FIELD EFFICACY OF TWO COMMERCIAL PREPARATIONS OF ENTOMOPATHOGENIC NEMATODES AGAINST LARVAE OF DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE) IN ALFISOL TYPE SOIL

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ABSTRACT

Spring and fall field trials were conducted to determine the efficacy of two species of entomopathogenic nematodes for the control of larvae of Diaprepes abbreviatus in a citrus grove with alfisol type soil (sandy clay loam). Both Steinernema riobrave (Bio Vector 355) as a water-dispersible granule and Heterorhabditis indica (Grubstake™ 100) as a paste on sponge at rates from 22-108 IJ's/cm² failed to reduce larval populations in the tree rhizosphere at 25 d post-treatment. Larval parasitism by entomopathogenic nematodes in baited screen cages was sporadic over time, with the only significant treatment effect occurring at the highest rate (108 IJ's/cm²) of S. riobrave in the fall at 7 d post-treatment. Possible constraints to nematode efficacy are discussed.

Key Words: biological control, citrus root weevils, Diaprepes, entomopathogenic nematodes, Pachnaeus

RESUMEN

Se llevaron a cabo pruebas de campo en la primavera y el otoño para determinar la eficacia de dos especies de nemátodos entomopatógenos para el control de larvas de Diaprepes abbreviatus en huertos de cítricos con suelo de tipo alfisol (marga arcilla arenosa). Las dos especies, Steinernema riobrave (Bio Vector 355) en la forma granular dispersable en agua, y Heterorhabditis indica (Grubstake™ 100) en la forma de una pasta puesta encima de una esponja al porcentaje de 22-108 IJ's/cm², no redujeron la población de larvas en la rizosfera a 25 d después del tratamiento. El parasitismo de larvas por nemátodos entomopatógenos en jaulas de tela metálica con cebo fue esporádico durante el tiempo del estudio, el único efecto significativo del tratamiento sucedió en la concentración más alta (108 IJ's/cm²) de S. riobrave en el otoño a los 7 d después del tratamiento. Se discuten las posibles restricciones a la eficacia de nemátodos.

Several species of polyphagous root weevils, particularly Diaprepes abbreviatus L. and Pachnaeus spp., are important field and nursery pests of citrus, ornamentals, and some agronomic crops in Florida (McCoy 1999). Larval feeding injury to roots by D. abbreviatus can have a devastating effect on citrus trees since all stages feed on the roots for most of the year. Root injury appears to be cumulative, and most importantly, feeding sites can serve as infection courts for root rot diseases (Graham et al. 1996), thereby exacerbating economic loss. Tree decline can be particularly bad in poorly drained groves when water stress affects root health. There is no estimate of the total economic loss to the growers from larval root injury to citrus, but the end result can frequently be tree death. Current integrated pest management (IPM) strategies for weevil control include sound horticultural practices, fungal disease control, adult weevil monitoring, and a mix of suppressive tactics to control larvae and adults (McCoy & Duncan 2000).

Native and introduced entomopathogenic nematodes are infectious to all larval stages and possibly adults (Adair 1994, Beavers et al. 1983, Schroeder 1990). Naturally occurring species within the genera Heterorhabditis and Steinernema have been found in citrus groves throughout Florida infecting as much as 38-68% of caged D. abbreviatus larvae in the summer in deep sandy soils found on the central ridge and the sandy clay loam soils of the coastal and interior flatwoods (Beavers et al. 1983, McCoy et al. 2000). The density and distribution of endemic nematode populations, regardless of species, vary from grove to grove, within a grove and within a season. Inundative releases of mass-produced entomopathogenic nematodes (EPN) for larval control have been pursued by private industry for about 15 yrs (Duncan et al. 1999, Schroeder 1987). During this time, four nematode species have been sold in Florida to control D. abbreviatus in citrus: Heterorhabditis bacteriophora Poinar; Heterorhabditis indica Poinar, Karunarke and David; Steinernema
carpocapsae (Weiser); and Steinernema riobrave (= riobravis) Cabanillas, Poinar and Raulston (Shapiro & McCoy 2000a). Currently, H. indica (Grubstake™ 100, Integrated BioControl Systems, Aurora, IN) and S. riobrave (Bio Vector 355, Certis Corporation, Columbia, MD) are sold commercially for use on Florida citrus.

The label rate for Bio Vector 355 is 4.9 × 10^2 viable IJ's/treated hectare at 250 trees/grove hectare, that is, 2,000,000 IJ's/tree (Knapp 2000). The label rate for Grubstake™ 100 is one-half the BioVector rate. These field rates can be highly variable since the area of soil treated per hectare can change according to tree size and method of application. To circumvent this problem, private industry suggests that growers apply Grubstake™ 100 at 11 IJ's/cm^2 and Bio Vector 355 at twice that rate. To date, no published field research has shown that these rates are effective against Diapheromera. Field trials by Bullock & Miller (1994), Bullock et al. (1999), and Schroeder (1990), showed that rates of 2-5 million IJ's of S. carpocapsae or S. riobrave per tree applied within a ~0.3 m^2 area surrounding the base of the tree in the spring significantly reduced adult emergence of both D. abbreviatus and Pachnaeus litus (German). In addition, field trials in groves on the central ridge using S. riobrave and H. bacteriophora showed that rates of 120-250 IJ's/cm^2 reduced larval populations significantly within 4 weeks post-treatment (Downing et al. 1991, Duncan et al. 1996, Duncan & McCoy 1996). In three separate trials, McCoy et al. (2000) showed that nematode parasitism by either S. riobrave or H. indica at 22 IJ's/cm^2 or less was no different than parasitism in the untreated control in a flatwoods grove. In fact, 108-216 IJ's/cm^2 of S. riobrave were required to increase parasitism to 40-60%.

Although published data cited above clearly show that higher rates of entomopathogenic nematodes result in (i) higher parasitism, (ii) greater suppression of larvae in the soil, and (iii) reduced adult emergence from the soil, data also suggest that efficacy is influenced by other unknown factors relating to nematode, host and/or environment (Kaya & Gaugler 1993). For example, S. carpocapsae at rates greater than 100 IJ's/cm^2 gave no control in central ridge and coastal flatwoods groves (Adair 1994; Bullock et al. 1999; Duncan et al. 1996). Laboratory studies have shown that S. riobrave is more effective at warmer soil temperatures, and host age also affects susceptibility of D. abbreviatus larvae to the nematode (Shapiro & McCoy 2000b; Shapiro et al. 1999). In addition, soil type can affect virulence and persistence of S. riobrave and H. bacteriophora (Shapiro et al. 2000), whereas culture and formulation method have no effect on larval mortality for S. riobrave (Shapiro & McCoy 2000a).

The objectives of this study were to further test the efficacy of a commercial formulation of S. riobrave (Bio Vector 355) in a flatwoods-like grove with alfisol type (sandy clay loam) soil at different rates per soil surface area and, for the first time, test the field efficacy of high rates of H. indica (Grubstake™ 100) in the same soil. Tree destruction and baited traps were used to assess larval population survival and nematode parasitism, respectively.

**Materials and Methods**

Experimental Site

Two field trials were conducted near Poinciana, FL, (Osceola County) in a declining mature planting of Hamlin oranges grafted to Swingle citrumelo rootstock. The grove was planted on two row beds with a setting pattern of 6.1 × 8.5 m. The grove was equipped with under-tree micro-jet sprinkler irrigation. The alfisol soil type for the grove was classified as Floridan fine sand 68.8% sand, 11.8% silt, 19.4% clay. The surface layer was about 35.6 cm loam and the subsurface layers about 76.2 cm grey fine sand followed by clay. The soil was poorly drained with a low to moderate organic content and a pH of 4.8. The trials were conducted within 40 m of each other. Adult weevil injury to the leaf (McCoy 1999) was evident in all trees throughout the grove.

In trial one, 40 single tree plots were arranged on two row beds in a completely randomized design with four experimental treatments and 10 replications. Beds were separated by a drainage ditch ~7.6 m in width. In trial two, 48 single tree plots were arranged on two row beds in a completely randomized block design with eight experimental treatments and six replications. In both trials, an in-row variable tree buffer was also established to prevent treatment interference.

Nematode Viability and Application

Two species of entomopathogenic nematodes, S. riobrave and H. indica, formulated as a water-dispersible granule (WDG) and a paste, respectively, were used in these field trials. Regardless of formulation, nematodes were kept cool (~20°C) both in storage and in the field prior to tank mixing. Within 2-3 h of field application, the viability (nematode mobility) of each preparation was determined microscopically by counting the number of mobile and dead infective juveniles (IJ's) in a fixed number of fields at 60× magnification. Samples of nematode preparations used for viability determination were held for a minimum of 2 h with and without aeration before counting. In both trials, viability of the water-dispersible granules of S. riobrave averaged only 57.1 and 46.2%, respectively, and therefore, an adjustment in quantity of preparation was made to achieve the desired field rate for experimentation. In trial...
two, the viability of the paste formulation of *H. indica* averaged 94.7% and no adjustments were necessary to achieve the desired field rate.

Since the WDG formulation of *S. riobrave* used in trial one had poor viability, infectivity (virulence) of the preparation was compared to an *in vivo* laboratory culture that was passed through *Diaprepes* six times and an untreated control. The bioassay procedure was identical to that described by Shapiro & McCoy (2000a). Eighth instar *D. abbreviatus* were obtained from the USDA-ARS Horticultural Laboratory (Fort Pierce, FL). One laboratory assay was performed in 50-dram plastic containers filled with Candler sand with soil moisture of about 8% by weight. A single larva was placed on the bottom of each container prior to adding sand, then 500 IJ's were applied to the soil surface. The experiment was arranged in a randomized design with each treatment replicated 10 times. The experiment was conducted at 24°C for 10 d post-inoculation. Nematode parasitism was confirmed via microscopy. Control parasitism was 0%, *in vivo* culture parasitism 40%, and WDG parasitism 80%, suggesting that the viable nematodes in the preparation were highly infectious.

For nematodes used in trial two, a similar laboratory assay was conducted and compared the WDG formulation of *S. riobrave*, the paste formulation of *H. indica*, an *in vivo* laboratory culture of *S. riobrave* that had been passed through *Diaprepes* 16 times, and an untreated control. In this case, 30 replicates were conducted per treatment. Larval mortality in the control was 3.3%, whereas it was 63.3% for the paste formulation, 50.0% for the WDG, and 53.3% for the *in vivo* culture.

In trial one, *S. riobrave* was applied to the soil beneath 10 trees at rates 0, 22, 54, and 108 IJ's/cm² on 16 April 2000, from 3:30-6:00 p.m. under overcast skies. Water-dispersible granules were pre-mixed in 1 liter of water and the appropriate volume of nematode suspension then added to the spray tank. Prior to and after nematodes were applied to the soil, irrigation was applied for about 3 h to assure soil moisture in the top 30 cm and again for 1 h the following day.

### Field Efficacy

In trial one, larval suppression in the soil rhizosphere was determined at 23 ± 1 d post-treatment using a tree removal-soil sampling procedure. Initially, trees were topped using a chain saw, then the roots along with the surrounding soil were removed using a backhoe. Most of the soil adhering to the roots was removed by shaking and/or probing with a shovel. The soil within the root crown was generally wet and compact, while the surrounding soil was moist and easy to process. Using a shovel, soil from the roots and beneath the tree was then placed in buckets for subsequent sieving. Approximately 0.4 m³ of soil was collected per tree to a depth of 30 cm according to the procedures of Duncan & McCoy (1996). All developmental stages of *D. abbreviatus* except larvae less than fifth instar were visually detectable and recovered from the soil using a motor-driven shaker and 0.64-cm mesh sieve. The number of larvae, pupae, and adults were recorded per tree. All larvae exhibiting normal behavior were recorded as live. Each dead larva was placed in a disposable Petri dish (50 × 9 mm) on a moistened filter paper. Cadavers were examined microscopically every other day for 7 d to detect characteristic changes typical of bacterial, fungal, or nematode infection. Differences in mean larval population density between treatments were tested on square root transformed data by analysis of variance.

In trial two, larval suppression in the soil rhizosphere was determined at 26 ± 1 d post-treatment using the previously described sampling procedure. Soil moisture in relation to the tree was similar to trial one, except where the sandy layer appeared at the surface in Block F causing low soil moisture within a small area within the grove. The experiment was analyzed using a three-way analysis of variance for nematode species, application rate, and block (SAS Institute, Cary, NC). Larval counts were transformed using a square root transformation prior to analysis. Because of the obvious difference in soil texture for Block F, data were analyzed with and without the block included.

In addition to estimating differences in native populations of *Diaprepes* among treatments by tree removal, larval-baited traps were used to measure post-treatment parasitism by nematodes. Our intention here was to determine if levels of larval parasitism by nematodes were comparable to changes in wild larval populations.
In the field, a hand held auger was used to make a circular hole in the soil beneath the tree canopy midway between the trunk and canopy margin to a depth of 30 cm for insertion of a baited cylindrical cage (McCoy et al. 2000). The cage made from an in-line liquid filter (7 × 3 cm diam.) with stainless steel screen (mesh size 225) was filled partially with excavated soil. Then a single 8th instar larva of *Diaprepes* produced on synthetic diet in the laboratory was placed in the cage in the soil. Additional soil was then added to fill the cage before capping. In trial one, four traps per tree were buried at the compass points beneath 10 trees (n = 40) where they remained for 7 d. Within 12 h of retrieval from the soil, each cage was opened and recovered larvae examined for nematode infection. Healthy and dead larvae were processed and diagnosed for parasitism in the manner described for wild larval collections. This procedure was conducted at 1, 2, and 3 weeks post-treatment. In trial two, two traps per tree (n = 12) were buried in the above manner and the procedure was conducted at 1 week pre-treatment and 1, 2, and 3 weeks post-treatment. For each field test, a contingency table analysis using Chi-square test (SAS Institute, Cary, NC) was performed to compare statistically the effect of field rates of nematodes on larval survival and parasitism in the soil.

**RESULTS**

**Trial One**

In the spring trial, the density of larvae (> 5th instar) recovered from the soil (0.4 m³/tree) via sieving was variable but quite high, ranging from 11 to 73 with a mean of 35.8 ± 20.5 in the control (Table 1). A few scattered pupae, usually encased in soil, and adults were also recovered at the time of tree extraction. Although larval location in the soil was not quantified, they were more prevalent in the root crown in close proximity to the roots, often lodged in compact soil surrounding the crown roots or in association with moist soil at any depth to 30-40 cm. However, all developmental stages were recovered from dry soil. Dead and diseased larvae and adults were rarely observed. However, the sieving process was most likely destructive to cadavers. Arthropod predators were rare with only an occasional fire ant mound detected. The total root system on all trees was severely damaged by larval feeding over time, and many trees were infected with root rot diseases. Trees were virtually devoid of fibrous roots.

As shown in Table 1, there was no significant difference (P = 0.316) in larval density between the different rates of Bio Vector 355 and the control according to a one-way analysis of variance. The greatest number of late instar larvae were recovered from the highest rate of nematodes. Larval density among trees was variable (pooled S.D. = 1.337).

No difference in larval survival of caged 8th instars of *D. abbreviatus* was found among treatments after exposure for 1 week at 7 d (P = 0.24) (Table 2), 14 d (P = 0.09), or 21 d (P = 0.31) post-application. From all treatments, 34, 20, and 9 larval cadavers, respectively, were recovered from baited cages buried for 7 d at 4, 14, and 21 d post-application. All remaining larvae recovered from the different treatments were healthy. Nematodes recovered from cadavers were identified as bacterial feeding rhabditids only.

**Trial Two**

The density of larvae (> 5th instar) recovered from the soil via sieving in the fall trial was also high, but numerically lower than the spring. Larval recovery from all treatments ranged from 5 to 50 per tree with a mean of 17.8 ± 10.1 (n = 50). Interestingly, the different developmental stages of *D. abbreviatus* recovered from the soil in the fall trial were similar to those found in the spring, that is, mostly mid-instar larvae, a few scattered pupae, and adults. Root systems of all trees sam-

*Means based on 10 single tree replication/treatment.*

**TABLE 1. Larval, Pupal, and Adult Recovery of Diaprepes abbreviatus from the Rhizosphere after 25 D Exposure Following Treatment with Different Rates of Steinernema riobrave (Bio Vector 355) in the Spring at Poinciana, Florida.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate JF/scm²</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Adults</th>
<th>Mean no larvae/0.4cm³ ± SDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>358</td>
<td>7</td>
<td>7</td>
<td>35.8 ± 20.5</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>22</td>
<td>443</td>
<td>17</td>
<td>8</td>
<td>44.3 ± 14.1</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>54</td>
<td>375</td>
<td>13</td>
<td>7</td>
<td>37.5 ± 14.1</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>108</td>
<td>485</td>
<td>5</td>
<td>4</td>
<td>48.5 ± 12.3</td>
</tr>
</tbody>
</table>

F = 1.22,  
P = 0.316
pled were severely damaged by weevils and showed root rot symptoms.

The effect of the different rates of Bio Vector 355 and Grubstake™ 100 on the reduction of larvae of *Diaprepes abbreviatus* is presented in Fig. 1. The overall analysis of variance produced highly significant results (3-way ANOVA, $F = 3.56$, $df = 12, 35, P = 0.0016$). However, neither the main effect for nematode species nor for application rate was significant (nematode species, $F = 2.83, df = 1, 35, P = 0.1013$; application rate, $F = 1.43, df = 3, 35, P = 0.2510$), whereas the main effect for blocks was highly significant ($F = 6.98, df = 5, 35, P = 0.0001$). The species by dose interaction was also not significant ($F = 0.24, df = 3, 35, P = 0.8707$).

The data were also analyzed after pooling the results for the three application rates within species. Again, the overall analysis of variance produced a highly significant result (3-way ANOVA, $F = 5.08, df = 8, 39, P = 0.0002$). However, the main effects for nematode species and for application rate failed to reach significance (nematode

### TABLE 2. CONTINGENCY TABLES, CHI-SQUARE ANALYSIS FOR SURVIVING CAGED 8TH INSTAR LARVAE OF *DIAPREPS ABBRVIATUS* AFTER 1 WEEK EXPOSURE TO FIELD SOIL TREATED WITH DIFFERENT RATES OF *STEHNERNEMA RIOBRAVE* (BIO VECTOR 355) IN THE SPRING AT POINCIANA, FLORIDA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate LJ’s/cm²</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>50.0</td>
<td>72.4</td>
<td>90.4</td>
</tr>
<tr>
<td>Bio Vector 22</td>
<td>62.1</td>
<td>85.5</td>
<td>78.3</td>
<td></td>
</tr>
<tr>
<td>Bio Vector 54</td>
<td>45.6</td>
<td>64.9</td>
<td>85.5</td>
<td></td>
</tr>
<tr>
<td>Bio Vector 108</td>
<td>45.0</td>
<td>70.7</td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>Chi-square value</td>
<td></td>
<td>4.20</td>
<td>6.42</td>
<td>3.59</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>0.24</td>
<td>0.09</td>
<td>0.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate LJ’s/cm²</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>50.0</td>
<td>72.4</td>
<td>90.4</td>
</tr>
<tr>
<td>Bio Vector 22</td>
<td>62.1</td>
<td>85.5</td>
<td>78.3</td>
<td></td>
</tr>
<tr>
<td>Bio Vector 54</td>
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<td>85.5</td>
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</tr>
<tr>
<td>Bio Vector 108</td>
<td>45.0</td>
<td>70.7</td>
<td>80.0</td>
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</tr>
<tr>
<td>Chi-square value</td>
<td></td>
<td>4.20</td>
<td>6.42</td>
<td>3.59</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>0.24</td>
<td>0.09</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*Means based on 15 single tree replicates per treatment; 4 cages/replicate.*

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*Scientific names italics only*
As previously mentioned, Block F produced extremely low numbers of larvae (range, 0-26; mean, 6.5) in nearly all treatments. Therefore, we repeated the original analysis but omitted the extreme replicate. In this case, the overall analysis of variance failed to achieve significance (3-way ANOVA, $F = 1.88$, $df = 11, 28$, $P = 0.0862$). However, repeating this analysis without this extreme replicate and after pooling the three lowest application rates produced a significant result (3-way ANOVA, $F = 2.98$, $df = 7, 32$, $P = 0.0158$). Nonetheless, again, the main effect for nematode species was not significant ($F = 0.81$, $df = 1, 32$, $P = 0.3738$) and the main effect for application rate narrowly missed significance ($F = 3.45$, $df = 1, 32$, $P = 0.0723$). The main effect for blocks was highly significant ($F = 4.15$, $df = 4, 32$, $P = 0.0080$). The species by dose interaction were also not significant ($F = 0.16$, $df = 4, 32$, $P = 0.6960$). The species by dose interaction were also not significant ($F = 0.16$, $df = 1, 32$, $P = 0.6960$).

In the fall field trial, larval survival of caged 8th instar larvae of *D. abbreviatus* following 7 d exposure to pre-treated soils ranged from 81.8 to 100% (Table 3) and from 66.7 to 100% (Table 4). No significant difference was found among treatments receiving either *S. riobrave* ($P = 0.58$) or *H. indica* ($P = 0.115$). Larval survival of *D. abbreviatus* after 7 d exposure to soil treated with different rates of *S. riobrave* at 7, 14, and 21 d post-treatment was significantly lower for the highest rate of *S. riobrave* at 7 d ($P = 0.016$), while no difference between treatments was found at 14 ($P = 0.363$) and 21 d ($P = 0.279$) (Table 3). Larval survival of *D. abbreviatus* following exposure for 7 d to soil treated with different rates of *H. indica* at 7, ($P = 0.279$) 14, ($P = 0.271$), and 21 d ($P = 0.271$) post-treatment was not significantly different from the control (Table 4). Nematodes recovered from cadavers were identified by the junior author. Twelve infected larvae produced *Steinernema* sp. close to *S. riobrave*, 10 infected larvae had *H. bacteriophora*, 6 infected larvae had *Heterorhabditis* sp., and several *Cephalobus* sp. (bacterial feeders) were also recovered.

### DISCUSSION

Spring and fall applications of *S. riobrave* and *H. indica* at commercial rates (10-20 IJ's/cm²) and higher (54-108 IJ's/cm²) failed to reduce larval populations and increase nematode parasitism appreciably in the soil. When compared to previous field trials, these data further substantiate the broad variability in field efficacy experienced by previous researchers when applying entomopathogenic nematodes as biopesticides to different citrus groves (Duncan et al. 1999, McCoy & Duncan 2000).

Variation in efficacy can be caused by multiple factors relating to the nematode, its host, and the environment (Kaya & Gaugler 1993). A number of contributing factors such as larval age, soil temperature, nematode virulence, culture method, and soil characteristics have been recognized in experimentation with entomopathogenic nematodes as biological control agents of *Diaprepes* in citrus soils (Shapiro & McCoy 2000a, 2000b, 2000c; Shapiro et al. 1999). In addition, sampling methods for assessing nematode efficacy in the field have differed widely among researchers and no doubt have contributed somewhat to the variation in larval control (Bullock & Miller 1994, Duncan & McCoy 1996, McCoy et al. 2000).

Recent field and microcosm experiments, designed to determine the effect of soils of different composition and texture on nematode efficacy, strongly suggest that field failures reported herein were soil-related (Duncan et al. 2001).

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### TABLE 3.
Table: Contingency Table, Chi-square Analysis for Surviving Caged 8th Instar Larvae of *Diaprepes abbreviatus* After 1 Week Exposure to Field Soil Treated with Different Rates of *Steinernema riobrave* (Bio Vector 355) in the Fall at Poinciana, Florida.

