarity to one another in amino acid sequence. Fortunately the IDGF2 member has had the crystal structure described from X-ray crystallography (Varela et al. 2002). Studies have shown that computational predictions of protein structures to elucidate functions and interactions have reached an acceptable level of accuracy, especially when there are related proteins that have been well characterized (Martelli et al. 2003; Aloy & Russell 2003; Bell & Ben-Tal 2003; Valencia 2003). So far, IDGF proteins have been reported from the lepidopterans *Bombyx mori* (Tsuzuki et al. 2001) and *Pieris rapae* (Asgari & Schmidt 2004), and the dipterans *Drosophila melanogaster*, *D. simulans*, and *D. yakuba* (Zurovcova & Ayala 2002). Rigorous evaluations of current hypotheses explaining growth promoting activation by IDGFs are still awaiting evidence from biochemical studies that will define the specificity of the ligand binding of these novel invertebrate growth factors; however, Varela et al. (2002) has postulated that the observed stimulation of imaginal disc cell proliferation by the cooperation of IDGFs and insulin may be a requirement to achieve optimal signaling of the insulin receptor. Invertebrate imaginal discs express an insulin receptor which is homologous to that of vertebrates (Garofalo & Rosen 1988; Fernandez et al. 1995; Ruan et al. 1995) and which is required for normal growth (Chen et al. 1996). An in-depth review is provided by Held (2002).

Herein we present the discovery of a coleopteran IDGF and its full length *idgf* transcript which was isolated and cloned from the *Diaprepes* root weevil, DRW. The genetic information from DRW will aid our understanding of the developmental and biological pathways important to the survival of DRW, and supports the development of

new management strategies against DRW by identifying genes critical in developmental pathways.

MATERIALS AND METHODS

Diaprepes Root Weevil Rearing and Collection

Larvae of DRW were obtained from a colony maintained at the U.S. Horticultural Research Laboratory (USHRL), Ft. Pierce, Florida. Individuals were reared in cups containing artificial diet at 26°C as described by Lapointe & Shapiro (1999). Callow teneral adults recently emerged from the pupal exuvium were selected and separated by gender for processing.

Library Construction

Seventeen whole teneral female DRW were used in the construction of an expression library. The insects were ground in liquid nitrogen and the total RNA extracted with guanidinium salt-phenol-chloroform procedure as previously described by Strommer et al. (1993). Poly(A)+RNA was purified with Micropoly(A) Pure™ according to the manufacturer's instructions (Ambion, Austin, TX, USA). A directional cDNA library was constructed in the Lambda Uni-ZAP® XR vector with Stratagene's ZAP-cDNA Synthesis Kit (Stratagene, CA, USA). The resulting DNA was packaged into Lambda particles with Gigapack® III Gold Packaging Extract (Stratagene, CA, USA). An amplified library was generated with a titer of 1.0×10^9 plaque-forming units per mL. Mass excision of the amplified library was accomplished by Ex-Assist® helper phage (Stratagene, CA, USA). An aliquot of the excised, amplified library was used for infecting XL1-Blue MRF' cells with subsequent plating on LB agar containing 100 µg/mL ampicillin. Bacterial clones containing excised pBluescript SK(+) phagemids were recovered by random colony selection.

Sequencing of Clones and Computer Analysis

pBluescript SK(+) phagemids were grown overnight at 37°C and 240 rpm in 96-well culture plates containing 1.7 mL of LB broth/well, supplemented with 100 µg/mL ampicillin. Archived stocks were prepared from the cell cultures with 75 µL of a LB-amp-glycerol mixture and 75 µL of cells. These archived stocks are held at the USHRL in an ultra low temperature freezer set at -80°C. Plasmid DNA was extracted by using the Qiagen 9600 liquid handling robot and the QIAprep 96 Turbo miniprep kit according to the recommended protocol (QIAGEN, Inc., Valencia, CA, USA). Sequencing reactions were performed with the ABI PRISM® BigDye™ Primer Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) along with a universal T3 primer. Reac-

tions were prepared in 96-well format with the Biomek2000™ liquid handling robot (Beckman Coulter, Inc., USA). Sequencing reaction products were precipitated with 70% isopropanol, resuspended in 15 µL sterile water, and loaded onto an ABI 3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Base confidence scores were calculated by TraceTuner® (Paracel, Pasadena, CA, USA). Low-quality bases (confidence score <20) were trimmed from both ends of sequences. Quality trimming, vector trimming, and sequence fragment alignments were executed by Sequencher® software (Gene Codes, Ann Arbor, MI, USA). cDNA sequences arising from rRNA and mitochondrial DNA were identified with BLASTN and were excluded from analysis along with sequences less than 200 nucleotides in length after both vector and quality trimming. Additional ESTs that corresponded to vector contaminants were removed from the dataset. To estimate the number of genes represented in the library and the redundancy of specific genes, ESTs were assembled into "contigs" by Sequencher®. Contig assembly parameters were set with a minimum overlap of 50 bases and 95% identity match.

Sequence Analysis and Structure Prediction

The *idgf* cDNA sequence was covered in eight overlapping clones and then bi-directionally sequenced five more times for sequence validation. The status of the gene sequence was determined based on TBLASTX homology searches by the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.nih.gov) with the protein sequence comparisons made to protein databases (BLASTP). CLUSTALX was used for multiple alignments of amino acid sequences of the homologous proteins (Thompson et al. 1994), and an unrooted phylogenetic tree was constructed from this alignment with PAUP 4.0 and 1,000 bootstraps (Swofford 2002). The theoretical molecular weight and pI value of the predicted protein was calculated by ExPASy (http://au.expasy.org). SignalP 3.0 server (http://www.cbs.dtu.dk/services/SignalP) was used to analyze the presence and location of potential signal peptide cleavage sites in the amino acid sequence. Structural modeling of the IDGF protein was performed with ROBETTA (http://www.robetta.com). ROBETTA is a full-chain protein structure prediction server which also performs domain parsing, 3-D modeling, fragment library generation, and protein-protein interaction studies by using an interface alanine scanning method. The programs utilize the Ginzu domain parsing and fold detection method developed by Dylan Chivian, David Kim, Lars Malmstrom, and David Baker, and the ROSETTA fragment insertion method (Simons et al. 1997). ROBETTA scans protein

chains to identify homologs with PDB-BLAST, FFAS03, 3D-Jury, and the Pfam-A protein family databases and assigns z-scores by MAMMOTH, a computer program that provides consistent protein model quality ranking by comparing modeled structures and their experimental counterparts (Ortiz et al. 2002). PSI-BLAST multiple sequence alignment then assigns regions of increased likelihood of including an adjoining domain with sequence clusters (Bowers et al. 2000; Rohl & Baker 2002). Loop regions are assembled from fragments and optimized to fit the aligned template structure. Protein domain predictions were identified by using the full prediction protocol (Kim et al. 2004). Graphic rendering of the predicted 3-D structures were done by the PDB file created by ROBETTA and Protein Explorer (http://www.molvis.sdsc.edu/protexpl/ frntdoor.htm).

A phylogenetic tree was generated by bootstrap neighbor joining, 1000×, PAUP 4.0, unrooted (Swofford 2002). Topology was similar when analyzed by maximum parsimony (branch-and-bound search method); data not shown. The phylogenetic tree of amino acid sequences was constructed with five members of *Drosophila* IDGF, and a bacterial chitinase.

<center>RESULTS</center>

Isolation of IDGF-DRW

An initial 10,000 clones were sequenced from the 5' end. These sequences were trimmed of vector and low-quality sequence and filtered for minimum length (200 contiguous bp, Phred 20 quality score) producing the final set of 8,480 high-quality ESTs. These ESTs were assembled by the program Sequencher® (Gene Codes, Ann Arbor, MI 48105) to produce 5,508 contiguous sequences (contigs) with 1,240 ESTs remaining as singlets. Of the assembled sequences analyzed, one contig (representing eight ESTs) was similar to the known IDGF protein sequences based on the TBLASTX results. The nucleotide sequence of this contig contained an intact ORF and was bidirectionally sequenced for sequence validation, providing an average 5× coverage for 90% of the transcript sequence, which was registered in GenBank (accession no. AY821658). The mRNA transcript was designated as *idgf-DRW*. A second cDNA library produced from DRW adults caught in the field also identified the *idgf-DRW* transcript (Hunter, data not shown).

Sequence Analysis of IDGF-DRW

Analysis of the nucleotide sequence revealed an ORF in *idgf-DRW*, consisting of 1329 bases and encoding a putative protein of 442 amino acid residues (Fig. 1) with a predicted molecular weight of 49.5 kDa and a pI value of 6.68. TBLASTX com-

parison and multiple sequence alignment of the *idgf-DRW* cDNA sequence (Fig. 2) indicated that the deduced amino acid sequence for the IDGF-DRW protein (accession no. AAV68692) had 43% similarity with *Drosophila melanogaster* IDGF1 (accession no. AAC99417), 48% with *Drosophila melanogaster* IDGF2 (accession no. AAC99418), 43% with *Drosophila melanogaster* IDGF3 (accession no. AAC99419), 51% with *Drosophila melanogaster* IDGF4 (accession no. AAC99420), 44% with *Drosophila melanogaster* IDGF5 (accession no. AAF57703), 51% with *Drosophila melanogaster* DS47 (accession no. AAC48306), 51% with *Pieris rapae* IDGF (accession no. AAT36640), and 51% with *Bombyx mori* IDGF-like protein (accession no. BAB16695). SignalP analysis of the predicted IDGF-DRW showed the most likely signal sequence cleavage site was between Ser_{23} and Ala_{24} (Fig. 1, ●), which will generate a signal peptide of 23 amino acid residues and a predicted mature protein with a calculated molecular weight of 47.1 kDa and a pI value of 6.76. Presence of a signal peptide was common to all IDGF proteins described to date although the length of the signal peptide varied (Fig. 2; refs for variation in signal sequence length: Chou 2002; Martoglio & Dobberstein 1998). Additionally, a single consensus motif for N-linked glycosylation (Asn_{227}), as previously reported for DS47 (Asn_{233}), also was identified (Fig. 1, ●).

Phylogenetic Relationship Between IDGF-DRW and Other Members of the IDGF Family

Analysis of the sequence and predicted protein structure of IDGF-DRW strongly supports its classification as a new member of the growth-promoting glycoproteins IDGF family. The IDGF proteins, encoded by members of the *idgf* gene family, were the first soluble polypeptide growth factors to be reported from invertebrates. These proteins are structurally related to chitinases rather than to known growth factors, and were shown to possess no catalytic activity (Kirkpatrick et al. 1995; Kawamura et al. 1999). The tree topology revealed that the *Drosophila* IDGF1, IDGF2, and IDGF3 members form a clade separate from the other insect IDGF4, IDGF5, and chitinase (Zurovcova & Ayala 2002) (Fig. 3). Herein, a phylogenetic tree of the multiple amino acid sequence alignment of IDGF-DRW, *Drosophila* IDGF, *P. rapae* IDGF, and *B. mori* IDGF-like proteins is shown (Fig. 3). In pairwise comparisons, these IDGF proteins showed 38% to 79% similarity. The phylogenetic tree showed that the three protein ortholog groups (IDGF1, IDGF2, and IDGF3) were more similar to each other than to the other six.

Structure was predicted by using ROBETTA, which is a full-chain protein structure prediction server (Fig. 4). The view of conserved residues (in green space-fill) predicted to be essential to main-

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AATTCGGCACGAGGATTGACATGGAGTGTGTAAAAATTGTCCTCTTGGCAATTTTTGCCTTGGCGAGTTTCACCG    75

               M   E   C   V   K   I   V   L   L   A   I   F   A   L   A   S   F   T   G    19

GCAAGACAGAATCAGCAACAGATAGCAAATTGGTGTGTTACTATGATAGCAGAGCATATAATAGACCAGGAAATG   150

  K   T   E   S ●   A   T   D   S   K   L   V   C   Y   Y   D   S   R   A   Y   N   R   P   G   N   G    44

GTAAATTCGACATTCCGTTCTTGGAACCCGCTTTGCAATTCTGCACTCACTTAATTTACGGATATGCAGGAATCA   225

  K   F   D   I   P   F   L   E   P   A   L   Q   F   C   T   H   L   I   Y   G   Y   A   G   I   R    69

GAGAGGACAATTTCAAAATATCACCATTGAACGAACCCCTGGACATCAACAAACAAAACTATAGACATATCACTG   300

  E   D   N   F   K   I   S   P   L   N   E   P   L   D   I   N   K   Q   N   Y   R   H   I   T   D    94

ATTTGAAGAGGAAATACCCTGGCTTGAGAGTTCTTCTTTCCGTTGGAGGAAATAATGATGTTACCGGAGAAGGCA   375

  L   K   R   K   Y   P   G   L   R   V   L   L   S   V   G   G   N   N   D   V   T   G   E   G   S   119

GTGAAAAGAATTTGAAATATAGAACTTTGCTAGAATCTGTAGAAAGCAGGTTAGCCTTCGTCAACTCTGCTCACG   450

  E   K   N   L   K   Y   R   T   L   L   E   S   V   E   S   R   L   A   F   V   N   S   A   H   D   144

ATTTAGTGAAAAACTATGGCTTTGATGGACTTGATCTCTCTTGGGAATTCCCAGAAAATAAACCAAAGAAAATCC   525

  L   V   K   N   Y   G   F   D   G   L   D   L   S   W   E   F   P   E   N   K   P   K   K   I   R   169

GTAACGCAGTTTCTTCATGGTTTTCCAAAATCAAACATAAAATTGTTGGTGAATCAGTAGTTGATGAAAAAGCCG   600

  N   A   V   S   S   W   F   S   K   I   K   H   K   I   V   G   E   S   V   V   D   E   K   A   E   194

AAGAACATAAAGAACAATTTACTGCTTTAGTTCGAGAACTTAAGAACGTATTCAGACATGATGGACTTCTATTAA   675

  E   H   K   E   Q   F   T   A   L   V   R   E   L   K   N   V   F   R   H   D   G   L   L   L   T   219

CAGTCTCTGTATTACCAAATGTTAACAGTTCAGTATACTTTGACCCCCGTCAGCTTGCTCCAAATATTGATTTTG   750

  V   S   V   L   P   N   V   N ●   S   S   V   Y   F   D   P   R   Q   L   A   P   N   I   D   F   A   244

CTACACTTGAAGCTTTTGACTACAGAACTCCTCAACGCAATCCTAAAGAATTAGATTATGTCGCTCCACTTTACG   825

  T   L   E   A   F   D   Y   R   T   P   Q   R   N   P   K   E   L   D   Y   V   A   P   L   Y   E   269

AACTTTTGGACAGAAAAGTTGATGAAAATGCTGATTACCAAGTTAGATATTGGTTGGGAGGTGGTTTACCAGCCA   900

  L   L   D   R   K   V   D   E   N   A   D   Y   Q   V   R   Y   W   L   G   G   G   L   P   A   N   294

ATAAACTTATCCTTGGCATTCCAACCTACGGTCGCGCCTGGAAACTTAATGATGACTCTGGACTGACTGGCGTTC   975

  K   L   I   L   G   I   P   T   Y   G   R   A   W   K   L   N   D   D   S   G   L   T   G   V   P   319

CACCGCTACTTACAGATGGGGCTGCAGATCCTGGCCCTTACAGCAATGAAGCTGGGCTATTAAGCTATCCAGAAA  1050

  P   L   L   T   D   G   A   A   D   P   G   P   Y   S   N   E   A   G   L   L   S   Y   P   E   I   344

TTTGCAGTAAAATTGCCACTCCAAAGGAAATTCAAGCCGGATATCTTGGAAAGTTAAGGAAAACTAATGATCCAA  1125

  C   S   K   I   A   T   P   K   E   I   Q   A   G   Y   L   G   K   L   R   K   T   N   D   P   T   369

CAAAAAGATATGGTTCATATGCATATCGTCTACCCGACAGTAATGGTGAAAATGGTATTTGGGTAGGATTTGAAG  1200

  K   R   Y   G   S   Y   A   Y   R   L   P   D   S   N   G   E   N   G   I   W   V   G   F   E   D   394

ATCCTGACACCGTTGGAAATAAAGCTGCATATTCCAAGGCTAAAGGACTTGGAGGTATTGCTATCGTTGACCTAA  1275

  P   D   T   V   G   N   K   A   A   Y   S   K   A   K   G   L   G   G   I   A   I   V   D   L   T   419

CACTAGATGATTTTAGAGGAACATGTTCACAAGATCATTTCCCATTGTTAAGAGCTGCGAAGTTTAGATTATAAA  1350

  L   D   D   F   R   G   T   C   S   Q   D   H   F   P   L   L   R   A   A   K   F   R   L   *       442

TACCCCATTTCAATTTTTTTTATGTTAAATGTTAATAATTTCATATTATATCA                          1403
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Fig. 1. Nucleotide and deduced amino acid sequences of cDNA encoding IDGF-DRW. The deduced amino acid sequence is shown below the nucleotide sequence and numbered accordingly. The protein sequence is numbered starting from the first **Met** residue. The signal peptide cleavage site is indicated by ● after Ser_{23}. The signal peptide is shown by underline. A single N-linked glycosylation site is indicated by ● at Asn_{227}. The start codon is bolded and encodes the residue Met. The termination codon is indicated in bold and asterisk in the nucleotide and protein sequence, respectively.

tain the barrel folding are shown. Residues are Gly_{109}, Gly_{110}, Asp_{152}, Gly_{153}, Leu_{218}, Asp_{242}, Lys_{295}, Gly_{412}, and Asp_{421}. The residue at position 159 is Glu (in brown space-fill) which is commonly re-

placed by Gln in other known IDGF proteins. There are two disulfide bridges, Cys_{31}-Cys_{58} and Cys_{345}-Cys_{427}, in the predicted IDGF-DRW structure which are conserved in all known IDGF fam-

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                  ←β1→            ←α1→        ←β2→    β2→  ←α2→   ←

DRW  IDGF        MECVKI---VLLAIFALASFTGKTESATDSKLVCYYDSRAYNRPGNGKFDIPPLEPALQFCTHLIYGYAGIREDNFKISPLNEPLDINK--QN  89
D.m. IDGF2       MKAW-----IWFTFVACLFAAS---TEAASNLVCYYDSSSYTREGLGKLLNPDLEIALQFCSHLVYGYAGLRGENLQAYSMNENLDIYK--HQ  84
D.m. IDGF1       MRFQ-----LFYLLGL-LSVTS--LTHAASNLICYYDSNSYLRQGLAKMHTNELDLALQFCTHLVYGYAGLKSGTLELFSLN--VDLDM--FY  82
D.s. IDGF1       MRFQ-----LFYILGL-LSVTS--LTQAANNLCYYDSTSYLRQGLAKMHTNELDLALQFCTHLVYGYAGLKSGTLELFSLN--VDLDM--FY  82
D.y. IDGF1       MRFQ-----LCYLLGL-LSVTS--LSHAASNLICYYDSTSYLRQGLAKMHTHELDLALQFCTHLVYGYAGLKAGTLELFSLN--VDLDM--FY  82
D.m. IDGF3       MTGS-----LWLSLALSLAVLAQFKVSAAPNLVCFYDSQGSQRQGLAQFSMIDIELALQFCTHLVYGYAGVNADNYEMQSINKRLDLEQ--RH  87
D.s. IDGF3       MSGS-----LWLSLALSLAVLAQFKVSAAPNLVCFYDSQGSQRQGLAQFSITDIELALQFCTHLVYGYAGVNADNYEMQSINKRLDLEQ--RH  87
D.y. IDGF3       MTGS-----LWLSLALSLAVLAQFKVSAAPNLVCFYDSQGFQRQGLAQFSMTDMELALQFCTHLVYGYAGVNADNYEMQSINKRLDLEQ--RH  87
D.m. IDGF4       MKLYAL---FSLLVGSLAIGQISAAGS--HHLLCYYDGNSFVREGLSKLILTDLEPALQYCTHLVYGYAGINPSSNKLVSNNEKPDLDLGSSL  88
D.m. IDGF5       MMWIQKNPPLGLLLCSFLAFFQSTYAEVG-KLVCFYDAQSFVREGPAQMSLAELEPALQFCNFLVYGYAGIDAVTYKIKSLDPSLTNDR--QH  91
D.m. DS47        MIIKALAI-VSLCLASIQASKVGAPQLPKKHLVCYYDSASFVKEGLGKLVIDELEPALQFCDYLVYGYAGIERDSHKAVSLNQQLDLDLGKGL  92
P.r. IDGF        MK-------IILTLAGVLALAVSTPTPAQSKVVCYYDSKSYVRESQARMLPLDLDPALSFCTHLVYGYAGIQPDTYKMVPLNENLDVDRSHAN  86
B.m. IDGF-like   MK-------LFIALVGLLALAKALPAVTHSKVLCYYDSRSYVRESQARMLPLDLDPALSFCTHLLYGYAVIQPDTYKLVSLNENLDIDRTHDN  86
                                          *  **                     **  *   ****

                 α2'→    ←β3→               ←αa→   ←  α3    →  ←β4→

DRW  IDGF        YRHITDLKRKYPGLRVLLSVGGNNDVTGEGS--EKNLKYRTLLESVE-SRLAFVNSAHDLVKNYGFDGLDLSWEFPENKPKKIRNAVSSWFSK  178
D.m. IDGF2       FSEVTSLKRKYPHLKVLLSVGGDHDIDP-----DHPNKYIDLLEGEKVRQIGFIRSAYELVKTYGFDGLDLAYQFPKNKPRKVHGDLGLAWKS  171
D.m. IDGF1       YKDITALRQKFPQLKILLSVGGDRDVDE-----AHPNKYVELLEANRTAQQNFIDSSMILLKRNGFDGLDLAFQLPRNKPRKVHGSLGSYWKS  169
D.s. IDGF1       YKDITALRQKFPQLKILLSVGGDRDVDE-----AHPNKYVELLEANRTAQQNFIDSSMILLKRNGFDGLDLAFQLPRNKPRKVHGSLGTYWKS  169
D.y. IDGF1       YKEITALRQKFPQLKILLSVGGDRDVDE-----AHPNKYVELLEANRTFQQNFIDSSMILVKRNGFDGLDLAFQLPRNKPRKVHGSLGTYWKS  169
D.m. IDGF3       LAQITSMKERYPHIKFLLSVGGDADTN------EGNQYIKLLESGQQGHRRFIESARDLVRRYNFDGLDLALQLPRNKPRKVHGDVGSAWKS  172
D.s. IDGF3       LAQYTSMKERYPHIKFLLSVGGDADTN------EGNQYIKLLESGQQGHRRFIESARDLVRRYNFDGLDLALQLPRNKPRKVHGDVGSAWKS  172
D.y. IDGF3       LAQVSSLKERYPHIKFLLSVGGDADTN------EGNQYIKLLESGQQGHRRFIESARDLVRRYNFDGLDLALQLPRNKPRKVHGDVGSAWKS  172
D.m. IDGF4       PRQVTGLKRKYPALKVLLSVGGDKDTVDPEN-----NKYLTLLESSNARIPPINSAH SLVKTYGFDGLDLGWQFPKNKPKKVHGSIGKFWKG  175
D.m. IDGF5       YRHITALRKKYPHVRFLLSVGGDRDVNSEGV--ADSDKYLRLLEQSE-HRKSFQASVLAELNNNGFDGIDLAWQFPKNRPKLQQGVFKRVWGS  188
D.m. DS47        YRTVTRLKRKYPNVKILLSVGGDKDIELDKDAKELPNKYLELLESPT-GRTRFVNTVYSLVKTYGFDGLDVAWQFPKNKPKKVHSGIGNLWKG  184
P.r. IDGF        YRAITNFKSKYPGLKVLLSVGGDADK-------EEEQKYNLLLESSPQ-ARTAFVNSGMLLAEQNGFDGIDLAWQFPARIKPKKVRSTWGSIWHG  171
B.m. IDGF-like   YRAITSLKAKYPGLTVLLSVGGDADT-------EEPEKYNLLLLESQQ-ARTAFINSGVLLAEQYGFDGIDLAWQFPRVKPKKIRSTWGSLWHG  171
                      *  ******         *  ***      *             *  **   *

                    ←  σ4    →  ←β5→  αb→ α5→  β6→

DRW  IDGF        IKHKIVGESVVDEKAEEHKEQFTALVRELKNVFRHD-GLLLTVSVLPNVNSSVYFDPRQLAPNIDFATLEAFDYRTPQRNPKELDYVAPLYEL  270
D.m. IDGF2       IKKLFTGDFIVDPHAALHKEQFTALVRDVKDSLRAD-GFLLSLTVLPNVNSTWYFDIPALNGLVDFVNLATFDFLTPARNPEEADYSAPIYHP  263
D.m. IDGF1       FKKLFTGDFVVDPQAEQHKSQFTDLVGNIKNAFRSA-NLMLSLTVLPNVNSTWYFDVPKLHPQFDYINLAAFDFLTPLRNPEEADFTAPIFFQ  261
D.s. IDGF1       FKKLFTGDFVVDPLAEQHKSQFTDLVGNIKNAFRSA-NLMLSLTVLPNVNSTWYFDVPKLHPQFDYINLAAFDFLTPLRNPEEADYTAPIFFQ  261
D.y. IDGF1       FKKLFTGDFVVDPLAEQHKSQFTDLVGNLKNAFRSA-NLMLSLTVLPNVNSTWYFDVPKLHPQFEYINLAAFDFLTPVRNPEEADFTAPIFFQ  261
D.m. IDGF3       FKKFFTGDFIVDTESETHKGQVTALIKDLSAALKQN-DLLLSLTVLPNVNSSWWYDAPSIAPSLDFINLGTFDFLTPQRNPEEADFSAPTYEA  264
D.s. IDGF3       FKKFFTGDYIVDTESETHKGQVTALIKDLSAALKQN-DLLLSLTVLPNVNSSWWYDAPSIAPSLDFINLGTFDFLTPQRNPEEADFSAPTYEA  264
D.y. IDGF3       FKKFFTGDFIVDTDSETHKGQVTALIKDLSAALKQN-DLLLSLTVLPNVNSSWWYDAPSIAPSLDFINLGTFDFLTPQRNPEEADFSAPTYEA  264
D.m. IDGF4       FKKIFSGDHLVDEKAEEHKEAFTALVRELKNAFRPD-GYILGLSVLPNVNSSLFFDVPAIINNLDYVNLHTYDFQTPERNNEVADFPAPIYEL  267
D.m. IDGF5       LR-GWFSSSSVDEKSEEHKEQFATLLEELQSDLRRG-GQLLTVSMLPHVSAELFIDVPKVLSNVDFVNLGTYDFQTPERDPKVADLPTPLYAM  271
D.m. DS47        FKKVFSGDSIVDEKSEEHKEQFTALLRDVKNAFRPD-NLLLSTTVLPNVNSSLFYDIPAVVNYLDFVNLGTFDFFTPQRNPEIADYAAPIYEL  276
P.r. IDGF        IK-KTFPGTTLVDEKAEEHREGYTALVRELKQAMTLK-QMQLSISVLPNVNSSIYYDVPAIINLVDIVNLHAYDYYTAARNPKEADYSAPTYKP  262
B.m. IDGF-like   IK-KTFPGTTPVDEKESEHREGFTALVRELKQALIHKPKMQLGVTVLPNVNSTIYHDVPAIINLVDYVNVGAYDYYTPTRNNKEADYTAPIYTP  263
                         **   **                      *  **            **   *

                   ← α6   →   ←β7→←βa→           βb→←αc→

DRW  IDGF        LD---RKVDENADYQVRYWL--GGGLPANKLILG--IPTYGRAWKLNDDSGLTGVPPLLTD-GAADPGPYSN-EAGLLSYPEICSKIATP-KE  353
D.m. IDGF2       DGSKDRLAHLNADVQVEYWL--SQGFPSNKINLG--VATYGNAWKLTKDSGLEGVPVVPETSGPAPEGFQSQ-KPGLLSYAEICGKLSNPQNQ  351
D.m. IDGF1       DE-ENRLPHLNVEFQINYWL--QNHCPGQKLNLG--IASYGRAWKLSKGSGLSGAPIVHETCGVAPGGIQIQSAEGLLSWPEICSKLSQNASA  349
D.s. IDGF1       DE-QNRLPHLNVEFQVNYWL--QNHCPGQKLNLG--IASYGRAWKLSKESGLSGAPIVHETCGAAPGGIQIQSAEGLLSWPEICSKLSQNASA  349
D.y. IDGF1       DE-QNRLPHLNVEFQVNYWL--QNNCPGQKLNLG--IASYGRAWKLSKGSGLSGAPIVQETCGAAPGGIQIQSADGLLSWPEICSKLSQNASA  349
D.m. IDGF3       VG-QNRLGHYNLNFQMEHWL--IATYGRSWMKSKDSGDSGMPVVPSTQGFAPAGPQSK-QEGLLNWAEICSLMPNPSNT  351
D.s. IDGF3       VG-QNRLGHYNLNFQMEHWL--LQRVPANKINIG--IATYGRTWKMSKDSGDSGMPVVSSTQGPAPAGPQSK-KEGLLNWAEICSLMPNPSNS  351
D.y. IDGF3       VG-QNRLGHYNLNFQTEHWL--LQRVPANKINIG--IATYGRTWKMTKDSGDSGMPVVPSTQGPAPAGPQSK-KEGLLNWAEICSLMPNPGNT  351
D.m. IDGF4       NE---RNPEFNVNYQVKYWT--GNRAPAAKINVG--IATYGRAWKLTKDSGLTGLPPVAEADGVAPAGTQTQ-IPGLLSWPEVCAKLPNPANQ  351
D.m. IDGF5       YD---RDPSHNVQYQVQYWMMNQTSEISVHKLHVG--VTSYGRAWNMTRNSGITGYPPIPAANGAAPPGRQTV-TPGLLSWPEICDLLQ---QQ  355
D.m. DS47        SE---RNPEFNVAAQVKYWL--RNNCPASKINVG--VATYGRPWKLTDDSGDTGVPPVKDVKDEAPVGGNTQ-VPGIYSWPEVCALLPNQNNA  361
P.r. IDGF        QN---RNELLNVDAAISYWL--TGGAPSSKIVVG--IATYARTWKLDSDSEISGVPPLHTD-GPGEAGPYTK-TEGLLSYPEVCAKLINP-NH  344
B.m. IDGF-like   QN---RNPLQNADAAVTYWL--TSGAPSQKIVLSXRLRSYLETGX---DSEIAGVPPIHTD-GPGEAGPYVK-TEGLLSYPEVCGKLINP-NQ  344
                       *  *                     **         **   ** *     * *