<table>
<thead>
<tr>
<th></th>
<th>Mean % larval survival, day post-treatment</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>100.0</td>
<td>100.0</td>
<td>90.9</td>
<td>83.3</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>11</td>
<td>81.8</td>
<td>100.0</td>
<td>83.3</td>
<td>100.0</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>54</td>
<td>91.7</td>
<td>91.7 a</td>
<td>75.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Bio Vector</td>
<td>108</td>
<td>90.9</td>
<td>63.6 b</td>
<td>60.0</td>
<td>83.3</td>
</tr>
<tr>
<td>Chi-square value</td>
<td></td>
<td>1.97</td>
<td>10.26</td>
<td>3.19</td>
<td>3.84</td>
</tr>
<tr>
<td>Probability</td>
<td></td>
<td>0.58</td>
<td>0.016*</td>
<td>0.363</td>
<td>0.279</td>
</tr>
</tbody>
</table>

n.s. n.s. n.s.

*Means based on 12 cages/treatment.*
When soil (entisol type) from a deep sandy ridge grove (Lake Alfred) with a percent sand:silt:clay ratio of 97.6:1.5:0.9 was compared to sandy clay loam soil (alfisol type) from our experimental site (Poinciana) with a ratio of 68.8:11.8:19.4, *S. riobrave* applied at 20 IJ's/cm² killed 70-80% of the larvae of *D. abbreviatus* buried to a depth of 30 cm in the sandy soil (Lake Alfred), but only 4-17% of the larvae in sandy clay loam soil (Poinciana) suggesting that higher clay soil with finer texture reduced host contact or affected the infection process.

This marked difference in nematode efficacy between sandy and sandy clay loam soils is suggested in the published literature. For example, in two groves on the central ridge with a deep sandy soil (entisol type), *S. riobrave* applied at 20 IJ's/cm² killed 70-80% of the larvae of *D. abbreviatus* buried to a depth of 30 cm in the sandy soil (Lake Alfred), but only 4-17% of the larvae in sandy clay loam soil (Poinciana) suggesting that higher clay soil with finer texture reduced host contact or affected the infection process.

In recent microcosm studies comparing the efficacy of *S. riobrave* at a rate of 20 IJ's/cm² in eight autoclaved soils from citrus groves including Poinciana, Duncan et al. (2001) found that larval mortality of *D. abbreviatus* was positively correlated with the proportion of sand in the soils, but was inversely related to the percentage of fine sand. The strongest correlation with efficacy was with percentage of medium and coarse sand in soils. Both nematode emergence from the cadaver and recycling in cadavers were favored coarse sandy soils.

The importance of soil texture in relation to soil compaction as determined by Duncan et al. (2001) is supported by field observations we made on soil compaction within the tree rhizosphere at the time of tree removal. Soil surrounding the roots was very fine in texture resulting in extreme compaction on the roots and within the rhizosphere. Soil was so compact within the root crown of the tree, it was virtually impossible to remove with a probe. When larvae adjoining the roots were removed with a probe, invariably they were healthy suggesting the soil was so compact nematode penetration of the soil was infrequent.

As previously mentioned in the methods, nematode mobility and viability of WDG formulation of *S. riobrave* was generally lower in these studies compared to formulations from earlier field studies (Duncan & McCoy 1996; Duncan et al. 1996). It might be argued that nematode vigor was a factor in explaining poor field efficacy in these trials. Although this could be true for *S. riobrave*, it cannot explain our results with *H. indica*, where nematode viability was excellent.

Both larvae used in baited cages to measure nematode parasitism (8th instar) and the larval instars recovered from the native soil (6th-10th instar) fall within the age group (i.e., 100 d old) reported by Shapiro et al. (1999) as being least

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### Table 4. Contingency Table, Chi-square analysis for surviving caged 8th instar larvae of *Diaprepes abbreviatus* after 1 week exposure to field soil treated with different rates of *Heterorhabditis indica* (Grubstake™ 100) in the fall at Poinciana, Florida.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate IJ's/cm²</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>66.7</td>
<td>83.3</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Grubstake</td>
<td>11</td>
<td>100.0</td>
<td>91.7</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Grubstake</td>
<td>54</td>
<td>90.9</td>
<td>83.3</td>
<td>83.3</td>
<td>83.3</td>
</tr>
<tr>
<td>Grubstake</td>
<td>108</td>
<td>72.7</td>
<td>60.0</td>
<td>91.7</td>
<td>91.7</td>
</tr>
<tr>
<td>Chi-square value</td>
<td></td>
<td>5.92</td>
<td>3.74</td>
<td>3.91</td>
<td>3.911</td>
</tr>
<tr>
<td>Probability</td>
<td>n.s.</td>
<td>0.115</td>
<td>0.291</td>
<td>0.271</td>
<td>0.271</td>
</tr>
</tbody>
</table>

*Means based on 12 cages/treatment.*
susceptible to nematode infection by both *S. riobrave* and *H. indica*. In view of their findings, it is reasonable to assume that host age can influence nematode parasitism in the field.

The results of these field studies supported by the studies of Duncan et al. (2001) pose important implications relating to the biological control of larvae of *D. abbreviatus* with entomopathogenic nematodes in Florida citrus. Foremost, soil characteristics appear to be important determinants of field efficacy. Current nematode products appear most efficacious in deep sandy soils common to the central ridge of Florida; however, efficacy is affected substantially by different soils, particularly the sandy clay loams. The issue of optimal rate appears variable and is likely influenced by host age and edaphic factors. Finally, further research is warranted on nematode species selection and the dynamics of edaphic conditions in relationship to field performance.

ACKNOWLEDGMENTS

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RELATIVE ATTRACTIVENESS OF ENRICHED GINGER ROOT OIL AND TRIMEDLURE TO MALE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT
This study describes field experiments that compare the relative attraction of male Mediterranean fruit flies (or medfly), Ceratitis capitata (Wiedemann), to trimedlure and ginger root oil, which contains the natural attractant \(\alpha\)-copaene. The ginger root oil was embedded in a paste-like matrix, and the concentration of \(\alpha\)-copaene was enhanced 20-fold above natural levels (hence the term “enriched” ginger root oil or EGRO). In tests conducted in a mixed fruit orchard in Waimanalo, Hawaii, 8 Jackson traps (4 baited with trimedlure, 4 baited with enriched ginger root oil) were placed in a circle (40 m radius) about a central point from which 500 males were released per replicate. Trap catches were scored 48 h after male release. In experiments using fresh (non-aged) lures, the amount of trimedlure used per trap was constant (1 ml), but the amount of EGRO-containing paste used in traps was 1, 10, or 20 drops. Significantly more males were captured in the trimedlure traps than the EGRO traps over all doses of EGRO. Similar experiments conducted in a small citrus grove yielded the same results. Additional experiments revealed that female medflies showed no attraction to either trimedlure- or EGRO-baited traps and that immature and mature males showed equal, short-range attraction to trimedlure and EGRO-baited traps.

RESUMEN
Este estudio describe los experimentos de campo para comparar la atracción relativa de los machos de la mosca mediterránea de la fruta, Ceratitis capitata (Wiedemann) al trimedlure y al aceite de la raíz de jengibre los cuales contienen un atrayente natural \(\alpha\)-copaene. El aceite de la raíz de jengibre fue embutido en una pasta a manera de matriz y la concentración de \(\alpha\)-copaene fue mejorada 20-veces por encima del nivel natural (de aquí el término “aceite de la raíz de jengibre “enriquecido” o EGRO por su sigla en ingles). En pruebas llevadas a cabo en huertos de frutas mezcladas en Waimanalo, Hawaii, 8 trampas de tipo Jackson (4 con el cebo de trimedlure, 4 con el aceite de la raíz de jengibre enriquecido) fueron colocadas en un circulo (40 m de radio) alrededor de un punto central de donde liberaron 500 machos por cada replicá. El contenido de las trampas fue contabilizado 48 h después de liberar los machos. En experimentos usando atrayentes frescos (no viejos), la cantidad de trimedlure usado por trampa fué constante (1 ml), pero la cantidad de pasta embutida con EGRO usada en trampas fue 1, 10, ó 20 gotas. Una cantidad significativamente mayor de machos fueron capturados en trampas de trimedlure que en las trampas de EGRO con las diferentes dosis de EGRO. Experimentos similares llevados a cabo en huertos pequeños de cítricos produjeron los mismos resultados. Experimentos adicionales revelaron que las hembras de la mosca mediterránea no mostraron ninguna atracción a ninguna de las trampas con cebo de trimedlure- o de EGRO y los machos maduros e inmaduros mostraron una atracción de corto alcance igual hacia las trampas con cebo de trimedlure como las de EGRO.

Females of the Mediterranean fruit fly, Ceratitis capitata (Wied.) (medfly), may lay several hundred eggs over their lifetime and infest a wide variety of fruits and vegetables (Christenson & Foote 1960). Given this high intrinsic rate of population growth, early detection of incipient medfly outbreaks is critical for successful suppression or eradication. Following intensive screening of potential attractants in the 1950’s (Beroza & Green 1963), trimedlure, tert-butyl 4 (and 5)-chloro-trans-2-methylcyclohexane-1-carboxylate, emerged, and still prevails, as the standard attractant used to detect and monitor medfly populations (Beroza et al. 1961).

Despite its wide use, however, trimedlure is not the most attractive compound known for male medflies. In the aforementioned screening efforts, botanically derived compounds were tested, and oil from the seeds of the angelica flower, Angelica archangelica L. was found to be highly attractive to C. capitata males. The active components were subsequently identified as \(\alpha\)-copaene and \(\alpha\)-ylangene, two structurally related tricyclic sesquiterpenes (Guitto et al. 1972). Field tests (Flath et al. 1994a,b) revealed that male medflies displayed greater attraction to \(\alpha\)-copaene than \(\alpha\)-ylangene and that \(\alpha\)-copaene was actually more attractive than trimedlure (when presented in equal volumes). \(\alpha\)-copaene was subsequently identified as a minor component in the essential oils of many host plants of C. capitata, including orange, Cit-

Although attractive, α-copaene is not used in medfly detection programs, because 1) it occurs in very low concentrations in plants, making extraction impractical, and 2) it has a complex chemical structure, making synthesis laborious and expensive (Flath et al. 1994b). Ginger root, Zingiber officinalis Roscoe, oil is an inexpensive and readily available source of α-copaene, and a recently developed distillation procedure can greatly increase the concentration of this compound. α-copaene comprises 8% of this so-called enriched ginger root oil (hereafter EGRO) compared to only 0.4% in commercially available oil (Citrus and Allied Essences, Ltd., Lake Success, NY; F. Webster, personal communication).

The primary purpose of the present study was to compare trap captures of male medflies to trimedlure- and EGRO-baited Jackson traps in field tests. Similar tests were conducted in a mixed fruit orchard that contained orange, guava, and mango trees. Jackson traps, containing a sticky insert, were placed singly in the canopies of 8 trees (2 m above ground) arranged in a circle (radius 40 m) about a central tree, which served as the release point. The Jackson traps contained trimedlure in 4 trees and EGRO in the remaining 4 trees (see below). The lures were applied to the traps at the laboratory (4 km from the study site), and the traps were then immediately transported to and placed in the test trees. At a given tree, the bait used was alternated between successive replicates, and for a given replicate, adjacent test trees contained different baits.

For the trimedlure-baited traps in all experiments, 1 ml of the lure was applied to a cotton wick (2.5 cm long), which was then placed in a perforated, plastic basket that was, in turn, suspended within the Jackson trap. This dose is ½ of that used in area-wide survey and detection programs (California Department of Food and Agriculture 1995). Trimedlure is composed primarily of eight isomers of which one (designated C) is the most attractive (McGovern et al. 1987), and 1 ml of trimedlure contains approximately 0.358 g of isomer C (trimedlure purity × concentration of C isomer × weight of 1 ml trimedlure = 0.965 ⋅ 0.399 ⋅ 0.929g; J. Knapp, personal communication). For the EGRO-baited traps, the amount of lure used varied between experiments (see below) and was placed on a small piece of aluminum foil directly on the sticky insert within the Jackson trap. The oil was embedded within a paste-like matrix (Last Call™, IPM Technologies, Inc.), comprised primarily of tinuvin, that was applied with a calibrated pump (1 drop of paste = 50 µl or 0.05g). EGRO comprised 20% of the paste (by weight), α-copaene constituted 8% of the EGRO (by weight), and thus one drop of paste contained 0.0008 g of α-copaene (J. McLaughlin, personal communication). It should be noted that the distillation procedure used to increase the concentration of α-copaene increases the concentration of other sesquiterpenes as well (e.g., α- and β-ylangene; F. Webster, personal communication) and that the combination of all sesquiterpenes is more attractive to male medflies than α-copaene alone (T. W. Phillips, personal communication).

Five hundred males were released per replicate for all experiments. As wild flies were rare at the study site, released flies were not marked, and we assumed that all captured flies were from the releases. Successive replicates were run a minimum of 2 d apart to allow previously released flies time to disperse from the test area. Ten replicates were performed per experiment. Flies were re-

Field Experiments – Waimanalo

Most of the field experiments were conducted at the University of Hawaii Agricultural Experiment Station in Waimanalo in a mixed fruit orchard that contained orange, guava, and mango trees. Jackson traps, containing a sticky insert, were placed singly in the canopies of 8 trees (2 m above ground) arranged in a circle (radius 40 m) about a central tree, which served as the release point. The Jackson traps contained trimedlure in 4 trees and EGRO in the remaining 4 trees (see below). The lures were applied to the traps at the laboratory (4 km from the study site), and the traps were then immediately transported to and placed in the test trees. At a given tree, the bait used was alternated between successive replicates, and for a given replicate, adjacent test trees contained different baits.

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leased between 1000-1200 hours by placing 4 buckets (volume 5 liters and each containing 125 flies) on the ground beneath the release tree and gently removing the screen cover from the bucket. The buckets were not tapped or shaken, and the flies exited the bucket on their own volition. Trap catches were counted 2 d later. Field work at Waimanalo was conducted during September-December, 2000, and daily minimum and maximum temperatures ranged from 21-23°C and 24-28°C, respectively.

The number of males captured at trimedlure-baited traps was compared with traps baited with 1 (50 µl), 10 (0.5 µl), or 20 (1 ml) drops of the paste containing EGRO. When multiple drops of EGRO-bearing paste were used, the individual drops were placed close together but were not overlapping. To investigate temporal decline in lure attractiveness, male catches were compared between trimedlure-baited traps and traps baited with 1 drop of the EGRO-containing paste using baits that had been aged 5 d prior to use (baits were placed in Jackson traps and placed outdoors in a covered area several km from the study site). In a final experiment, the female attraction to trimedlure-baited traps and traps baited with 1 drop of the paste containing EGRO was compared following the procedures outlined above for males.

Following the trimedlure-EGRO comparisons, we ran 2 additional experiments (using the protocol described above) that examined the potential attractiveness and repellency, respectively, of the paste in which the EGRO was delivered. In the first case, we compared male captures in 4 traps baited with 1 drop of the EGRO-containing paste versus traps baited with the one drop of paste to which no EGRO was added. In the second, we placed 0.5 ml of ginger root oil (Citrus and Allied Essences, Ltd., Lake Success, NY) on wicks for all 8 traps. In 4 of the traps, we also placed 10 drops of paste (lacking EGRO) on a piece of aluminum foil fastened to the sticky insert, while in the remaining 4 traps, we placed the foil only and no paste. Six replicates were run of each of these 2 experiments.

Field Experiments—Pearl City

To examine potential habitat differences in lure attractiveness, a second set of field experiments was conducted in a small citrus grove (1 ha) at the University of Hawaii’s Urban Garden Center in Pearl City. Fieldwork at this site was conducted during June-July, 2001, and daily minimum and maximum temperatures ranged from 22-26°C and 30-33°C, respectively. The protocol used was identical to that described above, except that 1) trimedlure plugs containing 2 g of trimedlure (Farma Tech International, Fresno, CA), were used in place of liquid trimedlure and 2) only 4 traps, 2 baited with trimedlure and 2 baited with EGRO, were used per replicate. Trimedlure-baited traps were compared only with traps baited with 1 or 10 drops of the EGRO-containing paste, and no experiments were run involving aged baits or females. Eight replicates were performed per experiment.

Field-Cage Experiments—Waimanalo

Four experiments were conducted during June-September, 2001, at Waimanalo using field-cages (height 2.5 m, diameter: 3.0 m) that contained rooted guava trees. In the first, we investigated age-dependent attraction of males to trimedlure by comparing trap catches of immature (1 d old) and mature (9-13 d old) males. Groups of 100 immature and 100 mature males were released between 0900-1100 hours in a field-cage containing 1 baited (treated) and 1 unbaited (control) trap, and trap catches were recorded 24 h later. For the purpose of identification, males were marked by age group by cooling them for several minutes and then placing a small dot of enamel paint on the thorax. Immature males were marked in the late afternoon of the day of adult emergence to allow hardening of the exoskeleton; mature males were marked 1-2 d before testing. Traps were rectangular pieces of white cardboard (9 by 16 cm) coated on both sides with Tanglefoot™ and suspended in the canopy (1.5 m above ground) with wire hooks. A wick containing 1 ml of trimedlure was placed in the center of one side of the treated trap, and a cotton wick (without trimedlure) was applied to the control trap. The trimedlure used had a deep red color, and consequently we added a small amount of diluted red food coloring (McCormick & Co., Inc.) to the wick on the control trap to equalize visual stimuli. The same trap sites were used over all replicates, but the positions of baited and control traps were alternated between successive replicates. The second experiment examined age-dependent response of males to EGRO following the same procedures described above. In this case, 1 drop of EGRO-containing paste was placed on a small piece of aluminum foil, which was then placed in the center of one side of the trap. For the control trap, aluminum foil was placed on the trap, but no paste was applied. In the third and fourth experiments, we investigated close-range attraction of females to trimedlure and EGRO, respectively. The same protocol was followed except that only 1 group of 100 females (9-13 d old) was released per replicate, and their numbers on baited and control traps were compared. Seven replicates were conducted for each of the 4 field-cage experiments.

Statistical Analyses

Pairwise comparisons of trap catches were made using the Mann-Whitney test (test statistic
T), a nonparametric equivalent of the Students t-test. Multiple comparisons were made using the Kruskal-Wallis (test statistic H), and if significant variability was detected, the multiple comparison Tukey test (test statistic q) was used to assess pairwise differences. Nonparametric tests were employed to avoid assumptions of normality and equal variance for the sampled populations. Analyses were conducted using SigmaStat Statistical Software (Version 2.0).

RESULTS

Field Experiments – Waimanalo

On average, the trimedlure-baited traps captured more males than the EGRO-baited traps in all experiments (Table 1A). With fresh (non-aged) baits, the trimedlure-baited traps captured approximately 1.6-2.6 times more males, on average, than did the EGRO-baited traps. When aged baits were used, the trimedlure-baited traps caught 19 times as many males as the EGRO traps (experiment 4). The number of males captured in trimedlure-baited traps did not differ significantly across the 4 experiments (H = 2.7, df = 3, P > 0.05), indicating that the 5-d aging period did not reduce trimedlure’s attractiveness. In contrast, male numbers in EGRO-baited traps varied significantly among experiments (H = 23.4, df = 3, P < 0.001), with the male catch for aged EGRO-baited traps being significantly lower than those recorded for any of the freshly-baited traps (P < 0.001 in all cases). Among traps having fresh EGRO-containing paste, the number of males captured varied independently of the amount of paste used (H = 3.5, df = 2, P > 0.05).

Neither trimedlure nor GRO was attractive to females. Not a single female was caught in any trap over 10 replicates.

The paste used to deliver EGRO was not attractive or repellent to male medflies. Similar to experiment 1, traps baited with 1 drop of EGRO-containing paste caught an average of 11.1 (range: 5-18) males per replicate. However, traps baited with paste that lacked EGRO captured no males at all over 6 replicates. In testing for potential repellency of the paste, we found no significant difference in captured males between traps with paste (x̄ = 11.3; range: 8-18) versus traps without paste (x̄ = 10.7; range: 7-14; T = 40.0, P > 0.05) adjacent to the wick containing ginger root oil.

Field Experiments - Pearl City

Results obtained at Pearl City were similar to those reported above for Waimanalo (Table 1B). The trimedlure-baited traps, on average, captured significantly more males than did the EGRO-baited traps. In relative terms, the difference between the baits was even more pronounced

| Table 1. Numbers of males captured in trimedlure- or EGRO-baited Jackson traps at Waimanalo and Pearl City study sites. |
|---|---|---|---|
| Experiment | Amount of EGRO-containing paste | Trimedlure | GRO | T |
| A. Waimanalo | | | | |
| 1 | no | 50 µl | 21.7A,a (11-27) | 8.3B,b (3-17) | 152.0*** |
| 2 | no | 0.5 ml | 18.5A,a (11-26) | 11.2B,b (4-18) | 143.0** |
| 3 | no | 1.0 ml | 18.4A,a (10-26) | 11.2B,b (4-16) | 141.0* |
| 4 | yes | 50 µl | 19.2A,a (6-30) | 1.8B,c (0-4) | 155.0*** |
| B. Pearl City | | | | |
| 1 | no | 50 µl | 24.1A,a (11-37) | 6.6B,b (4-17) | 98.0*** |
| 2 | no | 0.5 ml | 19.4A,a (6-27) | 7.7B,b (2-9) | 92.0** |

In all experiments, 1 ml of trimedlure was used in the trimedlure-baited traps. Values represent mean numbers of males captured per replicate; ranges are given in parentheses. Ten and 8 replicates were conducted for experiments conducted at Waimanalo and Pearl City, respectively. T values were computed for Mann-Whitney tests comparing male captures for the 2 attractants within a given experiment (row). For a given location, values in the same row followed by the same uppercase letter were not significantly different, and values in the same column followed by the same lowercase letter were not significantly different following the Kruskal-Wallis or Tukey test (P = 0.05). Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001.
at Pearl City, where the average trap catches were 3-4 times higher for trimedlure-baited traps than EGRO-baited traps. Additionally, no difference in male captures was detected between traps having 1 or 10 drops of the EGRO-bearing paste over the two experiments (T = 75.0, P > 0.05). Male captures were also similar for the trimedlure-baited traps across the 2 experiments (T = 78.0, P > 0.05).

Inter-habitat comparisons revealed that, for trimedlure-baited traps, the number of males captured per trap did not differ significantly between Waimanalo and Pearl City (T = 431.5, n₁ = 30, n₂ = 16, P > 0.05). For the EGRO-baited traps, however, trap catches were significantly greater at Waimanalo (x = 10 males/trap) than at Pearl City (x = 6.5 males/trap; T = 245.5, n₁ = 30, n₂ = 16, P < 0.05).

Field-Cage Experiments – Waimanalo

No difference was detected in the number of immature and mature males captured on treated versus control traps for the experiments involving trimedlure or EGRO (Table 2). Data pooled for immature and mature males revealed that, on average, treated traps captured approximately 13 times as many males as control traps in a given replicate for both trimedlure (80/6) and EGRO (66/5). Consistent with the field experiments, the average number of males captured per replicate (age groups combined) was higher for trimedlure than EGRO-baited, although this difference was not statistically significant (T = 64.0, P > 0.05). In the third and fourth experiments, females showed no short-range attraction to trimedlure or EGRO. On average, the trimedlure-baited trap captured 2.7 (range: 0-6) females per replicate compared to 2.4 (range: 1-5) for the control trap (T = 54.5, P > 0.05). Similarly, the EGRO-baited trap captured 3.7 females per replicate compared to 2.7 females for the control trap (T = 63.5, P > 0.05).