                  ←βc→     ←βd→ ←βe→   ←  α7   →  ←β8→      ← α8 →

DRW  IDGF        IQAGYLGKLRKTNDPTKRYGSAYRLPDSNGENGIWVGFEDPDTVGNKAAYSKAKGLGGIAIVDLTLDDFRGTCS-QDHFPLLRAAKFRL--  442
D.m. IDGF2       FLKGNESPLRRVSDPTKRFGGIAYRPVDGQITEGIWVSYDDPDSASNKAAYARVKNLGGVALFDLSYDDFRGQCS-GDKYPILRAIKYRL--  440
D.m. IDGF1       QYRGELAPLRKVTDLTQKYGNYALRPADDNGDFGVWLSFDDPDFAGLKAVYAKGKGLGGIALFDLSYDDFRGLCT-GQKYPILRSIKYFMG-  439
D.s. IDGF1       QYRGELAPLRKVTDLTQKYGNYALRPADENGDFGVWLSFDDPDFAGIKAVYAKSKGLGGIALFDLSYDDFRGLCT-GQKYPIVRSIKYFMG-  439
D.y. IDGF1       QYRGEMAPLRKVTDLTQKYGNYALRPADDNGDFGVWLSFDDPDFAGIKAAYAKGKGLGGVAIFDLSYDDFRGLCT-GQKFPIVRSIKYFMG-  439
D.m. IDGF3       NARGPNAPVKRVVDPTKRYGSYAFRAADENGDHGLWISYDDPDSASSKAMYARARNLGGVALFDLTQDDFRGQCT-NDRFPMLRAIKYRLL-  441
D.s. IDGF3       NARGPNAPVKRVVDPTKRYGSYAFRAADENGDHGLWISYDDPDSASSKAMYARARNLGGVALFDLTQDDFRGQCT-GDRFPMLRAIKYRLL-  441
D.y. IDGF3       NARGPSAPVKRVLDPTKRYGSYAFRAADENGDHGLWISYDDPDSASSKAMFARVRNLGGVALFDLTQDDFRGQCT-NDRFPMLRAIKYRLL-  441
D.m. IDGF4       HLKGADGPLRKVGDPTKRFGSYAYPSADDSGENGVWVGYEDPDTAAIKAEYVKREGLGGIAVVDLSFDDFRGGCT-GHDKFPILRQVKSKL-  442
D.m. IDGF5       PQDREVPHLRKVGDPTKRFGIYAYRAADDQGENGLWVGYEDPLTAAIKAGFVHAQGLGGVAFHDLSMDDFRGQCA-GEKFPILRSIKFKL--  444
D.m. DS47        YLKGANAPLIKVQDPAKRFGSYAYRAADKKGDNGIWVSFEDPDTAADKAGYVRTENLGGVALFDLSYDDFRGLCT-NEKYPILRAIKYRLTN  452
P.r. IDGF        -QKGMRPHLRKVTDPSKRFGTYAFRLPDDNGDPGLWVSYEDPDTAGQKASYVKSKNLGGVSIVDLSMDDFRGLCT-GDKYPILRAAKYRL--  433
B.m. IDGF-like   -QKGMRPHLRKVTDPSKRFGTYAFRLPDDNGEGGIWVSYEDPDTAGQKAAYVKSKNLGGVAIVDLSLDDFRGLCT-GDKYPILRAAKYRL--  433
                  *  *  *          **      **     ***   *     ** ***    *    * *
```
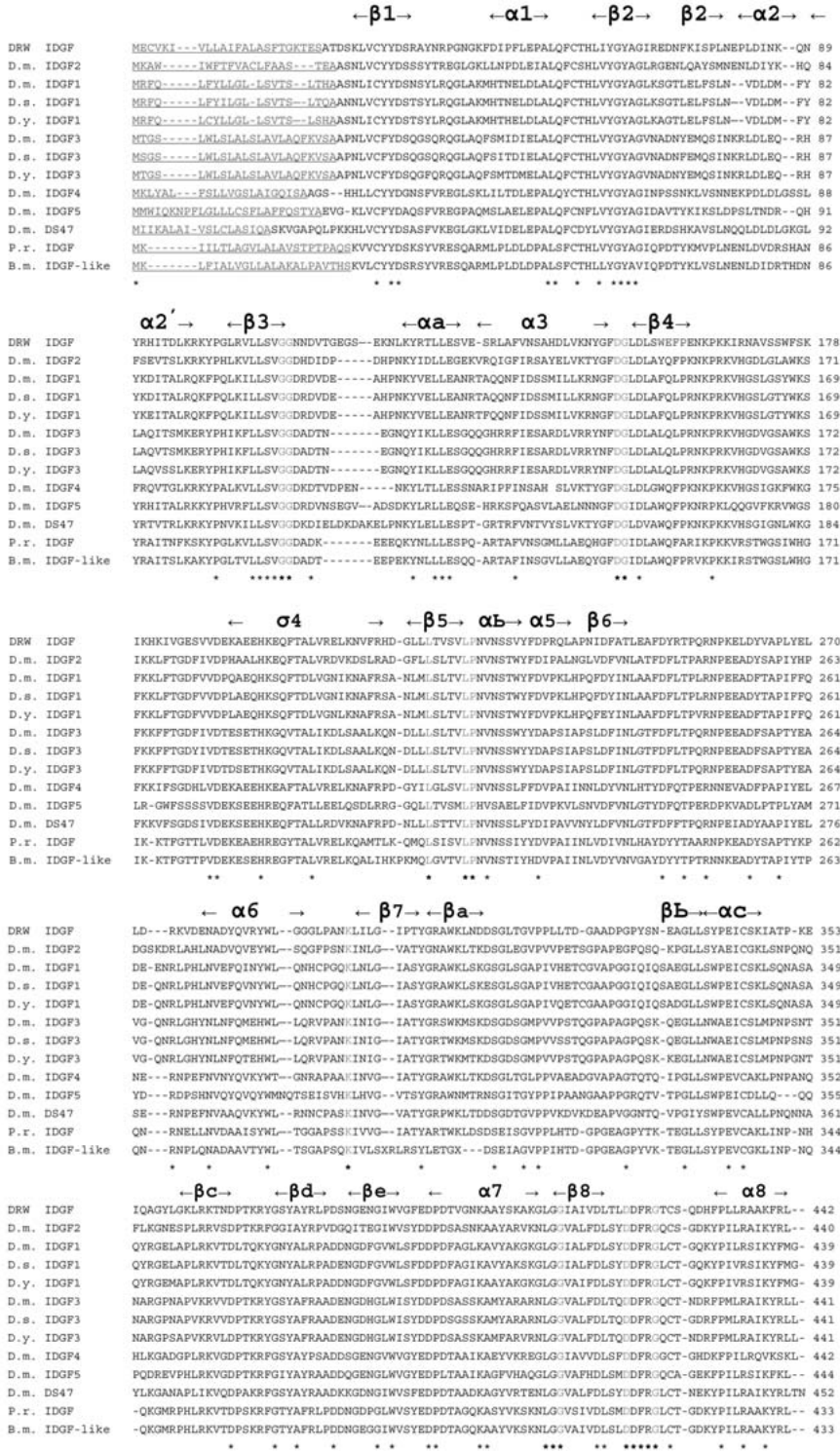
Fig. 2. Multiple sequence alignment of IDGF-DRW and all IDGF family members. *Diaprepes abbreviatus*. DRW, *Drosophila melanogaster*, D.m., *Drosophila simulans*, D.s., *Drosophila yakuba*, D.y., *Pieris rapae* P.r., and *Bombyx mori*, B.m. The signal peptide is underlined. The red characters show the conserved residues, * in bold are predicted to be necessary for barrel formation in IDGF's. The strictly conserved residues in all IDGF are shown by asterisk. IDGF-DRW secondary structure elements are depicted on top as assigned IDGF2 after Varela et al. (2002).
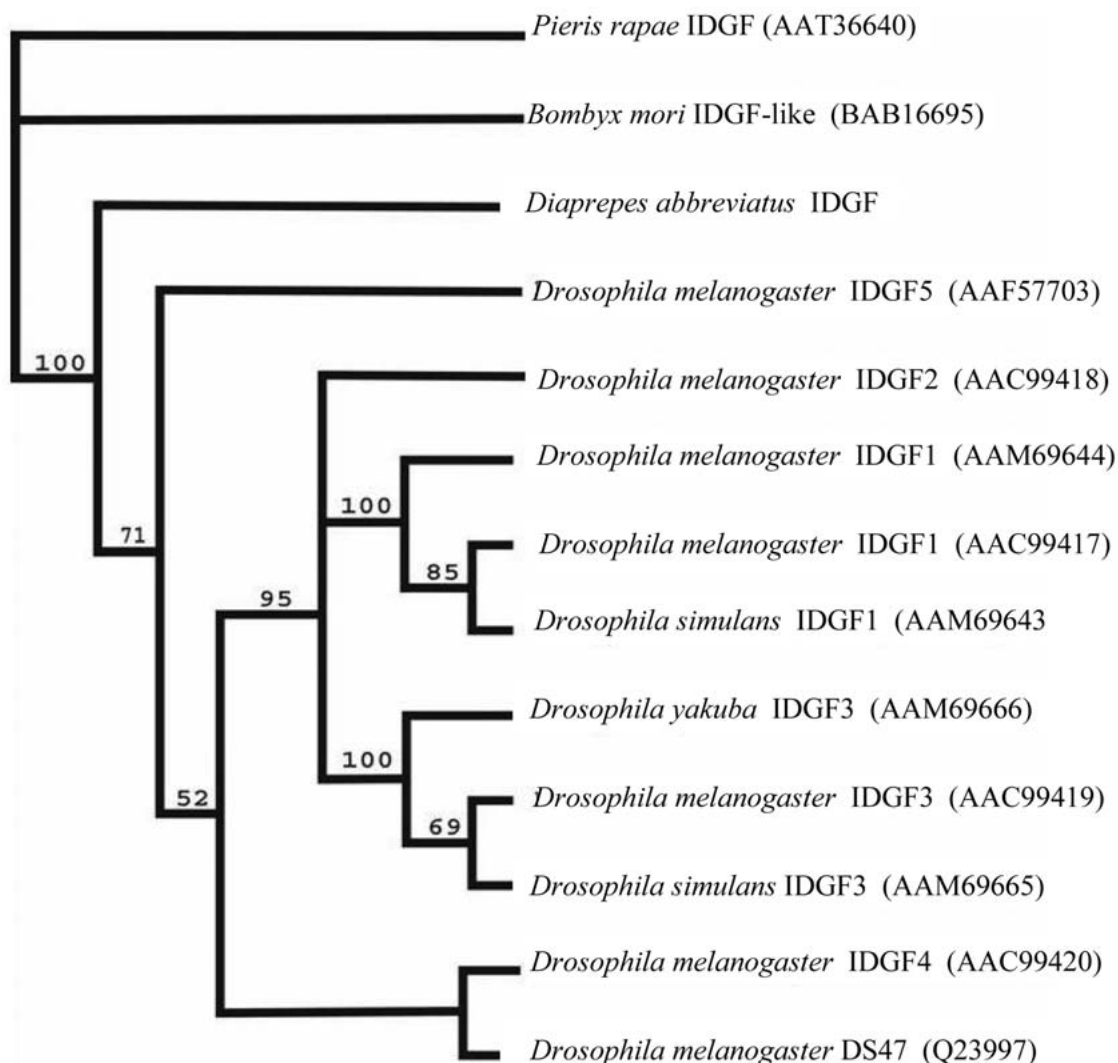
Fig. 3 Phylogenetic tree of the IDGF family. The accession number for sequences is shown inside parentheses. Phylogenetic tree was generated by bootstrap neighbor joining, 1000×, PAUP 4.0, unrooted (Swofford 2002). (*Diaprepes abbreviatus*, IDGF- DRW accession no. <u>AAV68692.1</u>).

ily members (Fig.2), in human chitotriosidase, and in mammalian chitinase-like proteins with no chitinase activity, but are not found in family 18 glycosyl hydrolases from plants or bacteria (Varela et al. 2002). The putative binding site of IDGF2 (Varela et al. 2002) is composed of the ten residues, Tyr_{65}, Asp_{111}, His_{112}, Gln_{159}, Phe_{160}, Lys_{162}, Asp_{250}, Tyr_{303}, Phe_{416}, and Tyr_{420}. These residues are not strictly conserved in all known IDGF family members, however, the predicted binding site of IDGF-DRW contains a subset of these residues (Tyr_{65}, Phe_{160}, Asp_{250}, and Tyr_{303}) and the remaining residues are replaced by the same or complementary category of amino acids.

DISCUSSION

Overall Structure of IDGF-DRW

The structure of IDGF-DRW was modeled against *Drosophila* IDGF2 (Varela et al. 2002). The formation of the clade of IDGF members from *Drosophila* species separate from the other insects, implies an evolutionary divergence of all the *Drosophila* sequences after the evolutionary divergence of the insects for which IDGF sequence data is presented. This would suggest a very recent expansion of the IDGF gene family. Further examination is needed before statements
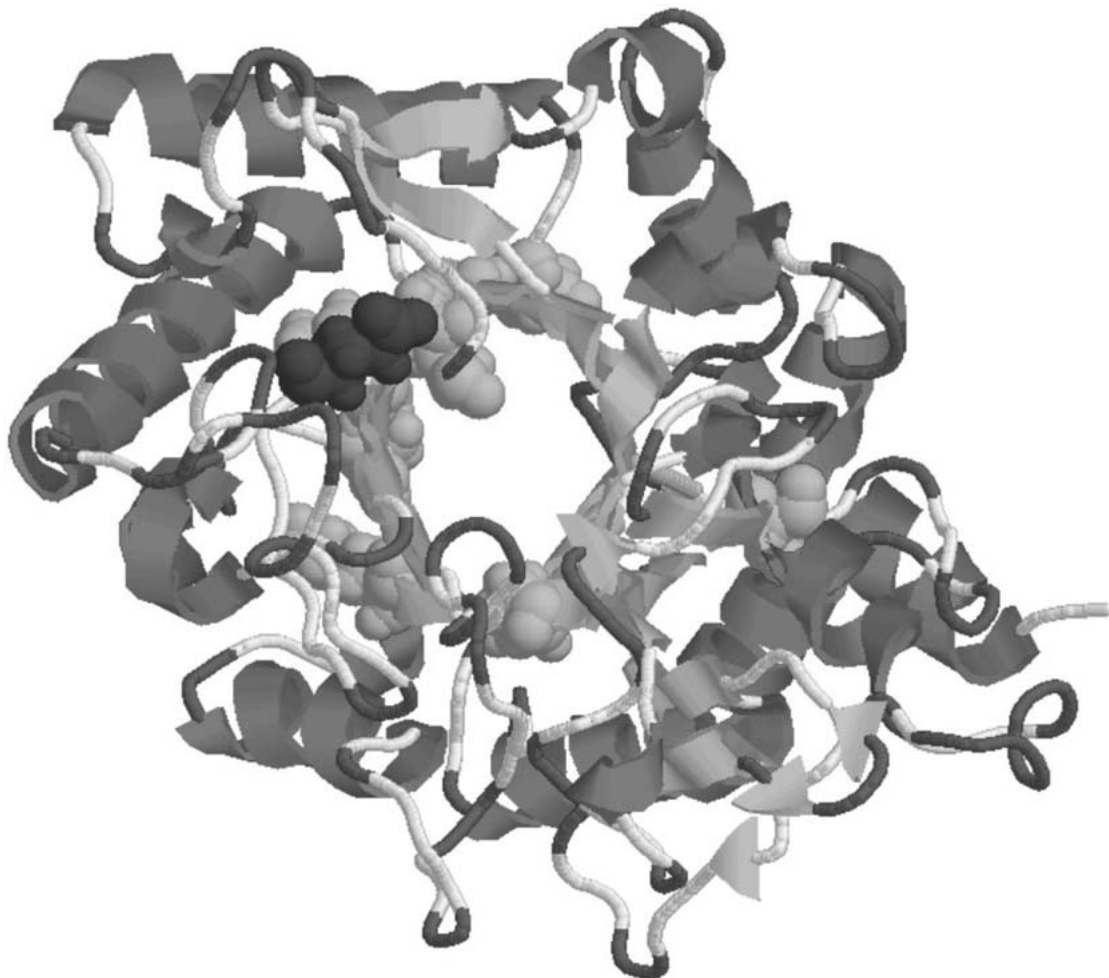
Fig. 4. Basic view of IDGF-DRW protein 3-D structure. The beta barrel motif shown in yellow in center. Structure was predicted with ROBETTA, a full-chain protein structure prediction server. View of conserved residues (in green space-fill) are apparently essential to maintain the barrel folding as predicted by interface alanine scanning (Van Scheltinga et al. 1996). Residues are Gly_{109}, Gly_{110}, Asp_{152}, Gly_{153}, Leu_{218}, Asp_{242}, Lys_{295}, Gly_{412}, and Asp_{421}. The residue at position 159 is Glu (in brown space-fill), which is replaced by Gln in other known IDGF proteins. Structure key: alpha helix (pink), beta strand (yellow), turn (blue) and other (grey).

of evolutionary divergence within the remaining insect species, DRW, *P. rapae*, and *B. mori* can be ascertained.

The structure of IDGF-DRW displays the characteristic fold of the family 18 glycosyl hydrolases. An insertion (Gly_{304} to Phe_{392}) in the beta barrel motif between strand β_7 and helix α_7 forms an additional $\alpha+\beta$ domain similar to that of *Serratia marcescens* chitinases A and B (Perrakis et al. 1994; Van Aalten et al. 2000). The feature is common to other IDGF proteins and to most chitinase-like proteins described to date, although the insertion length varies (Varela et al. 2002). A few conserved residues, present in family 18 glycosyl hydrolases and other IDGF proteins, also were found in IDGF-DRW and are apparently essential

to maintain the barrel folding residues shown in red (Fig. 2). Characteristically in IDGF2, there are three *cis* peptide bonds—Gly_{64}-Tyr_{65}, Pro_{319}-Val_{320}, and Phe_{416}-Asp_{417}. The first and third are conserved in all family 18 members and appear to be necessary for correct folding of the barrel. The second is located in the inserted $\alpha+\beta$ domain and is not conserved in *S. marcescens* chitinase A or B (Perrakis et al. 1994; Varela et al. 2002). The corresponding peptide bonds in IDGF-DRW are Gly_{64}-Tyr_{65}, Pro_{319}-$Pro_{320,}$ and Val_{416}-Asp_{417}. A conserved amino acid change in the third peptide bond, $Phe_{416} \rightarrow Val_{416}$, may preserve its function as valine is an aliphatic-hydrophobic amino acid and capable of binding substrate. Additionally, the aromatic residues (Tyr_{65} and Phe_{416}) involved in the

two conserved *cis* peptide bonds have been reported to be important for binding of substrates in all glycosyl hydrolases with triosephosphate isomerase barrel folds (Jabs et al. 1999). Thus the changes in amino acids were predicted to not affect the binding site structure.

The cooperation between IDGFs and insulin in promoting cell proliferation makes these interactions a possible genetic target for disruption, especially in a long-lived insect like DRW which has a subterranean larval stage. Disruption of critical developmental pathways, such as reduction of the signaling of the insulin receptor, may provide a means for the development of novel management methods to reduce DRW growth and/or survival.

Our work has identified a new member of growth-promoting glycoprotein IDGF protein family, IDGF-DRW. The *idgf-DRW* sequence was amplified from both cultured and field caught adult DRW. Further investigations are needed to examine the expression of these proteins during development to identify when they are maximally expressed, and their interactions with downstream effector signals in developmental pathways. Identification of the genes and proteins functioning in developmental pathways increases our understanding and aids the development of essential tools to conduct future experiments on the functions and interactions of growth-factor like receptors in the DRW.

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A NEW SPECIES OF *MOLCHINA* AMYOT AND SERVILLE, 1843, FROM ECUADOR (HETEROPTERA: COREIDAE: SPARTOCERINI)

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ABSTRACT

A new species, *Molchina xantha,* is described from Ecuador. Comments and new distribution data are provided for *M. compressicornis* (Fabricius), *M. hopei* (Perty), and *M. linnei* Stål. *Molchina molitor* Breddin is synonymized with *Molchina granulata* Stål. A key to the known species of *Molchina* is provided, along with illustrations of the habitus of *M. xantha* and the pronotum of all known species.

Key Words: Insecta, Heteroptera, Coreidae, Spartocerini, *Molchina*, new species, Ecuador

RESUMEN

Una nueva especie, *Molchina xantha*, recolectada en Ecuador es descrita. Comentarios y nuevos datos distribucionales son incluidos para *M. compressicornis* (Fabricius), *M. hopei* (Perty) y *M. linnei* Stal; *Molchina molitor* Breddin es sinonimizado con *Molchina granulata* Stal. Una clave para reconocer las especies de *Molchina* es incluida, asi como ilustraciones del pronoto de cada una de las especies conocidas y de *M. xantha* en vista dorsal.

Translation provided by the author.

Amyot & Serville (1843) proposed the generic name *Molchina* to include *Lygaeus compressicornis* Fabricius, and they originally placed this genus in the Mictides. They were followed by Stål (1870) (Mictina), but three years later, he placed *Molchina* in the Spartoceraria. Both Bergroth (1913) and Blöte (1936) included *Molchina* in Spartoceridae. O'Shea (1979) redescribed the genus and discussed its relationship with Nematopodini and Acanthocerini, but ultimately left *Molchina* as unplaced until more American coreid genera could be studied.

In this contribution, *Molchina* is once again included in Spartocerini based on the subquadrate head, the prominent antenniferous tubercles, situated close together and projecting distinctly anteriorly of tylus, the postocular tubercle forming a smooth curve with the eye, the pronotum steeply declivent, with anterolateral margins, especially the posterior third, nodulose, and the mesosternum without a median carina. The pronotum is illustrated in all known species in Figs. 1-5.

Previously, six species of *Molchina* were known: *M. compressicornis*, *M. granulata* Stål, *M. hopei* (Perty), *M. linnei* Stål, *M. molitor* Breddin, and *M. obtusidens* Blöte. In this contribution, one new species, *M. xantha,* collected in Ecuador is described; *M. molitor* is synonymized with *M. granulata*; new distributional records are given; and a key to the known species is included (except for *M. obtusidens*).

All measurements are given in millimeters. Acronyms used are: AMNH (American Museum of Natural History, New York); CAS (California Academy of Sciences, Golden Gate Park, San Francisco, California); CMNH (Carnegie Museum of Natural History, Pittsburgh, Pa.); CUIC (Cornell University Insect Collection, Ithaca, New York); DEI (Deutsches Entomologisches Institut, Eberswalde, Germany); INPA (Instituto de Pesquizas da Amazonia, Manaus, Brazil); MNKM (Museo de Historia Natural, Noel Kempff Mercado, Santa Cruz, Bolivia); NRE (Naturhistoriska Riksmuseet, Stockholm, Sweden); PUCE (Pontificia Universidad Católica del Ecuador); UMSM (Universidad Nacional Mayor de San Marcos, Museo de Historia Natural, Lima, Peru); UNAM (Instituto de Biología, Universidad Nacional Autónoma de México); UNCB (Universidad Nacional de Colombia, Instituto de Investigaciones en Recursos Biológicos, Alexander von Humboldt, Santa Fé de Bogotá); NMNH (National Museum of Natural History, Smithsonian Institution, Washington, D.C.); and USUL (Utah State University, Logan, Utah).

Molchina compressicornis (Fabricius)

Lygaeus compressicornis Fabricius 1794: 138

Diagnosis. Endocorium with black discoidal spot located near middle third; posterior lobe of pronotal disk with black discoidal spots, as well as greenish or bluish tints; humeral angles with short, stout projection (Fig. 3).

Distribution. This species was originally described from French Guiana (Cayenne). Dallas (1852) reported this species from Brazil (Para),
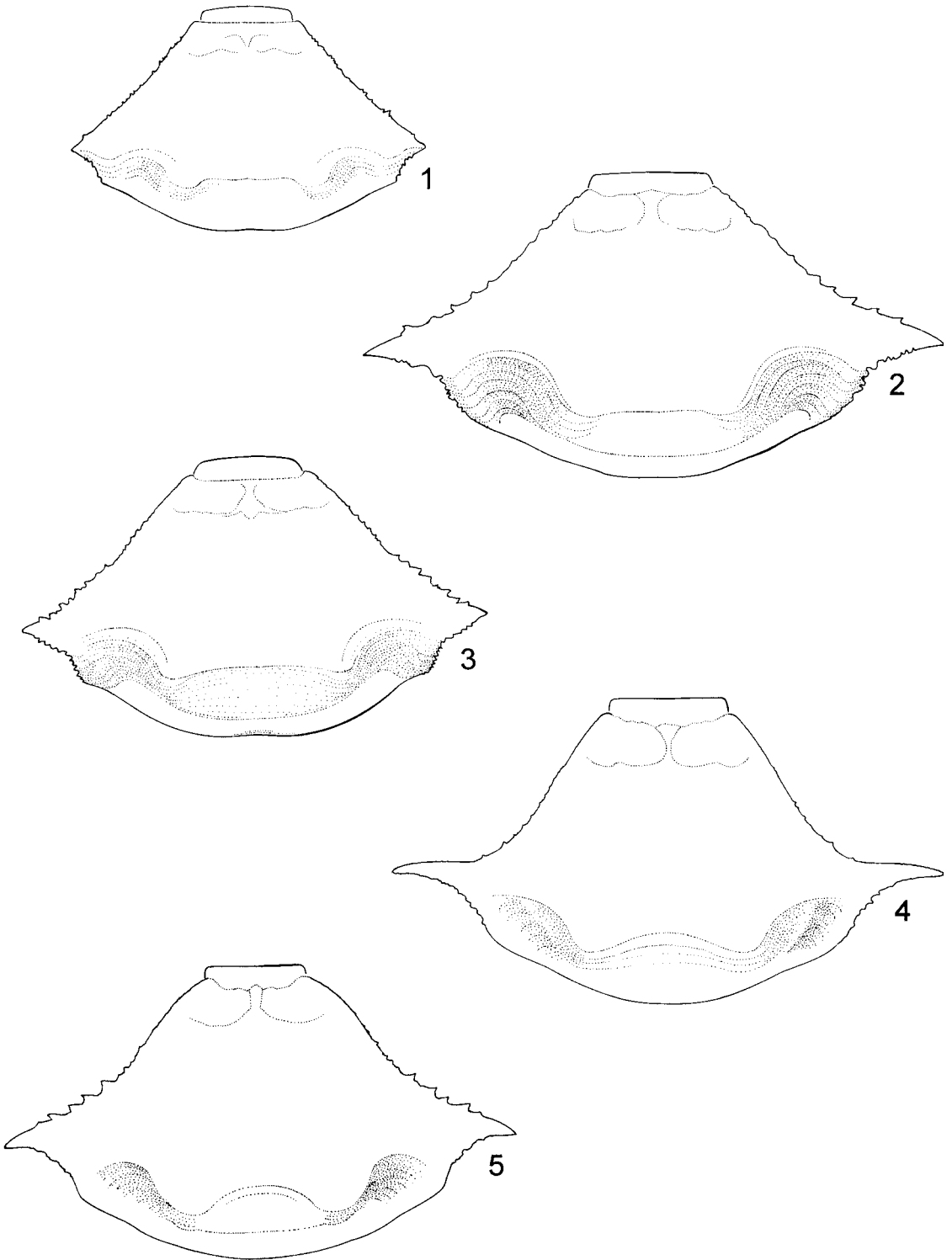
Figs. 1-5. Pronotum of *Molchina* spp. 1. *M. linnei* Stål. 2. *M. granulata* Stål. 3. *M. compressicornis* (Fabricius). 4. *M. hopei* (Perty). 5. *M. xantha* Brailovsky.

Stål (1870) from Suriname and northern Brazil, and Blöte (1936) from Brazil (Corumba, Matto Grosso) and Peru (Marcapata).

Material examined. 1 male, BRAZIL, Santarem, VII-1919, S. M. Hages (CMNH); 1 male, 1 female, BRAZIL, Para, Serra Norte, Campo Cururu, 5-VI-1983, Pimentel (UNAM); 1 male, 1 female, BRAZIL, Para, Rio Xingu, 60 km S Altamira, 8-12-X-1986, P. Spangler and O. Flint (NMNH); 1 male, BRAZIL, Mato Grosso, X-1974, M. Alvarenga (AMNH); 3 males, 2 females, BRAZIL, Rondonia, 62 km S of Ariquemes, vic Fazenda Rancho Grande, 15-22-III-1991 and 6-16-XI-1996, W. J. Hanson (USUL); 1 male, COLOMBIA, Meta, Guatiquia, 430 m, VII-1948, L. Richter (UNCB); 1 female, COLOMBIA, Amazonas, 21-III-1977, R. Restrepo (UNCB). 1 female, ECUADOR, Provincia Napo, 58 km E, Mishualli, 450 m, 28-XII-1987, M. Huybensz (UNAM). 1 female, PERU, Middle Rio Ucayali, II-1926 (AMNH). 1 female, VENEZUELA, Bolivar, Rio Guaniano, 25-28-V-1979 (UNAM); 1 male, VENEZUELA, La Caja, km 105 S de El Dorado, 3-VIII-1961 (UNAM).

Molchina granulata Stål

Molchina granulata Stål 1870: 131

Molchina molitor Breddin 1898: 151-153. **Nov. Syn.**

Diagnosis. Endocorium lacking black to dark reddish brown discoidal spot; corium and clavus violaceus to purple without greenish or bluish tints; humeral angles acute (Fig. 2); antennal segment I black, II and III black with basal third yellow, and IV black with basal joint yellow.

Distribution. This species was originally described from northern Brazil. Breddin (1898) cited this species from Bolivia (without data).

Material examined. *Mochina molitor* Breddin (1898). Holotype female: Bolivia (without data) (DEI). 1 female, BOLIVIA, Departamento Cochabamba, Provincia Chapare, Palmar, 1000 m, I-1951, Steinbach (CMNH); 1 male, 1 female, BOLIVIA, Departamento Santa Cruz, Provincia Velasco, Parque Noel Kempff Mercado, 4-IV-1992, J. Justiniera (MNKM); 1 female, BOLIVIA, Departamento Santa Cruz, Provincia Andres Ibáñez, Urubo, 7-IX-1991, L. Baco (MNKM). 1 male, PERU, El Campanario, Colonia Berene, 11-VI-1920 (CUIC).

Molchina hopei (Perty)

Pachylis hopei Perty 1830: 171

Diagnosis. Endocorium with black discoidal spot near middle third; posterior lobe of pronotal disk with greenish or bluish tints, and with or without black discoidal spots; humeral angles pro-

duced into elongate, slender spines, directed laterally and with apex directed backward (Fig. 4).

Distribution. This species was originally described from the "Amazons" (without data). Signoret (1861) reported this species from Peru (Jurimaguas), Stål (1870) from northern Brazil, and Blöte (1936) from French Guiana (Cayenne), Colombia (Umbria, and Guinea River), and Peru (Marcapata).

Material examined. 1 male, BRAZIL, Amazonas, Manaus, X-1945 (CMNH); 1 male, 1 female, BRAZIL, Amazonas, Manaus, Reserva Ducke, 4-XI-1959, V-1968, E. V. Silva and A. Faustino (INPA). 1 male, BRITISH GUIANA, Arakaka (whitout date) (CMNH). 1 male, COLOMBIA, Meta, VIII-1935, Restrepo (UNCB). 1 male, PERU, Madre de Dios, Boca Rio La Torre, 300 m, 19-II-1982, G. Lamas (UMSM); 2 males, 1 female, PERU, Loreto, Iquitos, I-1977, 8-12-VIII-1985, W. Kinzey, and G. Burrows (AMNH, UNAM); 1 female, PERU, Distrito Putamayo, road La Chorrera-La Sombra, 31-VIII-1930 (AMNH); 1 male, PERU, Rio Santiago, 17-VI-1924 (AMNH); 1 female, PERU, Loreto, Rio Nanay Mishna, Estación de Biología Callicebus, 150 m, 12-I-1980, G. Lamas (UMSM).

Molchina linnei Stål

Molchina linnei Stål 1859: 451

Diagnosis. Endocorium with black to reddish brown discoidal spot; posterior lobe of pronotal disk dark to pale reddish brown, lacking black discoidal spots, and green or blue tints; humeral angles with small lateral spine (Fig. 1).

Distribution. This species was described by Stål from northern Brazil.

Material examined. 2 males, 2 females, BRAZIL, Chapada, V-1919, XII,1919 (CMNH); 1 female, BRAZIL, Mato Grosso, Corumba (without date) (AMNH); 1 female, BRAZIL, Mato Grosso, Cerrado, II-1968, B. E. Freeman (UNAM); 1 female, BRAZIL, Mato Grosso, 11-20-XI-1961, B. Malkin (CAS); 4 males, 2 females, BRAZIL, Mato Grosso, Barra do Tapirape, 26-XII-1962, 9-I-1963, B. Malkin (CAS, UNAM).

Molchina obtusidens Blöte

Molchina obtusidens Blöte 1936: 24-25

Blöte (1936) based the original description on one male. He indicated that *M. obtusidens* was similar to *M. compressicornis* and *M. hopei*. I was unable to obtain any material of this species for study and cannot provide a diagnosis beyond Blöte's original description.

Distribution. This species was described from Panama (Chiriqui). No other records are known.

Molchina xantha, **NEW SPECIES**
Figs. 5-6

Description. Holotype male. Dorsal coloration: head dark reddish brown with tylus and antenniferous tubercles shiny orange; antennal segment I shiny orange with apical joint dark reddish brown, segment II with basal half shiny yellowish orange, and apical half dark reddish brown, III shiny yellowish orange with apical third dark reddish brown, and IV dark reddish brown with basal joint dark orange. Anterior lobe of pronotal disk dark reddish brown; posterior lobe orange castaneus; humeral angles, including spine, posterolateral and posterior borders black to dark reddish brown. Scutellum dark reddish with lateral borders black and apex yellow; clavus, corium, and connexivum shiny yellowish orange; hemelytral membrane dark amber with basal angle almost black; dorsal abdominal segments
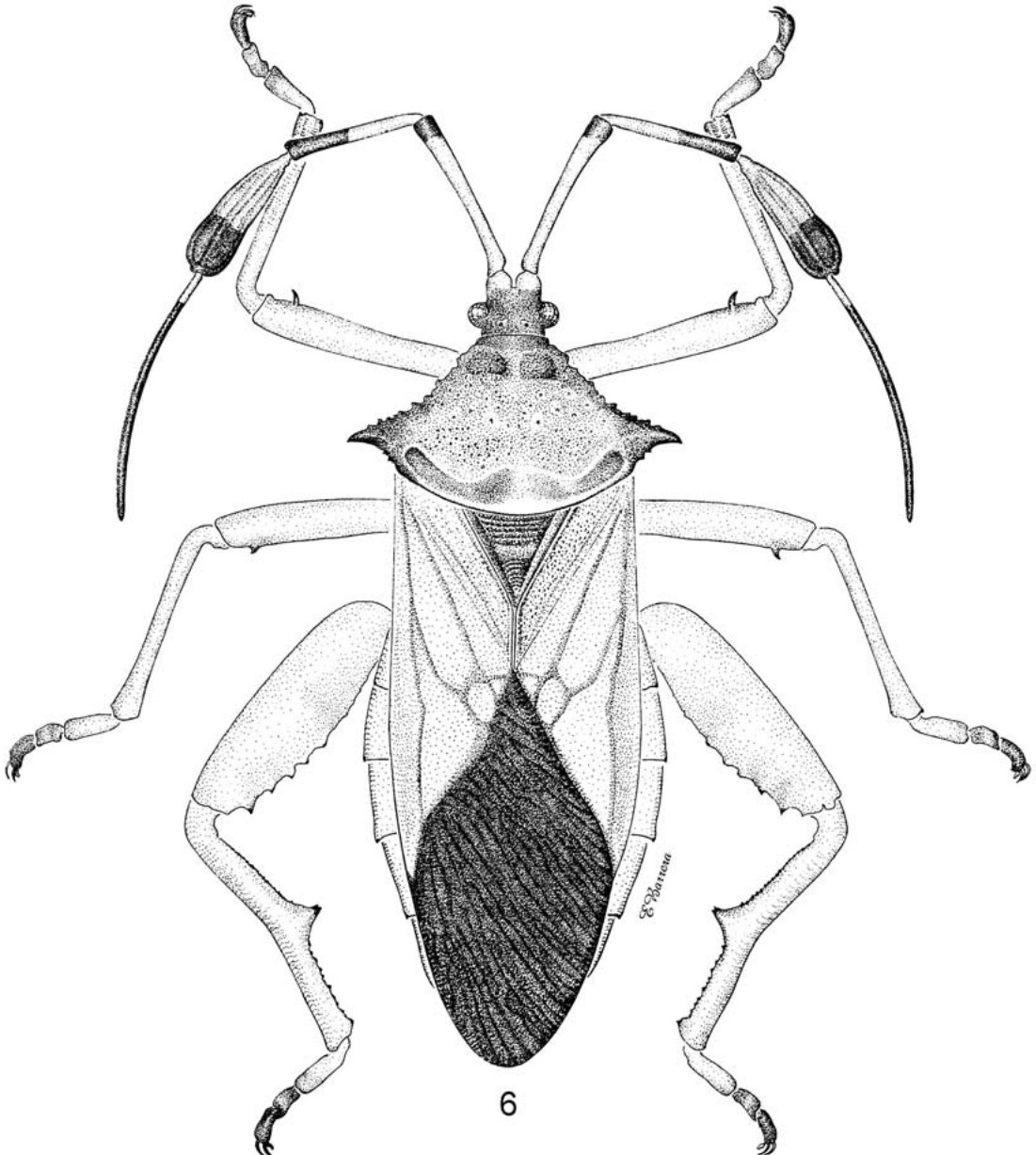
Fig. 6. Dorsal view of *Molchina xantha* Brailovsky

dark reddish except segment VII black. Ventral coloration: head black; rostral segments I to III shiny orange, and IV black; prothorax dark reddish brown, with posterior margin dark orange castaneus; mesothorax dark reddish brown with anterior surface shiny orange castaneus and with single black discoidal spot located on outer third of anterior border; mesothorax dark reddish brown with acetabulae dark orange castaneus; anterior and posterior lobe of metathoracic peritreme black, adjacent areas dark reddish brown with black margin; coxae, trochanters, femora, tibiae, and basal segment of each tarsus shiny orange; middle and distal segments of each tarsus dark reddish brown; abdominal sterna and genital capsule dark reddish brown tinged with dark orange castaneus. Head ventrally, thorax, and abdominal sterna covered with thick whitish tomentum. Structure: head subquadrate; antenniferous tubercles prominent, nearly contiguous at the apex, and projecting distinctly anteriorly to the tylus: antennal segment I cylindrical, relatively stouter and curved; segment II cylindrical and slender; segment III dilated on both sides for the whole length; antennal segment IV curved, fusiform, and very long; rostrum reaching the middle third of mesosternum; pronotum steeply declivent; collar distinct; anterolateral margins spinose to nodulose especially at posterior third; frontal angles obtuse; humeral angles produced into short, broad spine, directed laterally and slightly backwardly (Fig. 5); posterior pronotal disk with scattered small tubercles; calli slightly convex; hind trochanter armed with small spine; femora armed with distal spines on ventral surface; hind femur conspicuously incrassate; fore and middle tibiae cylindrical and sulcate; inner margin of hind tibiae spinose and expanded into large and broad spine, located near middle third; abdominal sterna III and IV armed medio-ventrally with large tubercles. Genital capsule: posteroventral edge simple, slightly concave.

Measurements. Male. Head length 1.74; width across eyes 3.19; interocular width 1.76; interocellar width 0.62. Length of antennal segments: I, 6.84; II, 5.09; III, 5.32; IV, 8.96. Pronotum: Total length 6.68; maximum width across anterior lobe 4.80; maximum width across posterior lobe 11.40.

Scutellar length 3.11; width 3.34. Total body length 29.16.

Female. Coloration: similar to male holotype. Dorsal coloration: pronotum entirely black; posterior pronotal lobe with black discoidal spots; scutellum black with apex dark orange; connexival segments VIII and IX shiny orange. Ventral coloration: head (buccula dark orange castaneus), thorax, and abdomen black; anterior and posterior lobe of metathoracic peritreme, and adjacent areas black; coxae shiny orange with reddish brown marks; trochanter, femora, tibiae and tarsi shiny orange; metatarsi with basal segment shiny orange and middle and apical segments shiny reddish brown; gonocoxae I dark reddish brown; paratergite VIII with basal half reddish brown, and apical half shiny orange; paratergite IX shiny orange. Head, pronotum, scutellum, thorax, and abdominal sterna covered with thick whitish tomentum. Structure: hind trochanters without spine; femora armed with distal spines on ventral surface; hind femora incrassate (less than males); fore and middle tibiae cylindrical and sulcate; hind tibiae cylindrical, sulcate and unarmed; abdominal sterna III and IV without tubercles.

Measurements. Female. Head length 1.97; width across eyes 3.34; interocular width 1.90; interocellar width 0.70. Length of antennal segments: I, 6.08; II, 4.25; III, 4.71; IV, 8.05. Pronotum: Total length 7.06; maximum width across anterior lobe 5.77; maximum width across posterior lobe 12.54. Scutellar length 3.64; width 3.82. Total body length 30.10.

Holotype: Male, ECUADOR, Provincia Napo, Rio Hollin, 1100 m, 5-XII-1996, N. Vieira (PUCE).

Paratype: 1 Female, ECUADOR, Zamora, Chinchipe-Zamora, 78°45'22"W-03°49'42"S, 18-II-2000, A. Iglesias (UNAM).

Discussion. This species can be recognized by having antennal segment I (except apical joint which is dark reddish brown), clavus, corium, connexivum, legs, and rostral segments I to III yellowish orange to shiny orange. In all other species each of these structures is black and with or without reddish brown marks.

Etymology. From the Greek *xanthos*, meaning yellow, referring to the yellowish clavus and corium.

KEY TO SPECIES OF THE GENUS *MOLCHINA**

1.   Antennal segment I shiny yellowish orange with apical joint black; clavus, corium,
      connexivum and legs shiny orange; rostral segments I to III shiny orange. . . . . *xantha*, **new species**

1'. Antennal segment I black; clavus, corium, connexivum, and legs black, tinged or not
      with reddish brown; rostral segments I to III black 2

2.   Endocorium with black to dark reddish brown discoidal spot located near middle third 3

2'. Endocorium lacking black to dark reddish brown discoidal spot . . . . *granulata* Stål

3.   Connexival segments III to VII pale shiny orange with basal margin reddish brown;
      posterior lobe of pronotal disk dark to pale reddish without black discoidal spots, and

green or blue iridescence; antennal segment IV relatively stout and shorter than 5.5 mm; humeral angles with small lateral spine (Fig. 1) . . . . *linnei* Stål

3'. Connexival segments III to VII entirely black to dark reddish brown; posterior lobe of pronotal disk black to dark reddish brown, with several black discoidal spots, as well as green to blue iridescence; antennal segment IV elongate, slender, and longer than 8.8 mm; humeral angles with robust or elongate spine (Figs. 3-4) 4

4. Each humeral angle of pronotum with elongate, slender projection, this directed laterally and with the apex directed posteriorly (Fig. 4) . . . . *hopei* (Perty)

4'. Each humeral angle of pronotum with short and stout projection directed laterally (Fig. 3) . . . . *compressicornis* (Fabricius)

Molchina obtusidens Blöte is excluded.

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DESCRIPTION OF A NEW GENUS AND SPECIES OF WEEVIL PARASITOID FROM HONDURAS (DIPTERA: TACHINIDAE)

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ABSTRACT

Lixadmontia franki, **new genus** and **new species**, is described in the Blondeliini (Diptera: Tachinidae). This parasitic fly attacks the larvae of the weevil *Metamasius quadrilineatus* Champion which infests bromeliads in tropical montane cloud forests of Honduras, and it is a potential biological control agent of the bromeliad-eating weevil *Metamasius callizona* (Chevrolat) in Florida. It is most similar to members of the genera *Admontia* and *Lixophaga*. A key to the species of *Admontia* and *Lixadmontia* in North and Central America is given. *Admontia dubia* Curran is placed in synonymy with *Admontia pollinosa* Curran **new synonymy**.

Key Words: *Lixadmontia franki*, *Metamasius quadrilineatus*, *Admontia*, taxonomy, biological control

RESUMEN

Se describe *Lixadmontia franki* nuevo género y nueva especie en la Blondeliini (Diptera: Tachinidae). La mosca parasítica ataca a las larvas del picudo *Metamasius quadrilineatus* Champion que infestan bromeliaceas en bosques nebulosos de Honduras, y es un agente potencial en el control biológico del picudo *Metamasius callizona* (Chevrolat), una plaga de bromeliaceas en Florida. Es más similar a los miembros de los géneros *Admontia* y *Lixophaga*. Se presenta una clave para las especies de *Admontia* y *Lixadmontia* en América del Norte y Central. Se pone *Admontia dubia* Curran en sinonomía con *Admontia pollinosa* Curran **nueva sinonomía.**

Translation provided by the authors.

In the course of searching for parasitoids of a bromeliad-infesting weevil, *Metamasius quadrilineatus* Champion (Coleoptera: Dryophthoridae), an undescribed tachinid fly was found in Honduras, which may prove to be useful as a biological control agent of the Mexican bromeliad weevil, *Metamasius callizona* (Chevrolat), a pest that was accidentally introduced into Florida from the Neotropical region (Frank & Thomas 1994). The fly appeared at first to belong to the genus *Admontia*, especially on characters of the head, but the female fore tarsi, the male genitalia, and other characters of the abdomen more closely resemble members of the genus *Lixophaga*. Females of both genera deposit eggs ready to hatch at the openings of burrowing or boring insect larvae. A few species of *Admontia* have been reared from larvae of crane flies (Diptera: Tipulidae) living in soil or rotting wood, while various species of *Lixophaga* have been reared from larvae of Lepidoptera and Coleoptera. In North America, *Lixophaga unicolor* Smith has been reared from both Sesiidae (Lepidoptera) and Cerambycidae (Coleoptera) larvae

boring in the same tree trunks. The best known species of *Lixophaga*, *L. diatraeae* (Townsend), develops in the larvae of a pyralid, the sugar cane borer, *Diatraea saccharalis* (F.). Much effort was expended in the early years of the last century on the introduction of this formerly Cuban tachinid into various parts of Latin America.

Although host records of species of *Admontia* are mostly tipulid larvae, Arnaud (1978) recorded some Lepidoptera, including *Spodoptera* sp. (Noctuidae) and *Grapholita* sp. (Olethreutidae), as hosts of *A. degeerioides* (Coquillett). Arnaud (1978) listed larvae of various Lepidoptera as hosts of various species of *Lixophaga*. Most hosts belonged to taxa with protected larvae, either in stems as borers or in webbing or rolled leaves, such as in Pyralidae, Tortricidae, and other microlepidoptera. *Lixophaga variabilis* (Coquillett), however, was recorded as parasitizing a wide array of hosts, including larvae of beetles, caterpillars, and even sawflies. There are only two records of *Lixophaga* being reared from weevils: *L. sphenophori* (Villenueve) has been reared from *Rhab-*

docoelis sp. as well as from *Diatraea*, and *L. parva* Townsend from *Lixus* sp. Some of these records may represent contaminants, i.e., parasitized larvae or puparia inadvertently included in the soil used for the insect that ended up being recorded as host. The wide host range of *L. variabilis* may also be a reflection of inadequate taxonomy of this particularly difficult genus.

Females of *Admontia* and *Lixophaga* have globose abdomens, presumably for the retention of a large number of embryonated eggs prior to oviposition. Deposition of these eggs on soil, rotten wood, or frass at the entrance to a burrow in a stem may be less efficient than laying eggs directly on the host. However, it allows these flies to successfully parasitize protected hosts, a tactic also used by members of the tribe Dexiini as well as by the eryciine genus *Lydella* that attacks the European corn borer, *Ostrinia nubilalis* (Hübner), in the corn stalk. *Admontia* and *Lixophaga* may not be the only blondeliines whose females deposit eggs near their hosts and rely on the searching capabilities of their neonate larvae to find the host. Females of *Chaetostigmoptera* and *Paracraspedothrix* also have globose abdomens like those of *Admontia* and *Lixophaga*, a condition that resembles that found among the Goniini that deposit microtype eggs on foliage to be eaten by potential hosts. These four genera may form a clade within the Blondeliini. To this group must also be added the new species described below, which is sufficiently unlike either *Admontia* or *Lixophaga* to be included readily in either. Although it runs to *Admontia* in keys to blondeliine genera (Wood 1985, 1987), the fore tarsus of the female resembles that of *Lixophaga* and lacks the expanded fore tarsus characteristic of *Admontia*. Therefore, *Lixadmontia,* a new genus, is proposed.

MATERIALS AND METHODS

Terminology used in the descriptions follows McAlpine (1981). Acronyms for collections in which types are deposited are as follows:

CNCI — Canadian National Collection of Insects, Ottawa, Ontario

EAPZ — Escuela Agrícola Panamericana, El Zamorano, Honduras

FSCA — Florida State Collection of Arthropods, Gainesville, Florida

IRRC — Indian River Research & Education Center, Ft. Pierce, Florida

USNM — United States National Museum, Washington, D.C.

Lixadmontia Wood and Cave **new genus**

Head (Fig. 1): Arista tapering evenly to apex (as in *Lixophaga*), thickest on basal third or less (basal half thickened in *Admontia*); first two aris-
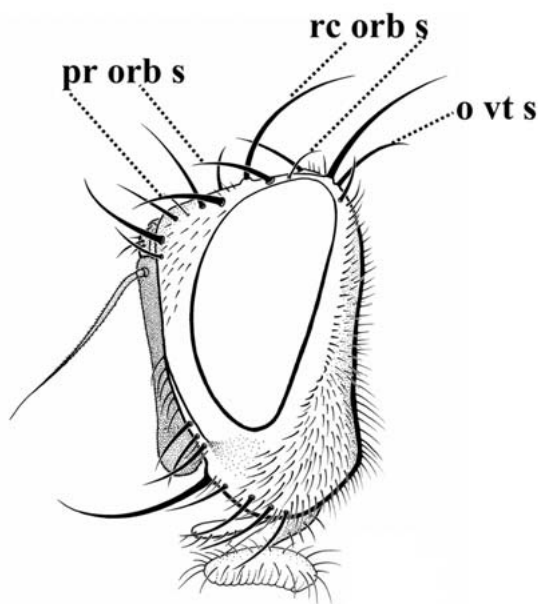

Fig. 1. Left lateral view of head of male *Lixadmontia franki*. *pr orb s*, proclinate orbital bristle; *rc orb s*, reclinate orbital bristle; *o vt s*, outer vertical bristle.

tomeres minute, not longer than wide (first aristomere longer than wide and second aristomere 2 to 5 times as long as wide in most species of *Admontia)*. Frons with two proclinate upper orbital bristles in both sexes (as in *Admontia*, but these bristles more widely separated from one another in *Lixadmontia*). Two reclinate upper orbital bristles in both sexes (as in *Admontia*), anterior bristle longer than posterior bristle, the former in line with frontal bristles rather than displaced laterally, the latter more in line with two large proclinate orbital bristles. Outer vertical bristle well-developed in both sexes, more than half as long as inner vertical bristle (a third or less as long in *Admontia*) and about twice as long as posterior reclinate orbital bristle (well-developed only in females of *Lixophaga*, and in males of *Admontia podomyia* Brauer and Bergenstamm and *A. cepelaki* (Mesnil)). Parafacial parallel-sided, with a few tiny scattered appressed setae below lowest frontal, not descending to level of uppermost bristle on facial ridge (more extensive and erect in most species of *Admontia*). Gena one-quarter height of eye. Facial ridge setose on lower third (usually lower half in *Admontia*), but bristles, especially those uppermost, more decumbent. Occipital setae extending down to lower cranial margin with scarcely any occipital dilation.

Thorax: Postpronotum with three bristles arranged in a triangle. Anterodorsal corner of anepisternum with two bristles, one below and behind the other (as in *Lixophaga retiniae* (Coquil-

lett) and *A. cepelaki*, but no other *Admontia*). Katepisternum with 2-3 bristles, middle katepisternal bristle small, absent in some specimens, when present arising almost directly below pleural suture and only slightly behind anterior katepisternal bristle. Apical scutellar setae closer to subapical scutellar bristles than to each other. Subapical scutellar bristles nearly parallel to one another or convergent (divergent in *Admontia* and *Lixophaga*). Lateral scutellar bristles less than half as long as subapical scutellar bristles and parallel to one another, curving medially (longer and divergent in most *Admontia* and *Lixophaga*, i.e., parallel to subapical scutellar bristles, but homoplastic in both genera). Fore tarsus of female slender (as in *Lixophaga*), not broadened or flattened (as in *Admontia*).

Abdomen: Abdominal syntergite 1+2 lacking median marginal bristles (present on all *Admontia* and *L. retiniae*) and with mid-dorsal depression not extending to hind margin of tergite. Tergites 3 and 4 each with a pair of median marginal bristles, but no discal bristles (which are present in all *Admontia* and a few species of *Lixophaga*). Fifth sternum of male with 2 to 3 small bristles on

posterolateral corner (a single large bristle in *Lixophaga*).

Genitalia (Fig. 2): Cerci of male fused medially for less than half their length, as in *Lixophaga* (in *Admontia* fused along more than half their length), and more widely separated from each other.

Type-species: *Lixadmontia franki* Wood and Cave **new species**.

Lixadmontia franki Wood and Cave **new species**

Male: Head: Arista brown, darker in color than base of first flagellomere; first aristomere minute, second aristomere not longer than wide. First flagellomere entirely gray-brown in male and nearly as long as height of eye, slightly shorter and paler on basal third to half in female. Parafrontal and parafacial silvery-gray. Genal groove dark reddish. Occiput gray, all occipital setae black except for a small midventral patch of pale hairs. Thorax: Scutum, scutellum, and pleuron uniformly gray pruinose. Abdomen: Abdominal syntergite 1+2 dark on basal half, reddish on apical half laterally and ventrally, with mid-dorsal
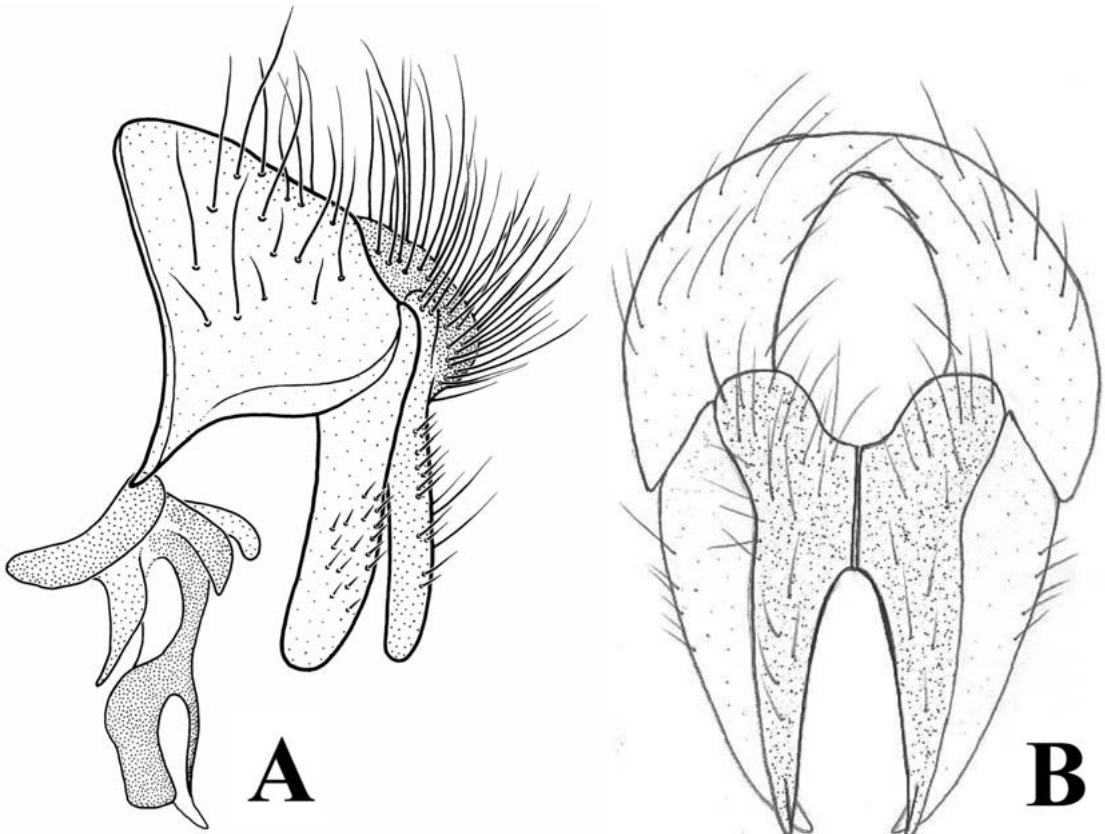

Fig. 2. Right lateral view (A) and caudal view (B) of male terminalia of *Lixadmontia franki*.

stripe about 1/3 width of abdomen. Tergite 3 reddish laterally and ventrally, with black mid-dorsal stripe continuous with that of syntergite 1+2, widening distally to nearly the full abdominal width. Tergite 4 entirely black dorsally except for reddish anterolateral corners, reddish ventrally on anterior half, black ventrally on posterior half. Tergite 5 black dorsally, reddish ventrally. Tergites 3 to 5 each with narrow transverse basal band of silvery pruinosity. Sides tapering towards apex.

Female: Similar to male except: syntergite 1+2 and tergites 3-5 black dorsally and laterally, tergites 3-5 narrowly reddish ventrally at middle or entirely black; abdomen more parallel-sided.

Holotype: male, [first label] HONDURAS, Fco. Morazán, Tatumbla, Montaña El Aguacate, 17 Oct 1995, rcol J. Ortega, [host plant] *Tillandsia oreogenes,* [second label] ex: larva de *Metamasius quadrilineatus* (Curculionidae), deposited in CNCI.

Paratypes: 1 male, same data as holotype (CNCI); 2 males, 2 females, same data as holotype except 13-IX-1995 (EAPZ); One male, HONDURAS, El Paraíso, Yuscarán, Montserrat, 6-III-1995 and 17-VIII-1995, same collector, host plant, and host as holotype (EAPZ). One female, HONDURAS, Francisco Morazán, Cerro La Montaña, 26-XI-1995, same collector and host as holotype (EAPZ). Two males and 1 female, HONDURAS, Francisco Morazán, Cerro Uyuca, IX-1995, D. Alvarez, same host as holotype (EAPZ). Five males and five females, from a laboratory colony at the Escuela Agrícola Panamericana, Honduras, received into UF/IFAS quarantine facility, Ft. Pierce 4-XI-2005 (FSCA, IRRC, USNM).

Etymology: The species is named for Dr. J. Howard Frank, Professor at the University of Florida, in recognition of his persistent and tireless efforts to find and study parasitoids of *M. callizona* in order to establish a biological control agent of this pest in Florida.

Biology: *Lixadmontia franki* lives in cool, moist montane cloud forests in central Honduras at elevations between 1,200-2,000 m. Cave (1997), Alvarez del Hierro (1997), and Alvarez del Hierro & Cave (1999) described the discovery, ecology, levels of parasitism in the field, and potential of *L. franki* (as *Admontia* sp.) as a biological control agent of *M. callizona*. Cave et al. (2003) noted that five tachinid larvae emerged from a single unidentified weevil host (probably *M. quadrilineatus*) collected by Barbra Larson on July 7, 2000 in Alta Verapáz, Guatemala; the larvae pupated on July 17 and later emerged as adults on August 6. These specimens were identified as being of the same species from Honduras. However, the specimens were lost and therefore are not included in the type material for *L. franki*.

In the key to genera in the Manual of Nearctic Diptera (Wood 1987), this species ought to key out to *Admontia* if the user begins at couplet 119, the first blondeliine couplet. *Lixadmontia* could be mistaken for a species of *Admontia*, except for its parallel to convergent subapical scutellar bristles. It is not likely to be mistaken for *Lixophaga* because of setulae on the parafacial, although *Lixophaga retiniae* (Coquillett 1897) has setulae present. Also, *Lixophaga* males do not have proclinate orbital bristles. There are no known Nearctic Blondeliini with convergent subapical scutellar bristles, this being rather exceptional in a few Neotropical genera, which are otherwise apparently Blondeliini. To facilitate the recognition of *L. franki* if it should be introduced successfully into Florida, the following key is presented.

KEY TO SPECIES OF *ADMONTIA* AND *LIXADMONTIA* OF NORTH AND CENTRAL AMERICA

1. Second aristomere two or more times as long as wide (Fig. 42 of Wood 1987)........................2

1'. Second aristomere about as long as wide..10

2. Lateral scutellar bristles about as long, as stout and as straight as sublateral bristles, and more or less parallel to them (unless distorted during preparation) (as in Fig. 185 of Wood 1987, but with apical scutellar bristles straightened and reduced in size)...................3

2'. Lateral scutellar bristles at most two-thirds as long as sublateral bristles, usually curved medially and subparallel to one another (as in Fig. 184 of Wood 1987)...........................9

3. Crossveins r-m and m-cu each surrounded by an area of darkened membrane several times wider than width of the crossvein. Currently known only from Cortes Pass, Mexico......................................*Admontia ducalis* Reinhard

3'. Crossveins r-m and m-cu not appreciably more infuscated than other veins.......................4

4. First and second aristomeres, and thickened base of third aristomere, pale orange. Palpus orange. Only 2 katepisternal bristles...5

4'. Arista and palpus brown or black. Three katepisternal bristles (except in some males of *A. pergandei*)..6

5. First flagellomere of female orange, especially in area at base of arista. First fore tarsomere widened apically, as wide at its apex as width of second fore tarsomere. Wing clear or lightly infuscated along veins........*Admontia rufochaeta* Curran

5'.   First flagellomere of female gray, darkened around area at base of arista.
         First fore tarsomere gradually dilated apically but narrower at its apex than
         width of second fore tarsomere. Wing distinctly infuscated beyond apex of vein
         R1 and crossvein r-m, especially along veins . . . .*Admontia nasoni* Coquillett

6.   Outer vertical bristle well-developed, as large as lateroclinate upper orbital
         bristle and both nearly as large as upper proclinate orbital bristle. Lower facial
         margin protruding, visible in profile. Europe to eastern Siberia and Yukon,
         presumably also Alaska. . . . . *Admontia cepelaki* (Mesnil)

6'.   Outer vertical bristle absent or weakly differentiated, hardly larger than one of
         the larger bristles of the occipital fringe. Lower facial margin not visible in profile. 7

7.   Parafacial wide, with patch of setae at least 3 irregular rows wide.
         British Columbia and Utah to California and Arizona . . . .*Admontia badiceps* Reinhard

7'.   Parafacial narrower, about width of first flagellomere, with setae arranged
         in single irregular vertical row confined to anterior half of parafacial or less. 8

8.   First fore tarsomere of female abruptly dilated near middle, its apex as wide
         as second fore tarsomere. Male without elongate upper occipital bristles.
         Abdominal tergites 3 and 4 each with a single pair of median marginal
         bristles. Widespread . . . . *Admontia pergandei* Coquillett

8'.   First fore tarsomere of female gradually widening apically. Male with
         one or more upper occipital bristles longer than others, and thus
         resembling upper vertical bristles, but usually separated from
         inner vertical bristle by a smaller seta. Europe to Yukon . . . .*Admontia grandicornis* (Zetterstedt)

9.   Abdominal tergites entirely tessellated gray pruinose, the bases of all
         setae each surrounded with a halo of glossier cuticle. Widespead . . . .*Admontia pollinosa* Curran

9'.   Abdominal tergites glossy black, except for a narrow basal band
         of silvery pruinosity, usually devoid of setae. Widespread . . . .*Admontia degeerioides* (Coquillett)

10.   Outer vertical bristle well-developed, as large as proclinate orbital bristles,
         and twice the size of posterior reclinate orbital bristle (Fig. 1). Parafacial setae
         few, sparse, and very small, easily overlooked. Subapical scutellar bristles
         parallel to convergent, apices of lateral bristles usually curving beneath
         them, apical setae closer to subapical scutellar bristles than to each other.
         Abdominal tergites 3 and 4 lacking discal bristles; lateral marginal
         setae well-developed on syntergite 1+2, median marginal
         setae lacking. Honduras . . . . *Lixadmontia franki* Wood and Cave **n. sp.**

10'.   Outer vertical bristle absent or equivalent in size to reclinate upper orbital bristle.
         Parafacial setae conspicuous. Subapical scutellar bristles divergent, apical setae
         as close to one another as to subapical scutellar bristles 11

11.   Arista, except for the tapered apex, orange 5

11'.   Arista brown 12

12.   First flagellomere mostly orange, grayish at apex; arista dark brown,
         contrasting with first flagellomere. Parafacial setae long and erect,
         as long as uppermost facial bristles and overlapping with them in
         middle of parafacial. Widespread in USA . . . . *Admontia tarsalis* Coquillett

12'.   First flagellomere gray, orange only at extreme base; arista pale brown.
         Parafacial setae shorter and recumbent. Arizona . . . .*Admontia offella* Reinhard

Note: *A. rufochaeta* may be conspecific with *Admontia duospinosa* West, but *duospinosa* has not been included for lack of specimens. In a footnote to his key, Curran (1927) indicated that he had not included this species.

Coquillett (1895) described *Degeeria washingtonae* based on a female, a year after his description of *A. pergandei* based on a male; it has not been possible to distinguish which are females of the latter or males of the former, and the two seem to belong to the same species. By describing them in different genera, it is evident that Coquil-

lett did not realize that they could be conspecific. However, synonymy is not warranted until a revision has been completed.

Curran (1927) separated *Admontia dubia* Curran from *A. pollinosa* on a tenuous character state; the facial ridge is bristled on its lowest 1/3 in *dubia*, and on the lowest 1/2 or more in *polli-*

nosa. Additional material has shown this separation to be untenable, and *A. dubia* is hereby synonymized with *A. pollinosa* **new synonymy**.

Many specimens from Central America resemble *A. tarsalis* and/or *A. offella* and some may belong to either of these species. Unfortunately, only a fragment of the holotype of *Admontia occidentalis* van der Wulp, the earliest name, remains, and it was not possible to decide which of various species might be referred to this taxon.

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MATING DISRUPTION AND ATTRACT-AND-KILL AS REDUCED-RISK STRATEGIES FOR CONTROL OF GRAPE ROOT BORER *VITACEA POLISTIFORMIS* (LEPIDOPTERA: SESIIDAE) IN FLORIDA VINEYARDS

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Abstract

Mating disruption and attract-and-kill (A&K) gels were evaluated for control of grape root borer (GRB), *Vitacea polistiformis* Harris (Lepidoptera:Sessidae), in Florida grape (*Vitis* sp.) vineyards. For mating disruption, pheromone twist-ties with leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae), pheromone were placed in vines at a rate of 635 per ha. Attract-and-kill (A&K) gels containing the GRB pheromone and a pyrethrin (botanical insecticide) were applied to vine trunks at a rate of 112.5 g per hectare. These treatments were compared with chlorpyrifos (Lorsban), an organophosphate insecticide, and an untreated control in a randomized complete block design. Two wing-style sticky traps with GRB pheromone were placed in each treatment to monitor male moth activity and determine levels of trap shutdown. Complete trap shutdown occurred in the twist-tie sections for both 2003 and 2004 suggesting disruption of mating. Traps placed in the A&K and the pheromone twist-tie sections caught significantly fewer GRB than the Lorsban treatments in 2003. In 2004, significantly fewer moths were caught in the pheromone twist-tie and Lorsban treatments than the A&K and untreated controls. The differences between 2003 and 2004 for A&K treatments were due to the use of the incorrect pheromone blend in the 2004 batch of A&K. Whereas the differences in Lorsban treatments between both years may be related to residual activity of the pesticide. The economics of adopting A&K and mating disruption with twist-ties containing leopard moth pheromone is discussed. Both A&K and mating disruption with the leopard moth pheromone show promise as reduced-risk control tactics to be used in a GRB integrated pest management program and warrant further study.

Key Words: grape root borer, *Vitacea polistiformis*, mating disruption, attracticides, off-blend pheromones, IPM

Resumen

Sistemas de confusión de atracción sexual y geles para atraer y eliminar, fueron evaluados para el control de *Vitacea polistiformis* Harris (Lepidoptera: Sessidae) en viñas de Florida. En el sistema de confusión de la atracción sexual, se utilizaron "twist-ties" con feromonas de *Zeuzera pyrina* L. (Lepidoptera: Cossidae). Seiscientos treinta y cinco unidades de este atrayente fueron usados por hectárea. El gel para atraer y eliminar usando una mezcla de feromonas de *V. polistiformis* y pyrethrin (insecticida botánico) se aplico en una dosis de 112.5 g/ha. Estos tratamientos fueron comparados con chlorpyrifos (Lorsban), un insecticida organofosforado, y con un control sin ningún tratamiento químico, usando un diseño de bloques completos al azar. Dos trampas adhesivas con feromona de *V. polistiformis* se colocaron en cada tratamiento para monitorear la actividad de los machos. Los tratamientos con "twist-ties" consiguieron confundir los machos al punto que no se capturo ninguno en las trampas en 2003 ó 2004 sugiriendo que no hubo cópula. Las trampas colocadas en el tratamiento con "twist-ties" y con gel para atraer y eliminar, capturaron significativamente menos machos que las trampas colocadas en los tratamientos con Lorsban en 2003. En 2004, significativamente menos machos fueron capturados en los tratamientos con Lorsban y con "twist-ties" que los tratamientos que usaron el gel o que en el control sin tratamiento. Las diferencias entre 2003 y 2004 para el tratamiento de atraer y eliminar se debió a la el uso de una mezcla incorrecta de feromonas en 2004. La diferencia de las capturas en los tratamientos con Lorsban puede estar relacionada con la actividad en el residuo del pesticida. Las ventajas y desventajas económicas de el uso de estas dos tecnologías son discutido en el articulo Ambos tratamientos, el gel y los "twist-ties" con feromonas de *Z. pyrina*, son promisorios como candidatos para incluir en un programa de manejo integrado de *V. polistiformis* y merece más estudios en el futuro.

Translation provided by the authors.

The grape root borer (GRB), *Vitacea polistiformis* (Harris) (Lepidoptera: Sesiidae), is the key pest of grapes (*Vitis* spp.) in Florida (Liburd et al.

2004). Larvae feed on the roots, resulting in reduced yields and eventually, death of the vine. In severe cases, entire vineyards are lost (Dutcher &

All 1979). Traditionally, chlorpyrifos (Lorsban 4E) has been used as a soil drench to control primarily first instars as they move to the soil surface and enter the soil seeking roots, and newly emerged adults. Lorsban, an organophosphate, is suspected of being carcinogenic (Food Quality Protection Act 1996) and its future use is not certain.

The potential for modifying an insect pest's behavior through the use of pheromones in order to control its impact on a crop has been investigated widely in the last 30 years. Mating disruption first proved to be successful in controlling cabbage looper moths, *Trichoplusia ni* (Hubner) (Shorey et al. 1967), and since then has been used successfully on a number of insect pests (Cardé & Minks 1995). Among the sesiids, some success has been obtained with the currant clearwing moth *Synanthedon tipuliformis* (Clerck) (Cardé & Minks 1995), the peachtree borer *Synanthedon exitosa* (Say), and the lesser peachtree borer *S. pictipes* (Grote and Robinson) (Yonce 1981).

Pheromone twist-ties with GRB pheromone, 99% (*E,Z*)-2,13-octadecadien-1-ol: 1% (*Z,Z*)-3-13-ocadecadien-1-ol [99% (*E,Z*)-2,13-ODDA: 1% (*Z,Z*)-3,13-ODDA] (Snow et al. 1987) have shown great potential as a viable control tactic for GRB management. Pearson & Meyer (1996) used 254 dispensers per ha in vineyards and examined females in the treatment plots. They found a significant reduction in the number of mated GRB females compared with females taken from the untreated controls. Also, Webb (1991) recorded a significant reduction in trap-catches, mated females, and pupal case counts in vineyards treated with GRB pheromones compared with the untreated vineyard, potentially indicating a high degree of mating disruption.

Some studies suggest that "off-blends" (incomplete pheromones) may work better at mating disruption than the synthetic pure pheromone blend that is most similar to the natural pheromone (Minks & Cardé 1988). The mechanisms by which off-blend pheromones function to inhibit mating are not well understood. However, one theory is that the off-blend camouflages the true female pheromone, rendering it indistinguishable from the background (Minks & Cardé 1988). Another theory is that by creating a sensory imbalance, the male becomes attuned to the more predominant off-blend, and the ratio in the true blend is interpreted as unnatural (Bartell 1982).

Attract-and-kill (A&K) is a promising new technology that involves an attractant such as a pheromone and a toxicant. Unlike mating disruption, which functions by "confusing" the insect, attract-and-kill technology attracts the insect to a pesticide laden gel matrix, which, upon contact, kills the insect. Attract-and-kill has been successfully used on several lepidopteran species including codling moth (Ebbinghaus et al. 2001), and Oriental fruit moth (Evenden & McLaughlin

2004). Recently, IPM Tech (Portland, OR) developed an attracticide for grape root borer, called Last Call™ GRB, which has not previously been tested under field conditions.

The overall goal was to evaluate the use of an off-blend pheromone for mating disruption as well as an attract-and-kill technique for the control of grape root borer in Florida vineyards.

MATERIALS AND METHODS

Four muscadine grape (*Vitis* sp.) vineyards with similar management practices were chosen for this experiment. All vineyards were pruned annually in early winter, had similar fertilization schedules, were treated with glyphosate 3-4 times a year in a 1.2-m band under the trellis, and were mowed between rows every 2-3 weeks. No other vineyards occurred within 16 kilometers, but areas of naturally occurring wild grapes were nearby.