Table 2. Numbers of 1-d old (immature) and 9-13 d-old (mature) males captured in control and treated (A-trimedlure; B-EGRO) panel traps in field-cages at Waimanalo.

<table>
<thead>
<tr>
<th>Trap type</th>
<th>Male Age</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Trimedlure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.6A (0-5)</td>
<td>3.0A (0-8)</td>
</tr>
<tr>
<td>Treated</td>
<td>42.8A (29-54)</td>
<td>37.0A (21-58)</td>
</tr>
<tr>
<td>B. EGRO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.2A (0-7)</td>
<td>3.1A (24-42)</td>
</tr>
<tr>
<td>Treated</td>
<td>32.5A (24-42)</td>
<td>34.6A (27-42)</td>
</tr>
</tbody>
</table>

*Values represent mean numbers of males captured per replicate; ranges are given in parentheses. Seven replicates were performed for all field-cage experiments. T values were computed for Mann-Whitney tests comparing captures for the 2 age groups within a given experiment (row). Values in the same row followed by the same uppercase letter were not significantly different (P = 0.05).
males as traps baited with fresh paste. Thus, data from both fresh and aged baits indicate that trimedlure is superior to EGRO as a detection and monitoring tool in medfly control programs.

Although only 2 sites were involved, the field experiments indicate, preliminarily at least, that the attractancy of EGRO-baited traps may vary more among different habitats than that of trimedlure-baited traps. Trap catches were similar between the 2 sites for trimedlure-baited traps, whereas a significantly higher number of males was captured at Waimanalo than Pearl City for the EGRO-baited traps. While this difference could reflect the small sample of sites included, it may also indicate differences in the ‘aromatic’ environment at the 2 sites that differentially affected (interfered with) the effectiveness of the 2 baits. For example, volatiles emanating from the citrus trees at Pearl City may have more closely resembled (or mimicked) those given off from EGRO-baited traps than trimedlure-baited traps. If so, the citrus trees might have effectively “swamped” or outcompeted the olfactory stimuli of EGRO-baited traps and thus effectively lessened the attractiveness of this bait to male medflies.

Females were not attracted to either bait in the field trials or the field-cage tests. The lack of female response has been documented previously for trimedlure (Delrio & Zumreoglu 1983, Howse & Knapp 1996) and α-copaene (Nishida et al. 2000). As with pure α-copaene, the non-attractance of females to EGRO was unexpected, because α-copaene, which is present in many medfly hosts (see aforementioned references), has been considered a rendezvous stimulus that brings the sexes together for mating (Nishida et al. 2001). Although not attractive to females by itself, α-copaene may still affect the mating system of the medfly by enhancing female response to the male pheromone. Dickens et al. (1990) reported that green leaf volatiles boost female response to male medfly pheromone, and it seems likely that particular plant compounds, such as α-copaene, may act in a similar way (see examples in Landolt & Phillips 1997).

Although differing in their overall attractiveness, trimedlure and EGRO were both attractive to immature and mature males. Initial experiments (Shelly 2001) showed that mature male medflies exposed to the aroma of ginger root oil had a mating advantage over non-exposed males in tests conducted 2 days after the exposure. Follow-up tests (Shelly 2001) further revealed that 1-day old males exposed to ginger root oil had a mating advantage over non-exposed males in tests conducted 8-10 days after exposure. Given these findings, it was not surprising that male attraction to EGRO was age independent: attraction to an α-copaene source appears to confer an immediate benefit to mature males and a delayed benefit to immature males. This explanation, however, does not appear applicable for trimedlure. Although exposing mature males to trimedlure boosts their mating success, the effect is short-lived, and mating enhancement was evident only within 24 h of exposure (Shelly et al. 1996). Although we have not exposed 1-day old males to trimedlure, it appears unlikely that such early exposure would affect mating performance a week following exposure. Consequently, the attraction of immature males to trimedlure cannot apparently be explained in the context of sexual selection.

ACKNOWLEDGMENTS

We thank Roger Coralis and Dale Sato for permission to work at the Waimanalo and Pearl City sites, respectively. We are also grateful to John McLaughlin, Tom Phillips, and Fran Webster for information on ginger root oil and α-copaene and Charmian Dang, Susan Kennelly, Courtney Ishimura, Eric Rutka, and Mindy Teruya for assistance in rearing and maintaining the flies. The EGRO-containing paste was kindly supplied by IPM Technologies, Inc., Portland, Oregon. Comments by Don McInnis and Grant McQuate improved the paper. We gratefully acknowledge the financial support of the California Citrus Research Board for this research (Agreement No. 5510-144).

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POTENCY, SPECTRUM AND RESIDUAL ACTIVITY OF FOUR NEW INSECTICIDES UNDER GLASSHOUSE CONDITIONS

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ABSTRACT

The toxicities of four classes of insecticides, emamectin benzoate (avermectin), chlorfenapyr (pyrrole), fipronil (phenylpyrazole), and tebufenozide (benzoylhydrazide) were compared using an artificial diet assay and a residual efficacy assay against several species of Lepidoptera. Emamectin benzoate was consistently the most toxic insecticide; it was 20- to 64,240-times more toxic than the other compounds tested. The LC90 values for emamectin benzoate ranged from 0.0050 to 0.0218 ug/ml for six species of Lepidoptera. Similarly, chlorfenapyr displayed consistent toxicity to all species, with LC90 values ranging from 1.9 to 4.6 ug/ml. The toxicities of fipronil and tebufenozide varied among the species tested. Fipronil LC90 values varied 501-fold (range, 0.64 to 321.3 ug/ml), while tebufenozide toxicity varied 113-fold (range, 0.24 to 27.1 ug/ml) among species tested. In residual efficacy tests conducted in the glasshouse, all compounds were effective (i.e., >90% mortality) at controlling Heliothis virescens on garbanzo bean at projected field rates and at 1/10 of projected field rates with fipronil and emamectin benzoate. Emamectin benzoate, chlorfenapyr and tebufenozide were effective at controlling Spodoptera exigua on sugar beet at projected field rates. However, mortality with fipronil was reduced to 20% or less at 7 to 14 days after treatment. All compounds at projected field use rates were effective against Trichoplusia ni on cabbage, although tebufenozide was the only compound effective at 1/10 of projected field rate for 14 days after treatment. However, tebufenozide was ineffective against Plutella xylostella at projected field use rates on cabbage while emamectin benzoate, chlorfenapyr, and fipronil were effective. The potential of these compounds for arthropod pest management are discussed.

Key Words: Chlorfenapyr, emamectin benzoate, fipronil, tebufenozide, residual assay

RESUMEN

La toxicidad de cuatro clases de insecticidas, emamectin benzoate (avermectin), chlorfenapyr (pyrrole), fipronil (phenylpyrazole), y tebufenozide (benzoylhydrazide) fueron comparadas utilizando un ensayo con dieta artificial y un ensayo de eficacia de residuo en contra de varios especies de Lepidoptera. Emamectin benzoate fue el insecticida más tóxico, (fué de 20- a 64,240 veces más tóxico que los otros compuestos probados). Los valores de LC90 para emamectin benzoate fueron de 0.0050 hasta 0.0218 ug/ml para seis especies de Lepidoptera. Similarmente, chlorfenapyr mostró toxicidad consistente para todas las especies, con valores de LC90 desde 1.9 hasta 4.6 ug/ml. Las toxicidades de fipronil y tebufenozide varían entre las especies probadas. Los valores de LC90 del Fipronil variaron 501-veces (de 0.64 hasta 321.3 ug/ml), mientras que la toxicidad de tebufenozide varió 113 veces (de 0.24 hasta 27.1 ug/ml), entre las especies probadas. En pruebas de eficacia de residuo llevadas a cabo en invernaderos, todos los compuestos fueron efectivos (i.e., >90% mortalidad) en el control de Heliothis virescens en el garbanzo aplicados a la taza proyectada en el campo (o sea a la misma concentración estipulada para el área del campo) y fueron efectivos al 1/10
The insecticide market has been dominated by the organophosphate, carbamate, and pyrethroid classes of insecticides. Recently, a number of new insecticide classes have been discovered and commercialized. Chlorfenapyr, a mitochondrial uncoupler (Black et al., 1994), is effective against both Acarina and Lepidoptera (Lovell et al., 1990, Wier et al., 1994, Ahn et al., 1996) in laboratory and field tests. Fipronil, an antagonist of the GABA-gated chloride channel (Bloomquist, 1994), has efficacy against a number of insect pests (Colliot et al., 1992, Burris et al., 1994, Hoy & Dunlap, 1995). Emamectin benzoate is a second generation avermectin with superior activity against lepidopterans compared with abamectin (Dybas et al., 1989, Jansson & Dybas, 1997). Tebufenozide, an ecdysone-receptor agonist (Retnakaran et al., 1995), has demonstrated activity against many lepidopterans (Chandler, 1994, Smagghe & Degheele, 1994, Ishaya et al., 1995).

The purpose of this research was to compare the potencies, spectrum, and residual effectiveness of these compounds against a broad panel of lepidopteran pests. These comparisons will help to provide information on the potential strengths and weaknesses of each compound in crop protection.

**MATERIAL AND METHODS**

**Chemicals**

Emamectin benzoate (Proclaim® 0.16 EC) was obtained from Merck & Co., Inc. (Rahway, NJ). Tebufenozide (Confirm 2F) was obtained from Merck & Co., Inc. and held at 11.0 °C and 50 ± 20% RH until needed. Eggs were shipped to Ricerca, Inc. on artificial diet to Ricerca, Inc. (Painesville, OH) and held at 24 ± 2°C and 50 ± 20% RH until needed. Eggs were shipped on artificial diet to Ricerca, Inc. and held at 11.0 ± 0.2°C until needed. All other eggs were shipped to Ricerca, Inc. and held at 11.0 ± 0.2°C until needed. Eggs were placed in disposable plastic cups with clipped foliage at 28 ± 2°C and 50 ± 20% RH two days before use. Larvae were tested as neonates (12-24 h) except P. xylostella, which were 6 d old due to the small size and delicacy of neonate P. xylostella.

**Diet Assay**

Methods were similar to those described previously (Jansson et al., 1998). Serial dilutions were made from formulated products in combination with a surfactant (0.01% Triton X-155) and deionized H2O. Controls consisted of 0.01% Triton X-155 in deionized H2O. Plutella xylostella diet was obtained from Southland Products (Lake Village, AR). Artificial diet for all Lepidopterans, except P. xylostella, was prepared using established methods (King and Hartley, 1985). Agar was heated in an autoclave (121°C) until dissolved and then added to a blender (3.8 liter) containing the dry ingredients. The agar and dry ingredients were blended for 1 min and transferred to a steam-jacketed kettle maintained at 70°C. The diet was dispensed (500 µl per well) into diet trays (C-D International, Inc., Pitman, NJ) using a semi-automated diet filler (Model MDF-100, C-D International, Inc., Pitman, NJ). Diet trays were cooled, wrapped in plastic, and used within 48 h after preparation.

An 50 µl aliquot of each dose of each test concentration was pipetted onto the surface of the diet in each of 16 individual wells per dose. Trays were shaken slightly to ensure that the aliquot evenly covered the surface of the diet. After treated diet was air dried, neonates were transferred onto the diet (one per well) and the wells
sealed using plastic adhesive strips. The tops of the plastic strips were pierced for ventilation. The criterion for death was the ability of the larvae to right itself. Mortality was recorded 6 d after application (Jansson et al. 1998).

Residual Efficacy Assays

Methods were similar to those described previously (Jansson et al. 1996, 1997). A custom built track sprayer system was used to apply insecticides. Treatments were applied using a calibrated double-nozzle (TJ8001E, Sprayer Systems, Wheaton, IL) track sprayer that delivered 100 ml of spray solution to 7-10 plants over 2 meters at 3.5 kg/cm². Plants 14-20 days old were sprayed with chlorfenapyr, emamectin benzoate, fipronil, and tebufenozide at estimated field use rates (224.2, 8.42, 56.0 and 140.0 g ai/ha, respectively) and 10% of these rates. Insecticides were applied in combination with the nonionic surfactant LeafAct 80 (PureGro, W. Sacramento, CA) at a rate of 0.58 l/ha (0.0625%). Controls consisted of the surfactant treatment alone. Plants were held in a glasshouse at 24 °C after treatment. Five replicate leaf cuttings from different plants were infested with larvae on 0, 4, 7, 10 and 14 days after treatment (DAT). Foliage was clipped and placed in Petri plates containing 20 ml of 1.8% water agar. Clippings were infested with 10-12 larvae and mortality was assessed after 4 d. Garbanzo bean, Cicer arietinum (L.) cv. Burpee Garbanzo 5024, and sugar beet, Beta vulgaris L. cv. USH-11, were used to test residual effectiveness at controlling H. virescens and S. exigua, respectively. Cabbage, Brassica oleracea var. capitata L. cv. Jersey Wakefield, was used to assess residual effectiveness against T. ni and P. xylostella. A compound was considered effective if mortality remained above 90%.

Data Analysis

Data from the diet assays were analyzed using probit analysis models in the POLO-PC program (Russell et al. 1977). Significant difference between LC values was based on overlap of 95% fiducial limits. The percentage mortality data of the residual efficacy assays was arcsine transformed and analyzed by ANOVA. Means within each rate range were separated by the Waller-Duncan K-ratio t-test (SAS Institute, 1993).

RESULTS

Diet Assay

Emamectin benzoate was the most toxic compound tested. The Lepidopteran species tested were 20- to 64,260-times more sensitive to emamectin benzoate than to the other three compounds (i.e., T. ni was 20-times more sensitive to emamectin benzoate than tebufenozide, and S. exigua was 64,240-times more sensitive to emamectin benzoate than fipronil). Fiducial limits for LC₉₀ values against most Lepidoptera overlapped, indicating that emamectin benzoate was equally potent against most Lepidoptera tested. Spodoptera exigua and P. xylostella were the most sensitive species to emamectin benzoate (LC₉₀ = 0.005 and 0.0053 ug/ml, respectively), while T. ni and P. includens were the least sensitive (LC₉₀ = 0.0125 - 0.0218 ug/ml, respectively). There was a 4-fold difference in LC₉₀ values between the least sensitive and most sensitive species.

Chlorfenapyr toxicity was consistent among species. The LC₉₀ values had a narrow range (1.9-4.6 ug/ml) with fiducial limits ranging from 1.6-6.5 ug/ml (Table 1). The slopes of the chlorfenapyr concentration responses against Lepidoptera were the steepest (3.9-8.2) among the four compounds tested.

In contrast to chlorfenapyr and emamectin benzoate, there was wide variation in sensitivity to fipronil among the six species tested. Plutella xylostella was over 10 to 502-times more sensitive to fipronil than the other five species tested (Table 1). LC₉₀ values for H. virescens, P. includens, T. ni and S. frugiperda ranged between 6.4 and 18.8 ug/ml and had overlapping fiducial limits. Spodoptera exigua was the least sensitive species to fipronil (LC₉₀ = 321.3 ug/ml).

There also was wide variation in sensitivity of Lepidoptera to tebufenozide with Trichoplusia ni being the most sensitive species (LC₉₀ = 0.24 ug/ml, Table 1). Pseudoplusia includens and S. frugiperda (LC₉₀ = 2.6 and 2.1ug/ml, respectively) were almost equally sensitive to tebufenozide. Spodoptera exigua and P. xylostella were approximately 33-times and 51-times more tolerant, respectively, of tebufenozide than T. ni. The Lepidopteran most tolerant of tebufenozide in these tests was H. virescens, which was 113-times more tolerant of tebufenozide than T. ni.

Residual Efficacy Assays

All compounds at projected field rates were effective at controlling H. virescens up to 14 DAT except tebufenozide, which was effective up to 10 DAT (Table 2). Emamectin benzoate also caused 100% mortality on all evaluation dates when applied at low rate (0.84 g AI/ha). Fipronil was effective at controlling H. virescens at all evaluation dates up to 14 DAT at low rate (5.6 g AI/ha). Mortality in these treatments was comparable to that produced by emamectin benzoate. The low rate of chlorfenapyr (22.4 g AI/ha) had lower percentage mortality on 7 DAT than the corresponding rates of emamectin benzoate and fipronil, but mortality increased to 100.0% on 14 DAT. This may have
TABLE 1. CONCENTRATION-MORTALITY RESPONSES OF VARIOUS LEPIDOPTERAN PESTS TO CHLORFENAPYR, FIPRONIL, EMAMECTIN BENZOATE AND TEBUFENOZIDE AND DIET AS- 

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Spodoptera exigua</th>
<th>Plutella xylostella</th>
<th>Trichoplusia ni</th>
<th>Pseudoplusia includens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorfenapyr</td>
<td>2.2 (1.8 - 2.6)</td>
<td>2.2 (1.9 - 2.9)</td>
<td>2.2 (2.7 - 3.8)</td>
<td>2.2 (2.7 - 3.8)</td>
</tr>
<tr>
<td>LC50 (ug/ml)</td>
<td>1.5 (1.3 - 1.7)</td>
<td>4.6 (3.7 - 6.5)</td>
<td>5.3 (8.2 + 1.6)</td>
<td>6.5 (1.6 - 2.9)</td>
</tr>
<tr>
<td>LC90 (ug/ml)</td>
<td>7.2 (6.8 - 2.0)</td>
<td>12.7 (6.8 + 1.6)</td>
<td>22.1 (8.2 + 1.6)</td>
<td>22.7 (4.6 - 2.0)</td>
</tr>
<tr>
<td>Slope</td>
<td>7.2 + 1.2</td>
<td>5.3 + 1.0</td>
<td>3.1 + 0.4</td>
<td>2.2 + 0.3</td>
</tr>
<tr>
<td>n = 319</td>
<td>(n = 311)</td>
<td>(n = 199)</td>
<td>(n = 349)</td>
<td>(n = 349)</td>
</tr>
</tbody>
</table>

| Emamectin Benzoate | 2.2 (1.8 - 2.6)   | 2.2 (1.9 - 2.9)    | 2.2 (2.7 - 3.8) | 2.2 (2.7 - 3.8)       |
| LC50 (ug/ml)       | 0.0034 (0.0023-0.0049) | 0.0086 (0.0039-0.0085) | 3.1 + 0.4 | 2.2 + 0.3 |
| LC90 (ug/ml)       | 3.1 (3.1 - 3.4)   | 3.6 (3.6 - 3.9)    | 5.4 (4.7 - 3.6) | 3.6 (3.5 - 3.6)       |
| Slope             | 3.1 + 0.4         | 3.6 + 0.4          | 3.6 + 0.4      | 3.6 + 0.4             |
| n = 324           | (n = 324)         | (n = 324)          | (n = 324)      | (n = 324)             |

| Fipronil | 2.2 (1.8 - 2.6) | 2.2 (1.9 - 2.9) | 2.2 (2.7 - 3.8) | 2.2 (2.7 - 3.8) |
| LC50 (ug/ml) | 5.8 (3.2 - 8.7) | 18.8 (12.0 - 52.3) | 25.0 + 0.3 | 22.2 + 0.3 |
| LC90 (ug/ml) | 95.2 (66.7 - 139.8) | 321.3 (18.0 - 15.8) | 0.4 + 0.1 | 2.2 + 0.3 |
| Slope       | 5.8 + 0.3        | 18.8 + 0.3        | 25.0 + 0.3   | 22.2 + 0.3          |
| n = 318     | (n = 318)        | (n = 318)         | (n = 318)    | (n = 318)            |

| Tebufenozone | 2.2 (1.8 - 2.6) | 2.2 (1.9 - 2.9) | 2.2 (2.7 - 3.8) | 2.2 (2.7 - 3.8) |
| LC50 (ug/ml) | 3.8 (2.2 - 3.4) | 12.8 (6.6 - 4.3) | 25.0 + 0.3 | 22.2 + 0.3 |
| LC90 (ug/ml) | 19.6 (9.2 - 26.3) | 78.0 (12.3 - 12.3) | 3.4 + 0.4 | 3.4 + 0.4 |
| Slope       | 3.8 + 0.4        | 12.8 + 0.4       | 25.0 + 0.3   | 22.2 + 0.3          |
| n = 318     | (n = 318)        | (n = 318)        | (n = 318)    | (n = 318)            |
TABLE 2. RESIDUAL ASSAY ON GARBANZO BEANS USING H. VIRESCENS. TREATMENTS WERE AT FIELD RATE AND 1/10 FIELD RATE OF INSECTICIDE WITH LEAFACT 80 (0.58 L/HA) AS AN ADJUVANT. FIVE REPLICATES WERE USED FOR EACH TREATMENT.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate 4 DAT</th>
<th>Rate 7 DAT</th>
<th>Rate 10 DAT</th>
<th>Rate 14 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g ai/ha</td>
<td>% Mort. (SEM)</td>
<td>% Mort. (SEM)</td>
<td>% Mort. (SEM)</td>
</tr>
<tr>
<td><strong>Projected Field Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>224.2</td>
<td>103.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>8.4</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Fipronil</td>
<td>56.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>140.0</td>
<td>100.0 a 0.0</td>
<td>98.0 a 2.0</td>
<td>97.1 a 2.9</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td>11.4 b 5.0</td>
<td>31.0 b 10.8</td>
<td>36.9 b 17.2</td>
<td>30.1 c 16.1</td>
</tr>
<tr>
<td><strong>1/10 Projected Field Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>22.4</td>
<td>85.5 a 9.2</td>
<td>76.5 b 9.6</td>
<td>85.3 ab 9.0</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>0.84</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Fipronil</td>
<td>5.6</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>94.3 a 5.7</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>14.0</td>
<td>89.2 a 6.6</td>
<td>90.0 ab 10.0</td>
<td>58.7 b 8.4</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td>11.4 b 5.0</td>
<td>31.0 ab 10.8</td>
<td>36.9 c 17.2</td>
<td>30.1 c 16.1</td>
</tr>
</tbody>
</table>

*aWaller-Duncan Ranking, values within rates and DAT having the same letter are not significantly different (P < 0.05).*
been due, in part, to the nutritional quality of the older leaves used on these DAT, as control mortality ranged from 30.1 to 36.9% on evaluations from 7 to 14 DAT. At the low rate (14.0 g AI/ha) tebufenozide caused mortality ranging from 68.7 to 90.0%, although mortality only differed from other insecticide treatments on 10 DAT. It should be noted that a number of dead and live tebufenozide-treated H. virescens larvae showed molting deformities characteristic of tebufenozide toxicity (Retnakaran et al. 1995).

The results of the residual efficacy tests on sugar beet using S. exigua were different from those on garbanzo bean. Chlorfenapyr, emamectin benzoate and tebufenozide resulted in complete or nearly complete control of S. exigua on sugar beet for up to 14 DAT when applied at the high rates (Table 3). Emamectin benzoate also caused 100% mortality for up to 14 DAT when applied at the low rate. Chlorfenapyr was not significantly different from emamectin benzoate up to 14 DAT. However, at 14 DAT chlorfenapyr treatments caused only 66.8% S. exigua mortality. Tebufenozide was effective for up to 4 DAT when applied at the low rates (14.0 g AI/ha). Phytotoxicity (i.e., chlorosis) was noted in sugar beets treated with chlorfenapyr at the projected field rate. As in the case of H. virescens, a number of dead and alive tebufenozide-treated S. exigua larvae showed molting deformities characteristic of tebufenozide toxicity. Fipronil at the projected field rate (56 g AI/ha) was effective at controlling S. exigua for up to 4 DAT. Mortality dropped markedly by 7 DAT and was similar to controls at 10 and 14 DAT (Table 3). Mortality caused by fipronil at the low rate was comparable to control mortality.

The high rates of chlorfenapyr, emamectin benzoate and fipronil resulted in 100% mortality of P. xylostella on cabbage for the duration of the test (Table 4). Emamectin benzoate and fipronil at the low rates (0.84 and 5.6 g AI/ha, respectively) caused 100% mortality up to 4 DAT. Emamectin benzoate also caused 95% mortality at 7 DAT. At the low rate, chlorfenapyr was ineffective from 4 to 14 DAT.

Tebufenozide was the only compound that was ineffective against P. xylostella when applied at the high rate (Table 4). The highest level of mortality caused by tebufenozide during the course of the test was 82.5% on 7 DAT. Mortality at the low rate of tebufenozide was comparable to controls between 4 and 14 DAT.

All compounds were effective against T. ni in the residual efficacy tests up to 10 DAT when applied to cabbage at their high rates. At 14 DAT, fipronil and tebufenozide caused less than 90% mortality, although these treatments were not significantly different from chlorfenapyr or emamectin benzoate. Emamectin benzoate was the only insecticide that caused 100% mortality of T. ni for the duration of the test (Table 5). However, differences among compounds were more apparent at their low rates. The low rate of tebufenozide (14.0 g AI/ha) caused 82.0-96.4% mortality of T. ni between 0-14 DAT. At 14 DAT, tebufenozide ranked higher than any of the other compounds tested (Table 5). At the low rate (0.84 g AI/ha), the efficacy of emamectin benzoate for control of T. ni started to diminish at 4 DAT. Control with emamectin benzoate at the low rate ranked lower than that from tebufenozide at its low rate (14.0 g AI/ha) on 10 and 14 DAT, and ranked lower than that from chlorfenapyr at its corresponding rate (22.4 g AI/ha) at 10 DAT. At the low rate, chlorfenapyr was ranked lower than tebufenozide at 14 DAT. Fipronil at low rate (5.6 g AI/ha) was only effective on 0 DAT, and at 4 DAT all the other insecticides outperformed fipronil. At 14 DAT only tebufenozide was different from control at the low rate.

**DISCUSSION**

Emamectin benzoate was consistently the most potent compound tested. It was at least 1-5 orders of magnitude more potent than all other compounds evaluated. Emamectin benzoate was potent against a wide spectrum of Lepidoptera species; toxicity differed by only 4-fold among the Lepidoptera tested.

Chlorfenapyr was the second most potent compound against most Lepidoptera, followed by tebufenozide and fipronil. Like emamectin benzoate, chlorfenapyr demonstrated broad spectrum activity, and was equally effective against all Lepidoptera tested. The spectrum of tebufenozide and fipronil were more variable. Of these three compounds, chlorfenapyr was the most potent to H. virescens, S. exigua and S. frugiperda, while tebufenozide was the most potent to T. ni and fipronil the most potent to P. xylostella.

Residual efficacy data under glasshouse conditions correlated with the spectrum and potency data. Emamectin benzoate and fipronil were particularly effective at controlling H. virescens when applied at high rates and at 10% of these rates. Tebufenozide and chlorfenapyr were effective at the field rate against H. virescens for 10 and 14 DAT, respectively. Emamectin benzoate and chlorfenapyr were more effective at controlling S. exigua than tebufenozide and, particularly, fipronil, which agreed with the diet bioassay data. It should be noted that a number of larvae treated with tebufenozide had molting deformities characteristic of tebufenozide toxicity. Some of these deformed larvae would have probably succumbed within a few days after the 4-day mortality assessment used in the residual efficacy test (Jansson et al. 1998). Tebufenozide was the least effective compound at controlling P. xylostella, which also concurred with the diet bioassay data.
<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate</th>
<th>0 DAT % Mort. (SEM)</th>
<th>4 DAT % Mort. (SEM)</th>
<th>7 DAT % Mort. (SEM)</th>
<th>10 DAT % Mort. (SEM)</th>
<th>14 DAT % Mort. (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Projected Field Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>224.2 g ai/ha</td>
<td>82.0 a 18.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>8.4 g ai/ha</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Fipronil</td>
<td>56.0 g ai/ha</td>
<td>83.3 a 8.8</td>
<td>98.0 a 2.0</td>
<td>5.3 b 2.2</td>
<td>20.7 b 9.5</td>
<td>10.5 b 5.1</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>140.0 g ai/ha</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>96.0 a 2.6</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td>10.5 b 6.1</td>
<td>10.0 b 10.0</td>
<td>0.0 c 0.0</td>
<td>7.3 b 2.3</td>
<td>4.0 b 4.0</td>
<td></td>
</tr>
</tbody>
</table>

**1/10 Projected Field Rate**

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate</th>
<th>0 DAT % Mort. (SEM)</th>
<th>4 DAT % Mort. (SEM)</th>
<th>7 DAT % Mort. (SEM)</th>
<th>10 DAT % Mort. (SEM)</th>
<th>14 DAT % Mort. (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorfenapyr</td>
<td>22.4 g ai/ha</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>80.0 ab 20.0</td>
<td>96.4 a 3.6</td>
<td>66.8 ab 13.7</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>0.84 g ai/ha</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Fipronil</td>
<td>5.6 g ai/ha</td>
<td>19.3 b 13.2</td>
<td>1.7 b 1.7</td>
<td>2.0 c 2.0</td>
<td>4.3 c 2.4</td>
<td>0.0 c 0.0</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>14.0 g ai/ha</td>
<td>100.0 a 0.0</td>
<td>96.7 a 3.3</td>
<td>45.9 b 16.2</td>
<td>53.8 b 20.2</td>
<td>58.9 b 19.0</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td>10.5 b 6.1</td>
<td>10.0 b 10.0</td>
<td>0.0 c 0.0</td>
<td>7.3 c 2.3</td>
<td>4.0 c 4.0</td>
<td></td>
</tr>
</tbody>
</table>

*Waller-Duncan Ranking, values within rates and DAT having the same letter are not significantly different (P < 0.05).*
TABLE 4. RESIDUAL ASSAY ON CABBAGE USING *P. xylostella*. TREATMENTS WERE AT FIELD RATE AND 1/10 FIELD RATE OF INSECTICIDE WITH LEAFACT 80 (0.58 L/HA) AS AN ADJUVANT. FIVE REPLICATES WERE USED FOR EACH TREATMENT.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate 0 DAT</th>
<th>4 DAT</th>
<th>7 DAT</th>
<th>10 DAT</th>
<th>14 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g ai/ha</td>
<td>% Mort.</td>
<td>% Mort.</td>
<td>% Mort.</td>
<td>% Mort.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(SEM)</td>
<td>(SEM)</td>
<td>(SEM)</td>
<td>(SEM)</td>
</tr>
<tr>
<td>Projected Field Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>224.2</td>
<td>100.0 a*</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>100.0 a</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>8.4</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>100.0 a</td>
</tr>
<tr>
<td>Fipronil</td>
<td>56.0</td>
<td>98.5 ab</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>100.0 a</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>140.0</td>
<td>81.8 b</td>
<td>74.4 b</td>
<td>82.5 a</td>
<td>50.0 b</td>
</tr>
<tr>
<td>Control</td>
<td>LeafAct 80</td>
<td>14.3 c</td>
<td>4.4 c</td>
<td>2.5 c</td>
<td>12.0 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/10 Protected Field Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>22.4</td>
<td>89.5 a</td>
<td>50.7 b</td>
<td>46.9 b</td>
<td>55.8 ab</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>0.84</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>95.0 a</td>
<td>72.0 a</td>
</tr>
<tr>
<td>Fipronil</td>
<td>5.6</td>
<td>100.0 a</td>
<td>100.0 a</td>
<td>82.5 a</td>
<td>56.0 ab</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>14.0</td>
<td>52.9 b</td>
<td>6.2 c</td>
<td>15.7 c</td>
<td>9.5 b</td>
</tr>
<tr>
<td>Control</td>
<td>LeafAct 80</td>
<td>14.3 c</td>
<td>4.4 c</td>
<td>2.5 c</td>
<td>12.0 b</td>
</tr>
</tbody>
</table>

*Waller-Duncan Ranking, values within rates and DAT having the same letter are not significantly different (P < 0.05).*
**TABLE 5. Residual Assay on Cabbage Using T. ni.** Treatments were at field rate and 1/10 field rate of insecticide with Leafact 80 (0.58 L/ha) as an adjuvant. Five replicates were used for each treatment.

<table>
<thead>
<tr>
<th>Insecticide</th>
<th>Rate</th>
<th>0 DAT</th>
<th>4 DAT</th>
<th>10 DAT</th>
<th>14 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g ai/ha</td>
<td>% Mort. (SEM)</td>
<td>% Mort. (SEM)</td>
<td>% Mort. (SEM)</td>
<td>% Mort. (SEM)</td>
</tr>
<tr>
<td><strong>Projected Field Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>224.2</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>94.7 a 5.3</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>8.4</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
</tr>
<tr>
<td>Fipronil</td>
<td>56.0</td>
<td>100.0 a 0.0</td>
<td>96.0 a 4.0</td>
<td>98.5 a 1.5</td>
<td>85.6 a 9.9</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>140.0</td>
<td>100.0 a 0.0</td>
<td>100.0 a 0.0</td>
<td>97.5 a 2.5</td>
<td>80.0 a</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td></td>
<td>8.2 b 6.5</td>
<td>4.3 b 2.7</td>
<td>6.0 b 4.0</td>
<td>0.0 b 0.0</td>
</tr>
<tr>
<td><strong>1/10 Projected Field Rate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorfenapyr</td>
<td>22.4</td>
<td>98.0 a 2.0</td>
<td>80.0 a 12.2</td>
<td>74.3 a 13.1</td>
<td>13.3 b 13</td>
</tr>
<tr>
<td>Emamectin Benzoate</td>
<td>0.84</td>
<td>100.0 a 0.0</td>
<td>83.6 a 8.4</td>
<td>21.3 b 13.6</td>
<td>2.5 b 2.5</td>
</tr>
<tr>
<td>Fipronil</td>
<td>5.6</td>
<td>98.0 a 2.0</td>
<td>36.5 b 10.2</td>
<td>5.8 b 2.4</td>
<td>2.2 b 2.2</td>
</tr>
<tr>
<td>Tebufenozide</td>
<td>14.0</td>
<td>96.4 a 3.6</td>
<td>86.0 a 9.3</td>
<td>98.5 a 1.5</td>
<td>82.0 a 13.2</td>
</tr>
<tr>
<td>Control LeafAct 80</td>
<td></td>
<td>8.2 b 6.5</td>
<td>4.3 c 2.7</td>
<td>6.0 b 4.0</td>
<td>0.0 b 0.0</td>
</tr>
</tbody>
</table>

*Waller-Duncan Ranking, values within rates and DAT having the same letter are not significantly different (p < 0.05).*
Emamectin benzoate was the only compound that resulted in complete control (i.e., 100% mortality) of *T. ni* through 14 DAT when applied at the high rates, although control achieved with the other insecticides at equivalent rates was also acceptable (Table 5). Tebufenozide was superior to all other compounds at controlling *T. ni* when applied at the low rates.

A number of factors, such as photostability, translaminar uptake, and leaf nutritional status, will affect residual efficacy (Verkerk & Wright 1996). In our studies the concentration-response appeared to correlate with residual efficacy in the two cabbage pests. At field rate, emamectin benzoate was equally effective at controlling *T. ni* and *P. xylostella*, causing 96.7-100.0% mortality to both insects for the duration of the test. Emamectin benzoate was only effective up to 4 DAT against *T. ni* at the low rate (Table 5), while against *P. xylostella* it was effective for up to 7 DAT (Table 4). In diet assays, *T. ni* was approximately 2-times more tolerant of emamectin benzoate compared with *P. xylostella* (Table 1). This may be the reason for the different response in the residual efficacy assays at the low rate between the two species.

Fipronil was effective against both species at the field rate. At the low rate, fipronil was effective for up to 7 DAT against *P. xylostella* (Table 4), but it was ineffective against *T. ni* after 0 DAT (Table 5). Again, these data confirm diet assay results. *Plutella xylostella* was the most sensitive species to fipronil based on LC values, while *T. ni* was less sensitive to fipronil (Table 1).

Chlorfenapyr was effective against both species for the duration of the test when applied at field rate (Tables 4 and 5). These data agree with results from the diet assay, which showed no difference in LC90 values between these two species.

Tebufenozide was efficacious against *T. ni* and remained effective at controlling this insect at both rates for the duration of the test (Table 5). However, tebufenozide was ineffective at both rates against *P. xylostella*, even on 0 DAT. These data confirm diet assay results. *T. ni* was the most sensitive species to tebufenozide based on LC50 values, whereas *P. xylostella* was 51-times more tolerant of tebufenozide compared with *T. ni* (Table 1).

Collectively, these data show that all four compounds have potential for controlling Lepidoptera pests. Emamectin benzoate and chlorfenapyr controlled a broader spectrum of lepidopteran pests and for this reason should have a broader utility in crop protection. Tebufenozide and fipronil controlled a narrower range of lepidopteran pests, but have already demonstrated utility under field conditions against certain lepidopteran pests.

**Acknowledgments**

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ECLOSION, MATING, AND GROOMING BEHAVIOR OF THE PARASITOID FLY PSEUDACTEON CURVATUS (DIPTERA: PHORIDAE)

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1Section of Integrative Biology, Brackenridge Field Laboratory, Fire Ant Laboratory
University of Texas, Austin, TX 78712
2USDA-ARS, Center for Medical Agricultural and Veterinary Entomology, P.O. Box 14565
Gainesville, FL 32604

ABSTRACT

Phorid flies from the genus Pseudacteon are parasitoids of Solenopsis ants. Recent efforts of controlling imported fire ants in the United States have focused on rearing and releasing these flies as biocontrol agents. We studied eclosion, mating, and grooming behavior of Pseudacteon curvatus Borgmeier in an effort to increase understanding of its biology. The sex ratio of eclosing flies in the lab was 1:1. The flies emerged only in the morning and were protandrous. Mating in the lab occurred on the substrate and did not require disturbed ants. Males and probably also females mated multiply.

Key Words: Biocontrol, Solenopsis invicta, Phoridae, Mass Rearing

RESUMEN

Las moscas del género Pseudacteon (Phoridae) son parasitoides de las hormigas Solenopsis. Esfuerzos recientes para controlar a la hormiga de fuego importada (Solenopsis invicta) en los Estados Unidos han estado enfocados en la cria y liberación de estas moscas como agentes de control biológico. Nosotros estudiamos la eclosión, apareamiento y el comportamiento de acicalamiento de Pseudacteon curvatus Borgmeier en un esfuerzo para aumentar nuestro entendimiento de su biología. La proporción de nacimiento de hembras y machos en el laboratorio fue 1:1. Las moscas emergieron solamente en la mañana y fueron protandrosas (los machos nacen más temprano que las hembras). El apareamiento en el laboratorio sucedió sobre el substrato y no requería que las hormigas fueran perturbadas. Los machos y probablemente las hembras se aparearon varias veces.

Until recently, attempts at controlling the red and black imported fire ants, Solenopsis invicta Buren and Solenopsis richteri Forel, respectively, have been almost entirely by chemical means. The consequences of early practices were quite disastrous in some cases (Carson 1962). Fortunately, more ecologically sound and sustainable methods have been developed (Drees et al. 1996). One of the methods currently under investigation is the use of Pseudacteon phorid flies as agents of biological control, first suggested for the control of attine ants in Weber (1972).

The female Pseudacteon phorid fly attacks and lays an egg in an adult worker ant. The larva ecloses, consumes its host’s internal organs, and then pupates in the ant’s head capsule (e.g., Porter 1998). The supposed biological control effect lies not so much in direct mortality, for each mature Solenopsis nest contains hundreds of thousands of worker ants, but in the indirect effect the fly’s presence has on normal fire ant behavior: fire ants stop foraging and defending resources in the presence of phorid flies, which potentially has a detrimental effect on the colony’s fitness (e.g., Orr et al. 1995, 1997).


There are 18 described species of phorid flies that attack fire ants in South America (Porter & Pesquero 2001). Such diversity can only be supported if the species of parasitoids have differing habitat preferences, foraging ecologies, and host preferences. Indeed, this has been shown to be the case (Porter & Briano 2000, Orr et al. 1997, Gilbert & Morrison 1997, Pesquero et al. 1996, Porter 1998a, Porter et al. 1995a, Fowler et al. 1995, Borgmeier 1922). For instance, some phorid flies attack fire ant minors, whereas others attack the
majors; some attack during the heat of the day, and others attack during the cooler parts of the day; and some attack at fire ant foraging trails, whereas others attack at disturbed mounds.

Because the ecology of the various species of parasitoids differ, the introduction of more than one species is likely to have a greater effect than if a single species is introduced. Further, to have the greatest effect, the suite of species released should have complementary biologies. This paper adds to what is known about the basic biology of P. curvatus. Much of this information will assist mass rearing and release efforts.

**MATERIALS AND METHODS**

A general description of eclosion behavior was obtained by observing five newly-eclosed flies from the time that the pupal cap opened until the fly was able to fly. For these observations, flies were kept in individual containers (capped 1.2 × 7.5 cm test tubes) and observed through a dissecting microscope.

A general description of mating was generated from observations of >20 matings. The mean duration of mating was timed from mounting until dismounting. Actual duration of copulation was probably less than “mating duration,” but we were not able to discern when actual intromission occurred.

To determine if multiple mating occurred, five virgin females and one virgin male were put into a container and left together for 30 h (n = 3). At the end of the 30 hours, all living flies were squashed and a phase contrast microscope was used to look for sperm.

The description of grooming was generated from observations of flies as they groomed while in the mating observation arena or during eclosion. We observed >20 grooming bouts.

**RESULTS**

In the lab, P. curvatus flies have been observed to emerge between 0300 and 1000 h. Emergence in the late morning was probably due to exposure to artificial light the evening before they emerged. In the field, emergence probably occurs within a few hours of dawn depending on the temperature and light cycle. In the lab, flies emerged over a period of about 2 h with males emerging about 30 ± 5 (SE) min earlier on average than females (ANOVA, $F_{1,12} = 35.3, p < 0.0001, n = 45$ and 59, respectively). Emerging flies required about 10 min to expand their wings. When temperatures were warm (26°C) flight can occur within an hour of emergence and mating about an hour later. However, cool temperatures (<22°C) can delay flight and mating until late morning or even into the afternoon. The sex ratio of males to females did not differ significantly from 1:1 (Porter 2000).

Average duration of mating was 22.3s ± 8.6 (mean ± SD, n = 10). There was considerable variation in mating time, ranging from 12 to 39 s. Because mating was terminated by the female kicking the male off of her back, the variation was probably not due to actual time required for mating, but to changes in female motivation during mating.

The following is a detailed description of eclosion and post-eclosion behavior of one female fly. The emerging fly popped open the pupal cap and climbed out of the ant head capsule onto the substrate, a process that usually only requires a few seconds. On first emerging, the fly’s abdomen was distended and elongated, and the ovipositor was extended. The cuticle of the legs, head and thorax was a dusty tan, the abdomen was a pale whitish color, and the wings were white and dull. The eyes and ovipositor appeared to be sclerotized. Almost immediately, the fly started pumping her abdomen to fill and expand her wings. After about 10 min, the wings appeared to be fully expanded and the abdomen had reduced considerably in size but was still elongated. About this time, the abdominal pumping stopped. Over the next several minutes, fluid returned to her abdomen, which became distended again, and she began pumping her abdomen again. About 15 min after eclosion, the costal margins of her wings began to sclerotize and her entire body began darkening about 10 min later. She continued to pump her abdomen from time to time and her cuticle became darker and darker until about 75 min after eclosion, when she finally retracted her ovipositor. Although her body seemed sclerotized and she began walking about 10 min later, her wings were still somewhat opaque, and she did not fly until almost 2h 15 min after eclosion.

Before mating, the females remained stationary for the most part, usually moving only if disturbed by another fly. Females groomed themselves while sitting. Males were also stationary and groomed some of the time, but were much more mobile than females; often walking about on the substrate and flying around the arena. While males were in flight, they sometimes tussled briefly with one another, but there were no discernible territories, nor were males particularly aggressive.

Visual cues and probably chemical cues were important to males for locating a mate. Males investigated small bumps in the substrate and were attracted to empty head capsules. One male vigorously attempted to copulate with an empty ant head capsule.

Females that had sat unnoticed for some time suddenly became attractive to males. We could not discern any difference in behavior of females that were attractive versus those that were not, so presumably they emitted some mate attraction chemical. There did not seem to be any mate choice by females. Mating was determined by scramble com-
petition. Often, 2 or 3 males simultaneously attempted to copulate with the same female.

The male flew toward the female, approaching her posteriorly. The male sat on top of the female and struggled to hold onto the back of the female’s abdomen with his hind legs. Once the male had a hold on the female’s abdomen, the adeagus was extended and curved around the back of the female’s abdomen to one side of the ovipositor. The female’s cloaca was just in front of the ovipositor.

Toward the end of the mating, the female began to walk around on the substrate and sometimes pushed off the male with her hind legs.

Following mating, the male and female sat within a few cm of each other and groomed, first the abdomen, then the hind legs, thorax, head, and legs. They did not groom their wings. After grooming, the female became more active and flew about the arena. The male also sometimes flew about, but tended to become less active.

Squashes of single virgin males kept with five virgin females clearly showed multiple mating by males. Of the female flies recovered alive after 30 h, 5/5, 3/3 and 2/4 were mated. All 3 males were still positive for sperm at the end of the test.

While making observations for mating duration, it was common to see more than one male attempting to mount a female both simultaneously and sequentially. However, whether females functionally mate more than once is not clear.

The abdomen, ovipositor, and wings are all groomed with the third pair of legs. To groom the abdomen, the inside surfaces of the hind tibiae and fibiae are drawn over the ventral and lateral surfaces of the abdomen. The wings are drawn to the side of the abdomen with the hind legs and the ventral surface of the hind leg is rubbed over the dorsal surface of the wing. The underside of the wing is groomed by rubbing the dorsal surface of the third leg along the ventral surface of the wing.

The posterior portion of the thorax is groomed using the femur of the third leg, whereas the forelegs are used to groom the posterior portion of the thorax and the head. The head is turned to each side and ventrally in order for each of the surfaces to be rubbed. Much more care is given to grooming the aristae than to any other part of the body. The fly often goes over each one with its forelegs several times.

The legs are groomed by rubbing against other legs. The forelegs are rubbed together, starting at the base, and moving toward the tarsi. The hind legs are rubbed together in similar fashion. To groom the middle legs, the forelegs are drawn back to the side to be groomed and rubbed over the middle leg. The hind legs are also used to groom the middle legs in similar fashion. It is possible that these grooming movements also groom the fore and hind legs respectively. Similar leg-cleaning movements are described and illustrated for D. melanogaster in Szébenyi (1969).

**Discussion**

Our observations revealed that *P. curvatus* flies mate on the ground in the morning several hours after eclosion from the puparium. This information will be useful to researchers interested in releasing *P. curvatus* flies for biocontrol of imported fire ants because field releases of this fly should be timed for late morning or afternoon, as mating does not occur in the air while females are trying to oviposit.