Each vineyard consisted of four treatments and was divided into four, 0.4-ha plots. Treatments included: 1) Mating disruption with pheromone twist-ties (Shin-Etsu Chemical Co. Ltd. Tokyo, Japan), 2) Attract-and-kill with Last Call™ GRB (IPM Tech Portland, OR), 3) Chemical control with chlorpyrifos (Lorsban 4E) (Dow AgroSciences LLC, Indianapolis IN), and an untreated control. A 15-m buffer zone was left between treatments. Experimental design was a randomized complete block (blocked by vineyard) with four treatments and four replicates. Two wingstyle sticky traps baited with the GRB pheromone [99% (*E,Z*)-2,13-ODDA: 1% (*Z,Z*)-3,13 ODDA] (Great Lakes IPM, Vestaburg, MI) were hung on the trellis wire at 1.0 to 1.5 m above the ground in each treatment at least 20 m apart to monitor populations of male GRB moths. This study was initiated in the 2003 grape-growing season and repeated in 2004.

Pheromone Twist-Ties

Pheromone twist-ties emitting the leopard moth, *Zeuzera pyrina* L. (Lepidoptera: Cossidae) pheromone (95% (*E,Z*)-2,13-ODDA: 5% (*E,Z*)-3,13-ODDA) (70 mg of active ingredients per unit) were applied to 0.4-ha treatment plots at a rate of 635 per ha (254 per 0.4 ha), approximately one twist-tie per vine. The dispensers were evenly distributed throughout the plot, and hung from the vine near the trellis wire at roughly 1 to 1.5 meters above the ground.

The leopard moth pheromone has not been previously tested in GRB mating disruption experiments. We chose to use this off-blend pheromone because it contains the same major component as the GRB pheromone and it is commercially available and significantly cheaper. Also, the findings of Johnson & Mayes (1980); Johnson et al. (1981, 1986); and Pearson & Meyer (1996) showed that it

is possible to cause mating disruption with attractants other than the complete blend.

Attract-and-Kill with Last Call-GRB

The Last Call™ GRB used in the 2003 and 2004 field trials contained 0.16% GRB pheromone, 6.0% pyrethrins (CAS 8003-34-7), and 93.984% inert ingredients. We applied 2,250 drops per ha (900 drops for 0.4 ha). Each drop contained 50 µl Last Call-GRB matrix. To achieve a uniform distribution of drops throughout the vineyard, the 900 drops were divided by the number of vines per 0.4 ha plot, approximating 2-4 drops per vine. Last Call™ GRB drops were applied to the trunks of vines ~0.5 to 1.5 meters from the ground through a calibrated hand-pump, manufactured by IPM Tech specifically for attract-and-kill gels. The pump fits in the palm of the hand and can be operated with one hand. Attract-and-kill was reapplied every 6 weeks for the duration of the season, according to the protocol determined by IPM Tech.

Chemical Control with Lorsban

Lorsban 4E® (chlorpyrifos) (Dow Chemical U.S.A., Midland, MI) (44.9% a.i.) was applied once per season at the labeled rate of 1.06 L to 378 L of water to treat 200 vines. It was applied closest to the period of greatest GRB emergence, based on earlier findings (Webb et al. 1992).

Statistical Analysis

Trap catches were counted weekly and recorded from the beginning of the season until the end of the GRB flight. Total mean number of GRB captures was analyzed by repeated measures Analysis of Variance (ANOVA), and differences among means were determined by Tukey's multiple comparison test ($P < 0.05$) (SAS Institute 2004).

RESULTS

During 2003, the number of male moths captured in traps in the areas treated with leopard moth pheromone twist-ties was significantly fewer than the male moths captured in the untreated control and the Lorsban treatments ($F = 9.81$; $df = 3, 264$; $P < 0.0001$) (Table 1). The number of male moths captured in traps in the twist-tie sections was not significantly different from those captured in traps in the areas treated with A&K. Traps deployed in the A&K plots caught significantly fewer male moths than traps deployed in the Lorsban sections. There were no significant differences in trap catches between the Lorsban and the untreated control sections (Table 1).

During 2004, significantly fewer GRB were captured in areas treated with leopard moth pheromone twist-ties compared with the A&K and untreated control sections (Table 1) ($F = 11.42$; df = 3,234; $P < 0.0001$). There were no differences between the twist-tie and Lorsban treatments or between the attract-and-kill and the untreated control sections (Table 1).

Figure 1 shows the mean number of GRB males caught per week per treatment for 2003 and 2004. The GRB captures in the untreated control sections were similar during each year. For the A&K treatments, larger numbers of GRB were captured later in the season than earlier for 2003 (Fig. 1A). Also, a relatively high number of GRB were caught in the traps in the Lorsban section in 2003. During 2004, the periods of low captures coincided with periods of extreme weather such as hurricane activity (early September). Also, trap catches in the A&K treatments were more erratic with periods of high and low captures occurring throughout the season (Fig. 1B). Grape root borer trap catches in the Lorsban treatment of 2004 was much lower than the previous year, especially later in the season during the 4-week period when Lorsban was active (Fig. 1B).

DISCUSSION

Mating Disruption

Wing traps were used to measure male moth activity throughout the vineyards and consequently, mating disruption success by trap shutdown. If GRB males were not able to locate the trap pheromone source, it is assumed that it

TABLE 1. WEEKLY MEAN ± SEM NUMBER OF GRAPE ROOT BORERS PER TRAP FOR MATING DISRUPTION, ATTRACT-AND-KILL, LORSBAN, AND UNTREATED CONTROL TREATMENTS IN FOUR FLORIDA VINEYARDS FOR 2003 AND 2004.

	Weekly mean trap capture ± SEM	
Treatment	2003	2004
Mating disruption	0.00 ± 0 c	0.00 ± 0 b
Attract-and-kill	1.07 ± 0.44 bc	3.50 ± 0.76 a
Lorsban	3.07 ± 0.73 a	0.84 ± 0.32 b
Untreated control	2.49 ± 0.62 ba	3.00 ± 0.75 a

Means in columns followed by the same letter are not significantly different ($P = 0.05$, Tukey's test)
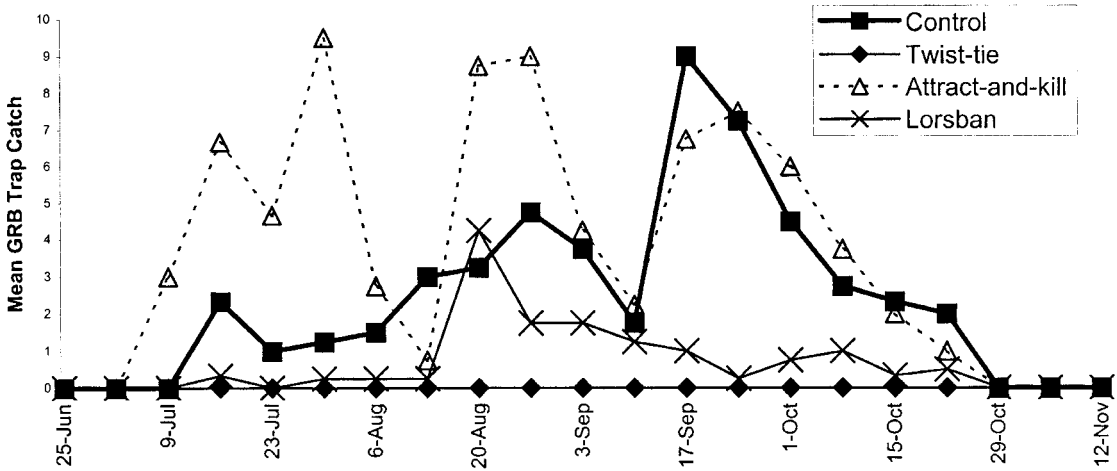
A   **2003**
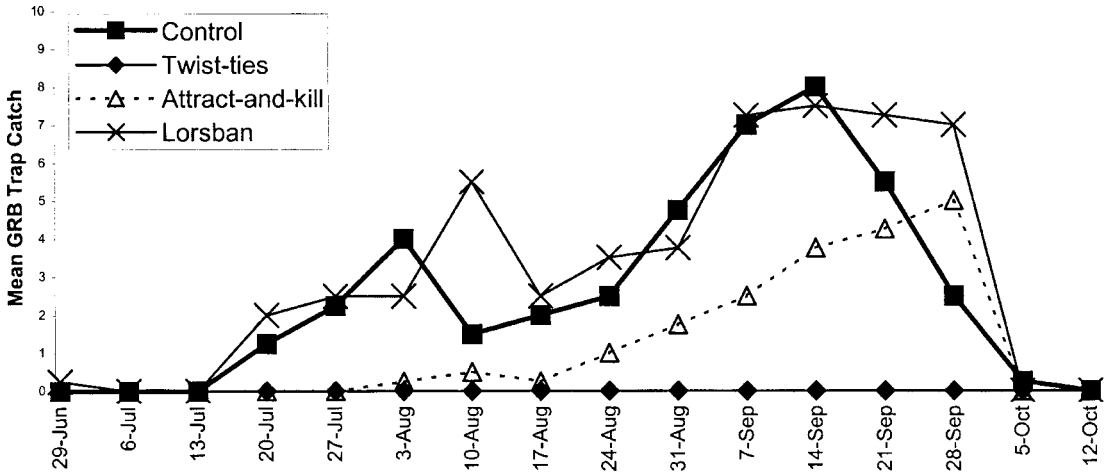
B   **2004**

Fig. 1 A&B. Mean number of grape root borer males caught in monitoring traps per week for four treatments: untreated control, pheromone twist-ties, attract-and-kill gels, and Lorsban 4E for 2003 and 2004.

would be unlikely for them to find a calling female. In an experiment by Webb (1990), males were unable to locate caged calling females in traps in a vineyard saturated with synthetic pheromone. In our study, complete trap shutdown was achieved in all of the pheromone twist-tie treatments for both years, indicating that males were unable to orient to the female pheromone source. Therefore, it is reasonable to assume that disruption of mating occurred.

It is possible that some mating may still occur despite complete trap shutdown. In addition, gravid females have been observed immigrating into pheromone-saturated vineyards and laying eggs (Johnson et al. 1986), This behavior is unexplained since Pearson & Schal (1999) demonstrated a weak attraction to the GRB pheromone by mated females. It is not known how often or to what extent gravid females enter pheromone-saturated vineyards, but it can have serious consequences on a mating disruption program.

Trap shutdown alone does not prove that mating disruption has occurred. Previous studies have confirmed mating disruption success in pheromone-saturated vineyards by other methods in addition to trap shutdown including deter-

mining if males could locate caged calling females, counting pupal skins, and production of fertile or infertile eggs by females caught within the vineyards. Webb (1990) showed a significant degree of mating disruption by trap shutdown as well as the reduction of pupal skin counts indicating a correlation between the two. Johnson et al. (1991) and Yonce (1981) also showed a correlation between reductions of pupal skin counts and trap shutdown. Attempts were made to recover pupal skins but a shortage of labor and inclement weather coupled with low recovery of pupal skins forced us to cancel this activity.

We used twist-ties with the pheromone of the leopard moth *Zeuzera pyrina* L. (Lepidoptera: Cossidae) 95% (*E,Z*)-2,13-ODDA: 5% (*E,Z*)-3,13-ODDA. It shares the major component with the GRB, although in a smaller percentage (95% rather than 99%), and has a different minor component. Due to the fact that it was possible to cause mating disruption with off-blends (Hodges et al. 1984; Minks and Cardé 1988), and the fact that the leopard moth pheromone was commercially available and significantly cheaper, it was chosen as our disruption tool.

Attract-and-Kill

Trap catches in the A&K treatment plots during 2003 were not significantly different from the twist-tie sections, indicating low activity by male moths in the area treated with A&K. It is unclear whether the success of the A&K was due to insecticide poisoning of the males because of contact with A&K or mating disruption. The A&K treatment did not work as well in 2004 when traps showed no difference from the untreated control. Traps in the A&K treatments caught 48% of the total GRB captured in 2004, compared with only 13% in 2003. Initially, the reason for this was unclear. Further investigation revealed that the producers of A&K failed to include the correct pheromone blend in the 2004 batch. Analysis of the gel revealed the ratio to be 95:5 (*E,Z*)-2,13-ODDA: 4.5%(*Z,Z*)-3,13-ODDA instead of 99:1. Previous studies demonstrated that a 95:5 ratio was mostly unattractive to male GRB (Snow et al. 1987).

We observed that the A&K drops often deteriorated quite rapidly under Florida weather conditions. During the summer growing season, Florida vineyards usually experience powerful storms with pelting rain, intense heat, and solar radiation. These conditions may affect the stability and longevity of the A&K. The protocol for A&K indicates that it must be reapplied every 6 weeks. However, we noticed that many drops were almost dry after 3 weeks and totally missing during the 4th through 6th week. Only in a few instances did we observe drops that lasted the entire 6-week period. Attract-and-kill warrants further investigation to determine the frequency of appli-

cation under Florida conditions and its overall effectiveness.

The Lorsban treatment was included in the study as a standard chemical treatment for GRB control. Lorsban primarily controls first instars as they emerge from eggs and burrow to the roots. It can also reduce the number of adults as they emerge from their cocoons. In 2003, there were no significant differences in the number of male moths caught in traps between the untreated (control) and the Lorsban-treated sections. However, during 2004, traps in the vineyards treated with Lorsban caught significantly fewer GRB than the untreated controls. The reason for the differences between 2003 and 2004 is not clear.

Costs

At our current application rates, LastCall-GRB costs $250 per hectare and it requires roughly 2.5 h/ha to apply and 3-4 applications per season (under Florida conditions). This could be labor-intensive, depending on the size of the vineyard. Future studies should evaluate how many drops per hectare would provide effective control. For instance, instead of 900 drops per 0.4 ha, perhaps the drops (1 drop = 0.05 g) could be consolidated into larger amounts and applied on fewer vines.

Our pheromone twist-tie application rate of 635 units per ha is fairly high. Practically, 635 twist-ties per ha may be expensive for farmers ($287/ha compared with $32-$65/ha for Lorsban). It takes an average worker 3 h/ha to deploy the twist-ties. However, the pheromone lasts the entire season under normal conditions.

Future studies should focus on different deployment tactics as well as rates of application (number of twist-ties per ha). It may be important to compare the leopard moth pheromone to the true GRB blend in further mating disruption studies. This was beyond the scope of this study, but future studies should also incorporate the counting of pupal skins as a means to determine GRB reductions from the treatments.

Mating disruption with the use of the leopard moth (*Zeuzera pyrina*) pheromone may be an effective, reduced-risk strategy for controlling GRB, and a good alternative to conventional chemical control. Attract-and-kill technology may also be a potentially effective strategy for GRB control, but more research is needed.

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MOLECULAR DIAGNOSTICS OF *ENAPHALODES RUFULUS* (COLEOPTERA: CERAMBYCIDAE)

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Abstract

Oak-hickory forests in northwestern Arkansas, eastern Oklahoma and southern Missouri have recently experienced an oak decline event with widespread oak mortality. The oak mortality is associated with an outbreak of a native wood-boring cerambycid, *Enaphalodes rufulus* (Haldeman), the red oak borer. Taxonomic identification, below the family level, of larval Cerambycidae through traditional morphological methods is not usually possible. We employed molecular diagnostics, with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), to distinguish *E. rufulus* from other closely related species of cerambycids. A portion of the mitochondrial DNA 16S rRNA gene, isolated from legs or thoraxes of adult museum specimens, was amplified and digested with *Alu* I and *Hind* III restriction enzymes. Both restriction enzymes independently produced fragments for *E. rufulus* that were significantly different from any other cerambycid tested. *Alu* I had one restriction site for *E. rufulus* and two restriction sites for all other cerambycids tested, while *Hind* III did not cut for *E. rufulus* but did cut at one restriction site for all other cerambycids. Eggs, larvae, and pupae of *E. rufulus* along with an unknown cerambycid larva and pupa were successfully amplified and digested by this method to verify validity of this technique for multiple life stages.

Key Words: PCR-RFLP, genetics, red oak borer, longhorn beetle, native insect pest, taxonomic identification

Resumen

De manera continua en los bosques de roble de "nuez dura" de la region de Ozark National Forests en el norte de Arkansas, el este de Oklahoma y el sur de Missouri, se presentan eventos de disminución de las poblaciones de robles. La mortalidad de los robles está asociada con el aumento de las poblaciones de *Enapholodes rufulus*, el perforador rojo del roble, un cerambícido nativo perforador de Madera. Para distinguir *E. rufulus* de otras especies de Cerambycidae muy relacionadas, se utilizaron polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Una porción de DNA mitocondrial del gen 16rRNA fue aislada de las patas o tórax de adultos de especímenes de museos, amplificada y digerida con las enzimas de restricción *Alu I* y *Hind III*. Ambas enzimas produjeron fragmentos para *E. rufulus* que fueron significativamente diferentes a otros cerambícidos probados. *Alu I* tuvo un sitio de restricción para *E. rufulus* y dos sitios de restricción para todos los otros cerambícidos probados, mientras *Hind* III no presentó ninguna restricción para *E. rufulus* y no se encontró otro sitio de restricción para ninguno de los otros cerambícidos. Estos resultados indican que este método podría ser usado para determinar la presencia de *E. rufus* en otros eventos de disminución de las poblaciones robles, cuando se establece que éste es un factor que contribuye en la mortalidad de los árboles.

Translation provided by the authors.

The red oak borer, *Enaphalodes rufulus* (Haldeman) (Coleoptera: Cerambycidae), is an important wood-boring species native to eastern hardwood forests of the United States (Donley & Acciavatti 1980). A variety of oak species are attacked by *E. rufulus*, but trees in the red oak group *Erythrobalanus* are preferred, especially black oak, *Quercus velutina* Lam., scarlet oak, *Q. coccinea* Muenchh., and northern red oak, *Q. rubra* L. (Hay 1974). Since *E. rufulus* attacks and reproduces in living trees, significant degrade in lumber quality is an important issue in commercial stands (Hay 1964). Damage caused by borers often goes unnoticed until trees are felled and sawn for timber, and by this time as much as 40% of the 120-year value of the tree may be lost (Donley & Worley 1976). In comparison, damage caused by defoliators is much more noticeable, but defoliation typically causes only a 15-20% reduction in value (Donley & Worley 1976). Donley and Acciavatti (1980) estimated that 38% of oak wood used for lumber, cooperage and veneer in the Eastern United States is affected by *E. rufulus*.

Enaphalodes rufulus population densities historically have been documented at low levels. Hay (1969) found an average of 3.7, 2.8, and 2.5 attacks on the bottom 1.8 m of black oak, northern red oak, and scarlet oak, respectively in Ohio, and Donley and Rast (1984) found an average of 2.0 attack sites per red oak in Pennsylvania and 3.6

in Indiana. Recently, however, an unprecedented outbreak with significant economic and ecological impacts has occurred in the Ozark oak/hickory forests of northern Arkansas and southern Missouri (U.S.A.) (Stephen et al. 2001; Starkey et al. 2000). Analysis of recent data from the Ozark National Forest reveal an average of 599 active attacks (or current generation galleries) and 77 live larvae per tree in northern red oak (Fierke et al. 2005). USDA Forest Service estimates 450,000 ha of forest in the Ozark Mountains will be impacted by *E. rufulus* with an estimated 68,000 m^2 of timber loss or degradation (Guldin et al. 2005).

Removal of infested trees is a recommended control method for *E. rufulus* (Donley 1981, 1983), but diagnosis of infestation may be delayed as easily identifiable adults emerge only every two years and other identification methods are difficult and unreliable. Historically, attack sites were identified by observing frass (Hay 1969), but this method is limited by observer ability, weather conditions, and multiple insects with similar life histories. Larval keys for North American cerambycid species exist (Craighead 1923) but are difficult to follow and outdated. In addition, morphological differences among closely related cerambycid larvae are often minute. Larval identification is also important for detecting *E. rufulus* in tree hosts related to red oaks, which may harbor several cerambycid species, such as *Elaphidion* spp., *Goes* spp., or *Noeclytus* spp. (Yanega 1996). Molecular genetic techniques are an alternative to traditional methods for distinguishing *E. rufulus* larvae from other cerambycids.

The objective of this research was to develop a molecular diagnostic technique for all life stages of *E. rufulus* with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Genetic research has never been conducted on *E. rufulus* and molecular diagnostics have been re-ported for only one other cerambycid, *Anoplophora glabripennis* (Motschulsky) (Kethidi et al. 2003). The *A. glabripennis* diagnostic technique utilizes sequence characterized amplified regions (SCARs) derived from randomly amplified polymorphic DNA (RAPD). While the end result of this technique is a very simple polymerase chain reaction diagnostic, development of this procedure is time-consuming and expensive. On the other hand, PCR-RFLP is a simple, inexpensive, established and reliable technique (Taylor & Szalanski 1999) that has been used to identify many economically important insects, including termites, *Reticulitermes* spp. (Isoptera: Reticulitermatidae) (Szalanski et al. 2003), screwworm flies, *Cochliomyia* spp. (Diptera: Calliphoridae) (Litjens et al. 2001), and corn rootworm, *Diabrotica* spp. (Coleoptera: Chrysomelidae) (Clark et al. 2001).

MATERIALS AND METHODS

Specimen Collection

Enaphalodes rufulus adults were collected from two areas, Fly Gap and White Rock, in the Ozark Mountains of northwestern Arkansas with standard black-lighting techniques during the flight period between mid-June and late July of 2003 (UTM Zone 15–S NAD83: Fly Gap—0431660, 3954978, White Rock—412668, 3949429). Beetles were placed in 100% ethanol immediately upon capture.

Adult cerambycids, other than *E. rufulus*, that are common to the Ozark Mountain region and one closely related *Enaphalodes* species were collected during concurrent research at the University of Arkansas. Voucher specimens are stored in the Forest Insect Collection at the University of Arkansas Forest Entomology Lab (Table 1). Specimens were caught in clear plexiglass, passive flight intercept panel traps during the summer of

TABLE 1. LIST OF ADULT SPECIMENS, COLLECTION LOCATION, COLLECTION DATE, AND NUMBER USED IN THIS STUDY.

Scientific name	Common name	Collection location (county, state)	Collection date	Number of specimens used
Enaphalodes rufulus	Red Oak Borer	Franklin Co., AR	19-VI-2001	29
Enaphalodes atomarius	N/A	Franklin Co., AR	21-VIII-2001	4
Distenia undata	N/A	Franklin Co., AR	10-VIII-2001	1
Orthosoma brunneum	Brown Prinoid	Franklin Co., AR	07-VIII-2001	2
Bellamira scalaris	N/A	Franklin Co., AR	03-VII-2001	1
Elaphidion mucronatum	Spine Bark Borer	Franklin Co., AR	06-VII-2001	3
Eburia quadrigeminata	Ivory Marked Beetle	Franklin Co., AR	07-VIII-2001	3
Neoclytus a. acuminatus	Red-headed Ash Borer	Franklin Co., AR	20-VII-2001	2
Goes tigrinus	White Oak Borer	Franklin Co., AR	10-VII-2001	1
Aegomorphus morrisii	N/A	Franklin Co., AR	10-VII-2001	1
Urographis fasciatus	N/A	Franklin Co., AR	27-VII-2001	3
Purpuricenus humeralis	N/A	Franklin Co., AR	10-VII-2001	3
Prionus imbricornis	Tile-horned Prionus	Franklin Co., AR	10-VIII-2001	2
Dorcaschema wildii	Mulberry Borer	Franklin Co., AR	06-VII-2001	1

TABLE 2. RESTRICTION FRAGMENT LENGTH POLYMORPHISMS FROM THE 16S rRNA GENE OF 14 CERAMBYICD SPECIES. PATTERNS A, B, AND C INDICATE DISTINCTIVE FRAGMENT LENGTHS.

Restriction enzyme	Species	Size of PCR Amplicon	Restriction site	Fragment(s)	Pattern
*Alu*I	*Enaphalodes rufulus*	414	291	291, 123	A
(AGCT)	*Enaphalodes atomarius*	415	168, 291	168, 124, 123	B
	Distenia undata	412	212, 288	212, 124, 76	C
	Orthosoma brunneum	417	217, 293	217, 124, 76	C
	Bellamira scalaris	413	216, 292	216, 121, 76	C
	Elaphidion mucronatum	413	215, 289	215, 124, 74	C
	Eburia quadrigeminata	414	215, 290	215, 124, 75	C
	Neoclytus a. acuminatus	418	218, 294	218, 124, 76	C
	Goes tigrinus	414	215, 290	215, 124, 75	C
	Aegomorphus morrisii	417	214, 289	214, 128, 75	C
	Urographis fasciatus	412	215, 290	215, 122, 75	C
	Purpuricenus humeralis	414	216, 290	216, 124, 74	C
	Prionus imbricornis	411	214, 287	214, 124, 73	C
	Dorcaschema wildii	412	213, 286	213, 126, 73	C
Hind III	*Enaphalodes rufulus*	414	—	414	A
(AAGCTT)	*Enaphalodes atomarius*	415	166	249, 166	B
	Distenia undata	412	210	210, 202	C
	Orthosoma brunneum	417	215	215, 202	C
	Bellamira scalaris	416	214	214, 202	C
	Elaphidion mucronatum	413	213	213, 200	C
	Eburia quadrigeminata	414	213	213, 201	C
	Neoclytus a. acuminatus	418	216	216, 202	C
	Goes tigrinus	414	213	213, 201	C
	Aegomorphus morrisii	417	212	212, 205	C
	Urographis fasciatus	412	213	213, 199	C
	Purpuricenus humeralis	414	214	214, 200	C
	Prionus imbricornis	411	212	212, 199	C
	Dorcaschema wildii	412	211	211, 201	C

2001. Specimens were collected in 50% ethylene glycol. Upon return to the lab, specimens were transferred to 95% alcohol until pinning. Morphological identification of adult specimens was made by Dr. J. K. Barnes, Arthropod Museum Curator at the University of Arkansas.

Eggs of *E. rufulus* were collected from a lab colony in March 2005. Larvae and pupae were collected from red oak trees harvested between October 2002 and March 2005. Eggs and early instars were frozen, and late instars and one pupa were stored in 95% alcohol until used. An unknown larva and pupa were collected from a white oak tree and stored in 95% alcohol until used.

PCR-RFLP Protocol

DNA was extracted from one leg or thorax of *E. rufulus* and the other 13 adult cerambycids used in this procedure. DNA extraction was accomplished by the protocol of the Qiagen DNeasy tissue kit (Valencia, CA). Re-suspended DNA was stored at -20°C until used. An approximately 420-bp portion of the 16S rRNA gene was amplified using the primers 16S-r (5'-CGCCTGTTTAT-CAAAAACAT-3') (Simon et al. 1994) and 16S-f (5'-TTACGCTGTTATCCCTAA-3') (Kambhampati & Smith 1995). PCR reactions were conducted with 1 µl of extracted DNA as per Szalanski et al. (2000) with a thermocycler profile consisting of 40 cycles of 94°C for 45 s, 46°C for 45 s, and 72°C for 45 s. PCR products were purified and concentrated with the Wizard SV Gel and PCR clean-up kit (Promega, Madison, WI). One sample from each adult was sent to the University of Arkansas for Medical Sciences DNA Sequencing Core Facility (Little Rock, AR), for direct sequencing in both directions. Consensus sequences for each species were acquired by manual alignment and editing of forward and reverse sequences in BioEdit (Hall 1999). GenBank accession numbers are DQ417758 to DQ2417771.

Webcutter 2.0 (Heiman 1997) was used to predict restriction sites from DNA sequence data. Amplified DNA was digested according to manufacturer's (Promega, Madison, WI) recommendations with the enzymes *Alu* I or *Hind* III. Fragments were separated by 2% agarose gel electrophoresis. Gels were stained with ethidium bro-

mide and photographed with the UVP BioDoc-it documentation system (Upland, CA).

RESULTS

The rRNA 16S amplicon ranged from 411 to 418 bp in all cerambycids studied (Table 1). Web-cutter 2.0 analysis of the 16S sequences revealed that either *Alu* I or *Hind* III could effectively distinguish *E. rufulus* from all other species (Table 2). Either enzyme was also capable of distinguishing *E. atomarius* (Drury), a less common but closely related species. All other species produced similar restriction profiles.