Males readily and successfully mated multiple times in our tests, indicating that laboratory-reared females will rarely lack sufficient mates for mating.

Very little is known about mating behavior of other *Pseudacteon* flies. Published reports of mating behavior for this genus are primarily field studies reporting male *Pseudacteon* presence at aggregations or trails of *Solenopsis* ants (Williams 1980, Feener 1987, Feener & Brown 1992, Porter et al. 1995a). The most detailed account is for *P. tricuspis* (Porter et al. 1997). From this, we know that mating behavior differs considerably between *P. tricuspis* and *P. curvatus*. Although males of *P. tricuspis* are attracted to disturbed fire ants, males of *P. curvatus* are not. Mating for *P. curvatus* takes place on the ground and takes more than 20 seconds on average; whereas for *P. tricuspis*, mating is initiated on the wing and is extremely brief (<1s, Porter et al. 1997).

Mating in other kinds of phorids often occurs in mating swarms (reviewed in Disney 1994). Not surprisingly, the most common type of mating swarm was composed of lekking males. However, there are species in which there are both sexes in one swarm, another with both sexes forming separate swarms, and one species with female mating swarms to which males were attracted (reviewed in Disney 1994). The preponderance of species using swarms for mating probably only indicates the ease with which swarms can be observed versus other mating strategies that are less likely to be stumbled upon.

The duration of mating among phorids ranges from <1s (*P. tricuspis*, Porter et al. 1997) to 8 minutes (*Dohrniphora cornuta*, Barnes 1990). How this interspecific variation relates to sperm competition and/or mate selection by females would be an interesting research direction.

**Acknowledgments**

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**References Cited**


A SHORT-TERM AUXILIARY DIET FOR THE PREDACEOUS STINK BUG, 
PERILLUS BIOCULATUS (HEMIPTERA: PENTATOMIDAE)

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ABSTRACT

Perillus bioculatus (F.) can be maintained in the laboratory on a diet of Heliothis virescens F. larvae if supplemented with Colorado potato beetle (CPB) eggs. Here we demonstrate that an artificial diet can replace the CPB eggs and maintain the colony for at least three generations. This enables us to maintain our Perillus colonies at high numbers independent of the normal fluctuations in our CPB colony. Survival of nymphs, adult longevity, start of ovipositioning, total number of eggs per female, total number of clutches per female, and percent hatch were equivalent between the two rearing regimes for three generations. Fecundity on the artificial diet was greatly reduced by the sixth generation, leading to the collapse of the colony during the seventh generation.

Key Words: Perillus bioculatus, artificial diet

RESUMEN

Perillus bioculatus (F.) puede ser mantenido en el laboratorio con una dieta de larvas de Heliothis virescens F. si son suplementados con huevos del escarabajo de Papa de Colorado (CPB), (Leptinotarsa decemlineata Say). Aquí demostramos que una dieta artificial puede reemplazar los huevos de CPB y mantener la colonia por lo menos en tres generaciones. Este nos permite mantener poblaciones altas de nuestra colonia de Perillus independientemente de las fluctuaciones normales en nuestra colonia de CPB. La sobrevivencia de las ninfas, la longevidad de los adultos, el inicio de la oviposición, el número total de huevos por hembra, el número total de grupos de huevos por hembra, y el porcentaje de eclosión fueron equivalentes entre los dos regímenes de cria por tres generaciones. La fecundidad de las hembras alimentadas con la dieta artificial fue grandemente reducida en la sexta generación, causando la caída total de la colonia durante la septima generación.

Perillus bioculatus (F.) is a predaceous pentatomid endemic to North America. P. bioculatus feeds on a number of insect orders under both field and laboratory conditions, but has an intrinsic preference for the Colorado potato beetle (CPB), Leptinotarsa decemlineata (Say) (Saint-Cyr & Cloutier 1996). CPB is the major defoliator of potato (reviewed Ferro 1985, Hare 1990, Cloutier et al. 1996). CPB is the major defoliator of potato (reviewed Ferro 1985, Hare 1990, Cloutier et al. 1996). With its strong preference for CPB, P. bioculatus has drawn attention as a possible biocontrol agent for CPB in North America (Biever & Chauvin 1992) and Europe (Jermy 1980).

Currently there is no published optimized artificial diet for P. bioculatus. Therefore, P. bioculatus must be reared on CPB or a secondary host such as Trichoplusia ni (Biever & Chauvin 1992) or Heliothis virescens supplemented with CPB (Adams 2000). Currently we are maintaining our P. bioculatus colony on H. virescens larvae, and feeding only first and second instar nymphs on CPB egg masses (Adams 2000). Even with decreased dependence on CPB, problems with the CPB colony have led to loss of our P. bioculatus colonies, or to insufficient numbers of CPB egg masses to undertake planned experiments. We investigate here whether a simple, inexpensive diet for another predaceous insect, Chrysoperla rufilabris (Cohen & Smith 1998), could replace the CPB eggs to maintain P. bioculatus for several generations. This would serve as a bridging diet when CPB is unavailable, while avoiding the cost of incorporating an additional prey insect colony into our research program.

MATERIALS AND METHODS

Rearing

P. bioculatus were reared as described by Adams (2000). Stock colonies were reared in a walk-in environmental chamber set to 16 h light:8 h dark, 25 ± 2°C and 65% relative humidity. Both nymphs and adults were fed 5th instar H. virescens larvae. The H. virescens larvae were frozen and stored at -25°C until needed. Approximately 300 P. bioculatus eggs were used to start each cage of nymphs. First and second instars received three CPB egg masses and one H. virescens larva daily. Third through fifth instar nymphs received
seven larvae per day. All nymphal colonies also received two water wicks with a potato leaf inserted in each.

Adults were removed daily from the nymphal colonies and placed into egging cages which had three water wicks with an inserted potato leaf. Each egging cage contained 40 females and 10 males, and 10 *H. virescens* larvae were provided daily. The females deposited their eggs on any hard substrate in the cage. Eggs were collected on Mondays, Wednesdays and Fridays and placed into hatching tubs.

**Diet Preparation**

The diet was prepared according to Cohen & Smith (1998), except that antibiotics were not included. The diet contained: 100 g 70% lean ground beef, 100 g beef liver, 100 g hen’s eggs, 15 g sucrose, 5 g honey dissolved in 20 ml water, 10 g brewer’s yeast, 5 ml 10% acetic acid and 45 ml water. Because we were not able to get the diet mixture to solidify as described by Cohen & Smith (1998), the diet was fed to stink bugs in Parafilm domes (Adams 2000).

**Experimental Treatment**

In the colony-hatching containers the first instar nymphs aggregated on the water wicks and fed minimally, if at all, on the CPB egg masses. Therefore, for ease of handling, all experiments were set up using unfed (water only) second instar nymphs. The nymphs were set up in plastic containers (25 cm diameter by 7 cm high, Pioneer Packaging, Dixon, KY) with a 5 cm hole in the lid covered by nylon screen (Sefar America, Kansas City, MO). The nymphs were reared at 16 h light:8 h dark and 30 ± 0.5°C in a reach-in environmental chamber (Conviron, model I25L, Winnipeg, Canada). Three replicates with 30 nymphs each were used for each treatment group.

**Developmental Experiments**

The control nymphal colony was given three CPB egg masses per day for the first three days, and three water wicks with potato leaves. The diet treatment nymphs received 2 Parafilm domes of artificial diet and three water wicks without potato leaves. The artificial diet domes were changed every other day. All treatment groups received two *H. virescens* larvae per day for the first eight days, after which the number was increased to three per day.

**Fecundity Experiments**

Potato leaves were not used, because the developmental experiment showed no significant difference in development of *P. bioculatus* with or without potato leaves. The control adults received *H. virescens* larvae, three CPB egg masses per day for the first three days and three water wicks. The artificial diet-treatment group received the artificial diet, *H. virescens* larvae and three water wicks throughout their life cycle. Adults were removed daily from the rearing containers, sexed and weighed. Individual mating pairs were placed into plastic wide-mouth containers (10 × 8.5 × 9.5 cm) (Consolidated Plastics, Twinsburg, OH), with a 6.5 cm hole in the lid covered by Nitex screen (Tetko, Lancaster, NY). Each mating container had a roll of paper (6 × 64 cm) for ovipositioning and one water wick. The adults were fed one *H. virescens* larvae per day. The cups were checked daily for eggs, which were subsequently removed, counted and placed in 1 oz. plastic cups (Fill-Rite, Newark, NJ) with lids (Stan-Pac, Lewiston, NY) to measure percent hatch.

**Statistical Analysis**

Proportions of adult emergence were compared using constant statements in PROC LOGISTIC (SAS Institute 1989). Longevity, start of oviposition, total number of eggs per female, total number of clutches per female, and percent hatch were analyzed using ANOVA with the means being separated using Student-Newman-Keuls test, SAS Institute (1989). The level of significance in all tests was 0.05. The developmental rate for each treatment group was estimated by using the median adult emergence time computed from Kaplan-Meier estimates of survival produced by the surfvit function of S-Plus (S-Plus 2001). For the Fecundity Experiments the initial experimental design called for collecting data from the first, third, sixth, ninth and twelfth generations.

**Results**

Preliminary experiments demonstrated that the Cohen-Smith artificial diet alone could not support nymphal development (results not shown). There was a significant decrease in survival from 65% to 36% for *P. bioculatus* nymphs fed only *H. virescens* larvae ($\chi^2 = 9.6747, n = 6, df = 1, P < 0.0019$), as compared to control nymphs, which received both *H. virescens* larvae and CPB eggs. Substituting the artificial diet for CPB eggs had no significant effect upon survival of the nymphs to adult emergence, compared to controls at the 0.05 level (Table 1). Increasing the availability of the artificial diet from the first 3 days to 6 days for all nymphal instars did not increase survival of the nymphs compared to the controls at the 0.05 level (Table 2). Nymphs fed the artificial diet had median adult emergence times equivalent to the controls (Table 3). Of nymphs receiving only *Heliothis* larvae, insufficient numbers survived to analyze their median adult emer-
There was no significant difference in the weights of day 1 adults for the first generation. However, by the third generation there was a significant increase in the weight of females receiving the artificial diet (Table 4). There was no observed difference in weight of males in the course of these experiments. Females that were fed *H. virescens* larvae supplemented during the 2nd instar with Colorado potato beetle eggs (Control) for the first 3 days (3D), six days (6D) or throughout nymphal development (15D) with artificial diet. There were no significant differences in female longevity, onset of oviposition, total number of eggs, number of clutches per female, or percent hatch at the 0.05 level. There was a marked decrease in the number of eggs laid by the females during the sixth generation of continuous rearing on the artificial diet plus larvae, and loss of the colony during the seventh generation.

**DISCUSSION**

The data presented here clearly demonstrate that a diet regime of the modified Cohen-Smith artificial diet for *C. rufilabris* (Cohen & Smith 1998) plus *H. virescens* larvae is as efficient as CPB eggs and *H. virescens* larvae for supporting development for at least three generations. Supplementing *H. virescens* larvae with the Cohen-Smith artificial diet (Cohen & Smith 1998) produced three generations of *P. bioculatus* phenotypically indistinguishable (except for increased weight of female adults) from those on the control diet of larvae plus CPB eggs. This demonstrates that an artificial diet and a suboptimal secondary prey, which by themselves cannot support normal development, can be used in combination as a bridging diet to maintain a research colony during periods when the primary prey is unavailable.

However, while early generations seemed healthy, the experimental colony suffered from reduced fecundity by the sixth generation, and completely collapsed during the seventh. This raises intriguing questions as to what biochemical and molecular changes have occurred over the seven generations. Insects will express a particular subset of genes in order to survive environmental stresses such as heat shock (Roberts & Feder 1999), cold shock (Yocum 2000), and desiccation (Tammarielo et al. 1999). As with other stresses, low food quality or quantity will induce a number of physiological and behavioral changes in insects (reviewed, Slansky & Scriber 1985; Wheeler 1996). We are currently investigating unique gene expression associated with feeding on suboptimal diets. Our bridging diet fed to *P. biocula-

**TABLE 1. EFFECT OF NYMPHAL DIET ON SURVIVAL OF NYMPHS TO ADULT EMERGENCE.***

<table>
<thead>
<tr>
<th>Diet</th>
<th>Number of Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae only</td>
<td>10.8 ± 2.1*</td>
</tr>
<tr>
<td>Larvae + CPB eggs (control)</td>
<td>19.5 ± 1.4*</td>
</tr>
<tr>
<td>Larvae + artificial diet</td>
<td>17.3 ± 2.0*</td>
</tr>
</tbody>
</table>

*Data are expressed as means ±SE of six replicates of 30 nymphs each. Nymphs were fed *H. virescens* larvae only (Larvae only), or *H. virescens* larvae supplemented during the 2nd instar with either Colorado potato beetle eggs (Larvae + artificial diet), Larvae + artificial diet. Means followed by the same letter are not significantly different at the P ≤ 0.05 level (PROC LOGISTIC).

**TABLE 2. EFFECT OF DURATION OF FEEDING ON ARTIFICIAL DIET UPON NYMPH SURVIVAL TO ADULT EMERGENCE.***

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>23.0 ± 2.5</td>
</tr>
<tr>
<td>3D</td>
<td>22.7 ± 1.7</td>
</tr>
<tr>
<td>6D</td>
<td>24.5 ± 1.4</td>
</tr>
<tr>
<td>15D</td>
<td>24.0 ± 1.8</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ±SE of six replicates of 30 nymphs each. Nymphs were fed *H. virescens* larvae supplemented during the 2nd instar with Colorado potato beetle eggs (Control) for the first 3 days (3D), six days (6D) or throughout nymphal development (15D) with artificial diet. PROC LOGISTIC analysis revealed no significant effects of diet on the number of adults obtained.

**TABLE 3. EFFECT OF NYMPHAL DIET ON THE MEDIAN EMERGENCE TIME OF ADULT *P. BIOCULATUS*.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nymphs¹</th>
<th>Adults²</th>
<th>Median adult emergence (D)</th>
<th>0.95 LCL</th>
<th>0.95 UCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>360</td>
<td>255</td>
<td>21</td>
<td>20</td>
<td>21</td>
</tr>
<tr>
<td>3D</td>
<td>360</td>
<td>243</td>
<td>22</td>
<td>21</td>
<td>22</td>
</tr>
<tr>
<td>6D</td>
<td>180</td>
<td>147</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>15D</td>
<td>180</td>
<td>144</td>
<td>21</td>
<td>21</td>
<td>22</td>
</tr>
</tbody>
</table>

¹Data are expressed as median adult emergence times (days) ± lower (LCL) and upper (UCL) 95% confidence limits. Nymphs were fed *H. virescens* larvae supplemented during the 2nd instar with Colorado potato beetle eggs (Control) or for the first 3 days (3D), six days (6D) or throughout nymphal development (15D) with artificial diet.

²Total number of 2nd instar nymphs at the start of the experiment.

²Total number of adults that emerged.
TABLE 4. EFFECTS OF PROLONGED FEEDING ON THE ARTIFICIAL DIET ON DAY 1 ADULT *P. BIOCULATUS* WEIGHT. * 

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Generations</th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Weight (mg)</td>
<td>N</td>
<td>Weight (mg)</td>
</tr>
<tr>
<td>Control</td>
<td>F1</td>
<td>18</td>
<td>47.8 ± 1.5*</td>
<td>27</td>
<td>60.1 ± 1.1*</td>
</tr>
<tr>
<td>Diet-fed</td>
<td>F1</td>
<td>18</td>
<td>47.8 ± 1.3*</td>
<td>24</td>
<td>63.8 ± 1.2**</td>
</tr>
<tr>
<td>Diet-fed</td>
<td>F3</td>
<td>14</td>
<td>48.9 ± 1.9*</td>
<td>18</td>
<td>65.2 ± 2.1b</td>
</tr>
</tbody>
</table>

*Data are expressed as means ±SE of the weights of N number of day 1 adults. Means followed by different letters are significantly different from each other at P ≤ 0.05 as determined by Student-Newman-Keuls test. Nymphs were fed *H. virescens* larvae supplemented with either Colorado potato beetle eggs during the 2nd instar (Control) or the artificial diet throughout development (Diet-fed). ANOVA analysis revealed no significant effects of diet on female longevity (days), start of oviposition, total number of eggs per female, number of clutches per female, or percent hatch at the P ≤ 0.05.

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INFLUENCE OF MALE DIET ON MALE MATING SUCCESS AND LONGEVITY AND FEMALE REMATING IN THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT

The purpose of the present study was to investigate the effect of dietary protein on the mating behavior and survival of male Mediterranean fruit flies (medflies), Ceratitis capitata (Wied.), as a means of enhancing the effectiveness of mass-reared males in sterile release programs to suppress wild populations. Conducted in the laboratory, our study addressed three main questions: 1) Does the inclusion of protein in the adult diet affect mating success of wild and mass-reared males? 2) Are copulation duration and remating tendency of wild females affected by the strain (wild versus mass-reared) and diet (protein-fed versus protein-deprived) of their initial mating partner? 3) Does the inclusion of protein in the adult diet affect the longevity of mass-reared males? In mating trials involving wild flies, protein-fed males had a mating advantage over protein-deprived males. However, the addition of protein to the diet did not boost the mating success of mass-reared males in competition with wild or mass-reared males for wild females. The inclusion of protein in the male diet had no apparent effect on female remating tendency, copulation duration, or male longevity. Independent of male diet, we found no difference between wild and mass-reared males in the duration of copulations with wild females, and wild females mated initially to wild and mass-reared males displayed similar remating propensity. The implications of these findings for SIT are discussed.

RESUMEN

El propósito del presente estudio fue para investigar el efecto de la proteína dietética en el comportamiento del apareamiento y la sobrevivencia de machos de la mosca mediterránea, Ceratitis capitata (Wied.), como una manera de mejorar la eficacia de la producción en masa de los machos en programas de liberaciones de machos esteriles para suprimir las poblaciones silvestres. Nuestro estudio, conducido en el laboratorio, se enfocó en tres preguntas principales: 1) Si la inclusión de proteína en la dieta del adulto afecta el éxito en el apareamiento de los machos silvestres y de los machos criados en masa? 2) Si la duración de la cópula y la tendencia de reaparearse las hembras silvestres con la pareja inicial son afectadas por la raza (silvestres versus criados en masa) y la dieta (alimentadas con proteína versus privadas de proteína)? 3) Si la inclusión de proteína en la dieta del adulto afecta la longevidad de los machos criados en masa? En pruebas de apareamiento con moscas silvestres, los machos alimentados con proteína tuvieron una ventaja de apareamiento sobre los machos privados de proteína. Sin embargo, la adición de proteína a la dieta no aumentó el éxito de aparearse en los machos criados en masa en competencia con machos silvestres con las hembras silvestres. La inclusión de proteína en la dieta del macho no tuvo un efecto aparente sobre la tendencia de las hembras para reaparearse, la duración de la cópula, o la longevidad del macho. Independientemente de la dieta del macho, no encontramos una diferencia en la duración de la cópula entre machos silvestres y machos criados en masa al aparearse con hembras silvestres, y las hembras silvestres apareadas inicialmente con machos silvestres o con machos criados en masa mostraron una tendencia similar para aparearse de nuevo. Se discuten las implicaciones de estos resultados para técnica del insecto estéril (SIT).

The sterile insect technique (SIT) is widely used in suppression or eradication programs against the Mediterranean fruit fly, Ceratitis capitata (Wied.) (Hendrichs et al. 1995). To a large degree, the success of SIT depends on the ability of mass-reared, sterile males to compete successfully against wild males in obtaining copulations with wild females (unless otherwise indicated, males derived from large-scale, production facilities are hereafter referred to as 'mass-reared' regardless of whether they were irradiated (sterilized) prior to study or release). Unfortunately, the mass-rearing environment, and, in particular, the high density at which adults are held, imposes strong selection factors that may alter courtship behavior and subsequently lessen
the competitive ability of sterile males in the wild (Leppla & Ozaki 1991; Cayol 2000). For example, Briceno and Eberhard (1998) observed that mass-reared males displayed shorter courtships than wild males and suggested that accelerated courtship evolved in response to frequent disturbances resulting from dense crowding.

Genetically based changes in sexual behavior and life history traits appear to be an inherent consequence of mass-rearing and are therefore difficult (if not impossible) to avoid or mitigate. Because of this, there is a persistent need to develop procedures that boost the performance of mass-reared males via simple, easily incorporated, and inexpensive modifications to the standard mass-rearing protocol. For example, a recent study on the medfly (Shelly & McInnis 2001) demonstrated that exposure to the odor of ginger root oil dramatically enhanced the mating success of mass-reared males. In the absence of chemical exposure, wild males outcompeted mass-reared males and obtained 74% of all matings. However, following exposure to ginger root oil, the mating frequencies were reversed, and mass-reared males achieved 75% of all matings.

Modification of the pre-release, adult diet may represent another simple approach to increase the effectiveness of male medflies in SIT. Current programs (e.g., California, Israel) feed newly emerged adult medflies with sucrose-containing agar exclusively. However, recent studies showed that the addition of protein hydrolysate to the diet resulted in a significant increase in male mating success. In noncompetitive conditions, mass-reared male medflies fed a protein-sugar mixture mated more frequently than males fed sugar only (Blay & Yuval 1997). Likewise, in direct competition for females, mass-reared males given the sugar-protein diet signaled (pheromone-called) and mated more frequently than mass-reared males that were protein-deprived (Kaspi & Yuval 2000; Taylor & Yuval 1999). Similar findings were reported for tests involving wild medflies as well (Kaspi et al. 2000; see also Papadopoulos et al. [1998]).

Although these findings clearly support the addition of protein to the pre-release diet, this modification may have costs in terms of reduced male survivorship after release. Following 24 h of starvation on the fifth day of life, mass-reared males fed a protein-containing diet for the preceding 4 d were far more likely to die than were males fed sugar only during the first 4 d of life (Kaspi & Yuval 2000). Thus, development of a pre-release, protein-containing diet that optimizes male effectiveness in SIT programs requires additional information on the balance between heightened sexual competitiveness and reduced survivorship.

The purpose of the present study was to investigate further the effect of dietary protein on the mating behavior and survival of male medflies. Based on laboratory observations, our study addressed three main questions: 1) Does the inclusion of protein in the adult diet affect mating success of wild and mass-reared males? 2) Are copulation duration and remating tendency of wild females affected by the strain (wild versus mass-reared) and diet (protein-fed versus protein-deprived) of their initial mating partner? 3) Does the inclusion of protein in the adult diet affect the longevity of mass-reared males? Although field cages would have provided a more natural environment, we chose to conduct this study in the laboratory, because we were able to measure copulation duration, female remating and male longevity with greater accuracy and obtain larger sample sizes than possible from field tests.

MATERIALS AND METHODS

Mating Experiments

Wild flies were reared from the fruits of Jerusalem Cherry (Solanum capsicum L.) collected in the Hawaii Volcanoes National Park, HI. Fruits were held over vermiculite, and larval development proceeded in situ. Pupae were sifted from the vermiculite 7-9 d after fruit collection, and adults were separated by sex within 2 d of eclosion, well before reaching sexual maturity (T. E. S., unpublished data). Mass-reared males were obtained from the Hawaii Fruit Fly Rearing Facility, Waimanalo, HI. Pupae were exposed in air to 150 Gy of gamma radiation from a $^{137}$Cs source 2 d before eclosion and then delivered to our laboratory. Males were collected within 12 h of eclosion. Both wild and mass-reared adults were held in plastic buckets covered with nylon screening (volume 5 liters; 100-200 flies per bucket). Room temperature was maintained at 22-25°C and relative humidity at 65-90%, and flies were exposed to natural and artificial light in an approximately 12:12 h light:dark cycle. Wild and mass-reared males were separated into 2 dietary regimes: “protein-deprived” males were given only sugar (sucrose) plus water, and “protein-fed” males were given a 3:1 mixture (by volume) of sugar and protein hydrolysate plus water. Wild females were given the sugar-protein mixture plus water. Samples (n = 30 individuals) of wild and mass-reared males maintained on the two diets and wild females were weighed (wet weight) to the nearest 0.1 mg with an electronic balance. In all cases, adults were weighed when 5 d old.

Three experiments were performed that compared the mating frequency of 1) protein-fed versus protein-deprived wild males, 2) protein-fed versus protein-deprived mass-reared males, and 3) protein-fed or protein-deprived mass-reared males versus protein-fed wild males. Wild females were used in all cases. Mating tests were conducted in the same manner for all experiments. Sixty males of each diet type (or strain type for ex-
experiment 3) and 60 females were placed in transparent plexiglass cages (30 × 30 × 40 cm with screen-covered openings on the top and sides). Although this density far exceeds natural levels (Shelly et al. 1994), females nonetheless had ample space for movement and were clearly capable of moving away from (i.e., rejecting) courting males. Wild flies were used at 10-14 d of age, and mass-reared males were 6-9 d old when tested (the different ages reflect differing maturation rates between wild and mass-reared males). Males were marked 1 d before testing by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no adverse effects, and males resumed normal activities within minutes of handling.

Flies were placed in the cages between 0730-0800 hours and monitored continuously for the next 5 h. Cages were placed adjacent to east-facing windows and thus received both natural and artificial light. Mating pairs were collected by gently coaxing couples into vials, which were then labeled and placed on holding trays. Vials were monitored continuously, and times of collection (i.e., mating start) and break-up (i.e., mating stop) were recorded for each pair. When mating ceased, males were removed and discarded, and females were held in plastic buckets for the remating trials (see below; data on copulation duration and female remating were collected only for experiments 1 and 2). All unmated flies were discarded. Two or 3 cages were run on each of 5 days for all experiments. Eleven replicates (cages) were run for each experiment.

Mated females were provided with food and water as above and a perforated, plastic vial (containing a sponge soaked with lemon juice) for oviposition. Two days after their initial mating, groups of females that mated with males of the same diet type were placed in glass cages (30 cm cubes with a cloth sleeve covering one side) with an equal number of protein-fed, wild males between 0730-0800 hours. Cages were monitored for 5 h, and copulation durations were recorded as noted above. Females that remated 2 d after the initial mating were discarded, and the remaining females were held and re-tested in the same manner 4 d after the initial mating. Females that failed to mate at this time were held and tested a final time 7 d after the initial mating. Males used in the different remating trials were 11-19 d old. Female mortality was relatively low and varied independently of the strain or diet of the initial mate. Over all females, 95% survived from the initial mating to the 2-d test, 96% survived from the 2-d test to the 4-d test, and 91% survived from the 4-d to the 7-d test.

Male Longevity

Two experiments were performed to investigate the effect of adult diet on the longevity of mass-reared males. First, the probability of surviving the first week of adult life was compared between protein-fed and protein-deprived males. Emerging males were collected between 0600-0900 hours, and groups of 50 individuals were placed in screen cages (30 cm cubes) and given continuous access to water plus sugar only or the protein-sugar mixture. Dead flies were removed daily. Twenty-one cages were run for each diet type. The second experiment examined the effect of dietary protein on male longevity following food removal. Newly emerged males were protein-fed or protein-deprived for 4 d following eclosion. On the morning of the fifth day, groups of 50 males from a given diet were placed in screen cages with water only, and deaths were recorded every morning over the next 4 d (by which time all individuals in both treatments were dead; see below). Nine cages were run for each diet type.

Statistical Analyses

The Mann-Whitney test (test statistic T) was used for pairwise comparisons involving male weights, the number of matings achieved by competing male types, and the number of surviving males from the two diet groups. With respect to sexual competition, this test does not explicitly test for deviation from random mating (i.e., each male type accounts for 50% of the total matings), so a binomial test (using the normal approximation and Z scores with Yates correction for continuity) was performed with data pooled over all replicates. Remating frequencies were compared using the log-likelihood ratio for contingency tables (test statistic $\chi^2$, where $df = (\text{rows}-1) \times (\text{columns}-1)$) with Yates correction for continuity. Because sample sizes were relatively small (especially for females initially mated to mass-reared males) and because remating was a relatively rare event, comparisons were made using data pooled over all groups of females initially mated to the same male type. Copulation durations were compared among male types using the Kruskal-Wallis test (test statistic H). Statistical procedures followed Zar (1986).

RESULTS

Adult Body Weight

Among wild males, diet had no apparent effect on weight; protein-fed males had an average weight of 7.9 mg (SD = 1.2; range: 5.4-9.8 mg) compared to 7.5 mg (SD = 1.0; 5.4-9.4 mg) for protein-deprived males (T = 799, P > 0.05, n1 = n2 = 30 in this and subsequent weight comparisons). Among mass-reared males, however, protein-fed males ($\bar{x} = 7.8 \text{ mg}$; SD = 0.9; range: 6.0-9.7 mg) were significantly heavier, on average, than protein-deprived males ($\bar{x} = 7.3$; SD = 0.7; range: 6.0-
8.6 mg). Wild and mass-reared males fed protein-containing diets did not differ significantly in body weight (T = 926.5, P > 0.05), but protein-fed, wild males were significantly heavier than protein-deprived, mass-reared males (T = 1078.0, P < 0.05). The average weight of wild females was 8.2 mg (SD = 1.3; range: 5.0-10.7), which did not differ significantly from that of protein-fed, wild (T = 986; P > 0.05) or mass-reared (T = 999.0; P > 0.05) males.

**Male Mating Success**

In the experiment using wild males exclusively, diet had a marked effect on male mating success, and protein-fed males obtained an average of 16.5 matings per replicate compared to only 10.2 for protein-deprived males (Table 1). Protein-fed, wild males obtained 61% (182/296) of the total matings observed over all replicates (Z = 4.0; P < 0.001).

Diet had a lesser effect on the outcome of mating competition involving mass-reared males only (Table 1). On average, protein-fed males obtained a greater number of matings per replicate than protein-deprived males (6.6 versus 4.5, respectively), but this difference was not statistically significant. However, pooling data over all replicates, we found that protein-fed, mass-reared males obtained a disproportionately larger number of matings than expected by chance alone (73/123 = 59%; Z = 2.2; P < 0.05). Combined over both diet types, the total number of matings recorded per replicate was significantly higher for wild than mass-reared males (26.7 vs. 11.2, respectively; T = 184.0; n1 = n2 = 11; P < 0.001).

In the final experiment, where wild and mass-reared males competed directly, diet had no detectable influence on the relative mating success of mass-reared males (Table 1). When both strains were protein-fed, wild males obtained an average of 19.0 matings per replicate compared to 5.4 for the mass-reared males. In this case, wild males obtained 78% (209/269) of the matings over all replicates (Z = 10.9; P < 0.001). When the mass-reared males were protein-deprived, the wild males obtained an average of 18.4 matings per replicate compared to only 4.1 for the mass-reared males. Wild males obtained 82% (202/247) of the total matings recorded over all replicates (Z = 13.2, P < 0.001). In competition with protein-fed wild males, there was no significant difference in the proportion of total matings obtained by protein-fed and protein-deprived mass-reared males ($\chi^2 = 1.4, P > 0.05$).

**Female Remating**

In tests involving wild males, female remating tendency was generally independent of the diet of the mating partner in tests conducted 2, 4, or 7 d after the initial mating. Approximately 10% of the females that mated with protein-fed (18/173) or protein-deprived (11/107) males remated 2 d after the initial mating ($\chi^2 = 0.3; df = 1$ in this and all subsequent pairwise comparisons of frequency; $P > 0.05$). In tests conducted 4 d after the first mating, 4% (6/150) and 2% (2/95) of females mated to protein-fed and protein-deprived males remated, respectively ($\chi^2 = 0.4; P > 0.05$). In tests conducted 7 d after the first mating, 7% (9/131) and 6% (5/79) of females mated to protein-fed and protein-deprived males remated, respectively ($\chi^2 = 0.2; P > 0.05$). Combining data over diet types, we found significant variation in the incidence of remating over the different intervals tested ($\chi^2 = 10.6, P < 0.01$), reflecting primarily the large difference in remating probability between 2 d (29/280 = 10%) and 4 d (8/245 = 3%) after the initial mating.

In tests involving mass-reared males, female remating tendency was also independent of the diet of the mating partner. Remating frequencies 2 d after the initial mating were 3% (2/67) for females that mated with protein-fed males and 6% (3/46) for females that mated with protein-deprived males ($\chi^2 = 0.6; P > 0.05$). In tests conducted 4 d after the first mating, none of the females that initially mated to protein-fed or protein-deprived males remated ($\chi^2 = 0; P > 0.05$). In tests conducted 7 d after the first mating, 7% (4/

**Table 1. Influence of Male Diet and Strain on Mating Success. Matings Values Represent Average (±1 SD) Number of Matings per Replicate (N = 11 in All Cases).**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Male strain</th>
<th>Male diet</th>
<th>Matings</th>
<th>T</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wild</td>
<td>Protein-fed</td>
<td>6.5 (3.8)</td>
<td>161.0</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Wild</td>
<td>Protein-deprived</td>
<td>10.2 (3.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mass-reared</td>
<td>Protein-fed</td>
<td>6.6 (2.6)</td>
<td>147.0</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Mass-reared</td>
<td>Protein-deprived</td>
<td>4.5 (2.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a</td>
<td>Wild</td>
<td>Protein-fed</td>
<td>19.0 (4.2)</td>
<td>187.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mass-reared</td>
<td>Protein-fed</td>
<td>5.4 (2.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3b</td>
<td>Wild</td>
<td>Protein-fed</td>
<td>18.4 (5.2)</td>
<td>189.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Mass-reared</td>
<td>Protein-deprived</td>
<td>4.1 (2.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
55) and 5% (2/39) of females mated to protein-fed and protein-deprived males remated, respectively ($\chi^2 = 0.2; P > 0.05$). Combining data over diet types, we found that the probability of female remating varied significantly over the intervals tested ($\chi^2 = 9.0; P < 0.05$), owing chiefly to the absence of any remating in tests conducted 4 d after the initial mating.

Combining data over diet types, we found no difference in remating frequency between females mated to wild or mass-reared males for any time interval tested (2 d: wild - 29/280, mass-reared - 5/113, $\chi^2 = 3.4; P > 0.05$; 4 d: wild - 8/245, mass-reared - 0/99, $\chi^2 = 3.0; P > 0.05$; 7 d: wild - 14/210, mass-reared - 6/94, $\chi^2 = 0.2, P > 0.05$). Using data combined across male diet and male strain, we found significant variation in the incidence of female remating over the intervals tested ($\chi^2 = 14.8, P < 0.001$). Over all females, remating frequencies were 9% (34/393) at 2 d, 2% (8/344) at 4 d, and 6% (20/304) at 7 d after the initial mating.

Copulation Duration

For initial matings, copulation duration varied independently of male strain and diet ($H = 6.1; df = 3; P > 0.05$; Fig. 1). Among wild males, mating times averaged 144.3 min (SD = 40.8; range: 31 – 275) and 141.5 min (SD = 46.3; range: 21 - 239) for the protein-fed (n = 182) and protein-deprived (n = 114) males, respectively. Among mass-reared males, mating times averaged 135.6 min (SD = 41.5; range: 11 - 256) and 134.4 min (SD = 39.8; range: 67 - 223) for the protein-fed (n = 73) and protein-deprived (n = 50) males, respectively. Independent of diet, there was no significant difference in copulation duration for initial matings with wild ($\bar{x} = 143.3$ min; SD = 42.3; n = 296) and mass-reared ($\bar{x} = 135.1$; SD = 40.6; n = 123) males ($T = 22.054.0; P > 0.05$).

Male Longevity

Among mass-reared males, survival probability to 7 d of age was independent of diet type. On average, 38.5 (SD = 4.5) protein-fed males per
cage survived the first week of life compared to 37.4 (SD = 4.0) protein-deprived males (T = 494.0; \( n_1 = n_2 = 11; P > 0.05 \)).

Survivorship following food removal was likewise independent of diet type. On average, 45.0 (SD = 3.7) protein-fed males per cage were alive 1 d following food removal compared to 46.7 (SD = 2.2) of the protein-deprived males (T = 97.0; \( n_1 = n_2 = 9; P > 0.05 \)). Two days after food removal, an average of 4.4 (SD = 3.6) protein-fed and 5.4 (SD = 5.0) protein-deprived males were alive per cage (T = 89.0; \( n_1 = n_2 = 9; P > 0.05 \)). Of the 900 males observed in this experiment, only 4 protein-fed and 3 protein-deprived males survived for 3 d after food removal, and none survived to 4 d in either treatment.

**DISCUSSION**

Consistent with previously conducted field-cage trials (Shelly et al. 2001), the laboratory data reported here revealed that, among wild male medflies in Hawaii, protein-fed individuals achieved significantly more matings than protein-deprived individuals in direct competition for wild females. In the field-cage trials, we found no difference in levels of pheromone-calling between protein-fed and protein-deprived, wild males (Shelly et al. 2001), and consequently it appears that the difference in mating frequency reported here does not simply reflect a diet-mediated difference in sexual signaling. This situation differs from other studies reporting increased calling and mating for mass-reared (Kaspi & Yuval 2000) and wild (Kaspi et al. 2000) male medflies fed protein as adults. Although diet-mediated variation in signaling activity may not be evident among wild males in Hawaii, the inclusion of protein in the adult diet may have resulted in qualitative differences in the composition, and hence attractiveness, of the male sex pheromone. In comparing female arrivals to artificially established leks in the field, we found that, while signaling activity did not vary noticeably with diet, approximately twice as many female sightings were made at leks composed of protein-fed males than at leks composed of protein-deprived males (Shelly et al. 2001).

In contrast to wild males, mating success was independent of adult diet among mass-reared males whether the competition involved mass-reared males only (experiment 2, although a mating advantage for protein-fed males was suggested when data were pooled over all replicates) or wild and mass-reared males (experiment 3). Although the results are preliminary, ongoing field-cage tests (T. E. S., unpublished data) similarly indicate that, relative to wild males, the inclusion of protein in the adult diet has no influence on the mating success of mass-reared males from a genetic sexing (temperature sensitive lethal, Franz & McInnis 1995) strain. Thus, contrary to other studies (Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi & Yuval 2000), our results do not support the notion that the addition of protein to the pre-release diet would, in general, enhance the mating competitiveness of mass-reared medfly males in SIT programs (but see below).

Male body weight had no obvious impact on the outcome of mating competition in any of the experiments. Among wild males, protein-fed and protein-deprived males were similar in body weight, yet protein-fed males enjoyed a significant mating advantage (experiment 1). Conversely, protein-fed, mass-reared males were heavier, on average, than protein-deprived, mass-reared males, yet there was no difference in mating frequency between them (experiment 2). When wild and mass-reared males competed directly, the protein-fed and protein-deprived, mass-reared males had similar mating success relative to wild males despite the fact that wild males were similar in weight to protein-fed, mass-reared males but significantly heavier than protein-deprived, mass-reared males (experiment 3).

The low level of mating observed in the experiment involving mass-reared males exclusively (experiment 2) suggests that mass-reared males were generally unacceptable to wild females independent of their diet. This interpretation, in turn, suggests that females of *C. capitata* select males largely on the basis of “absolute” criteria and not on a relative or “best-of-n” basis (Janetos 1980). That is, females may accept only males above a certain threshold level for a particular sexual signal(s) and reject other males. If true, *C. capitata* females are not choosing mates on the basis of between-male comparisons made over a sampling interval, and consequently the relative abundance of males of varying quality does not affect female choice. This, in turn, implies that mass-rearing facilities for SIT should concentrate, not simply on increasing production of males, but on maintaining sexual competitiveness of the males as well (Calkins 1984).

The frequency of female remating varied independently of male diet regardless of whether the initial mate was a wild or mass-reared male. This finding differs from that of Blay and Yuval (1997), who, in their study of mass-reared flies, found that females that first mated with a protein-fed male were less likely to remate (on the day following the initial mating) than females that first mated with a protein-deprived male. In that study, protein-fed males were significantly heavier than protein-deprived males, a difference that may have affected renewal of female receptivity (Blay & Yuval 1997; see also Bloem et al. 1993a). Although diet-related differences in male weight were detected in our study, the incidence of female remating was similar following initial mating to males of different sizes (e.g., protein-fed and protein-deprived, mass-reared males).
We also noted that, when data were combined over male diet types, wild females initially mated to wild or mass-reared males remated at approximately the same frequency. To our knowledge, no prior studies have drawn this comparison despite its potential importance for SIT programs. Several studies, however, have examined the effect of irradiation per se on female remating, and the results obtained are contrary to our finding. Working exclusively with laboratory flies, Katiyar and Ramirez (1970) and Bloem et al. (1993b) both found that females first mated to irradiated males were more likely to remate than females whose initial mate was non-irradiated.

Within wild and mass-reared strains of the medfly, male diet had no apparent effect on copulation duration. Taylor et al. (2000) likewise found no effect of male diet on copulation duration for matings involving flies from a wild population in Israel. However, male diet has been found to affect copulation duration in matings involving mass-reared flies (Taylor & Yuval 1999; Field & Yuval 1999), with copulations involving protein-fed males being shorter than those involving protein-deprived males. Independent of male diet, we also found no difference in copulation duration between matings (with wild females) involving wild or mass-reared males (see also Orozco and Lopez 1993). Using laboratory flies exclusively, several studies have examined the influence of irradiation on males on copulation duration with inconsistent results: Katiyar and Ramirez (1973) found no effect of irradiation, whereas Seo et al. (1990) reported that copulations involving irradiated males (and non-irradiated females) were shorter than those involving non-irradiated males.

Data from the present study contribute further evidence for global variation in copulation duration in the medfly. For example, copulation duration is similar between wild flies from Hawaii (x̅ = 143 min) and Israel (median = 145 min; Taylor et al. 2000) but is appreciably longer for wild flies in Argentina (x̅ = 172 min; Cayol et al. 1999). Conversely, copulation duration between mass-reared males and wild females is similar between Hawaii (x̅ = 135 min) and Argentina (x̅ = 134 min calculated using from Table 1 in Cayol et al. 1999) but is much longer for mass-reared flies in Israel (median = 160 - 180 min; Field et al. 1999; Taylor et al. 2001).

We also failed detect an effect of diet on the longevity of mass-reared males. In contrast, Kaspi and Yuval’s (2000) study that, after 4 d of feeding, protein-fed males were less likely to survive a 24 h period of starvation (on day 5) than protein-deprived males. Following the same protocol, we found that the number of males surviving the starvation period was nearly identical between protein-fed and protein-deprived males. Survivorship of protein-deprived males was similar between the two studies (approximately 10%), but protein-fed males had much higher mortality in Kaspi and Yuval’s (2000) study than in our experiment (approximate mortality after 1-d starvation: 50% versus 10%, respectively). Protein hydrolysate comprised 25% of the protein-containing diet in our study but only 9% in Kaspi and Yuval (2000), but whether this difference was responsible for the differential mortality is unknown.

In conclusion, the finding that dietary protein affected mating frequency in wild males indicates an important role for adult nutrition in mating competition in the Mediterranean fruit fly, a conclusion consistent with other studies (Blay & Yuval 1997; Taylor & Yuval 1999; Kaspi & Yuval 2000; Shelly et al. 2001). The absence of the same effect in Hawaiian mass-reared males might indicate inter-strain differences in the behavioral and physiological responses of males to dietary composition. However, it appears more likely that it derived from the low overall quality of the Hawaiian mass-reared strain tested, which essentially ‘overwhelmed’ any positive effect resulting from the inclusion of dietary protein. This, in turn, suggests that the potential benefits to SIT of including protein in the pre-release, adult diet will vary with the quality of the mass-reared strain, being greatest for males that compete relatively well against wild males independently of diet composition.

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CURRENT DISTRIBUTION OF THE FORMOSAN
SUBTERRANEAN TERMITE AND OTHER TERMITE SPECIES
(ISOPTERA: RHINOTERMITIDAE, KALOTERMITIDAE) IN LOUISIANA

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ABSTRACT
A statewide survey in Louisiana on the current distribution of the Formosan subterranean
termite, Coptotermes formosanus Shiraki, and other termite species was conducted with 91
pest control companies, city and state agencies, and the New Orleans Mosquito and Termite
Control Board from January 1999 to August 2002. A total of 812 samples were used in the
survey constituting all eight known termite species from Louisiana. The subterranean ter-
mite species identified were Reticulitermes flavipes (Kollar), R. virginicus (Banks), R. hageni
Banks, and C. formosanus. The drywood termite species identified were Incisitermes snyderi
(Light), I. minor (Hagen), Cryptotermes brevis (Walker), and Kalotermes approximatus (Sny-
der). Incisitermes minor was also collected in Mississippi and is a new record in that state.
The collective data on the flight season of each species was also recorded.

Key Words: Coptotermes formosanus, Rhinotermitidae, Kalotermitidae

RESUMEN
Un reconocimiento de la distribución actual de la termita subterránea formosana, Coptotер-
mes formosanus Shiraki y de otras especies de termitas fue llevado a cabo en Louisiana,
EE.UU con la colaboración de 91 compañías de control de plagas, las agencias estatales y
municipales, y el Buró de Control de Mosquitos y Termitas de Nueva Orleans desde enero de
1999 hasta agosto de 2002. Un total de 812 muestras fueron usados en el reconocimiento
constituyendo las ocho especies de termitas conocidas de Louisiana. Las termitas subtrár-
neas identificadas fueron Reticulitermes flavipes (Kollar), R. virginicus (Banks), R. hageni
Banks, y C. formosanus. Las termitas de madera seca identificadas fueron Incisitermes sny-
deri (Light), I. minor (Hagen), Cryptotermes brevis (Walker), y Kalotermes approximatus
(Snyder). Incisitermes minor fue también colectada en Mississippi y es un nuevo registro en
aquel estado. Los datos colectivos sobre la temporada de vuelos para cada especie también
fueron registrados.

Translation provided by author.
ducted in Georgia (Scheffrahn et al. 2001), Florida (Scheffrahn et al. 1988), Texas (Howell et al. 1987), and South Carolina (Hathorne et al. 2000). These surveys significantly contributed to our understanding of the current distribution of the economically important FST.

Because there have been many unconfirmed reports of the FST throughout the state, the main objective of this survey was to identify and confirm the current distribution of the FST in Louisiana with the help of the pest control industry, the Louisiana Department of Agriculture and Forestry, and mosquito control districts. In addition, the New Orleans Mosquito and Termite Control Board concurrently conducted a separate statewide survey for all subterranean and drywood termite species.

**MATERIALS AND METHODS**

**Pest Management Professional (PMP) Survey**

Beginning in January 1999, letters asking for participation in the survey were mailed to 589 PMPs and mosquito control districts throughout Louisiana, including a few pest control companies operating near the state line in Mississippi and Texas. Termite collecting packets were then prepared and sent to each company who returned the postcard with a response of willingness to participate. Each packet included individually numbered collection vials (13 ml polypropylene SnapSeal®, Corning Brand) containing 85% ethanol, corresponding vial data sheets, return padded envelopes, and a hand-held aspirator. Each participant was encouraged to collect termite alates and soldiers during routine inspections and treatments of residential and commercial structures. They were also encouraged to include any relevant information from each collection on the data sheet, which included date and location of collection, flight date (if applicable), and any additional comments and requests for more collection vials.