To validate the diagnostic, this procedure was tested on 29 adult *E. rufulus* and as many replicates as possible for the additional adult species (Table 1). All species were replicated except *Distenia undata* (Fabricius), *Bellamira scalaris* (Say), *Dorcaschema wildii* Uhler, *Goes tigrinus* (De-Geer), and *Aegomorphus morrisii* (Uhler). There was only one individual available from the collection for these five species. Samples digested with *Alu* I revealed clearly defined results (Fig. 1) as did samples digested with *Hind* III (Fig. 2).

All life stages of *E. rufulus* also were tested with this procedure. Five *E. rufulus* eggs, four *E. rufulus* early larval head capsules, six *E. rufulus* late larval incised head capsules and one *E.*

rufulus incised pupa head were used for confirmation of the use of this diagnostic procedure for multiple life stages. All samples produced positive results when either enzyme was used. One unknown cerambycid larva, and one unknown cerambycid pupa collected from a white oak tree also were tested. Results gave fragment lengths similar to those of all other cerambycids except *E. rufulus* and *E. atomarius*.

DISCUSSION

Results from this research show that *Enaphalodes rufulus* readily can be distinguished from all other cerambycids tested by PCR-RFLP and either of the two restriction enzymes, *Alu* I or *Hind* III. The ability to digest the PCR amplicon by either enzyme could be important for future studies where use of only one enzyme is possible.

PCR-RFLP is a well established means for species diagnostics of many organisms (Taylor & Szalanski 1999; Slade et al. 1993; Sperling et al. 1994; Roehrdanz 1997; Szalanski et al. 1997; Harrington and Wingfield 1995; Taylor et al. 1996). This method of identification requires less equipment and is less expensive than other practiced methods. It is also easy to repeat and can be used not only for diagnostics but also for phylogenetic analyses (Taylor & Szalanski 1999).
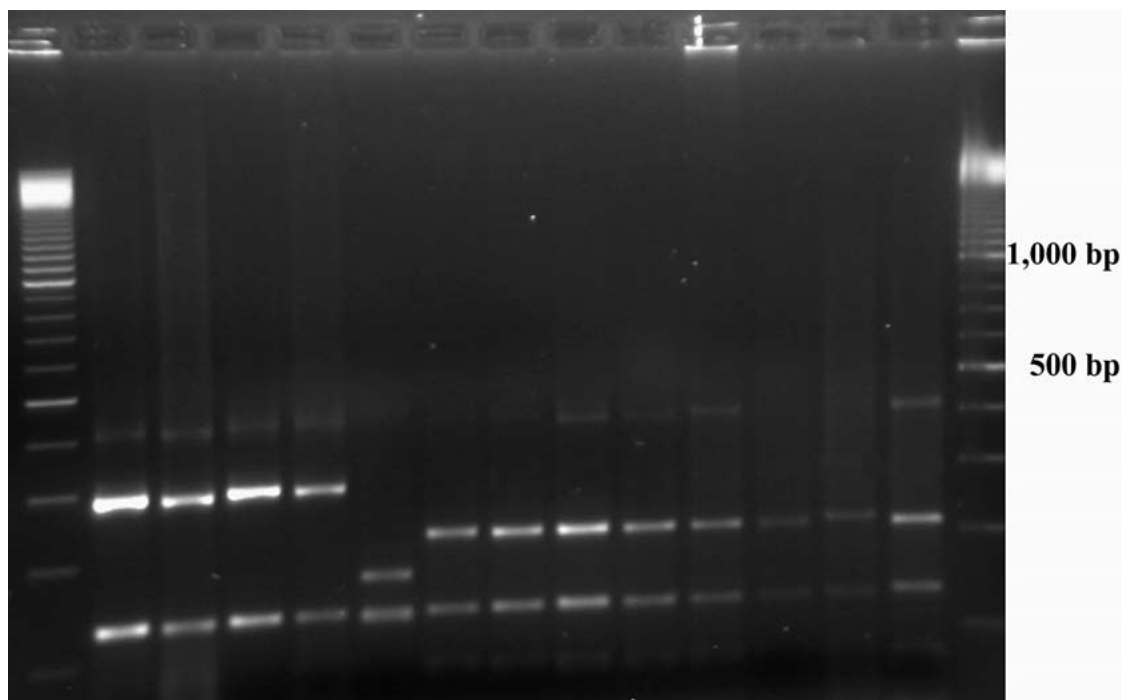

Fig. 1. *Alu* I digest of the 16S amplicon for 4 *Enaphalodes rufulus* (lanes 2-5), *Enaphalodes atomarius*, *Orthosoma brunneum*, *Purpuricenus humeralis*, *Prionus imbricornis*, *Elaphidion mucronatum*, *Eburia quadrigeminata*, *Urographis fasciatus*, *Neoclytus a. acuminatus*, and *Aegomorphus morrisii* on a 2% Agarose gel. The top band is undigested PCR product.
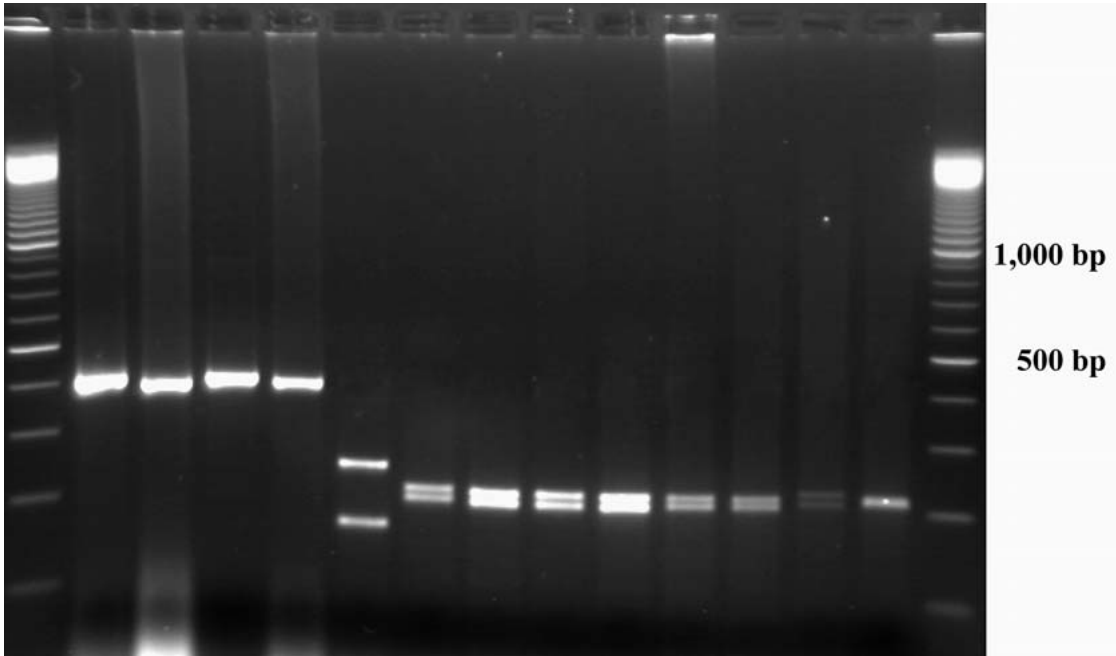
Fig. 2. *Hind* III digest of the 16S amplicon for 4 *Enaphalodes rufulus* (lanes 2-5), *Enaphalodes atomarius*, *Orthosoma brunneum*, *Purpuricenus humeralis*, *Prionus imbricornis*, *Elaphidion mucronatum*, *Eburia quadrigeminata*, *Urographis fasciatus*, *Neoclytus a. acuminatus*, and *Aegomorphus morrisii* on a 2% Agarose gel.

DNA extraction from dried adult legs or thoraxes worked well with Qiagen DNeasy extraction kit and PCR-RFLP. Extracting DNA from pinned, dried adult cerambycids could prove useful in future studies where genetic information may need to be extracted from stored museum specimens. Adults were used to create this procedure as they easily are identified by morphological characteristics, and positive identification was necessary for sequence comparisons.

Morphological identification of early instar cerambycid larvae is difficult if not impossible. PCR-RFLP is an established diagnostic tool for larval identification and easily could be used to distinguish morphologically similar, yet genetically distinct species of cerambycids that have similar life history strategies. This should prove useful in detecting *E. rufulus* as a contributing factor in other oak mortality events especially during the larval stage in which they spend about 90% of their two-year life cycle. Early detection of *E. rufulus* in other oak decline events should help foresters or landowners make informed management decisions in regard to harvest options and/or silvicultural remediation.

White oak mortality is prevalent throughout the Ozark Mountains in Arkansas. White oaks can harbor several species of cerambycids including *E. rufulus* and white oak borer, *G. tigrinus*. An unknown larva and pupa from a white oak tree, which may have been *G. tigrinus*, were tested to confirm the validity of this diagnostic technique and to show that this procedure works with larvae and pupae of species other than *E. rufulus*. The resulting larval and pupal fragments were similar to those of all other cerambycids tested, except *E. rufulus* and *E. atomarius*. A molecular diagnostic procedure possibly could be created for other cerambycids from the Ozarks. This would clarify to what extent *G. tigrinus* or other cerambycids are contributing to white oak mortality, and may offer insight into the prevalence of other immature cerambycids in economically important oak trees.

It was difficult to obtain samples from areas other than northern Arkansas as *E. rufulus* is normally at low population levels. However, this study provides a good foundation for *E. rufulus* identification with PCR-RFLP and our results can be expanded as specimens are collected from other regions of the eastern U.S.

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AUGMENTATIVE APPLICATIONS OF *STEINERNEMA SCAPTERISCI* (NEMATODA: STEINERNEMATIDAE) FOR MOLE CRICKET (ORTHOPTERA: GRYLLOTALPIDAE) CONTROL ON GOLF COURSES

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Abstract

The insect parasitic nematode, *Steinernema scapterisci* Nguyen and Smart, is a non-chemical alternative to pest mole cricket control in the southern United States. These ambush nematodes can become established after one application and spread into untreated areas through host movement in the soil. However, the nematode's persistence from previous inoculative applications in 1988 and 1989 and the effectiveness of subsequent augmentative applications on intensively managed golf courses were unknown. In 2001, two linear pitfall traps were placed in the roughs of 10 holes on each of two golf courses (20 traps per course) near areas of adult mole cricket activity, and half of the plots with traps were treated with *S. scapterisci*. Ten to 15% of mole crickets trapped before the augmentative nematode applications were infected by *S. scapterisci*. After this application, the percentage of infected mole crickets was higher than the baseline for 8 mo at one golf course and 17 mo at the other. The percentage of mole crickets infected on treated plots equaled or exceeded pretreatment levels about 4-8 wk post-application. The percentage of infected mole crickets in untreated areas at both sites equaled the percent infection in treated areas after about 5 mo. Mole cricket trap catches and percent of infection declined in the second year, but continued to fluctuate with mole cricket population density, age, and environmental conditions. Augmentative applications of *S. scapterisci* for pest mole cricket control can enhance mole cricket mortality on golf courses.

Key Words: insect parasitic nematodes, turfgrass, integrated pest management, augmentative biological control, predatory arthropods

Resumen

El nematodo entoparasitico, *Steinernema scapterisci* Nguyen and Smart, es una de las alternativas no químicas para el control de grillo topos en el sur de los Estados Unidos. Estos nematodos cazadores pueden establecerse después de una aplicación y moverse a áreas sin tratamiento con ayuda del huésped en el suelo. Sin embargo, la persistencia del nematodo debido a aplicaciones inoculativas entre 1988 y 1989, y la afectividad de aplicaciones aumentativas en campos de golf que han sido intensamente manejados, es desconocida. En 2001, dos trampas de caída se colocaron en las áreas periféricas de 10 hoyos en cada uno de dos campos de golf (20 trampas en cada campo) cerca de los focos de grillo topos, la mitad de los lotes con trampas fueron tratados con *S. scapterisci*. Después de las aplicaciones, el porcentaje de grillo topos infectados por el nematodo fue mayor que la línea base después de 8 meses en un campo y 17 meses en el otro. El porcentaje de grillo topos infectados igualaron o sobrepasaron los niveles del pretratamiento entre 4 y 8 semanas después de la aplicación. El porcentaje de grillo topos infectados en áreas sin tratamiento igualaron el porcentaje de infección de los lugares con tratamiento 5 meses después del inicio del experimento. El número de grillos capturados y el porcentaje de infección se redujeron en el segundo año, pero continuaron fluctuando con la población, edad y condiciones ambientales en las que se encontraban los grillo topos. Aplicaciones aumentativas de *S. scapterisci* para el control de grillo topos pueden incrementar los niveles de mortalidad en campos de golf.

Translation provided by the authors.

Pest mole crickets (*Scapteriscus* spp.) have been the targets of a classical biological control program during the past 25 years (Frank & Parkman 1999). Three species, including the tawny (*S. vicinus* Scudder), southern (*S. borellii* Giglio-Tos), and shortwinged (*S. abbreviatus* Scudder) mole crickets, were inadvertently brought without their natural enemies to the southern U.S. from South America via ship ballast in the early 1900s (Walker 1985). They became established and damaging in pastures and managed turfgrass areas, such as golf courses, athletic fields,

and home lawns. Their root-feeding and tunneling in the soil kills patches of turfgrass, which significantly reduces turfgrass quality and aesthetics. The importation, rearing, subsequent release, and dispersal of three natural enemies (*Larra bicolor* F., *Ormia depleta* (Wiedemann), *Steinernema scapterisci* Nguyen and Smart) has helped suppress mole cricket populations, but insecticides are still frequently used to provide control.

The insect parasitic nematode *Steinernema scapterisci* was discovered in Uruguay in the early 1980s, cultured in the U.S., and released in Florida in 1985 (Parkman et al. 1993b). This nematode infects older mole cricket nymphs and adults and recycles within the soil environment. The nematode, once inside the mole cricket host, releases *Xenorhabdus innexi* bacterium into the hemolymph (Lengyel et al. 2005). The bacterium reproduces and kills the mole cricket through septicemia, producing a nutrient rich bacterial soup that the nematodes consume. The infective juvenile nematodes then exit the body and infect other mole crickets in the soil (Nguyen & Smart 1991). *Steinernema scapterisci* (Nematac S®, Becker Underwood, Ames, IA) can be used in inoculative releases for mole cricket control (Parkman et al. 1994). Other insect parasitic nematodes (e.g., *Steinernema carpocapsae* Weiser; *S. feltiae* (Filipjev); *S. riobrave* Cabanillas, Poinar and Raulston; and *Heterorhabditis bacteriophora* Poinar) used against pest mole crickets have a broader host range and may infect non-target organisms.

Various methods to optimize the survival and establishment of insect parasitic nematodes have been tested. For example, commercial formulations may be sprayed onto turfgrass (Parkman et al. 1993a,b, 1994), chiseled, injected, or buried into the ground (Parkman et al. 1993b; Adjei et al. 2003), or target pests may be trapped into containers, treated with nematodes, and then released (Parkman & Frank 1992). To enhance the establishment of *S. scapterisci*, adult mole crickets can be attracted to a treated area with synthetic, electronic male mole cricket songs (Parkman & Frank 1992). Application timing is limited to when large nymphs and adults are present and actively tunneling through the soil, primarily late August to late October and March to May in the southern U.S. Usually only one application of *S. scapterisci* is necessary to successfully establish and recover *S. scapterisci* populations on a site (Parkman et al. 1993b), but annual applications are recommended (Lombardo et al. 1999).

Because nematode applications may be more expensive and labor-intensive than insecticide applications (Lombardo et al. 1999), spot treatments of nematodes may be more economical. Treating smaller areas of mole cricket damage should reduce costs, and it takes advantage of mole cricket behavior. Adult mole crickets infected with *S. scapterisci* can fly several kilometers before dying

(Walker 1985), thus spreading nematodes to uninfected sites. *Steinernema scapterisci* can also live in moist soil and survive without a host for at least 10 wk (Nguyen & Smart 1990), which increases its value in areas of low mole cricket density. The goals of this study were to assess the baseline level of mole cricket infection from a previous inoculative application and determine whether subsequent augmentative applications could increase mole cricket infection rates over time.

MATERIALS AND METHODS

Study Sites

The establishment and spread of *S. scapterisci* was monitored on two golf courses in Alachua Co., FL: Ironwood Golf Course and Gainesville Golf and Country Club. Ironwood Golf Course (IGC) was an 18-hole city-owned public golf course built in 1964. The roughs were bermudagrass (*Cynodon dactylon* Pers. × *C. transvaalensis* Burtt-Davy) var. Tifway, mowed at 4.7 cm. Gainesville Golf and Country Club (GGCC) was an 18-hole private course located 16.6 km from Ironwood Golf Course. Gainesville Golf and Country Club was built in 1962 and originally planted with bermudagrass var. Ormond and the roughs were mowed at 3.2 cm. Ironwood Golf Course and GGCC had been previously treated with *S. scapterisci* in the late-1980s and did not have any subsequent treatments. Soil texture was sandy loam on both golf courses. Pesticides were not applied to these plots during the study.

Treatment Application

Nematodes (1 billion/378.5 L of water) were applied in an aqueous suspension with a boom sprayer calibrated at 0.5 L/m² at Ironwood Golf Course on 31 October 2001 at ~1600 h and at Gainesville Golf and Country Club on 5 November 2001 at ~0700 h. The weather at application at GGCC was cloudy, 20°C air temperature, 64-72% relative humidity, 4.3 km/h, and 21°C soil temperature (10.2 cm depth). The weather during application at IGC was partly cloudy, 28°C air temperature, 48-55% relative humidity, 0-2.5 km/h, and 20°C soil temperature (10.2 cm depth). All treated plots were irrigated with 0.6 cm of water before and 0.6 cm after application.

Pitfall Trap Sampling

Two areas of mole cricket damage were located in the roughs adjacent to ten fairways on each golf course in September 2001. One area (20.1 × 20.1 m or 0.04 ha) was randomly assigned the nematode treatment and the other was the paired untreated control. A linear pitfall trap (modified from Lawrence 1982) was placed near the two areas per

hole, at least 80 m apart in September and early October 2001 (20 areas per golf course), before nematodes were applied. A 19-L plastic bucket was buried in the center with the top flush with the soil surface, and a 3.8-L bucket containing 3-5 cm deep sand was placed inside. Both buckets had water drainage holes. Four PVC pipes (3 m long, 7.6 cm diameter) were installed at right angles to the center of the bucket, with a 2.5-cm slit lengthwise along the top that was also flush with the soil surface. Each distal pipe end was capped. Before sampling, traps were cleaned, fresh sifted sand was added to the 3.8-L bucket, and all surface-active arthropods that had fallen into the 3.8-L bucket were collected 24 h later (Parkman et al. 1993a,b).

Pretreatment samples were collected from all 20 traps on 11, 18, and 25 October 2001. Samples were then collected weekly for 6 wk post-application, and once or twice a month thereafter for 1 year on Gainesville Golf and Country Club and for 2 years on Ironwood Golf Course. Traps were removed from GGCC after 1 year at the request of the golf course superintendent. Adult and juvenile mole crickets with pronotal lengths >4 mm (Hudson & Nguyen 1989a) were tested for nematode infection in the laboratory. Mole crickets were placed individually in 20-mL plastic scintillation vials (Fisher Scientific) with 1-2 drops of deionized water at an air temperature of 23°C

and 12:12 L:D. Mole crickets were examined 7 and 10 d after death under a dissecting microscope (10×) for the presence of nematodes. *Steinernema scapterisci* were identified by Dr. Khuong Nguyen, Entomology and Nematology Department, University of Florida. Potential natural enemies caught in the traps also were identified.

Statistical Analysis

Comparisons of percent infection between sites and years were subjected to analysis of variance and Tukey's studentized range test or Student's *t*-test (SAS Institute 2001). All comparisons were made at 0.05 significance level. Nontransformed means plus or minus one standard error of the monthly mean are presented.

RESULTS AND DISCUSSION

Spot treatments of *S. scapterisci* successfully increased the percentage of mole crickets infected in bermudagrass roughs within 4-8 wk after application at both golf courses, compared to pretreatment levels. The increased percentage of infection lasted for 17 months at IGC and 8 months at GGCC (Figs. 1 and 2). Likely because of mole cricket movement over time, infection rates in untreated plots (>80 m from treated areas) equaled
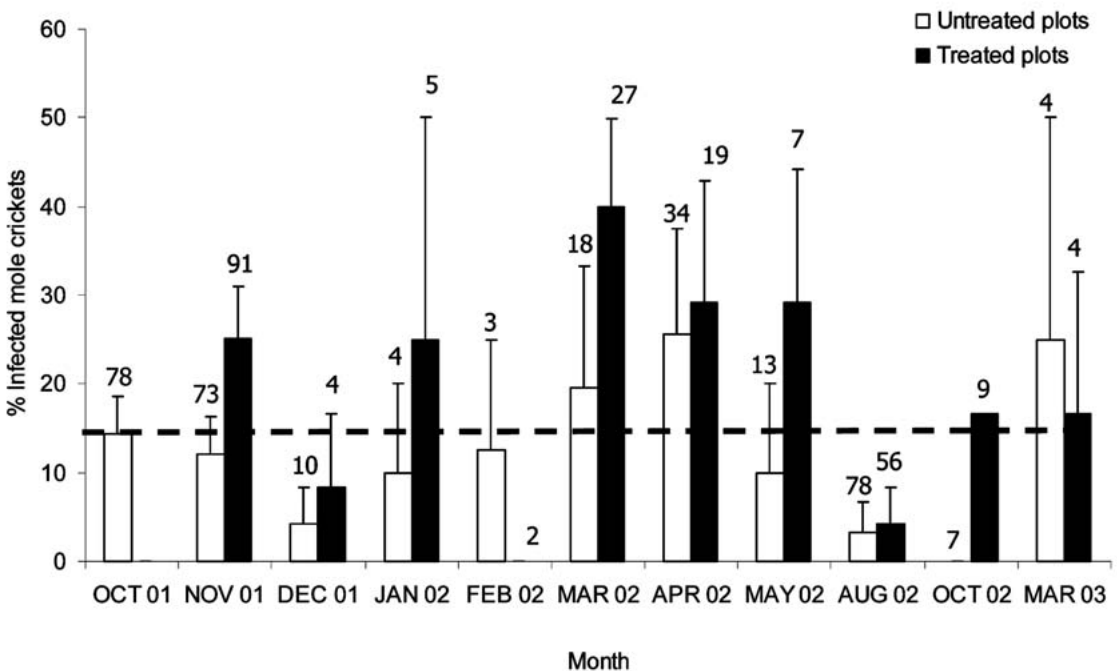

Fig. 1. Mean monthly (± SEM) percent infection of mole crickets collected in pitfall traps at Ironwood Golf Course from areas treated with *Steinernema scapterisci*. Only months with infection levels are presented. Untreated areas received no *S. scapterisci* and were >80 m from treated areas. Dashed line represents baseline pretreatment infection level. Data presented are for *Scapteriscus vicinus* and *Scapteriscus borellii* combined. Total numbers of mole crickets collected are presented above SEM bars.
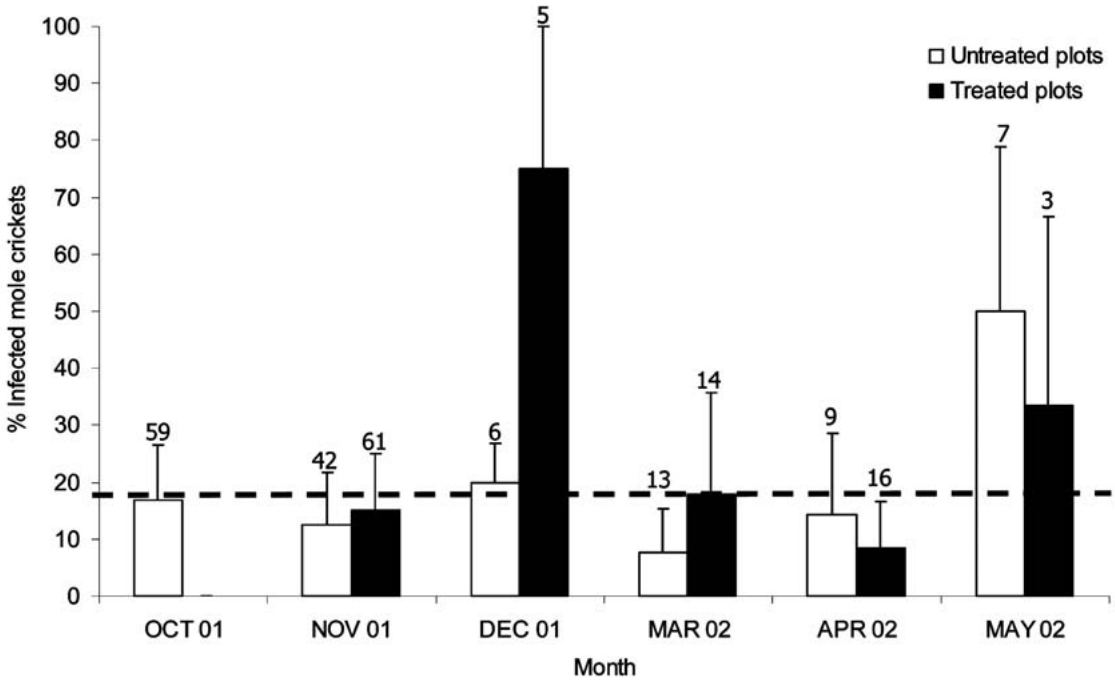
Fig. 2. Mean monthly (± SEM) percent infection of mole crickets collected in pitfall traps at Gainesville Golf and Country Club from areas treated with *Steinernema scapterisci*. Only months with infection levels <0 are presented. Untreated areas received no *S. scapterisci* and were >80 m from treated areas. Dashed line represents baseline infection level. Data presented are for *Scapteriscus vicinus* and *Scapteriscus borellii* combined. Total numbers of mole crickets collected are presented above SEM bars.

the percent infection in treated plots after ~5 months. Turfgrass density ratings (0-9 scale), which indicate the relative amount of damaged turfgrass, did not differ among nematode-treated and control plots in this test at each sampling time (Barbara 2005), and are thus not reliable indicators of nematode establishment.

Nematode infection levels seemed to fluctuate primarily with mole cricket population density, age, and environmental conditions over time (15-40% of mole crickets infected at IGC, 15-75% at GGCC). However, the mean cumulative percentages (± SE) of infection for mole cricket trap collections from 2001 to 2003 from the sites GGCC (22.1 ± 10.5%) and IGC (15.8 ± 4.6%) did not differ ($t = 2.00$; $df = 2, 56$; $P > 0.05$). Fewer mole crickets were collected in year 2 than in year 1 at IGC ($t = 2.47$; $df = 1, 37$; $P < 0.01$). Although *S. scapterisci* persisted throughout the study period, the percent infection also decreased in year 2 at IGC compared to year 1 ($t = 6.63$; $df = 1, 37$; $P < 0.01$). Mole crickets that were large enough to be vulnerable to *S. scapterisci* infection were present from late August to April, but cooler winter temperatures likely reduced mole cricket activity and possibly nematode infectivity (Molyneux 1985) from December to February. Similar to Parkman et al. (1994), we found more mole cricket adults infected

with *S. scapterisci* than nymphs. But, contrary to Parkman et al. (1994), more *S. vicinus* were collected in traps and infected than *S. borellii* (Table 1). Other factors that may have influenced mole cricket numbers, nematode infectivity, and/or sur-

TABLE 1. MEAN PERCENTAGE (± SEM) OF MOLE CRICKETS INFECTED BY *STEINERNEMA SCAPTERISCI* ON TWO GOLF COURSES IN GAINESVILLE, FL.

	Mean % of infected mole crickets (total # crickets collected)	
	GGCC[1]	IGC[2]
S. borellii		
Nymphs	0 (1)	0 (15)
Adults	0 (1)	3.3 ± 2.6 (20)
Total	0 (2)*	1.6 ± 1.3 (35)*
S. vicinus		
Nymphs	7.7 ± 4.0 (70)	9.7 ± 3.4 (208)*
Adults	13.9 ± 4.5 (163)	33.2 ± 7.0 (313)
Total	10.8 ± 3.0 (233)	11.9 ± 2.5 (521)

Pairs of means within columns followed by asterisks are different, *t*-test ($P > 0.05$).
[1]GGCC: $F = 8.57$; $df = 2,111$; $P = 0.0003$.
[2]IGC: $F = 14.05$; $df = 2,155$; $P < 0.0001$.

vival include pesticide use (Barbara & Buss 2005), exposure to ultraviolet light (Gaugler & Boush 1978), soil saturation after excessive rain or irrigation (Molyneux & Bedding 1984; Hudson & Nguyen 1989b), infection by or competition with other nematodes (i.e., *Heterorhabditis* spp., *Steinernema* spp.), infection by fungi (i.e., *Beauvaria bassiana*), microbial consumption of the nematodes, parasitism by *Larra bicolor* F. or *Ormia depleta* (Wiedemann), or predation. Over 8,400 predaceous arthropods were collected in the pitfall traps with the mole crickets (Table 2), and the most abundant families included Carabidae (>16.6%), Formicidae (>19.9%), and Staphylinidae (>24.8%). However, earwigs were specifically observed attacking mole crickets in the traps. Fluctuations in predator and parasitoid abundance with mole cricket numbers were not examined.

Steinernema scapterisci may become established after one application to turfgrass, but this is the first known study to demonstrate that augmentative spot treatments can also increase the percent of mole crickets infected by a nematode. Given the background infection levels on both golf courses, it is possible that *S. scapterisci* popula-

tions can either persist on intensively managed golf courses for ≥13 years (initial applications had been made in 1988 and 1989) (Parkman et al. 1994), and/or infected mole crickets may reintroduce nematodes periodically during mating flights from other areas. Because infected or septic mole crickets might not be as mobile as healthy mole crickets, and thus not fall into traps and be detected, the reported percentage of mole cricket infection may be an underestimate. It is possible that *S. scapterisci* can synchronously cycle with mole cricket density over time, since it can reproduce in its host. These nematodes can also kill adult mole crickets before they lay all of their egg clutches (Barbara 2005), which reduces the potential population size of the next generation. Insect parasitic nematodes are viable, environmentally-friendly alternatives to insecticides for long-term mole cricket management.

TABLE 2. RELATIVE ABUNDANCE OF PREDATORY ARTHROPODS IN TWO GOLF COURSE BERMUDAGRASS HABITATS, GAINESVILLE, FL 2001-2002.

Taxon	No. per site		% of total[1]	
	IGC	GGCC	IGC	GGCC
Arachnida	403	265	9.5	6.4
Chilopoda	9	8	0.2	0.2
Insecta				
Coleoptera				
Carabidae	927	690	21.8	16.6
Coccinellidae	2	2	0.1	0.1
Histeridae	69	180	1.6	4.3
Lampyridae	1	0	<0.1	0
Phengodidae	1	0	<0.1	0
Staphylinidae	1,056	2,031	24.8	48.9
Dermaptera				
Carcinophoridae	4	14	0.1	0.3
Forficulidae	0	1	0	<0.1
Labiduridae	98	114	2.3	2.7
Hemiptera				
Anthocoridae	1	3	<0.1	0.1
Geocoridae	2	0	0.1	0
Nabidae	29	4	0.7	0.1
Reduviidae	14	13	0.3	0.3
Hymenoptera				
Formicidae	1,646	825	38.6	19.9
Total	4,262	4,150	100.0	100.0

[1]Values represent percent of total collection at each site.

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REARING *DIOMUS TERMINATUS* (COLEOPTERA: COCCINELLIDAE) ON THE CORN LEAF APHID, *RHOPALOSIPHUM MAIDIS* (HOMOPTERA: APHIDIDAE)

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Diomus terminatus (Say) (Coleoptera: Coccinellidae), a native species, has demonstrated potential as an augmentation biological control agent for pest aphids (White et al. 2001). It occurs from Texas to Vermont, including the entire state of Florida (Gordon 1976), and also has been found in Bermuda (Hilburn & Gordon, 1989). *Diomus terminatus* survives in a wide range of habitats, feeds on a variety of aphids and, because it is native species, poses far less environmental risk than exotic natural enemies (Lenteren et al. 2004). Hall (2001) and Hentz and Nuessly (2002) raised *D. terminatus* on the yellow sugarcane aphid, *Sipha flava* (Forbes), and (Osborne, unpublished) raised it on the cotton aphid, *Aphis gossypii* Glover, and green peach aphid, *Myzus persicae* (Sulzer). Our goal was to rear *D. terminatus* on the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) and standardize the associated rearing methods.

Sorghum (*Sorghum bicolor* var. hybrid grain sorghum SS800) was grown during the summer in a greenhouse maintained at 23 ± 6°C and 70% RH with ambient light. It was used 4-6 weeks after planting when it attained a height of at least 40 cm. The corn leaf aphid colony was maintained on the sorghum in three Florida Reach-In® growth chambers (Walker et al. 1993) at the University of Florida, Entomology and Nematology Department. The chambers were programmed to maintain a constant temperature of 22°C, 50% RH, and an 18 h photophase (16 h full light, 1399 Lux, followed by 2 h reduced light, 334 Lux) and 6 h scotophase. Two 20-watt white fluorescent lamps (Sylvania® Cool White) were placed in the back of each growth chamber behind a Plexiglas® panel and supplemented with two 20-watt white fluorescent lamps (Sylvania® Cool White) and two 20-watt blue/white fluorescent lamps (Sylvania® Gro-Lux Aquarium) overhead. Inoculation with aphids was accomplished by inserting aphid-infested leaves directly into the whorls of the plants. This succulent part of the plants stimulated rapid aphid reproduction. A colony could be used 1-3 weeks after inoculation.

The *D. terminatus* colony was maintained in the growth chambers. For each generation, 30 adults were established per plastic container (21 cm diam × 7.5 cm high) (Pioneer Packaging, Dixon, KY). The beetles were not sexed due to their minute size. Each container was fitted with two (4 cm diam) or three (3 cm diam) ventilation holes in the lid. Tape was used to open or close the screened ventilation holes and maintain 70-90% RH. Low humidity could be raised by misting the lids of the containers with water or by providing moist cotton balls over the holes. Each container was fitted with a rack made of 1-cm mesh hardware cloth folded so that it stood approximately 4 cm above the bottom. Two rolled laboratory tissues (38.1 × 42.6 cm) (Kimwipes® Ex-L, Kimberly-Clark, Roswell, GA) were placed under each rack to absorb excess moisture and provide pupation sites. Several wax paper strips (22 cm x 2 cm wide) were provided to separate females and serve as an extra oviposition substrate. The beetles were fed by placing aphid-infested sorghum leaves directly on the hardware cloth rack. After five days, the adults were removed from the container and the remaining leaves, Kimwipes®, and wax paper were held until larvae emerged. For routine rearing, about five aphids were provided per beetle larva in the first and second instars, and seven in the third and fourth. By day 10, most of the larvae pupated on the Kimwipes®, with only a few on the desiccated leaves or the lids of the rearing containers. The adults that began to emerge were collected by aspiration. The rearing procedure is summarized in Fig. 1.

The preovipositon period of *D. terminatus* fed corn leaf aphid nymphs was determined by removing pupae from colony cages on a single day and collecting the emerging adults within a 24-h period. Five beetles of undetermined sex were placed in each of 12 containers (10 cm wide × 10 cm long × 7.5 cm high). Each container was supplied with 10 aphids per beetle per day, a drop of water, and two strips of wax paper (10 cm x 2 cm) before being sealed with Parafilm®. The wax paper, leaves, and containers were thoroughly examined daily for eggs, and fresh aphids and water were added.

To determine the effect of adult nutrition on fecundity, *D. terminatus* pupae were collected from the colony and emerging adults were given an excess of corn leaf aphids on sorghum leaves for 10 d. This was sufficient time for mating and pre-ovi-
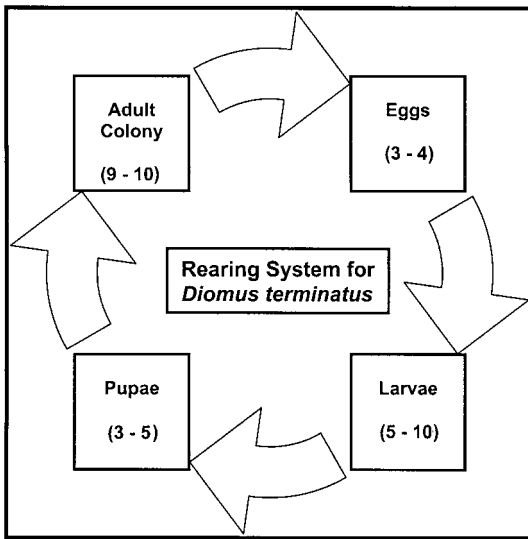
Fig. 1. Diagram of the standardized rearing system for *Diomus terminatus*. The adult colony is replenished by placing newly emerged beetles into mating/oviposition containers. The pre-oviposition period is 4-5 d and the females are held to produce eggs for 5 d. Eggs are collected daily and held for emergence of larvae in 3-4 d. Larvae are transferred to rearing containers and fed fresh corn leaf aphids daily until they pupate in 5-10 d. Pupae mature in 3-5 d and the adults emerge to complete the cycle.

position. Thereafter, individual beetles were isolated in Petri dishes (5 cm × 0.9 cm) and provided daily with 1, 4-6 or 7-10 large corn leaf aphid nymphs, a drop of water, and a small strip of wax paper (4 cm × 1 cm) for oviposition. The numbers of eggs deposited were recorded daily for 20 d. Every 3 d, the beetles were transferred to new Petri dishes because of the accumulation of frass. Comparisons were made of the number of eggs deposited/female/d with the General Linear Model (GLM) and LS means procedures in SAS (The SAS System for Windows v8, 2001).

These methods for rearing *D. terminatus* on corn leaf aphids produced results similar to those previously obtained with yellow sugarcane aphids (Hall 2001; Hentz & Nuessly 2002), and cotton aphids and green peach aphids (Osborne unpublished). Beetles held in large rearing containers and fed corn leaf aphids on sorghum leaves oviposited in 4 d. Females oviposited single eggs (0.68±0.001 mm long by 0.37±0.001 mm wide) mainly on the leaves but also on the wax paper and Kimwipes®.

The survival and reproduction of *D. terminatus* can be optimized by providing an abundance of aphids and adequate space. When provided with one aphid, only 53% of the females oviposited, averaging 0.5 ± 0.1 egg/15 females/d during the 5 d that the adult cages were maintained. After feed-

ing on 4-6 or 7-10 aphids, 100% of the females oviposited and produced 7.8 ± 0.5 and 14.1 ± 1.0 eggs/15 females/d, respectively. When Hall (2001) held 10 beetles in individual large tubes (15 cm × 2.2 cm), fecundity averaged 1.4 eggs/female/d. We maintained the beetles individually in Petri dishes (5 cm × 0.9 cm) and recovered about 1 egg/female/d. Osborne (unpublished) determined that each female produced a total of 86 eggs when fed cotton aphids and 37 on a diet of green peach aphids. Average daily and total consumption rates, respectively, were 13.5 and 425.5 cotton aphids, and 8.7 and 243.3 green peach aphids.

The results of *D. terminatus* rearing have been highly variable regardless of prey aphid or handling methods. When *D. terminatus* was fed yellow sugarcane aphids, both Hall (2001) and Hentz & Nuessly (2002) reported 3-4 d for embryogenesis and 4-5 d as pupae but different times for larval development; 10 versus 5 d, respectively. According to Hall (2001), *D. terminatus* females that consumed 5-10 yellow sugarcane aphids lived for 17-18 d, and produced a total of 43 eggs. Osborne (unpublished) determined that development of the egg, larva and pupa averaged 6.3, 9.4, and 6.4 d, respectively, on cotton aphids and 6.2, 7.4, and 4.1 d on yellow sugarcane aphids. In our rearing system, eggs were held for 3-4 d for larval eclosion, larvae developed in 5-10 d, and adults emerged from pupae within 3-5 d and lived for 30 d. The average egg to adult survival of 38.8 ± 0.1% (range of 7-93%) was consistent with the 39.4% reported by Hall (2001). Our *D. terminatus* colony was maintained on corn leaf aphids for 10 generations.

SUMMARY

A standardized tritrophic rearing system was developed for *Diomus terminatus* (Say) (Coleoptera: Coccinellidae) based on the sorghum-raised corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae). With 30 beetles per adult container and an ample supply of aphids, *D. terminatus* females had a minimum 4-d pre-oviposition period and 100% produced eggs. After oviposition began, the beetles were maintained for 5 d and each container yielded an average of 14.1 eggs/d. Survival from egg to adult averaged 39%, yielding 20-40 beetles per adult container. Presentation of aphids on sorghum leaves and large rearing containers supported continuous production of *D. terminatus* for 10 generations.

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ANTS (HYMENOPTERA: FORMICIDAE) IN WET LONGLEAF PINE SAVANNAS IN LOUISIANA

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Longleaf pine savannas once dominated the Southeastern landscape and are now among the most threatened ecosystems in the United States (Noss et al. 1995). Remaining communities form a patchwork of disconnected sites. Little is known about invertebrates in these communities (Folkerts et al. 1993). The goal of our study was to describe the ant fauna of wet longleaf pine savannas, compiled from two independent surveys, at the western edge of the East Gulf Coastal Plain.

Between 1997 and 2001, we surveyed ants in two wet savannas in the early stages of restoration in Southeastern Louisiana. The two savannas were being restored from dissimilar starting community types. One, Abita Creek Preserve (Abita), was a dense slash pine flatwoods that was converted to an open pine savanna with clearcutting and prescribed fire. The other, Lake Ramsay Preserve (Ramsay), was a relatively open longleaf pine savanna restored by prescribed fire. The two preserves are approximately 30 km apart.

Abita in St. Tammany Parish, Louisiana (30°30'N, 89°58'W) contains 338 ha. In 1998, slash pines were removed from sections of the preserve and the first prescribed fire was applied in the winter of 1997-98. Abita was burned again in May 2000. Ants were collected here with a combined flight intercept (FIT) and malaise trap (MT) (J.C. Hock Co., www.johnwhockco.com) and by baiting. Six FIT/MT traps were divided between clearcut (open) and closed canopy (wooded) sites. Traps were run for one week per month beginning in May 1999 and ending in April 2001 for a total of 19 months. No trapping was done in January or February. Baiting was done in July 2000 by placing 50 baited vials (60-ml plastic hinged-top) 2 m apart in a transect near each of the six FIT/MT traps for a total of 300 vials. Vials were baited with honey and Spam and left open for approximately 30 min.

Ramsay in St. Tammany Parish, Louisiana (30° 30' N, 90° 10' W) contains 526 ha. Because Ramsay has a history of wildfires the ground cover is rich in native species. The last wildfire was thought to have occurred in 1988. During our study, all sites within Ramsay received prescribed fire in August 1997 and some sites were burned again in August 1998. Ants were collected at Ramsay with pitfall traps, FITs, and by baiting. Pitfall traps (n = 144) consisted of two 100-ml round centrifuge tubes paired by a metal barrier. Each pitfall tube with collecting preservative was covered by a square of metal flashing held above tubes by nails. Sampling was conducted twice per month for 48 h each time. Sampling began during July 1996 and ended during August 1999 and resulted in 26 months of collecting. No sampling was done in four winter months of December-March. Twelve FITs were set up one week per month for 17 months starting in September 1997 continuing to August 1999 as above. Vials baited with peanut butter or honey were alternately placed 10 m apart (n = 240) along the same transects as pitfall traps for 1 h. We did this once per month for the 26 months pitfalls were run.

We collected 48 species of ants and 374,568 individuals in 5 subfamilies and 23 genera in the two wet savannas combined (Table 1). The number of ant species collected by all methods was similar at both sites with 38 species at Abita and 41 at Ramsay. Thirty-one species occurred at both locations. Sorenson's measure of species overlap between locations was high at 79%. Twenty-one species were newly reported for Louisiana (Table 1; Colby 2002; Dash 2004). Westward range extensions were detected for 4 native eastern or southeastern species (Table 1). No eastern extensions for western species were found.

We detected a large exotic component in the ant fauna. Seven or 15% of ant species collected were exotic (Table 1). Pooling across sites and collection methods, 98.5% of the ants collected were exotic when *S. invicta* was included and 43% were exotic when *S. invicta* was excluded. Baiting indicated extreme dominance by fire ants in open, grassy sites.

The majority of native ants collected were common, widespread species found throughout the southeastern region. Five species primarily associated with pine habitats are *Camponotus nearcticus* Emery, *Crematogaster ashmeadi* Mayr, *Cr. pilosa* Emery, *Pheidole dentigula* M. R. Smith, and *Temnothorax bradleyi* (Wheeler) (Colby 2002 and Dash 2004 and references therein). Nine species have been reported from more open, grassy habitats and may be typical residents of savannas. These include *Camponotus castaneus* (Latreille), *Camponotus impressus* (Roger), *Formica pallidefulva* Latreille, *Monomorium viride* Brown, *Pheidole dentata* Mayr, *Polyergus lucidus* Mayr, *Pseudomyrmex pallidus* F. Smith, *Temnothorax pergandei* (Emery), and *Trachymyrmex septentrionalis* (McCook) (see Colby 2002 and Dash 2004 and references therein). These subsets likely contain the best target or indicator species for management and restoration in this habitat. *Polyergus lucidus* is listed on the ICUN Red List as vulnerable because it occurs in small populations. Its presence at Ramsay is noteworthy.

TABLE 1. SPECIES AND ABUNDANCES OF ANTS COLLECTED AT ABITA AND RAMSAY BY TRAPPING METHOD.

Species[1]	Abita		Ramsay			Totals
	FIT/MT	Baits	FIT	Baits	Pitfalls	
Aphaenogaster carolinensis Wheeler[2]	3	30			2	35
Brachymyrmex depilis Emery	2		8		2	12
***Brachymyrmex musculus* Forel**	82		662	359	121	1,224
Camponotus castaneus (Latreille)	252				9	261
Camponotus nearcticus Emery[2]			2			2
Camponotus pennsylvanicus (DeGeer)	2	9				11
Camponotus impressus (Roger)	2					2
***Cardiocondyla wroughtonii* (Forel)**[2]	2					2
Crematogaster ashmeadi Mayr[2]	203	3	19	15	5	245
Crematogaster missuriensis Emery[2]	1			16		17
Crematogaster pilosa Emery[2,3]	119		36	412	122	689
***Cyphomyrmex rimosus* (Spinola)**	70		101	14	590	775
Formica pallidefulva Latreille[2]			32	92	33	157
Hypoponera opaciceps (Mayr)	21		98		213	332
Hypoponera opacior (Forel)	259		71		30	360
Lasius alienus (Foerster)[2]	8	1				9
Monomorium minimum (Buckley)[2]	5	2		32		39
Monomorium viride Brown[2]					2	2
Myrmecina americana Emery[2]	8		3		26	37
Paratrechina faisonensis (Forel)	114	5	66	1,172	376	1,733
Pheidole dentata Mayr[2]	11	40	4	61	81	197
Pheidole dentigula Smith				106	2	108
***Pheidole flavens* Roger**	30	112	140	1,523	438	2,243
Pheidole metallescens Emery	70	260	1	346	26	703
Polyergus lucidus Mayr[2]					6	6
Ponera pennsylvanica Buckley	8		2		12	22
Proceratium croceum (Roger)	1		2			3
Proceratium pergandei (Emery)	5					5
Proceratium silaceum Roger[2]	2					2
Pseudomyrmex ejectus (Smith)	6		2			8
Pseudomyrmex pallidus (Smith)	3		3	1		7
Pyramica clypeata (Roger)	1		2			3
Pyramica hyalina Bolton[2,3]			2			2
***Pyramica margaritae* (Forel)**	2		6		19	27
***Pyramica membranifera* (Emery)**	23		18		3	44
Pyramica metazytes Bolton[2,3]			2			2
Pyramica reflexa (Wesson & Wesson)[2]			2			2
Pyramica rostrata (Emery)			15			15
Pyramica talpa (Weber)	1		4		7	12
Solenopsis carolinensis Forel[2,3]	67	2	42	173	4	288
***Solenopsis invicta* Buren**	3,285	11,249	1,797	307,650	40,625	364,606
Solenopsis picta Emery	25		164			189
Strumigenys louisianae Roger[2]	24		7		27	58
Tapinoma sessile (Say)[2]	10	22		15		47
Temnothorax bradleyi (Wheeler)			1			1

[1]Exotic species indicated in bold.
[2]New Louisiana Record.
[3]Westward range extension.
[4]Trapping effort was estimated for FIT/MT by number of traps multiplied by number of days traps were open (t.d.), pitfalls for number of pitfall traps multiplied by number of days (p.d.), and for baits by number of bait vials used.

TABLE 1. (CONTINUED) SPECIES AND ABUNDANCES OF ANTS COLLECTED AT ABITA AND RAMSAY BY TRAPPING METHOD.

Species[1]	Abita		Ramsay			Totals
	FIT/MT	Baits	FIT	Baits	Pitfalls	
Temnothorax curvispinosus (Mayr)	2					2
Temnothorax pergandei (Emery)[2]	1		13			14
Trachymyrmex septentrionalis (McCook)	2				6	8
Total Number of Species	38	12	32	16	26	48
Total Number of Individuals	4,721	11,726	3,327	311,987	42,787	374,568
Trapping Effort[4]	399 t.d.	300 baits	1,428 t.d.	5,040 baits	14,976 p.d.	

[1]Exotic species indicated in bold.
[2]New Louisiana Record.
[3]Westward range extension.
[4]Trapping effort was estimated for FIT/MT by number of traps multiplied by number of days traps were open (t.d.), pitfalls for number of pitfall traps multiplied by number of days (p.d.), and for baits by number of bait vials used.

TABLE 2. SEASONALITY OF FEMALE REPRODUCTIVE FLIGHTS BY MONTHS POOLED ACROSS ALL SITES AND YEARS (DATA ARE NUMBERS OF ALATES CAUGHT IN FIT AND FIT/MT TRAPS).

Species[1]	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Totals
Aphaenogaster carolinensis				1			1				2
Brachymyrmex depilis				2		3					5
Brachymyrmex musculus			3	43	109	13	1	3		27	199
Cardiocondyla wroughtonii						2					2
Crematogaster ashmeadi				1							1
Cyphomyrmex rimosus			6	31	79	8	6	2	2		134
Hypoponera opaciceps		3		42	11	3	13	22	4		98
Hypoponera opacior				261	20	24	16	2			323
Lasius alienus				4							4
Myrmecina americana							1	8	1		10
Paratrechina faisonensis	60	8					2				70
Pheidole flavens	1		48	10	14	22	6			8	109
Pheidole metallescens				4	12					6	22
Ponera pennsylvanica							1	6	3		10
Proceratium croceum						1	1	1			3
Proceratium pergandei					5						5
Proceratium silaceum								1	1		2
Pseudomyrmex ejectus				1		2					3
Pseudomyrmex pallidus				3	1	1		1			6
Pyramica clypeata							2	1			3
Pyramica hyalina							2				2
Pyramica margaritae				2	3	1				1	7
Pyramica membranifera				21	11	5	2	1	1		41
Pyramica metazytes							1	1			2
Pyramica reflexa							1	1			2
Pyramica rostrata							13	2			15
Pyramica talpa							1	3	1		5
Solenopsis carolinensis				34	72	3					109
Solenopsis invicta		11	4	1	1						17
Solenopsis picta				107	46	12	2			2	169
Strumigenys louisianae				19	10					2	31
Tapinoma sessile				1		1					2
Temnothorax bradleyi					1						1

[1]Exotic species indicated in bold.

TABLE 2. (CONTINUED) SEASONALITY OF FEMALE REPRODUCTIVE FLIGHTS BY MONTHS POOLED ACROSS ALL SITES AND YEARS (DATA ARE NUMBERS OF ALATES CAUGHT IN FIT AND FIT/MT TRAPS).

Species[1]	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Totals
Temnothorax pergandei				11				1	1		13
Trachymyrmex septentrionalis					1		1				2
Total number of species	2	3	4	20	16	15	19	16	8	6	
Total number of individuals	61	22	61	599	396	101	73	56	14	46	1,429

[1]Exotic species indicated in bold.

Our FIT/MT traps allowed us to obtain rarely reported data on alate seasonality. Alates of most species were collected during warmer months of summer into early fall with a few species flying during all three seasons (Table 2). Alates of nine species were only collected in fall months. These were *Myrmecina americana* Emery, *Ponera pennsylvanica* Buckley, *Proceratium silaceum* Roger, and all but two *Pyramica* species (*P. margaritae* (Forel) and *P. membranifera* (Emery)). The two summer flying *Pyramica* were both exotics. *Paratrechina faisonensis* alates were collected primarily during early spring. Oddly, very few reproductives of the most abundant species, *S. invicta*, were captured in these traps.

SUMMARY

Forty-eighty species of ants were collected from two wet longleaf pine savanna sites in Southeastern Louisiana. Twenty-one were newly recorded species for Louisiana, 4 were westward range extensions, and 7 were exotics. The vast majority of ants captured (97%) were *Solenopsis invicta*.

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THE ESTABLISHMENT OF *ACERATONEUROMYIA INDICA* (HYMENOPTERA: EULOPHIDAE) IN THREE BIOGEOGRAPHICAL REGIONS OF ARGENTINA

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Aceratoneuromyia indica (Silvestri) is a gregarious eulophid parasitoid of tephritid larvae that was originally collected in India (Clausen 1978). Its natural range includes the Indo-Pacific region, but it has been introduced into Italy, South Africa, Australia, Mexico, Caribbean islands, and Central and South America as a biological control agent of various fruit fly species of economic importance (Graham 1991; LaSalle 1994). *Aceratoneuromyia indica* and the opiine *Diachasmimorpha longicaudata* (Ashmead) are the most widely employed exotic parasitoids for inundative releases against *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly, and *Anastrepha* species in the New World (Ovruski et al. 2000). *Aceratoneuromyia indica* was imported into Argentina from Mexico in 1961 and 800,000 were released between 1966 and 1977 in the northwestern provinces of Tucumán and Jujuy, in the northeastern provinces of Misiones and Entre Rios, and in the central province of Córdoba for control of both *Anastrepha fraterculus* (Wiedemann), the South American fruit fly, and *C. capitata* (Turica et al. 1971; Ovruski & Fidalgo 1994). This exotic parasitoid species was recovered immediately following releases in the following sites: Montecarlo (26°33' S, 54°47' W, 200 m) (Misiones province in northeastern Argentina), San Miguel de Tucumán (26°50' S, 65°13' W, 426 m), Quebrada de Lules (26°56' S, 65°21' W, 545 m), El Siambón (26°43' S, 65°27' W, 1,185 m) (Tucumán province), Calilegua (23°47' S, 64°46' W, 465 m) (Jujuy province in northwestern Argentina), Cruz del Eje (30°44' S, 64°49' W, 476 m), and Yacanto (32°03' S, 65°02' W, 1,208 m) (Córdoba province in Central Argentina) (Ovruski et al. 1999). A new introduction of *A. indica* into Argentina was made in 1986. Initial shipments originated in Hawaii, via Costa Rica. Although a laboratory colony was established in Tucumán's Research Center for Regulation of Noxious Organisms (CIRPON), no specimen was field-released (Ovruski et al. 1999). Even though Turica (1968), Nasca (1973), and Fischetti et al. (1978) considered it established,

there was no evidence of permanent establishment in any release locality prior to our data presented here.

Studies on the fruit fly parasitoids of Argentina have been largely neglected. The first major works concentrating on the different biogeographical regions of Argentina were those of Turica (Turica & Mallo 1961; Turica 1968). Recently, intensive fruit fly parasitoid surveys focused in ten localities of Tucumán province, including San Miguel de Tucumán, Quebrada de Lules, and El Siambón (Ovruski et al. 2004), and in four localities of the northwestern province of Salta (Ovruski et al. 2005) only revealed the existence of a native larval-pupal parasitoid guild. However, recent parasitoid surveys made in the provinces of Jujuy, Córdoba, and Misiones recorded the presence of *A. indica*.

Between January and February 1998, and during February 2001, 382 (= 12 kg) and 314 (= 10.3 kg) peaches (*Prunus persica* (L.) Batsch, Rosaceae) were collected in an untreated semicommercial peach orchard administred by the National University of Córdoba located at 31°48' S, 64°22' W and 425 m in Córdoba city (Córdoba province); during February 1999, 408 (= 16.9 kg), 223 (= 14.4 kg), and 387 (= 17.2 kg) guavas (*Psidium guajava* L., Myrtaceae) were collected in patches of disturbed wild vegetation adjacent to citrus orchards throughout the localities of Yuto (23°38' S, 64°28' W, 349 m), Caimancito (23°44' S, 64°36' W, 367 m), and Calilegua (Jujuy province), respectively; during February 2000, 400 (= 16.7 kg) guavas were collected in areas covered with wild vegetation in the locality of Montecarlo (Misiones province). The fruit samples consisted of fallen ripe fruit (60-70%) and ripe fruit still on the tree (30-40%). Peach samples collected in Córdoba city were processed in the Integrated Pest Management Department of the National University of Córdoba. Guavas samples collected in Misiones were processed in the Montecarlo's National Institute of Agricultural Technology Laboratory. Guava samples collected in Jujuy were

transported to the CIRPON's laboratory located in San Miguel de Tucumán. All fruit samples were placed in plastic containers with sand in the bottom as a pupation substrate. All pupae were removed weekly and the *A. fraterculus* and *C. capitata* pupae were separated by external pupal characters (White & Elson-Harris 1992). The pupae were placed in plastic vials containing sterilized humid sand until either a fruit fly or a parasitoid emerged. Fruit fly species were identified by S. Ovruski based upon Zucchi's (2000) taxonomic key. Parasitoid specimens were identified as species by S. Ovruski with the key from Wharton and Marsh (1978) for Braconidae, Graham's (1991) key for Eulophidae, Boucek y Rasplus' (1991) key for Pteromalidae, and the taxonomic description by Wharton et al. (1998) for Figitidae, Eucoilinae. Voucher specimens were placed in the entomological collection of the Fundación Miguel Lillo (FML) (San Miguel de Tucumán, Argentina).

A total of 231 fruit fly parasitoids was found representing one cosmopolitan species (*Pachycrepoideus vindemiae* (Rondani), Pteromalidae), five Neotropical species (*Doryctobracon areolatus* (Szépligeti), *D. brasiliensis* (Szépligeti), *Utetes anastrephae* (Viereck), *Opius bellus* Gahan (all Braconidae, Opiinae), and *Aganaspis pelleranoi* (Brèthes) (Figitidae, Eucoilinae)), and one exotic species (*A. indica*, Eulophidae). Table 1 summarizes parasitoid and fruit fly species abundance based on fruit samples collected in the different Argentinian localities.

The pupal parasitoid *P. vindemiae* was only obtained from *C. capitata*. This pteromalid species was introduced into Argentina for biocontrol of *C. capitata* and *A. fraterculus* and released in Córdoba province in the 1960s. However, it had been recorded about 30 years before under different scientific names (Ovruski & Fidalgo 1994). All native parasitoid species recovered in this study were only found attacking *A. fraterculus* larvae. These five Neotropical species have previously been recorded from *A. fraterculus* in both Las Yungas (Ovruski et al. 2004) and Paranaense biogeographical regions (Ogloblin 1937, Turica & Mallo 1961). They are solitary, koinobiont endoparasitoids of larvae of the genus *Anastrepha* (Sivinski et al. 2000; Ovruski et al. 2000). Of all the native parasitoids recovered in Jujuy and Misiones provinces, almost 42% and 26% were *D. areolatus* and *A. pelleranoi*, respectively (Table 1). A similar pattern of abundance was reported previously by Ovruski et al (2004) from collecting exotic and native fruit infested with *C. capitata* and/or *A. fraterculus* larvae in the northwestern province of Tucumán.

Aceratoneuromyia indica was recovered from *C. capitata* pupae that were obtained from peaches collected in Córdoba city and from *A. fraterculus* pupae obtained from guavas collected in Yuto, Calilegua, and Montecarlo localities. Thus, *A. indica* was recovered approximately

TABLE 1. NUMBER AND RELATIVE ABUNDANCE OF PARASITOID SPECIES REARED FROM *ANASTREPHA FRATERCULUS* AND *CERATITIS CAPITATA* PUPAE IN TWO HOST PLANT SPECIES IN ARGENTINA, 1998-2001.

Fruit	Collection localities (province)	Month and year collected	No. samples	Recovered fruit fly species				Parasitoid species[1], No. specimens and relative abundance (%)						
				A. fraterculus		C. capitata		A.i.	A.p.	D.a.	D.b.	O.b.	P.v.	U.a.
				No. pupae	No. adults	No. pupae	No. adults							
Peach	Córdoba city (Córdoba)	Jan-Feb 1998	6	—	—	1,285	1,055	4 (66.7)	—	—	—	—	2 (33.3)	—
		Feb 2001	3	—	—	456	375	7 (63.6)	2 (18.2)	—	—	—	2 (18.2)	—
Guava	Yuto (Jujuy)	Feb 1999	3	806	380	98	51	4 (8.9)	11 (24.4)	20 (44.4)	8 (17.8)	—	—	2 (4.5)
	Caimancito (Jujuy)	Feb 1999	2	754	329	136	71	—	2 (8.3)	14 (58.3)	7 (29.2)	—	—	1 (4.2)
	Calilegua (Jujuy)	Feb 1999	3	734	393	73	44	2 (2.4)	22 (26.5)	38 (45.8)	19 (22.9)	—	—	2 (2.4)
	Montecarlo (Misiones)	Feb 2000	4	1,100	529	—	—	4 (6.5)	24 (38.7)	26 (41.9)	6 (9.7)	1 (1.6)	—	1 (1.6)