As a result, 52 of the 91 participants returned collection vials for a total of 426 samples. All eight known termite species were collected (Table 1). The majority of these samples were collected from separate addresses. *Reticulitermes flavipes* was the most commonly collected species throughout the state (Table 1). The FST was the second most commonly collected species; however, the majority of the FST samples were collected from the New Orleans and Lake Charles areas (Table 1).

Each participant also included an exact or approximate date of dispersal flight whenever they collected alates. For the subterranean species,

**N. O. Mosquito and Termite Control Board (NOMTCB) Survey**

The senior author and other coworkers conducted a deliberate survey throughout Louisiana from 1999 to 2001. Termites were collected from live and dead trees, state parks, railroad ties, highway rest areas, private and public buildings, and any other type of wood found along highways and parish roads. We also traveled to addresses throughout the state to verify FST infestations and conduct further surveys in the surrounding areas. In addition, samples and FST locations were received from J. McPherson, Program Coordinator, Pesticide and Environmental Programs, Louisiana Department of Agriculture and Forestry, Baton Rouge, LA.

For both surveys, termite alates and soldiers were identified to species using termite keys developed by Banks & Snyder (1920), Miller (1949), Snyder (1954), Wessner (1965), Scheffrahn & Su (1994), and Hostettler et al. (1995). Samples containing only workers (*Reticulitermes* spp.) or pseudergates were identified to the family and/or genus level. Data from both surveys was entered into a computer database (FileMaker® Pro 3.0, Claris® Corporation). Longitude and latitude coordinates from the NOMTCB survey were recorded at each sample site using a Garmin GPS model 12 CX (Garmin International, Inc., Olathe, KS) hand-held global positioning receiver. Locations of each collection were plotted using ArcView GIS version 3.1 software (Environmental Systems Research Institute, Inc., Redlands, CA).

**RESULTS**

**PMP Survey**

Out of the original 589 survey letter mailings, 91 (15%) companies and individuals agreed to participate by collecting any type of termite they encountered during routine inspections and treatments of urban structures and trees. There was no response from 453 (77%) companies and 45 (8%) responded, but declined to participate. The majority of the companies who declined indicated that they do not conduct termite treatments.

Table 1. Total number of identified termite species from vials collected during the PMP survey.

<table>
<thead>
<tr>
<th>Termite species</th>
<th>Number of vials</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Reticulitermes flavipes</em></td>
<td>204</td>
</tr>
<tr>
<td><em>Coptotermes formosanus</em></td>
<td>118</td>
</tr>
<tr>
<td><em>Reticulitermes virginicus</em></td>
<td>40</td>
</tr>
<tr>
<td><em>Incisitermes snyderi</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Cryptotermes brevis</em></td>
<td>7</td>
</tr>
<tr>
<td><em>Incisitermes minor</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Kalotermes brevis</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Reticulitermes hageni</em></td>
<td>3</td>
</tr>
<tr>
<td>Workers/pseudergates only</td>
<td>39</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>426</strong></td>
</tr>
</tbody>
</table>
R. flavipes alates were recovered from Jan. 17 to April 19, R. virginicus alates from March 1 to May 17, R. hageni on Dec. 17, 2001 (single record), and the FST from April 12 to May 9. For the kalotermid species, I. snyderi alates were recovered from May 10 to July 22, C. brevis from May 9 to July 25, and K. approximatus from Oct. 10 to Nov. 1. Alate samples of I. minor were collected from Sept. 10 to Dec. 4 in Rayne, Cameron, and Le Moyeu, LA; however, monitoring of dispersal flights by the senior author in the New Orleans metro area occurred each year from late April to early June.

**NOMTCB Survey**

*Reticulitermes flavipes* was by far the most commonly collected termite species throughout Louisiana (Table 2). *Reticulitermes hageni* and *R. virginicus* were the two second most commonly collected species (Table 2). The number of FST collections only represents a few selected, confirmed sites throughout the state and does not include any samples taken from New Orleans. The distribution of FST infestations in Louisiana has significantly increased since 1966 (Table 3).

### Table 2. Total number of termite species and samples collected during the NOMTCB survey.

<table>
<thead>
<tr>
<th>Termite species</th>
<th>Number of collections</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Reticulitermes flavipes</em></td>
<td>177</td>
</tr>
<tr>
<td><em>Reticulitermes hageni</em></td>
<td>65</td>
</tr>
<tr>
<td><em>Reticulitermes virginicus</em></td>
<td>64</td>
</tr>
<tr>
<td><em>Coptotermes formosanus</em></td>
<td>40</td>
</tr>
<tr>
<td><em>Incisitermes snyderi</em></td>
<td>21</td>
</tr>
<tr>
<td><em>Incisitermes minor</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Cryptotermes brevis</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Kalotermes approximatus</em></td>
<td>2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>386</strong></td>
</tr>
</tbody>
</table>

*R. flavipes* alates were recovered from Jan. 17 to April 19, *R. virginicus* alates from March 1 to May 17, *R. hageni* on Dec. 17, 2001 (single record), and the FST from April 12 to May 9. For the kalotermid species, *I. snyderi* alates were recovered from May 10 to July 22, *C. brevis* from May 9 to July 25, and *K. approximatus* from Oct. 10 to Nov. 1. Alate samples of *I. minor* were collected from Sept. 10 to Dec. 4 in Rayne, Cameron, and Le Moyeu, LA; however, monitoring of dispersal flights by the senior author in the New Orleans metro area occurred each year from late April to early June.

### Table 3. Location of *Coptotermes formosanus* infestations in Louisiana, 1966-2001.

<table>
<thead>
<tr>
<th>Year</th>
<th>Parish</th>
<th>City</th>
</tr>
</thead>
<tbody>
<tr>
<td>1966*</td>
<td>Orleans</td>
<td>New Orleans, Algiers</td>
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<tr>
<td></td>
<td>Calcasieu</td>
<td>Lake Charles</td>
</tr>
<tr>
<td>1968*</td>
<td>Orleans</td>
<td>New Orleans, Algiers</td>
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<td></td>
<td>Calcasieu</td>
<td>Lake Charles</td>
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<tr>
<td></td>
<td>Jefferson</td>
<td>Grand Isle</td>
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<tr>
<td></td>
<td>La Fourche</td>
<td>Raceland</td>
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<tr>
<td>1986*</td>
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<td>New Orleans, Algiers</td>
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<tr>
<td></td>
<td>Calcasieu</td>
<td>Lake Charles, Westlake</td>
</tr>
<tr>
<td></td>
<td>Jefferson</td>
<td>Metairie, Gretna, Grand Isle</td>
</tr>
<tr>
<td></td>
<td>La Fourche</td>
<td>Raceland</td>
</tr>
<tr>
<td></td>
<td>St. Tammany</td>
<td>Slidell, Covington</td>
</tr>
<tr>
<td></td>
<td>Lafayette</td>
<td>Lafayette</td>
</tr>
<tr>
<td></td>
<td>East Baton Rouge</td>
<td>Baton Rouge</td>
</tr>
<tr>
<td>2001</td>
<td>Orleans</td>
<td>New Orleans, Algiers</td>
</tr>
<tr>
<td></td>
<td>Calcasieu</td>
<td>Lake Charles, Westlake, Moss Bluff, Sulphur</td>
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<td></td>
<td>Jefferson</td>
<td>Metairie, Gretna, Grand Isle, Kenner, Harahan, Westwego, Marrero</td>
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<tr>
<td></td>
<td>La Fourche</td>
<td>Raceland, Thibodaux, Larose, Cut Off, Galliano</td>
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<tr>
<td></td>
<td>St. Tammany</td>
<td>Slidell, Covington</td>
</tr>
<tr>
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<td>Lafayette</td>
<td>Lafayette</td>
</tr>
<tr>
<td></td>
<td>East Baton Rouge</td>
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<tr>
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<td>Prairieville</td>
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<td></td>
<td>St. Charles</td>
<td>Norco</td>
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<tr>
<td></td>
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<td>Pierre Part</td>
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<td></td>
<td>Terrebonne</td>
<td>Houma, Montegut</td>
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<td></td>
<td>St. Bernard</td>
<td>Chalmette</td>
</tr>
<tr>
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<td>Plaquemines</td>
<td>Belle Chase</td>
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<tr>
<td></td>
<td>Iberia</td>
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<tr>
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<tr>
<td></td>
<td>Sabine</td>
<td>Noble</td>
</tr>
<tr>
<td></td>
<td>Ouachita</td>
<td>Monroe, West Monroe</td>
</tr>
<tr>
<td>2002</td>
<td>Acadia</td>
<td>Rayne</td>
</tr>
<tr>
<td></td>
<td>St. Mary</td>
<td>Amelia</td>
</tr>
</tbody>
</table>

*La Fage 1987.*
In New Orleans, FST flight activity was monitored by the senior author using glue traps (TRAP-PEER® LTD, Bell Laboratories, Inc., Madison, WI) installed under lights near the French Quarter. Nightly observations and the number of FST alates recovered from glue traps reveal peak flight activity usually occurs from mid-May to early June, with some activity through mid-July (Table 4).

The majority of the I. minor and C. brevis samples were received from J. McPherson and local residents of New Orleans.

Location data from both surveys for the FST (Fig. 1), Reticulitermes species (Fig. 2), and kalotermitid species (Fig. 3) are presented on ArcView-generated maps.

**DISCUSSION**

The distribution of the FST in Louisiana has increased dramatically since the first confirmed reports in the mid-60s. However, many of these newer, confirmed infestations have remained relatively localized, and state officials have begun to target these areas for immediate treatment. Most of these localized introductions have occurred around structures, such as churches, or parks and campsites where FST-infested railroad ties were used as landscaping and/or building material. Future monitoring and confirmation of any new FST reports throughout the state is the first step to controlling human-aided spread.

**TABLE 4. COMBINED ALATE FLIGHT DATES FOR COPTOTERMES FORMOSANUS IN NEW ORLEANS, LOUISIANA, FROM 1998 TO 2001.**

<table>
<thead>
<tr>
<th>Date</th>
<th>Year</th>
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<th>Date</th>
<th>Year</th>
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<td>May 21</td>
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<td>2nd week of Feb.</td>
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<td>May 22</td>
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<td>June 27</td>
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<td>1st week of July</td>
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<td>July 7</td>
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<tr>
<td>July 13</td>
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<tr>
<td>3rd week of July</td>
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</tbody>
</table>

*Largest dispersal flight(s) each year.*
Outside the New Orleans and Lake Charles areas, *R. flavipes* and *R. virginicus* are the two most economically important subterranean termite species, with *R. flavipes* being the most common. The spatial distribution of all three *Reticulitermes* species is consistent statewide; however, *R. flavipes* seems to be more common in the extreme southern portions of the state. For example, samples of *R. flavipes* were collected from house pilings directly in the sand at Holly Beach on the Gulf of Mexico and from fishing camps around the Mississippi River delta basin.

During the PMP survey, *R. hageni* was rarely encountered in structures. In addition, *K. approximatus* was only collected from dead portions of trees and from alates flying into the vehicles of participants on two separate occasions. For both species, this confirms their general status as very limited structural pests (Weesner 1970, Schefrahn et al. 1988).

*Incisitermes snyderi* and *C. brevis* are the two most economically important kalotermitid species in Louisiana, with *I. snyderi* being the most common. *Cryptotermes brevis* is a non-endemic species and has only been recovered from structural lumber and furniture. *Incisitermes snyderi* is an endemic species commonly found in structural lumber and in dead portions of live trees throughout the southern half of the state.

The overall number of *I. minor* collections throughout the state was unexpected. Another interesting discovery was the number of public schools throughout the state with very active *I. minor* infestations, particularly in window framework. *Incisitermes minor* is endemic to CA, AZ, and Mexico, but has been introduced to many areas in the state, and in most cases, inside furniture. For example, a sample was taken from an infested pool table in Natchez, MS. In New Orleans, *I. minor* alates are usually collected from mid-April to mid-June during midday flights. However, alates were recovered after swarming from a window frame in an elementary school in Rayne, LA, during the second week of September 2001. In addition, *I. minor* alates were collected after swarming in a high school in Cameron, LA,
in late September 2001. Historical records reveal the flight season of *I. minor* usually occurs from July to December, and as early as May in the laboratory (Harvey 1934). In addition, *I. minor* flight records in California (Snyder 1954), Florida (Scheffrahn et al. 1988), and Georgia (Scheffrahn et al. 2001) revealed swarming usually occurs from September to November. An alarming discovery revealed *I. minor* alates swarming in a lumberyard near Le Moyeu, LA, in December 2001. This could lead to future introductions throughout the state.

In addition to the overall survey, a pictorial termite identification key was developed in 2001 to help PMPs, state officials, and termite researchers identify the FST and other economically important subterranean and drywood termite species currently present in Louisiana (Messenger 2002).

**ACKNOWLEDGMENTS**

We are grateful to E. S. Bordes, M. K. Carroll, and J. C. McAllister (NOMTCB) for reviewing the manuscript. Special thanks to John McPherson, Louisiana Department of Agriculture and Forestry, for providing samples and FST locations. The senior author would like to thank the following NOMTCB employees, Mike Schultz, Perry Ponseti, and Gus Ramirez, for their help in collecting termites. We would also like to thank the following individuals for advice, information, and collecting support: Dan Foster and Chris Castalano (Terminix—Houma), Eddie Martin and Vincent Palumbo (Terminix—Metairie), Zack Lemann (Audubon Institute), and Claudia Riegel (Dow AgroSciences LLC). We are very grateful to the following pest control companies for submitting termite samples: Al's Pest Control Service, Inc.; Louisiana Bug Doctors; Beasley Pest Control, Inc.; Pest Aid Co. of Alexandria, Inc.; Dial One Franklynn Pest Control; American Exterminating Co.; McKenzie Pest Control; Absolute Termite Control; Foti Exterminating Co.; Edgewood Pest Control, Inc.; Johnny Jones Pest Control Co.; Joyner's Pest Control; Fischer Environmental Services, Inc.; Terminix—Gretna; Al Latios Exterminating Co.; International Rivercenter; Responsible Pest Management LLC.; Sikes Pest Control, Inc.; J & R Pest Control, Inc.; Hubbards Pest Control; Tri-Parish Pest Control Co., Inc.; David Carter Exterminating Co., Inc.; Denney Exterminating Co.; Environmental Termite and Pest Control; Orkin Exterminating—Baton Rouge; E.A. Redd Pest Control, Inc.;

Fig. 2. Combined distribution data of *Reticulitermes* spp. in Louisiana from PMP and NOMTCB surveys.
Richard L. Robards Termite Services; Hookfin Pest Control Co., Inc.; Sugarland Exterminating Co., Inc.; Cohig Southern Environmental; Terminix—Slidell; Anti-Pest & Veitch, Inc.; Kevin's Pest Control, Inc.; Slug-A-Bug Exterminating Co.; E & G Pest Control, Inc.; Jerome Williams Pest Control Co.; Woods Pest Control; Sears Termite & Pest Control Inc.; Billiot Industries, Inc.; Vexcon Inc.; Stetler Pest Control; A Plus Exterminators, Inc.; Brent's Pest Control Services; Guardian Pest Control; Arceneaux Consulting; Calcasieu Parish Mosquito Control; East Baton Rouge Mosquito and Rodent Control; Mosquito Control, Inc.; St. Bernard Parish Mosquito Control; Louisiana Department of Agriculture and Forestry; and USDA-ARS SRRC. Partial funding for this project was provided by USDA-ARS under the grant agreement No. 58-6435-8-108. This article is Florida Agricultural Experiment Station Journal Series No. R-08828.

REFERENCES CITED


DISTRIBUTION AND NATURAL PARASITISM OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) EGGS AT DIFFERENT PHENOLOGICAL STAGES OF CORN

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3ESALQ-USP, Fitop e Zool. Agrícola, cx 09,13418-900, Piracicaba-SP, Brazil

**ABSTRACT**

The oviposition behavior of *Spodoptera frugiperda* (J. E. Smith) and natural parasitism of this pest by *Trichogramma* spp. at different phenological stages of corn were evaluated under field conditions. The distribution of *S. frugiperda* eggs varied according to the phenological stage of the corn. The preferred site for oviposition was the lower region of the plant and the abaxial leaf surface during the early development stages of the crop (4-6 leaves), changing to the middle and upper regions of the plant and the adaxial leaf surface at subsequent stages (8-10 and 12-14 leaves). A larger number of egg masses, and, therefore, of eggs was collected at the 4-6 and 8-10 leaf stages compared to plants in the 12-14 leaf stages. Natural parasitism was low, with a maximum of 2.21% eggs parasitized, especially on the lower and middle parts of the plant. The distribution and degree of parasitism by *Trichogramma* spp. on different regions of the plant were independent of the developmental stage of the crop. *Trichogramma pretiosum* Riley was the most frequent parasitoid, found in 93.79% of the parasitized eggs, followed by *Trichogramma atopovirilia* Oltman & Platner, with 2.07%.

Key Words: fall armyworm, *Trichogramma*, egg parasitoid, oviposition behavior, *Zea mays*

**RESUMEN**

Se evaluó el comportamiento de la oviposición de *Spodoptera frugiperda* (J. E. Smith) y en el parasitismo natural de esta plaga por *Trichogramma* spp. a diferentes etapas fenológicas de maíz bajo condiciones del campo. La distribución de los huevos de *S. frugiperda* varió según la etapa fenológica del maíz. El sitio preferida para la oviposición fue la región inferior de la planta y la superficie abaxial (dorsal o del envez) de la hoja durante las etapas tempranas de desarrollo del cultivo (4-6 hojas), cambiando a las regiones medianas y superiores de la planta y la superficie adaxial (ventral o del haz) de la hoja en las etapas sucesivas (8-10 y 12-24 hojas). Un mayor número de masas de huevos, y, por lo tanto, un mayor número de huevos fueron recolectados en las etapas de 4-6 y de 8-10 hojas en comparación con plantas en la etapa de 12-14 hojas. El parasitismo natural fue bajo, con un máximo de 2.21% de huevos parasitados, especialmente en las partes bajas y medias de la planta. La distribución y el grado de parasitismo por *Trichogramma* spp. en diferentes regiones de la planta fue independiente de la etapa de desarrollo del cultivo. *Trichogramma pretiosum* Riley fue el parasitoid más frecuentemente encontrado con el 93.79% de los huevos parasitados, seguido por *Trichogramma atopovirilia* Oltman & Platner, con el 2.07% de los huevos parasitizados.

In Brazil, *Spodoptera frugiperda* (J. E. Smith) is a common species in corn plantations. It is a pest of great economic importance, with production losses reaching 34% (Carnevalli & Florcovski 1995). It is found in corn from plant emergence up to the ear stage, within which it is often sheltered, and in many cases has become as important a pest as the corn earworm, *Helicoverpa zea* (Boddie) (Parra et al. 1995).

Morphological and physiological variations occur during the development and maturation of plants, often provoking changes in *S. frugiperda* egg distribution and egg mass characteristics, such as number of egg layers and scale density. These may be due to nutritional factors that vary as a consequence of physiological changes in the plants. *S. frugiperda* egg mass distribution on the host plant is influenced by the phenological stage of the crop, and can be concentrated within the lower, middle or upper plant regions and on different parts of the leaves and fruiting structures (Ali et al. 1989, Sifontes et al. 1988, Meneses et al. 1991). Sifontes et al. (1988) found that as the rice plant host grew older, *S. frugiperda* egg masses had a higher scale density, as well as a larger number of layers and eggs.
A possible control for *S. frugiperda* is the use of egg parasitoids like *Trichogramma*. These species, however, have difficulties in parasitizing egg masses of this pest because they are covered in scales and the eggs are deposited in layers (Toonders & Sánchez 1987, Cortez & Trujillo 1994). Thus, optimal use of *Trichogramma* spp. to control *S. frugiperda* requires information on egg placement by *S. frugiperda* during development of the corn crop, especially egg distribution in layers and the presence of scales, as well as the degree of natural parasitism by *Trichogramma* spp. The aim of this research was to evaluate the egg distribution and the natural parasitism of *S. frugiperda* eggs at different phenological stages of corn.

**Materials and Methods**

The trials were conducted at the Areão Farm, in Piracicaba, state of São Paulo, Brazil, at 22°42'00" South latitude, 47°38'00" West longitude and 546 meters altitude. Data were collected from March—July, 1998 and 1999, October—December, 1998, and from February—April, 2000. Each time a 2-hectare field, with approximately 50,000 corn plants per hectare was studied. The crop received conventional cultural practices recommended for corn, except that no insecticides were applied after the first leaves appeared.

Weekly, 250 plants were sampled, randomly distributed among 10 sampling spots, approximately 30 meters apart. In each spot, 25 plants in an “X” distribution were evaluated, with 5 plants in the center and 5 at each end of the “X”. Each group thus included 5 plants, approximately 5 meters from the next group. The plants were evaluated thoroughly and the plant height, the number of leaves per plant and the egg-laying sites were recorded. The eggs were grouped according to the plant region (lower, middle or upper) and leaf surface (abaxial or adaxial) and distribution and natural parasitism were studied at the corn plant phenological stages of 4-6, 8-10 and 12-14 leaves (Cruz & Turpin 1982). The eggs were collected and placed in labeled plastic tubes (3.5 × 1.0 cm), taken to the laboratory, and then kept in glass tubes (8.5 × 2.5 cm). Data were collected on the number of egg layers, the presence or absence of scales and the number of eggs, based on the number of larvae that hatched. Natural parasitism by *Trichogramma* spp. was determined by counting the number of parasitized eggs. The *S. frugiperda* eggs were placed in glass tubes (8.5 x 2.5 cm) in the laboratory for the observation of parasitoid emergence.

The results on the distribution and number of eggs found, the number of eggs and egg layers in each egg mass, and the number of parasitized eggs and their distribution at different phenological stages and on different parts of the corn plant were subjected to an analysis of variance with the means compared by the PLSD (Protected Least Significant Difference) test (alpha = 0.05). The treatments were arranged in a factorial scheme with two factors: plant age and plant region; the number of parasitized eggs and percentage *S. frugiperda* egg parasitism at different phenological stages were compared by the Tukey test (alpha = 0.05) with a completely randomized design, using SAS software for analysis.

**Results**

The *S. frugiperda* eggs were found at every phenological stage of the corn plant, and over 99% were found on leaves with only two egg masses found on the stem. A significant interaction was found between plant phenological stage and region (lower, middle and upper) (F = 2.79; P = 0.02) and corn leaf surface (abaxial and adaxial) (F = 7.79; P = 0.009). That is, the egg distribution among the different parts of the plant varied with the age and development of the corn plant.

At the early developmental stages of the crop (4-6 leaves), the majority of oviposition occurred on the 1st and 2nd leaves (60.4%) and on the abaxial surface of the leaves (83.4%). There were significant differences between the upper, medium and lower regions and the abaxial versus the adaxial leaf surface at the same developmental stage and among the various developmental stages (4-6, 8-10 and 12-14 leaves) (Table 1). As the plant developed to the 8-10 leaf stage, the preferred oviposition site changed and the eggs became more concentrated on the middle and upper plant regions (73.5%) and on the adaxial leaf surface (66.9% of the egg masses). At the 12-14-leaf stage, the greatest concentration of egg masses (61.4%) was observed on the middle region of the plant, significantly greater than on the lower and upper regions. However, there was no significant difference between the abaxial and adaxial leaf surfaces in terms of egg distribution. The middle part of the plant was, to a degree, constant as an oviposition site, with no significant difference between the phenological stages (Table 1).