Total				3,394	1,631	2,048	1,596	21 (9.2)	61 (26.4)	98 (42.4)	40 (17.3)	1 (0.4)	4 (1.7)	6 (2.6)

[1]A.i., *Aceratoneuromyia indica*; A.p., *Aganaspis pelleranoi*; D.a., *Doryctobracon areolatus*; D.b., *D. brasiliensis*; O.b., *Opius bellus*; P.v., *Pachycrepoideus vindemmiae*; U.a., *Utetes anastrephae*.

38 years after its first release in both Calilegua and Montecarlo sites. Similarly, in Montecarlo the permanent establishment of *D. longicaudata* was recently confirmed 40 years after its first release in Argentina (Schliserman et al. 2003). The presence of *A. indica* in the Yuto locality could be explained because this collection site is about only 36 km north of Calilegua, so that *A. indica* could spread north of the Jujuy province. The closest *A. indica* release sites to Córdoba city were Cruz del Eje, which is about 140 km north, and Yacanto, which is about 160 km south. These are the only documented release sites of this exotic parasitoid in Córdoba province. Three possible explanations for the presence of *A. indica* in central Córdoba can be drawn: (1) fruit infested with *C. capitata* or *A. fraterculus* larvae parasitized by *A. indica* could have been moved from north or south Córdoba to central Córdoba; (2) *A. indica* became established in north and/or south Córdoba and has spread to central Córdoba; (3) *A. indica* could have been released in central Córdoba without official knoweledge. If its presence in central Córdoba was the result of first releases, it would appear that *A. indica* has resided in the Córdoba province over 38 years. Thus, *A. indica* has become established in at least three different Argentinian biogeographical regions: Las Yungas region (including Jujuy province), Paranaense (including Misiones province), and Chacoan region (Córdoba province). The original native vegetation in the two former regions is a subtropical rain forest, while the Chacoan region is a subtropical dry forest. The climate in Las Yungas region is temperate-humid with a cold and dry winter. In the Paranaense region the climate is temperate-humid with a warm and rainy winter. The Chacoan region is dry-steppe with a cold and dry winter. For a thorough description of the Argentinian biogeographical regions, see Cabrera (1976) and Cabrera & Willink (1980).

SUMMARY

Specimens of the eulophid *Aceratoneuromyia indica* (Silvestri) were recovered from fruit fly pupae collected in three Argentinian biogeographical regions. A total of 11 *A. indica* specimens was obtained from pupae of the tephritid *Anastrepha fraterculus* (Wiedemann) in Las Yungas and Paranaense subtropical rain forest regions, and 10 *A. indica* specimens were recovered from pupae of the tephritid *Ceratitis capitata* (Wiedemann) in Chacoan subtropical dry forest region.

Thus, *A. indica* was recovered approximately 38 years after its first release in Argentina.

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FIELD EVALUATION OF A SYNTHETIC FEMALE SEX PHEROMONE FOR THE LEAFMINING MOTH *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE) IN FLORIDA CITRUS

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The leafmining moth, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in southern Florida in 1993 (Heppner 1993) and has since spread to all Florida citrus-growing counties and the states of Alabama, Louisiana, Texas in 1994, and California (Gil 1999) and Hawaii in 2000 (Nagamine & Heu 2003). Damage includes loss of photosynthetic capacity from mining, stunting and malformation of leaves, and potential damage from increased susceptibility of leafminer-damaged leaves to the citrus canker pathogen (Bergamin-Filho et al. 2000; Cook 1988).

Ando et al. (1985) found attraction in Japanese populations of *P. citrella* to traps baited with (*Z,Z*)-7,11-hexadecadienal. Attempts to show attraction of this material to populations in other countries were not successful (Sant'ana et al. 2003). Leal et al. (2006) found the three active compounds (*Z,Z,E*)-7,11,13-hexadecatrienal [*Z7Z11E*13-16Ald], (*Z,Z*)-7,11-hexadecadienal [*Z7Z11*-16Ald], and (*Z*)-7-hexadecenal [*Z7*-16Ald] by electroantennograms (EAG) from female pheromone gland extracts of a Brazilian population of *P. citrella* in a ratio of 30:10:1, respectively (Fig. 1). They also demonstrated that traps baited with a mixture of the two major constituents caught more males than traps baited with virgin female *P. citrella*.

Here we report the results from two field trials. The first documents attraction to a binary lure consisting of the two major EAG-active components. In the second trial, we deployed a factorial design to determine the influence of trap height in a mature citrus grove on trap catch and the relative attraction of a binary and a tertiary lure. For the first trial, six traps (Pherocon 1C Wing Trap, Trecé, Inc., Adair, OK) were deployed in a citrus grove at the experimental farm of the U.S. Horticultural Research Laboratory, Ft. Pierce, FL. Three traps were baited with rubber septa impregnated with a binary pheromone mixture con-
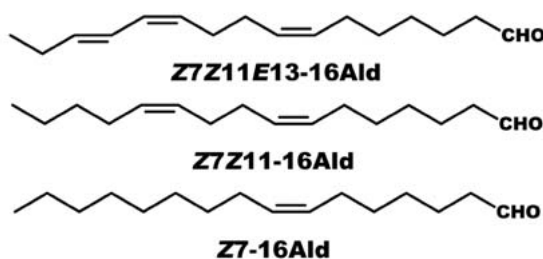

Fig. 1. Chemical structure of three semiochemicals isolated from pheromone gland extracts of the leafmining moth *P. citrella*.

sisting of *Z7Z11E*13-16Ald (96% pure) and *Z7Z11*-16Ald (98% pure) in a ratio of 3:1, and three traps were left without lures as controls. Septa were loaded with 50 μg of the major and 17 μg of the minor compound in 100 μl hexane per septum. Traps were randomly assigned to orange trees (approximately 2 m tall) within a section of 6 rows of 29 trees/row naturally infested with citrus leafminer. Traps were rotated daily between trees and the adhesive cards were removed daily and examined for the presence of *P. citrella*. Traps were deployed and counted for 3 d in August, 2005.

For the second trial, a factorial design ($3 \times 3 \times 6$) was used to investigate the effect of trap height and pheromone lure composition on trap catch over 6 d. Traps were deployed at heights of 1.3, 1.7, and 2.0 m within the same section of orange trees used in Trial 1. Traps were baited with septa impregnated with either the binary mixture as in Trial 1, a tertiary blend consisting of *Z7Z11E*13-16Ald and *Z7Z11*-16Ald and *Z7*-16Ald in a ratio of 30:10:1, or control septa impregnated with 100 μl hexane. Three replications were used for a total of 27 traps per d and 162 (3 height × 3 lures × 6 d) total traps counted over 6 d. Traps were randomly assigned to trees within the section. Spacing of the trees was 4.6 m within rows

and 7.6 m between rows. Adjacent trees were avoided to maintain a minimum of 9 m between traps. Traps with corresponding lures and fresh adhesive cards were randomly re-assigned to trees daily. Traps were deployed and counted for 6 d in August, 2005. During hurricane Katrina, traps and lures were removed; lures were stored at -80°C and re-deployed 3 d later. High levels of parasitization of leafminer larvae in the field, presumably by *Ageniaspis citricola* (Hymenoptera: Encyrtidae), interfered with our attempts to rear virgin female leafminers to compare with the synthetic lures.

Counts were made of the entire card (23 × 28 cm). We transformed data to normalize residuals before analysis by using natural log (x + 1). All tests of significance were based on transformed data. Untransformed means are presented. Data were analyzed by ANOVA. When significant differences were indicated by the F statistic at α = 0.05, means were separated by Fisher's protected least significant difference (LSD) (Abacus Concepts 1996).

No *P. citrella* adults were captured in the unbaited traps with the exception of one adult on d 3. A total of 391 *P. citrella* was captured in traps baited with the binary lure. There was no significant effect of d ($F = 2.73$; $df = 2, 12$; $P = 0.106$). More *P. citrella* were captured in traps baited

with the binary lure ($F = 9.92$; $df = 1, 16$; $P < 0.0001$). The binary lure attracted a mean (± SEM) of 43.4 ± 7.8 compared with 0.4 ± 0.3 adults/trap/d in the control traps.

In the second trial, a total of 6 adult *P. citrella* over 6 d was found in traps baited without pheromone. Traps baited with the binary mixture caught a total of 508 *P. citrella* and the tertiary mixture caught 605 *P. citrella*. There was no effect of trap height on trap catch ($F = 1.02$; $df = 2, 108$; $P = 0.366$). There was a significant effect of d on trap catch ($F = 6.90$; $df = 5, 144$; $P < 0.0001$) (Table 1) with a significant lure × day interaction ($F = 1.96$; $df = 10, 144$; $P = 0.042$). The effect of lure was significant ($F = 358.52$; $df = 2, 144$; $P < 0.0001$). There was no significant difference between the binary and tertiary lures for any of the days tested except for d 1 when there was a significant height effect ($F = 3.90$; $df = 2, 18$; $P = 0.039$) and a significant interaction between height and lure ($F = 3.90$; $df = 4, 18$; $P = 0.019$). This was due to a higher capture in the traps baited with the tertiary lure at 2 m (39.3 ± 7.4) compared with 13.7 ± 2.0 at 1.3 m and 17.7 ± 7.4 at 0.7 m. On all subsequent days there was no effect of height, no significant difference between binary and tertiary lures, nor was there a significant interaction between height and lure (α = 0.05) (Table 1).

SUMMARY

Traps baited with a binary mixture in the ratio of 30:10 of two EAG-active compounds, (*Z,Z,E*)-7,11,13-hexadecatrienal and (*Z,Z*)-7,11-hexadecadienal, attracted significantly more moths of the leafmining moth *P. citrella* compared with unbaited traps in a Florida citrus grove. The addition of a third EAG-active compound, (*Z*)-7-hexadecenal, did not increase trap catch. Trap height, at 0.7, 1.3, and 2 m did not significantly affect daily trap catch on 5 of 6 d. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable.

TABLE 1. MEAN (± SEM, $n = 3$) NUMBER OF *P. CITRELLA* ADULTS CAPTURED IN TRAPS BAITED WITH RUBBER SEPTA IMPREGNATED WITH A BINARY LURE, A TERTIARY LURE, OR UNBAITED (CONTROL) OVER 6 D AT FT. PIERCE, FL.

Day	Lure	Catch
1	Control	0.2 + 0.1 a
	Binary	15.0 + 2.4 b
	Tertiary	23.6 + 4.8 c
2	Control	0.2 + 0.1 a
	Binary	6.6 + 1.5 b
	Tertiary	7.0 + 1.5 b
3	Control	0.1 + 0.1 a
	Binary	8.4 + 1.1 b
	Tertiary	12.3 + 3.7 b
4	Control	0.0 + 0.0 a
	Binary	7.3 + 1.8 b
	Tertiary	6.9 + 1.6 b
5	Control	0.0 + 0.0 a
	Binary	10.4 + 1.6 b
	Tertiary	9.9 + 1.9 b
6	Control	0.1 + 0.1 a
	Binary	8.7 + 1.2 b
	Tertiary	7.6 + 1.2 b

Means grouped by d followed by the same letter are not significantly different (α = 0.05, Fisher's protected LSD).

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DIETARY PROTEIN AND MATING COMPETITIVENESS OF STERILE MALES OF THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE): MEASUREMENTS OF INDUCED EGG STERILITY IN LARGE FIELD ENCLOSURES

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The Sterile Insect Technique (SIT) is widely used to control infestations of the Mediterranean fruit fly (or medfly), *Ceratitis capitata* (Wied.) (Hendrichs et al. 2002). There is, however, considerable evidence (Lance et al. 2000) showing that mass-reared, sterile males are inferior to wild males in mating competition for wild females. Blay and Yuval (1997) demonstrated that the addition of protein (yeast hydrolyzate) to the standard sugar-agar diet increased the mating competitiveness of sterile males. However, research conducted in Hawaii failed to detect a similar protein effect (Shelly & McInnis 2003). Thus, the potential role of dietary protein in improving the effectiveness of medfly SIT remains uncertain.

This study compares levels of egg sterility in large field enclosures containing fertile flies and protein-fed or protein-deprived sterile males. In the aforementioned studies, mating trials were run over short intervals in laboratory cages or on single host trees and thus precluded potential effects of habitat heterogeneity and inter-tree movement on male mating competition. In addition, data on mating success do not necessarily mirror the level of egg sterility realized in the open field, because they ignore the possibility that females mate multiple times, even on the same day (Vera et al. 2003), and consequently that sperm competition may be occurring. While not a complete substitute for a long-term, open-field test, the protocol described below involved measurements of egg sterility over several days in enclosures holding multiple host trees, and the resulting data were presumably more reflective of natural conditions.

The flies used in this study were maintained following the protocol of Shelly et al. (2005). Owing to the limited availability of wild flies, we used flies from a recently established colony (REC) reared from field-collected coffee berries. Larvae were reared on artificial media, and adults were separated within 24 h of emergence and maintained on a sugar-protein mixture and water. When used, REC flies were 7-13 d old and 4-7 generations removed from the wild. Mass-reared flies were from a *tsl* strain (Vienna-7/Tol-99) produced by the California Department of Food and Agriculture Hawaii Fruit Fly Rearing Facility, Waimanalo, Oahu. Two d before eclosion, pupae were coated with fluorescent dye (standard marking procedure), irradiated, and placed in storage (so-called PARC) boxes. For a given trial, we placed

85 ml of pupae (60 pupae per ml) in each of 2 boxes, which yielded ≈4,080 flying adults (≈80% of *tsl* male pupae yield adults capable of flight, California Department of Agriculture, unpubl.). On the day of peak emergence, males in one box were provided with a slab of sugar-agar gel (standard size and formulation placed on the screened panel on the box lid), while males in the other box were provided with a sugar-agar slab plus the sugar-protein mixture. Boxes receiving this mixture had a hole drilled in one side through which we introduced 2 Petri dishes of the sugar-protein food (some mixture was invariably present on the day of male release, indicating that the supply of protein-containing food was not limited). The *tsl* males were held in the boxes for 4 d after peak emergence (i.e., 6 d after pupal placement) and then released in the field enclosures.

The methods and schedule of egg collection followed Shelly et al. (2005). Trials were conducted in 2 nylon-screened enclosures (16 by 6 by 2.5 m, l:w:h) erected in Waimanalo, Oahu, that contained 10 and 12 guava trees (*Psidium guajava* L.), respectively. Protein-deprived and protein-fed males were tested concurrently (1 treatment per enclosure), and treatments were alternated between the 2 enclosures in successive replicates. Trials were conducted during November 2004-March 2005, with daily maximum temperatures ranging between 23-28°C.

On d 1 of a replicate, we released 200 REC males, 200 REC females, and ≈4,080 *tsl* males of a given diet type (i.e., a ratio of ≈20:1, sterile:wild males) in the center of each enclosure between 0900-0930 h (males were released 20 min before females). Also, on d 1 food (sugar-agar) and water were placed at 4 locations in the enclosures and replaced daily. On d 2, 12 Granny Smith apples (*Malus domestica* Borkh.) were placed in each enclosure at 1000 h for oviposition (guava fruits were removed before the trials). Apples were suspended 1.5-2.5 m above ground by piercing the fruit with a nail and connecting the nail to a branch with wire. On d 3 and 4 at 1000 h, apples were collected and replaced with new ones. On d 5, apples were collected but not replaced, marking the end of the trial. Collected apples were returned to the laboratory, and eggs were removed with a scalpel and forceps. Eggs were placed on moistened blotter paper within Petri dishes, incubated at 27°C for 48 h, and then scored for hatching. Eight replicates

were conducted per diet regime, with successive trials separated by 7 d. Between trials, surviving flies were eradicated through trapping (trimedlure and food baits) and visual searching.

For each replicate (i.e., concurrent pair of trials), we also measured egg hatch of REC females mated exclusively to REC males in a smaller field-cage (3.0 m diameter, 2.5 m high) over a single guava tree adjacent to the large enclosures. One hundred individuals of each sex were introduced on d 1, and 2 apples were introduced on d 2 for 24 h. These data were used in the computation of Fried's (1971) competitiveness index (C; egg hatch in REC female by sterile male matings was assumed to be zero based on data from Shelly et al. (2005)).

There were no significant differences between diet treatments in the number of eggs collected for a given day or for an entire replicate (*t* test, *P* > 0.05 in all cases, Table 1). For both diets, however, the number of eggs collected differed significantly among days, with a steady decrease noted over time (Kruskal-Wallis test, *P* < 0.05 in both cases). There was no significant difference between diet treatments in the proportion of unhatched eggs collected for a given day or for an entire replicate (*t* test after arc sine transformed percentages, *P* > 0.05 in all cases, Table 1). Also, unlike egg number, the proportion of unhatched eggs varied independently of day for both diet treatments (ANOVA with arc sine transformed percentages, *P* > 0.05 in both cases).

In the field-cage containing REC flies only, 319.6 eggs (SE = 32.9) were collected per replicate of which 21% (SE = 2.6), on average, did not hatch. C values were computed for individual replicates for each diet treatment, and overall mean values of C did not differ between protein-deprived (mean = 0.27, SE = 0.07) and protein-fed (mean = 0.19, SE = 0.04) males (*t* test, *P* > 0.05).

Our results show no effect of dietary protein on the level of egg sterility induced by sterile males. Interpretation is potentially confounded by the likelihood that the sterile males ingested feces deposited on the box walls (as reported by Blay & Yuval 1997). Any supplementary nutrients so obtained by the protein-deprived males, in particular, might have acted to reduce behavioral differences arising from the experimental dietary treatments. This explanation is, of course, more important from a physiological perspective than an operational one, since it is the outcome (no diet-related difference in induced egg sterility), and not the mechanism, that is most relevant to program managers in medfly SIT. In addition, results should be interpreted with caution, because recently colonized flies were used in lieu of wild flies.

We thank R. Corrales for the temperature data, M. Teruya for laboratory assistance, and D. McInnis for comments.

SUMMARY

Competitive mating environments were established in large field enclosures by placing fertile medflies with protein-deprived or protein-fed sterile males. Based on the hatch rate of eggs collected from fruits over several days, the addition of protein to the adult diet had no effect on the mating competitiveness of sterile males.

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TABLE 1. NUMBER OF EGGS COLLECTED AND PROPORTION OF UNHATCHED EGGS PER DAY AND PER REPLICATE FOR FIELD ENCLOSURES CONTAINING FERTILE FLIES AND STERILE *tsl* MALES FED EITHER SUGAR-AGAR ONLY OR SUGAR-AGAR PLUS A SUGAR-PROTEIN MIXTURE. VALUES REPRESENT MEANS WITH STANDARD ERROR (SE) OF 8 REPLICATES.

Sterile male diet	Day[1]	No. eggs collected	% Unhatched eggs
Sugar only	2	640.6 (165.8)	85.3 (2.4)
	3	192.2 (41.0)	79.9 (5.4)
	4	100.6 (14.5)	85.9 (3.4)
	Total	933.5 (192.2)	84.3 (2.5)
Sugar + Protein	2	848.8 (170.3)	77.9 (4.1)
	3	296.2 (58.2)	80.2 (4.0)
	4	141.6 (25.7)	82.2 (4.6)
	Total	1286.7 (219.4)	79.0 (3.7)

[1]Day that groups of 12 apples were placed in enclosures; flies were released on d 1.

GOLDFLECK DAMAGE TO TOMATO FRUIT CAUSED BY FEEDING OF *FRANKLINIELLA OCCIDENTALIS* (THYSANOPTERA: THRIPIDAE)

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Flower thrips, *Frankliniella* spp., are important pests of tomatoes, *Lycopersicon esculentum* Mill., throughout the mid-Atlantic region. Several species cause direct damage to the leaves and fruits, and species such as the western flower thrips (WFT), *F. occidentalis* (Pergande), and the tobacco thrips, *F. fusca* Hinds, also vector tomato spotted wilt virus (Zitter et al. 1989).

Damage caused by *F. occidentalis* to tomato can appear in several forms. In greenhouses, *F. occidentalis* will feed on the leaves of young transplants, causing a spotting and desiccation of the leaves. Sclar (2000) states that *F. occidentalis* are attracted to pollen sources, and will feed extensively on flower tissues and degrade flower quality. This results in reduced length of bloom and flower tissue damage. Salguero Navas et al. (1991) report that small indentations appear in tomato fruit from female oviposition, and these indentations are sometimes surrounded by a light-colored halo. This damage can result in rejection of fruit and lowering of grade.

Although originally reported in the southwestern portion of the United States, WFT was first reported along the east coast in Georgia in 1981 (Beshear 1983). It is now present throughout the east coast, including New Jersey. Cosmetic damage to tomatoes, appearing as 'goldflecking' and or goldfleck rings on red tomatoes, began to occur simultaneously with the appearance of *F. occidentalis* in New Jersey. Ghidiu (1999) reported that up to 31% damage caused by goldfleck on tomato fruit was observed in fresh market tomatoes in southern New Jersey. Growers originally attributed the gold-fleck and goldfleck rings appearing on tomato fruit as damage caused either by environment, pesticide phytotoxicity, or a combination of these. However, Rice (1992) described a similar damage on nectarines as whitish skin patches caused by removal of cell contents by feeding of *F. occidentalis* that resembled spray residue which could not be washed off. The goldfleck rings often appear on the fruit where the skin was in contact with another object, such as another fruit, stem or leaf. Similar damage caused by *F. occidentalis* has appeared on nectarines throughout the region (Hogmire 1995), showing up as silverish spots or rings on fruit where the surface was in contact with another fruit or stem. He reported that *F. occidentalis* feed in protected areas such as in the flowers, under leaves, and between two fruit that are touching each other, resulting in a circular silver blemish.

The cosmetic fruit damage of goldfleck on tomato has become a serious economic problem throughout the mid-Atlantic region, resulting in the culling and downgrading of fruit. The purpose of this study was to determine if *F. occidentalis* causes the goldfleck spots and halo rings to green and red tomato fruit.

The studies were conducted in glass greenhouses in 2001. Tomatoes, 'Florida 47', were seeded to flats on 5 Feb., and on 6 Apr individual plants were transplanted into 7.6-liter pots containing a peat-vermiculite mix according to the Commercial Vegetable Production Recommendations for New Jersey (Anon. 2001). Twelve plants were placed on benches in each of two isolated, environmentally controlled, self-supporting enclosed greenhouse units on 7 Apr.

On 15 Apr., fruits on all plants were thinned to single fruit, or clusters of three fruit where the fruit were in contact with each other, and the remaining fruit and flowers were removed. An oversize yellow-orange ping-pong ball (Sears brand, 4.5 cm diam), simulating a small tomato, was attached with 20.3 cm long twist ties to each of the four green fruits and four red fruits on plants in both the treated (infested) and untreated (not infested) greenhouse units. Dennill & Erasmus (1992) found that infested, clustered avocado fruits were consistently more damaged by thrips than single fruits. The ping-pong balls were cleaned in distilled water and represented a non-organic object in contact with fruit to provide a protected area for the *F. occidentalis*. Each greenhouse had four plants each with at least one cluster of fruit (with fruit contacting each other), four plants each with at least one red fruit with a ping-pong ball attached, and four plants each with at least one green fruit with a ping-pong ball attached to it (the treated greenhouse contained infested plants, and the untreated greenhouse contained non-infested plants). *F. occidentalis* (identification confirmed by Dr. M. Parella, University of California) were reared on young asparagus ferns in a separate greenhouse and collected on 16 Apr with a white shake cloth and a Fisher aspirator. Approximately 50 *F. occidentalis* adults and nymphs were placed on the fruit of each treatment. Tomato fruit were harvested on 9 May, examined, and the number of fruit with "gold-fleck" damage was recorded on both green and red tomatoes in both greenhouses. Tomato pots were irrigated as needed by drip irrigation with micro-emitters.
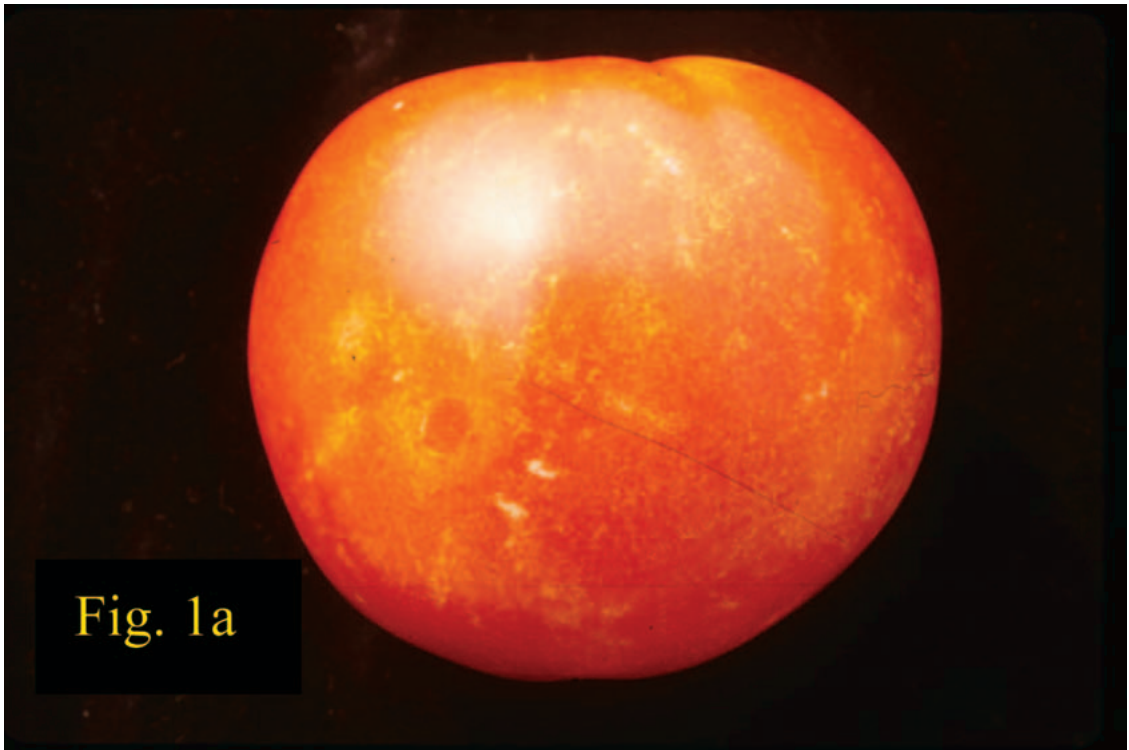
Fig. 1. (a) Goldflecks on tomato caused by feeding of *F. occidentalis* (Pergande). (b) Goldfleck rings on tomatoes at the spot they were touching each other, caused by feeding of *F. occidentalis* (Pergande).

Only tomato fruit (both green and red) that had been infested with *F. occidentalis* had visible "gold-fleck" damage on the surface of the fruit approximately 3 weeks after infestation (Fig. 1a, b); none of the red or green fruit in the uninfested greenhouse had any visible signs of goldflecking. The gold-fleck was observed on a higher percentage of infested red tomatoes (average 60% damaged red fruit) than on infested green tomatoes (average 25% damaged green fruit) for both the tomatoes that had a ping-pong ball attached and tomatoes that were in a cluster. *F. occidentalis* feeding damage on green tomatoes appeared as faint whitish flecks against a green background, making it difficult to see, while feeding damage on red tomatoes appeared as bright gold flecks against a red background. Gold-fleck damage appeared as both random individual flecks, or small spots, on the skin of fruit that were not touching another object, and also as circular gold rings of fleck on the skin of fruit that were touching another object, i.e., another fruit or the ping-ball ball. These rings outlined the border of the contact area between the two objects. Similar cosmetic damage of goldfleck rings was reported in nectarines and peaches by Hogmire (1995) and on plum (Lewis 1997).

No oviposition damage by females was observed on either the green or red fruit, indicating that gold-flecking is caused by feeding damage alone. Not all fruit infested with *F. occidentalis* showed damage, suggesting that either thrips mortality was high or feeding on the fruit was variable. The thrips used in this experiment were mixed populations of age and sex, which may have contributed to the increased variability.

SUMMARY

Although several species of thrips attack tomatoes in the mid-Atlantic region, results from this experiment show that the cosmetic fruit damage referred to as "gold-flecking" is caused by direct feeding of *F. occidentalis*. Further, the damage can occur on single green or red fruit, or on fruit that come in contact with another object, possibly leaves, stems or adjacent fruit. Further research is needed with the other tomato-infesting thrips species to determine if their feeding causes similar damage. Thrips management programs could then use feeding damage in the field to determine if the program needs adjustment to obtain better control of the thrips population.

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SERENDIPITOUS DISCOVERY OF AN RNA VIRUS FROM THE CRICKET, *ACHETA DOMESTICUS*

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Bioinformatic analysis of approximately 2000 expressed sequence tags (ESTs) from a monogyne *Solenopsis invicta* expression library identified 8 clones exhibiting significant homology to single-stranded RNA viruses (Valles et al. 2004). Three of these clones (3F6, 14D5, and 24C10) yielded a contiguous sequence fragment from which 3' and 5' rapid amplification of cDNA ends (RACE) reactions were conducted and ultimately led to the discovery of a new RNA virus (*Solenopsis invicta* virus-1 [SINV-1]) infecting the red imported fire ant, *S. invicta* (Valles et al. 2004; Valles & Strong 2005). Subsequent examination of fire ant ESTs revealed that clone 11F1 exhibited significant homology with the capsid polyprotein of the *Drosophila* C RNA virus and was distinct from the SINV-1 genome sequence. Thus, we were hopeful that EST 11F1 might similarly lead to a new fire ant virus discovery. However, when we conducted extensive field surveys (*n* > 50) of *S. invicta* nests by RT-PCR using oligonucleotide primers designed to the sequence of clone 11F1, all samples proved negative. Up to that point, sampling was conducted as had been done for the SINV-1; a scintillation vial was plunged into an ant nest and adults that fell into the vial were collected and used for RNA extraction and RT-PCR analysis. Failure to detect the sequence prompted a change in the collection protocol. We considered that perhaps if the 11F1 sequence was from a virus in the fire ant, that it could be infecting a stage other than adults. To retrieve a sample of brood for this analysis required collection of entire colonies. Our first collection was taken on a Friday, and because the floating method (Jouvenaz et al. 1977) to remove the ants and brood from the collection bucket would require several days to complete, we postponed the extraction process until the following Monday. This delay necessitated feeding the colonies, standard practice for fire ant nest collections. Each colony was provided with water, 10% sucrose solution, and approximately 30 frozen-killed crickets, *Acheta domesticus*, purchased from a cricket bait farm (Jerry's Bait Farm, Slocomb, AL).

RT-PCR analysis was conducted later the following week with RNA extracted from adults and brood (separately) of these colonies; every single sample tested positive. Although not immediately, we realized that perhaps the crickets were the actual source of the suspected virus, and ants were consuming the infected crickets and consequently testing positive. One-step RT-PCR (Invitrogen, Carlsbad, CA) analysis (1 cycle at 45°C for 30 min, 1 cycle at 94°C for 2 min, 35 cycles of 94°C for 15 s, 55°C for 15 s, 68°C for 30 s) of RNA extracted directly from crickets (*n* = 26 from three different lots) with clone 11F1-specific oligonucleotide primers (primer 60: 5' CAGATGGGTGCGAATAACT-TCAAATC, primer 61: 5' CACTTCGAAAAACA-ACTCAGTCTCCTG) produced an amplicon of anticipated size (311 bp) from 13 of the crickets (50% positive). Three of the amplicons were gel-purified and ligated into the pCR4-TOPO vector, transformed into TOP10 competent cells (Invitrogen) and sequenced by the Interdisciplinary Center for Biotechnology Research (University of Florida). The sequence was 99.7% identical (310/311) to the corresponding sequence of EST clone 11F1.

A 3'RACE reaction was conducted with RNA prepared from one of the crickets testing positive for the 11F1 sequence (GeneRacer kit, Invitrogen). cDNA was synthesized from 1 µg of total RNA with the GeneRacer Oligo dT primer (42°C for 50 min, 70°C for 15 min, held on ice for 2 min, RNase H added and incubated at 37°C for 20 min). The cDNA was subsequently used as template for PCR with a gene-specific primer, p393 (5'GCATCTACTGTACCCAATGTTCACCAGCGG TACAC) and GeneRacer 3' primer. The reaction produced a single amplicon of approximately 1300 bp that was gel purified, ligated, transformed, and sequenced as described above. Sequences were assembled with the NTI Vector software (Invitrogen) to produce a 1565 nucleotide contiguous fragment consistent with the corresponding regions of fire ant EST 11F1. The sequence was polyadenylated at the 3' end. The consensus assembly was deposited in the GenBank database under accession number DQ112164. Analysis of the sequence revealed a single large open reading frame (ORF) beginning at nucleotide 4 (start codon) and terminating at nucleotide 1439 (stop codon). The translated ORF yielded a predicted polypeptide comprised of 478 amino acids with a molecular mass of 52600 Da.

Protein-protein BLAST (Altschul et al. 1997) analysis showed that the predicted amino acid sequence exhibited significant similarity with the capsid polyproteins of positive-stranded RNA viruses in the GenBank database. Table 1 summarizes the level of sequence identity realized from the BLAST search. Specifically, the new sequence

TABLE 1. GENBANK SEQUENCES EXHIBITING SIGNIFICANT SIMILARITY WITH THE BAIT-CRICKET VIRUS SEQUENCE.

Virus	Abbreviation	GenBank accession number	Amino acid identity (%)	BLAST expectation score
Rhopalosiphum padi virus	RpV	NP046156	19.7	10^{-15}
Drosophila C virus	DCV	NP044946	18.6	10^{-15}
Cricket Paralysis virus	CPV	NP647482	16.0	10^{-14}
Kashmir Bee virus	KBV	NP851404	13.7	10^{-13}
Aphid Lethal Paralysis virus	ALPV	NP733846	16.8	10^{-11}
Acute bee paralysis virus	ABPV	NP066242	11.8	10^{-10}
Deformed Wing virus	DWV	NP853560	14.7	10^{-8}
Solenopsis invicta virus-1	SINV-1	YP164441	12.9	10^{-8}
Himetobi P virus	HPV	NP620561	14.1	10^{-7}
Black queen cell virus	BQCV	NP620565	13.8	10^{-4}

(referred to as the bait-cricket virus, or BCV) was compared with sequences corresponding to amino acids of the capsid polyprotein domain regions of other insect-infecting positive-stranded RNA viruses. Expectation values ranged from 10^{-15} (RpV) to 10^{-4} (BQCV); the most significant e scores were for the RpV (10^{-15}), DCV (10^{-15}), and CPV (10^{-14}). The data suggest that BCV is probably a member of the small RNA virus family *Dicistroviridae* that infect primarily insects.

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A NEW HOST RECORD FOR THE EGG PARASITOID *ANAGRUS NIGRIVENTRIS* (HYMENOPTERA: MYMARIDAE) OF THE CORN LEAFHOPPER, *DALBULUS MAIDIS* (HEMIPTERA: CICADELLIDAE)

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The corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott), is the most common leafhopper feeding on corn in Argentina. It causes great losses to corn crop in most tropical and subtropical Americas because of its ability to transmit three important pathogens: Corn stunt spiroplasma (CSS), Maize bushy stunt phytoplasma (MBSP), and Maize rayado fino virus (MRFV) (Nault & Ammar 1989; Oliveira et al. 1998). The diseases caused by these pathogens adversely affect the corn crop in Argentina (Giménez Pecci et al. 1998, 2002a, b; Virla et al. 2004).

Until now, six species of parasitoids were known from eggs of *D. maidis*: *Anagrus breviphragma* Soyka, *A. flaveolus* Waterhouse, *Anagrus* sp. (Mymaridae), and *Paracentrobia subflava* (Girault), *Paracentrobia* sp., and *Oligosita* sp. (Trichogrammatidae) (Marín 1987; De Santis et al. 1992; Gladstone et al. 1994; Triapitsyn 1997; Oliveira & Spotti Lopez 2000; Virla 1999, 2001).

Representatives of Mymaridae, particularly *Anagrus* spp., have been utilized in several instances for the biological control of crop pests. Twelve described species of *Anagrus* Haliday occur in Argentina (Triapitsyn 1997, 1999, 2002; Triapitsyn & Virla 2004). Of these, *A. breviphragma* and *A. flaveolus*, are mentioned as affecting *D. maidis* populations (Triapitsyn 1997; Virla 2001, 2004).

The eggs of *D. maidis* are imbedded in the corn tissues, mostly along the midrib on the top side of the leaf (Pitre 1967). Sentinel eggs of *D. maidis* were exposed to parasitization in a cornfield from December 2004 to April 2005 at "El Manantial" site (Tucumán Province, Argentina: latitude 26°49'50.2"S, longitude 65°16'59.4"W, elevation 495 m). Potted plants containing sentinel eggs were placed inside the cornfield at no more than 3 m from the edge of the field.

In the laboratory, 6-10 females of *D. maidis* were placed in Polyethylene-Terephtalathe cylindrical cages (35 cm high × 18 cm diameter) on corn leaves in order to obtain sentinel eggs. The *D. maidis* colony was maintained at room temperature (25 ± 4°C), 70-80% RH, with natural summer photoperiod. Potted corn plants in the vegetative stage (three to six leaves) were checked daily for eggs. Eggs less than 24 h old were exposed for 72-96 h. After eight days, the leaves with exposed eggs were cut from the plant and transferred to Petri dishes containing wet tissue paper on the bottom and covered with a clear plastic food wrap to avoid desiccation and to keep parasitoids from escaping. Parasitized eggs were checked daily to ensure leaf quality until the emergence of adult wasps.

In total, 13828 (58.1%) of 23781 eggs were parasitized. One of the parasitoids was the mymarid wasp *Anagrus nigriventris* Girault (with 7.2% of the total egg parasitism). It is the first record of the corn leafhopper as a natural host for this species of *Anagrus*. Due to the importance of the diseases vectored by the corn leafhopper in the Americas, *A. nigriventris* should be properly evaluated as a potential biological control agent against this leafhopper pest.

Anagrus nigriventris, *A. breviphragma*, and *A. flaveolus* can be distinguished with the keys by Triapitsyn (1997, 1999, 2002). *Anagrus nigriventris* is one of the most common mymarid species in the New World, and has been recorded from Argentina, Brazil, Canada, Chile, Mexico, Peru, Trinidad, Tobago, and USA (throughout, including Hawaii) (Triapitsyn 1997, 1999, 2002). Its other hosts include the leafhoppers, *Aceratagallia* spp., *Circulifer tenellus* (Baker), *Empoasca fabae* (Harris), *E. solana* DeLong, *Empoasca* spp., *Erythroneura comes* (Say), *Scaphytopius nitridus* (DeLong) (Triapitsyn & Moratorio 1998), and the mirid bug, *Pycnoderes quadrimaculatus* Guérin-Méneville (Triapitsyn 1997).

Voucher specimens of *A. nigriventris* resulting from this study are deposited in the collections of the Entomology Research Museum, University of California at Riverside, USA (UCRC) and Fundación e Instituto Miguel Lillo at San Miguel de Tucumán, Argentina (IMLA).

SUMMARY

A survey of the eggs parasitoids of the corn leafhopper, *Dalbulus maidis* (DeLong & Wolcott) was carried out in Tucumán Province, Argentina. Samples were collected during the summer of 2004-2005 with sentinel eggs. *Anagrus nigriventris* Gi-

rault was responsible for 7.2% of the total egg parasitism. That is the first record of this parasitoid reared from the eggs of *D. maidis*; *A. nigriventris* is one of three species of *Anagrus* known to affect populations of this leafhopper pest in Argentina.

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EFFECT OF PROPYLENE GLYCOL ANTIFREEZE ON CAPTURES OF MEXICAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) IN TRAPS BAITED WITH BIOLURES AND AFF LURES

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Multilure traps (Better World Manufacturing, Inc., Miami, FL) baited with BioLure MFF lures (Suterra LLC, Inc., Bend, OR) and containing water with propylene glycol antifreeze as the drowning agent were about 2× more attractive than similar traps baited with AFF lures (Advanced Pheromone Technologies, Marylhurst, OR) in orchard tests with irradiated Mexican fruit flies (*Anastrepha ludens* Loew) (Robacker & Czokajlo 2005). Although antifreeze originally was used in traps only to preserve the captured flies, Thomas et al. (2001) found that attraction of feral Mexican and Caribbean (*A. suspensa* (Loew)) fruit flies to McPhail-type traps baited with BioLure MFF lures doubled when antifreeze was added to the water. Thomas et al. (2001) did not establish whether or not antifreeze was attractive by itself. Hall et al. (2005) found that water with 10% propylene glycol was not more attractive than water but the two drowning agents were not tested in the same trap type so conclusive data about the attractiveness of antifreeze has not been published. The objectives of this work were 1) to determine if antifreeze is attractive to Mexican fruit flies, 2) to investigate whether antifreeze enhances attractiveness of the AFF lure; and 3) to compare efficacy of BioLures and AFF lures in traps containing water without antifreeze as the drowning agent.

Multilure traps were used to test the following treatments: 300 ml of water with 0.01% Triton X-100R (Fisher Scientific, Pittsburgh, PA) (hereafter referred to as water); 300 ml of water with 10% propylene glycol-based antifreeze (LowTox Antifreeze, Prestone Products Corp., Danbury, CT) (hereafter antifreeze); BioLure 2-component (ammonium acetate and putrescine) MFF lure (hereafter BioLure) with water; BioLure with antifreeze; AFF lure with water; and AFF lure with antifreeze. BioLures were deployed in traps by adhering the ammonium acetate patch and the putrescine patch separately on the inside wall of the plastic top. Two versions of the AFF lure, the standard lure and a smaller version made specifically for multilure traps, were used in separate experiments. For the standard lure, the plastic bags containing the AFF lure components were removed from the mesh bag provided by the manufacturer. The larger plastic bag was taped onto the inside wall of the trap top and the smaller one was put into the lure basket on the ceiling of the trap top. For the smaller version, both plastic bags were put into the lure basket of the trap top.

Tests were conducted with irradiated Mexican fruit flies from a laboratory culture started in 2000 from pupae collected from yellow chapote (*Casimiroa greggii*), a native host, from the Montemorelos area of Nuevo Leon in northeastern Mexico. Larvae were reared on artificial medium and pupae were irradiated with 70-92 Gy (Cobalt 60) 1-2 d before adult eclosion. Mixed-sex groups of 200 flies were kept in 473-ml cardboard cartons with sugar and water until released in test plots 3 to 8 d after eclosion.

Testing was conducted in a grapefruit (*Citrus paradisi*) (variety Rio Red) orchard near Weslaco, Texas. Three blocks of 6 consecutive trees were used in each of two rows for a total of 6 blocks. Traps were hung one to a tree, north of center, at 1-2 m height. Approximately 4000 flies were distributed equally onto trees in rows adjacent to the test rows during each week of the experiments. Each week, flies were removed and counted, water and antifreeze were changed, and the traps were rotated sequentially within blocks. Synthetic lures were not changed.

Two experiments were conducted that were identical except for the AFF lure type. Experiment 1 used the standard AFF lure and Experiment 2 used the smaller version of the AFF lure. Experiment 1 was conducted for 10 weeks (10 weeks × 6 blocks = 60 tests of each treatment) and Experiment 2 for 8 weeks. Replications over time (weeks) were treated like replications over space (blocks of trees) for statistical analyses. Counts of captured flies were transformed by square root to stabilize variance (Snedecor & Cochran 1967). Transformed data were subjected to analysis of variance by SuperANOVA (Abacus Concepts 1989).

The results of Experiment 1 with standard AFF lures are shown in Table 1. BioLure traps with antifreeze captured more than 2× as many males and females as BioLure traps with water. AFF lure traps with antifreeze also were significantly more attractive than AFF lure traps with water, but the difference was not as great as for the BioLure traps. Generally, BioLures and AFF lures performed comparably in traps with water. The results of Experiment 2 with smaller AFF lures (Table 2) were similar to those of Experiment 1.

TABLE 1. CAPTURE OF MEXICAN FRUIT FLIES IN MULTILURE TRAPS BAITED WITH BIOLURES OR STANDARD AFF LURES AND CONTAINING WATER WITH TRITON OR WITH ANTIFREEZE IN THE TRAP RESERVOIR.[1]

Lure/drowning agent	Males	Females	Total
none/water-Triton	0.2 ± 0.1 a	0.2 ± 0.1 a	0.3 ± 0.1 a
none/water-antifreeze	0.2 ± 0.1 a	0.3 ± 0.1 a	0.6 ± 0.1 a
BioLure/water-Triton	8.3 ± 0.8 b	9.2 ± 0.9 b	17.5 ± 1.7 b
BioLure/water-antifreeze	17.7 ± 2.3 d	20.6 ± 2.0 d	38.2 ± 4.2 d
AFF lure/water-Triton	9.2 ± 0.9 b	8.8 ± 0.8 b	18.1 ± 1.6 b
AFF lure/water-antifreeze	11.8 ± 1.2 c	12.6 ± 1.4 c	24.4 ± 2.4 c

[1]Means (± SE) in the same column followed by the same letter are not significantly different by Fishers protected LSD test ($P <$ 0.05) (males: $F = 154$; $df = 5,345$; $P < 0.0001$, females: $F = 166$; $df = 5,345$; $P < 0.0001$).

TABLE 2. CAPTURE OF MEXICAN FRUIT FLIES IN MULTILURE TRAPS BAITED WITH BIOLURES OR SMALL-VERSION AFF LURES AND CONTAINING WATER WITH TRITON OR WITH ANTIFREEZE IN THE TRAP RESERVOIR.[1]

Lure/drowning agent	Males	Females	Total
none/water-Triton	0.7 ± 0.1 a	0.8 ± 0.2 a	1.5 ± 0.2 a
none/water-antifreeze	0.7 ± 0.1 a	0.6 ± 0.2 a	1.3 ± 0.2 a
BioLure/water-Triton	7.0 ± 1.0 bc	10.0 ± 1.3 c	17.0 ± 2.1 bc
BioLure/water-antifreeze	17.8 ± 2.8 d	22.6 ± 3.4 d	40.4 ± 6.0 d
AFF lure/water-Triton	5.9 ± 0.8 b	7.3 ± 1.2 b	13.2 ± 1.9 b
AFF lure/water-antifreeze	9.4 ± 1.2 c	9.0 ± 1.3 bc	18.4 ± 2.4 c

[1]Means (± SE) in the same column followed by the same letter are not significantly different by Fishers protected LSD test ($P <$ 0.05) (males: $F = 61.6$; $df = 5,263$; $P < 0.0001$; females: $F = 67.3$; $df = 5,263$; $P < 0.0001$).

The results of these experiments indicate that antifreeze enhances the efficacy of AFF lures only slightly compared with the large enhancement effect with BioLure-baited traps. Differences in the effects of antifreeze in traps baited with BioLures and AFF lures may be related to differences in emissions from the two lures. Whereas both lures emit ammonia, putrescine, and 1-pyrroline, the lures differ in that BioLures also emit acetic acid and AFF lures emit methylamine (Robacker & Czokajlo 2005). In addition, AFF lures emit much more ammonia and 1-pyrroline than BioLures (Robacker & Czokajlo 2005).

agent enhanced attractiveness of BioLure-baited traps by more than twofold over traps containing water with Triton. Antifreeze increased attractiveness of traps baited with AFF lures by less than 50%. Because antifreeze had no attractiveness by itself, the effects reveal synergism. Reasons for the different interactions of antifreeze with BioLures and AFF lures were not determined.

SUMMARY

Multilure traps baited with AFF lures captured equal numbers of sterile Mexican fruit flies in a citrus orchard compared with traps baited with BioLure MFF 2-component lures, when water with Triton X-100R was used as the drowning agent. Use of 10% antifreeze as the drowning

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FIRST RECORD OF AN EGG PARASITOID FOR THE NORTH AMERICAN PROCONIINE SHARPSHOOTER *PARAULACIZES IRRORATA* (HEMIPTERA: CICADELLIDAE), WITH NOTES ON REARING TECHNIQUES

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Interest in the natural enemies of proconiine sharpshooters (Hemiptera: Cicadellidae: Cicadellinae: Proconiini) has increased since the accidental introduction and subsequent establishment of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), in California, Tahiti, Hawaii, and most recently Arizona. Previous surveys of egg parasitoids of proconiine sharpshooters from Florida indicated several species in the families Mymaridae (primarily *Gonatocerus* spp.) and Trichogrammatidae (Hymenoptera) (Triapitsyn et al. 1998; Triapitsyn & Hoddle 2001; Triapitsyn et al. 2002; Triapitsyn 2003; Tipping et al. 2005). However, no egg parasitoids have been reported to attack the eggs of the common proconiine sharpshooter *Paraulacizes irrorata* (Fabricius). This species has a distribution that includes central, northeastern and southeastern USA as well as northern Mexico (Young 1968).

The eggs of *P. irrorata* are deposited in masses into woody twigs and stems as well as the hardened petioles of a great variety of plant species. This method of oviposition results in great difficulty for researchers to find and identify egg masses in the field. Additionally, this species does not apply brochosomes to the site of oviposition. Other North American proconiine sharpshooter species, particularly those in the genera *Homalodisca* Stål and *Oncometopia* Stål, often powder oviposition sites with highly visible brochosomes.

Rearing *P. irrorata*

The first author of this communication initiated a colony of *P. irrorata* from females collected from crape myrtle, *Lagerstroemia indica* L. during the spring of 2004 at the University of Florida's North Florida Research and Education Center (NFREC) in Quincy, Florida. Individuals were kept in 1-m² wooden framed, screen-covered cages that were maintained in greenhouses. Greenhouse temperatures ranged between 25-32°C with indoor lighting to maintain a 16:8 light/dark photoperiod. Cages were provisioned with a combination of cotton (*Gossypium hirsutum* (L.), glabrous soybean (*Glycine max* (L.) 'D90-9216'), and basil (*Ocimum basilicum* L. 'Lemon'). The cotton and soybean plants were maintained in the cages after they had formed secondary growth on the stems and/or petioles, regardless of vigor, to provide oviposition sites for gravid *P. irrorata* females. The basil plants were replaced as they began to decline in vigor which occurred after flowering because nymphs and adults of *P. irrorata* would congregate on the younger basil plants. All plants used in the colony cages were potted in a 3:1:1 pine bark: sphagnum moss: sand mixture before placement into colony cages. The soil medium for all plants was watered to saturation twice daily. Declining plants often had newly deposited egg masses. The plant parts holding these eggs were trimmed to fit into Petri dishes (10 cm) filled with water agar and held until eclosion as described by Tipping et al. (2004).

Acquisition of Parasitoids

Thirty female *P. irrorata* were collected from several colony cages and placed into a separate cage that was provisioned with cotton plants that had secondary growth. After 48h, the plants were removed from the colony cage and placed in the field along a forest edge at NFREC for 5 d. Plants were then brought into the lab and covered with a clear plastic tube cage (15.2 cm by 45.7 cm) until parasitoids or leafhopper neonates were observed. Egg masses on stems and petioles were parasitized.

Several *P. irrorata* egg masses (<24 h old) were placed in agar-filled Petri dishes as described earlier and maintained at 25°C. Newly emerged parasitoids from the previous study were also placed in the dishes for 24 h and then removed. Twelve d later, adult parasitoids were observed in the dishes. Several parasitoids emerged from each egg of *P. irrorata*, exiting through two emergence holes in each end of the host egg. The parasitoids were preserved in 70% ethanol and sent to the second author for identification; they were then determined as *Gonatocerus fasciatus* Girault.

Gonatocerus fasciatus

This species was previously known only as an egg parasitoid of *H. coagulata* and *O. orbona* in the USA (Triapitsyn et al. 2003). Its distribution

includes Florida, Georgia, Illinois, Louisiana, Missouri, Tennessee, Texas, and Virginia (Triapitsyn et al. 2003), all within the range of *P. irrorata*. Recent biological observations on *G. fasciatus* revealed that it is a gregarious species (Triapitsyn et al. 2003). Following its introduction from Louisiana during 2002 (Triapitsyn et al. 2003), *G. fasciatus* has been mass-reared and released in California (CDFA 2005).

Material Examined

Gonatocerus fasciatus: USA, Florida, Gadsden Co., Quincy, 13-VI-2005, C. Tipping, numerous females and males (emerged from an egg mass of *P. irrorata* deposited on a soybean stem). All voucher material for this record (including two specimens of the host leafhopper) was deposited at UCRC (Entomology Research Museum, University of California, Riverside).

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SUMMARY

The mymarid wasp, *Gonatocerus fasciatus* Girault, was reared from egg masses of the proconiine sharpshooter *Paraulacizes irrorata* (Fabricius) maintained in culture at the University of Florida North Florida Research and Education Center in Quincy, Florida. This discovery is the first known host record of an egg parasitoid for *P. irrorata* and also for the genus *Paraulacizes* Young, members of which lay eggs in plant stems and twigs rather than in leaves.

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