The mean number of egg masses was significantly (F = 4.09; P = 0.01) different among the three developmental stages of the corn, with the highest concentration of egg masses at the early developmental stages (4-6 leaves) until approximately 60 days after planting, when the plants had 8-10 leaves, decreasing at the 12-14 leaf stage. However, the mean number of eggs did not differ among the three stages (F = 2.76; P = 0.01) (Table 2).

A significant interaction was found between plant-age and the number of egg layers in each *S. frugiperda* egg mass (F = 2.72; P = 0.01). At every phenological stage, most egg masses had more than one layer; the most frequent configuration was
### Table 1. Distribution of Spodoptera frugiperda Egg Masses on Corn Plants at Different Phenological Stages During the 1998-2000 Growing Seasons in Piracicaba, São Paulo State.

<table>
<thead>
<tr>
<th>Phenological stage</th>
<th>Plant age (days)</th>
<th>n</th>
<th>Plant region</th>
<th>Leaf surface</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Middle</td>
<td>Upper</td>
</tr>
<tr>
<td>4-6 leaves</td>
<td>14.0-38.0</td>
<td>8</td>
<td>9.7 ± 3.61aA</td>
<td>4.6 ± 0.94bA</td>
<td>2.0 ± 0.92cB</td>
</tr>
<tr>
<td>8-10 leaves</td>
<td>22.0-61.0</td>
<td>16</td>
<td>4.3 ± 1.06aB</td>
<td>6.1 ± 1.29aA</td>
<td>6.1 ± 1.31aA</td>
</tr>
<tr>
<td>12-14 leaves</td>
<td>36.0-90.0</td>
<td>16</td>
<td>2.0 ± 0.52bB</td>
<td>6.2 ± 1.67aA</td>
<td>1.9 ± 0.61bB</td>
</tr>
</tbody>
</table>

1. Data transformed into \((x + 1)^{0.1}\) and \(\log x + 1\), respectively.
2. Means followed by the same lower-case letter in rows and capital letters in columns do not differ by the PLSD (Protected Least Significant Difference) test (alpha = 0.05).

### Table 2. Number of Egg Masses, Eggs and Egg Masses with Varying Numbers of Layers (1, 2, 3, >4) of Spodoptera frugiperda at Different Phenological Stages of Corn, During the 1998-2000 Growing Season in Piracicaba, São Paulo State.

<table>
<thead>
<tr>
<th>Phenological stage</th>
<th>n</th>
<th>Average no. of egg masses</th>
<th>Average no. of eggs/collection</th>
<th>Layers/egg mass</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>4-6 leaves</td>
<td>8</td>
<td>16.2 ± 5.19a(^{1})</td>
<td>2,344.0 ± 882.22a(^{3})</td>
<td>0.8 ± 0.33bA(^{3})</td>
<td>6.4 ± 2.58aA</td>
</tr>
<tr>
<td>8-10 leaves</td>
<td>16</td>
<td>16.5 ± 2.89a(^{1})</td>
<td>3,647.7 ± 788.25a(^{3})</td>
<td>0.6 ± 0.15cA(^{3})</td>
<td>2.7 ± 0.15bB</td>
</tr>
<tr>
<td>12-14 leaves</td>
<td>15</td>
<td>10.1 ± 2.45b(^{1})</td>
<td>2,086.2 ± 471.13a(^{3})</td>
<td>0.6 ± 0.15bA(^{3})</td>
<td>1.5 ± 0.29abB</td>
</tr>
<tr>
<td>Overall average</td>
<td></td>
<td>10.7 ± 2.45b(^{1})</td>
<td>2,086.2 ± 471.13a(^{3})</td>
<td>0.6 ± 0.15bA(^{3})</td>
<td>1.5 ± 0.29abB</td>
</tr>
</tbody>
</table>

1. Data transformed into \((x + 1)^{0.1}\).
2. Means followed by the same lower-case letter in rows and capital letters in columns do not differ by the PLSD (Protected Least Significant Difference) test (alpha = 0.05).
3. Means followed by the same lower-case letter in rows and capital letters in columns do not differ by the PLSD test (alpha = 0.05).
three layers, with 91.56% covered by scales. At the 4-6-leaf stage the eggs predominantly had 2 or 3 layers and at the following stages - 8-10 and 12-14 leaves, those with 3 or 4 layers prevailed (Table 2).

With respect to parasitism, no significant interaction was observed between plant phenology and plant region (F = 1.49; P = 0.21), and no significant differences were found among the three developmental stages in the number (F = 0.3; P = 0.74) and percentage (F = 1.37; P = 0.25) of *S. frugiperda* egg masses parasitized by *Trichogramma* spp. (Table 3). However, significant differences in parasitism were detected among the three parts of the plant (F = 2.91; P = 0.05); the greatest number of parasitized eggs occurred on the lower and middle regions of the plant (Table 3). Also the number (F = 0.98; P = 0.38) and percentage (F = 1.84; P = 0.17) of parasitized *S. frugiperda* eggs did not differ among the three phenological stages of the corn (Table 4), showing that the distribution and degree of parasitism by *Trichogramma* spp. on different regions of the plant were independent of the developmental stage of the crop.

Overall natural parasitism was very low with a maximum of 2.21% of the eggs parasitized (Table 4), despite the fact that *S. frugiperda* eggs were available at every plant phenological stage. Parasitism remained constant over all densities of eggs observed at the 4-6 and 8-10 leaf stages (Table 2). Few *Trichogramma* species were found parasitizing the *S. frugiperda* eggs. *Trichogramma pretiosum* Riley was most abundant, comprising 93.79%. *Trichogramma atopovirilia* Oatman & Platner occurred in 2.07% of the samples. Multi-parasitism—a frequent phenomenon in *Trichogramma*—was observed, as *T. pretiosum* and *T. atopovirilia* appeared simultaneously in various egg masses. The remaining infested eggs (4.14%) were parasitized by other species of the genus *Trichogramma*. Only one individual of another genus of the family Trichogrammatidae was found. It was not identified because only one female emerged (the identification is based on males). In addition, this female failed to reproduce in eggs of the factitious host *Anagasta kuehniella* Zeller.

**DISCUSSION**

Changes in the oviposition behavior of *S. frugiperda* occurred as the host plant developed. Egg masses were most abundant on the lower region of the plant, and on the abaxial leaf surface, at the early 4-6-leaf stage; and on the middle and upper regions and the adaxial leaf surface at the subsequent stages (8-10 and 12-14 leaves). Pitre et al. (1983) showed that while in grasses such as sorghum and corn, *S. frugiperda* egg masses are more numerous and larger than in other hosts such as soybean and cotton. They also showed that the abaxial leaf surface, and the plant regions with greatest leaf mass, are preferred oviposition sites since they constitute a protected environment. The oviposition strategy of *S. frugiperda* on corn may focus on protection from natural enemies or suitable feeding sites for progeny, since females oviposit in areas near the feeding sites of the larvae. As the plant develops, egg laying shifts to the upper region of the plant, close to the spindle. When this site is not available after bolting, the middle region of the plant, close to the ear, becomes the main oviposition site. Labatte (1993) found that larvae migrate towards the upper region of the corn plant to feed at the 4-6 leaf stage. He indicated that up to the 10-leaf stage feeding occurs preferably among the whorl of young leaves, where the worms remain until the end of their development. After the 12-14-leaf stage, the worms migrate to the tassel and the middle region of the plant, during tasselling and ear formation, remaining in the leaves and ears. This egg laying and larva distribution pattern was also observed for *Ostrinia nubilalis* (Hub.) by Shelton et al. (1986) and Labatte (1991), who

**TABLE 3. MEAN NUMBER AND PERCENTAGE OF SPODOPTERA FRUGIPERDA EGG MASSES PARASITIZED BY TRICHOGRAMMA SPP. AT THREE DIFFERENT STAGES AND ON THREE DIFFERENT PARTS OF THE CORN PLANT, DURING THE 1998 - 2000 GROWING SEASONS IN PIRACICABA, SÃO PAULO STATE.**

<table>
<thead>
<tr>
<th>Phenological stage</th>
<th>Number of parasitized egg masses</th>
<th>Percentage parasitism</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-6 leaves</td>
<td>21</td>
<td>7.0 ± 3.01a</td>
</tr>
<tr>
<td>8-10 leaves</td>
<td>42</td>
<td>2.9 ± 1.14a</td>
</tr>
<tr>
<td>12-14 leaves</td>
<td>36</td>
<td>2.8 ± 1.15a</td>
</tr>
<tr>
<td>Plant region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower</td>
<td>33</td>
<td>2.0 ± 0.44a</td>
</tr>
<tr>
<td>Middle</td>
<td>38</td>
<td>1.5 ± 0.13a</td>
</tr>
<tr>
<td>Upper</td>
<td>29</td>
<td>0.6 ± 0.05b</td>
</tr>
</tbody>
</table>

*Means followed by the same letter do not differ by the F test among phenological stages or the PLSD (Protected Least Significant Difference) and F tests, respectively among plant regions (alpha = 0.05).*
stated that the phenological stage of the corn is the main factor affecting the pest’s behavior.

The decreased number of egg masses observed in our study as the plant grew older coincides with the population fluctuation of *S. frugiperda* adults and larvae observed by Mitchell et al. (1984) and Gutiérrez-Martínez et al. (1989). Surveys conducted by Gutiérrez-Martínez et al. (1989) showed that the first adults appear right after plant emergence, with the highest frequency from the 10th through the 41st day of emergence, when the plant is more susceptible, decreasing considerably up to the 72nd day, when fewer attacks on the corn crop occur. This phase also coincides with the period of greatest production losses, which may reach 19% when the plant achieves the 8-10 leaf stage (Cruz & Turpin 1982).

Although the number of egg masses decreased with plant age, the number of eggs found on the 8-10 and 12-14 leaf stages was higher than at the 4-6 leaf stage (Table 4). Meneses et al. (1991) also observed a high frequency of egg masses with three layers and an average of 385.6 eggs/egg mass in rice. Sifontes et al. (1988) found *S. frugiperda* with a higher number of layers and eggs in egg masses, and a higher scale density on older rice plants.

Studies by García & Sifontes (1987), Toonders & Sánchez (1987) and Hoffmann et al. (1995) revealed that *Trichogramma* spp. have difficulty in parasitizing *S. frugiperda* eggs, which is in accordance with our results. García & Sifontes (1987) reported that 46.9% of 47 egg masses parasitized by *Telenomus* and 4.3% parasitized by *Trichogramma* spp. together with *Telenomus*. Moreover, they considered only the number of egg masses parasitized and neglected the number of eggs parasitized, which obviously would give an even lower percentage of parasitism. Toonders & Sánchez (1987), on the other hand, counted the number of parasitized eggs, and observed that natural parasitism by *Trichogramma* spp. varied from 0 to 10% in samples examined in six different fields and when 30,000 parasitoids were released in 1.5 ha of corn the parasitism rate was only 4%.

The differences found in the degree of parasitism observed among the three parts of the plant (upper, middle and lower) do not indicate a preference of *Trichogramma* spp. for parasitizing the host eggs in the lower and middle portions, rather it may be associated with the oviposition behavior of *S. frugiperda* which overall oviposited more in these regions (average 5.37, 5.64 and 3.29 egg masses for the lower, middle and upper regions, respectively), allowing an increase in the number of parasitized eggs, although there were no differences in the percentage of parasitism (Table 3). These results are in accordance with those reported by Wang et al. (1997), who observed a greater preference of *T. ostriniae* for parasitizing *O. nubilalis* eggs on the lower and middle regions of the corn plant, as these are the sites with the greatest concentration of the host’s egg masses. However, a greater availability of eggs in a given region of the plant does not always result in increased parasitism by *Trichogramma*. For example, Romeis et al. (1998, 1999) observed that *Trichogramma chilonis* Ishii parasitized more *Helicoverpa armigera* eggs on pigeonpea leaves than on flowers and pods, even though more *H. armigera* eggs were available on the latter structures. These differences, in this case, can be explained by the presence of trichromes, which damage the parasite.

Records of parasitism by *Trichogramma* on *S. frugiperda* eggs are scarce and usually indicate low intensities, as found by García & Sifontes (1987) in the Sancti Spiritus region of Cuba, with 4.3% parasitized egg masses, similar to our data.

Knowledge about changes in the oviposition behavior of *S. frugiperda* at the various phenological stages could help in the planning of field-sampling methods, location of ovicide applications, and releases of *Trichogramma* spp. in augmentative biological control. However, doubts remain on the effectiveness of using *Trichogramma* to control this lepidopteran pest as natural infestation rates are quite low. This problem needs to be addressed before this parasitoid can be used as a viable alternative to chemical controls.

**ACKNOWLEDGMENTS**

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship grant which enabled this research, and Agricultural Engineer Raúl Barbosa for identifying the *Trichogramma* species.
REFERENCES CITED


QUALITY ASSESSMENT OF CHRYSOPERLA RUFILABRIS (NEUROPTERA: CHRYSOPIDAE) PRODUCERS IN CALIFORNIA

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ABSTRACT

Chrysoperla rufilabris (Burmeister) egg shipments from three commercial California insectaries were evaluated during a nine-month period. All three insectaries shipped similar numbers of eggs per unit weight (range 301.0 ± 10.3 to 315.4 ± 7.8 eggs/25 mg). Estimated total number of eggs per shipment for all three insectaries was between 1.80 and 3.30 times the number ordered (1,000). The estimated number of dead eggs per shipment ranged from 76.0 to 418.0 and the estimated number of larvae per shipment ranged from 0 to 9.9. Final hatch rates for all three insectaries were between 70.9% and 73.9%. Hatch began on the third day after shipment receipt and 70% of total hatch had occurred by the fourth day. Implications for timing of egg and larval releases are discussed.

Key Words: Chrysoperla rufilabris, lacewing, quality control, insectary rearing

RESUMEN

Los envíos de huevos de Chrysoperla rufilabris (Burmeister) de tres insectarios comerciales de California fueron evaluados durante un periodo de nueve meses. Todos los tres insectarios enviaron cantidades similares de huevos por unidad de peso (de 301.0 ± 10.3 hasta 315.4 ± 7.8 huevos/25 mg). El número total estimado de huevos por cada envío de los tres insectarios fue entre 1.83 y 3.31 veces mayor del número solicitado (1,000). El número estimado de huevos muertos por envío fue de 75.98 hasta 418 y el número de larvas estimadas por envío fue de 0 hasta 9.9. La taza de eclosión final para los tres insectarios fue entre 70.9% y 73.9%. La eclosión empezó en el tercer día después de recibir el envío y 70% de la eclosión total ocurrió en el cuarto día. Se discuten las implicaciones para el tiempo de liberar los huevos y las larvas.

Inundative and augmentative releases of natural enemies are widely used as non-disruptive alternatives to chemical control of arthropod pests. Chrysoperla (Neuroptera: Chrysopidae) species, commonly known as green lacewings, are among the most commonly marketed generalist insect predators (Tauber et al. 2000). Lacewings are applied in home gardens, row crops, orchards, and greenhouses on a variety of crops (Ridgway & Murphy 1984, Daane et al. 1998). Although Chrysoperla adults are not predaceous, all three larval stages are voracious eaters of soft-bodied arthropods and therefore are the desired stages for release (Tauber et al. 2000). High production costs, however, often make high volume larval purchases prohibitive. Alternatively, lacewing eggs can be as much as 17 times less expensive than larvae (Cranshaw et al. 1996). Eggs may be released upon receipt, or held until hatch and released as larvae. Substantial work has been done recently to develop, evaluate, and improve lacewing egg and larva field application methods (Morisawa & Giles 1995, Gardner & Giles 1996, Daane & Yokota 1997, Giles & Wunderlich 1998, Wunderlich & Giles 1999). Few studies, however, have evaluated the quality of commercial insectary egg shipments. The performance of natural enemy releases is only as high as the quality of organisms shipped by suppliers. In a self-regulated industry such as natural enemy mass-production, external product evaluations are important to maintain quality, which, in turn, positively promotes natural enemies as a potential tool for pest management.

O'Neil et al. (1998) evaluated post-shipment quality of four natural enemy species, including Chrysoperla carnea (Stephens). They found differences among C. carnea suppliers in the ratio of ordered eggs/received eggs, the number of larvae in egg shipments, survivorship of starved first instar larvae, and the sex ratio of reared adults. In addition, lacewing larvae reared to adulthood were identified as Chrysoperla rufilabris (Burmeister), not C. carnea. For the consumer planning lacewing releases, another important aspect of egg shipments, which O'Neil's group did not test, is...
when and how many eggs hatch into larvae. If consumers know what to expect in terms of shipment hatch, then they may be better able to coordinate release rates and timings to optimally target pest phenology and environmental conditions.

In this study, we evaluated *C. rufilabris* egg shipments from three California producers. Number of eggs per unit weight, estimated total number of eggs per shipment, and developmental stages of eggs upon receipt were measured and compared across the three insectaries. To compare egg quality among producers, timing and final percentage of eggs hatched were also determined. Among possible chrysopids, *C. rufilabris* was chosen for study because it was the only species produced by all three insectaries.

**MATERIALS AND METHODS**

Three insectaries in California were identified as producers of *C. rufilabris*: Beneficial Insectary (Oak Glen), Buena Biosystems (Ventura), and Rincon Vitova (Ventura). Throughout the experiment, larvae reared from egg shipments were identified as *C. rufilabris* by markings on head capsules and by setal patterns as described in Tauber (1974). For the sake of anonymity, each insectary was randomly assigned a unique number between one and three. Between March and November, 1999, ten shipments each of 1,000 eggs each were ordered and shipped overnight to our laboratory at the University of California, Riverside from Insectary 1 and Insectary 2. Four overnight shipments of 1,000 eggs each were received from Insectary 3 between July and November, 1999. Insectaries were aware that shipments were to be used in experimental evaluations of *C. rufilabris*, but were not told specifically that they were being evaluated on shipment quality.

Upon arrival, egg shipments were opened and the method of packaging noted. Total weight of each 1,000-egg shipment was recorded (Sartorius 1212 MP digital scale, Brinkman Instrument, Inc., Westbury, NY). A 25 mg sample was taken from each shipment, with the following exceptions: two samples were taken from the second shipment from Insectary 1, four from the ninth shipment from Insectary 2, and six and two from the third and fourth shipments, respectively, from Insectary 3. Using a dissecting microscope, the number of eggs per 25 mg sample was counted and eggs were categorized as either dead (ruptured or desiccated), green (indicating recent oviposition), or partially to completely brown with abdominal striping of the developing embryo visible (Gepp 1984). The number of hatched larvae, if any, was also recorded. Data for each category were analyzed for differences among insectaries using an unbalanced, nested ANOVA with unequal numbers of shipments per source and unequal numbers of samples per shipment. The specified model treated source as a fixed effect and shipments within source as a random effect (Sokal & Rohlff 1995, SAS Institute 1999). Tukey-Kramer’s test was used for comparison of least squares means. The level of significance for all tests was p = 0.05.

From each of the 25 mg samples taken from each egg shipment upon arrival, between one and four subsamples of 40 randomly selected eggs each were used in hatch rate determinations. Eggs were placed, one egg per well, in uncoated, plastic assay plates with rounded bottoms (96-well Assay Plates, Corning, Inc., Science Products Division, Acton, MA). Strips of clear adhesive tape were placed over the wells containing a single egg. To maintain relative humidity at a level (≥70%) conducive to *C. rufilabris* development (Tauber 1974), plates were placed on wet sponges and loosely enclosed in plastic boxes. Plastic boxes were kept under ambient laboratory temperature and light conditions, resulting in a temperature of 24 ± 1.5°C inside the boxes. Temperature and humidity were measured by HOBO Temp and HOBO RH, respectively (Onset Computer Corporation, Pocasset, MA). The number of emerged larvae, i.e. those completely separated from the chorion, were counted daily until the number of hatched larvae remained unchanged for two consecutive days. Cumulative percentage of hatched larvae was calculated daily. Thirty-six 40-egg subsample replicates were hatched out for Insectary 1, 33 replicates for Insectary 2, and eight replicates for Insectary 3. Arcsine (square-root) transformation was applied to daily cumulative hatch rates. Transformed data were analyzed for differences in daily cumulative percentage hatch among insectaries using a one-way ANOVA. Significant differences were further separated with Tukey’s test for comparison of means. The level of significance for all tests was p = 0.05.

**RESULTS**

All egg shipments arrived on time and were packaged in small plastic cups with tight-fitting lids, wrapped in paper. Both Insectaries 1 and 2 placed each paper-wrapped plastic cup into a styrofoam cooler with artificial ice packs, and the cooler was in turn packaged in a cardboard box for shipping. Insectary 3 shipped each paper-wrapped plastic cup in a cardboard box without styrofoam insulation or ice packs.

The mean weight of shipments from Insectary 2 (145.4 ± 9.2 mg) was significantly (F = 5.20; df = 2, 21; p = 0.015) less than the mean shipment weights from Insectaries 1 (247.5 ± 37.7 mg) and 3 (274.8 ± 47.6 mg). Variation in shipment weight for Insectary 2 (range 100 mg to 203 mg) was also less than that for either Insectary 1 (range 130 mg to 451 mg) or Insectary 3 (range 155 mg to 369 mg).
The mean number of eggs within a 25 mg sample was similar for all three insectaries, at just over 300 eggs (Table 1). Based on these counts, the estimated mean number of eggs per shipment was 3,046 for Insectary 1, 1,834 for Insectary 2, and 3,309 for Insectary 3.

Table 1 shows the composition of the 25 mg samples taken from each insectary’s egg shipments. For all insectaries, the majority of eggs (65%) in each sample had reached the striped stage, indicating imminent hatching. The estimated number of larvae per shipment ranged from 0 to 9.9 and the estimated number of dead eggs per shipment ranged from 76.0 to 418.0.

Timing of egg hatch was similar for all three insectaries (Fig. 1). There was little to no hatch on the first and second days. On day three, however, hatch for eggs from Insectary 1 and 3 were 13.2% and 21.6%, respectively, whereas a significantly lower percentage of eggs from Insectary 2 had hatched (3.7%). On days four through seven, percentage hatch for all three insectaries did not differ statistically. Final mean percentages of eggs hatched ranged from 70.9 to 73.9.

**DISCUSSION**

The similarity in hatch rates observed for all three insectaries suggests similar quality of eggs received from each. The approximately 30% of eggs from which larvae did not emerge was compensated for by the fact that shipments, on average, exceeded the ordered amount by between 83% and 231%. However, implications for shipments to the average consumer are not clear because the university shipping address may have biased insectaries to include extra eggs as a courtesy. This is underscored by comparing our results to those of O’Neil et al. (1998), who used a “blind ordering” system and found fewer lacewings than ordered in a majority of shipments received.

In addition to some level of egg mortality expected during the shipping and handling processes, the risk of egg mortality in lacewing shipments is higher due to the cannibalistic nature of their larvae (New 1975). Eggs held until release tend to be confined at high densities, increasing exposure to predation by newly emerging larvae as holding time increases (Daane & Yokota 1997, O’Neil et al. 1998). Without alternative prey provided, O’Neil et al. (1998) suggested immediate release of lacewing eggs to prevent cannibalism. Our findings suggest that losses to cannibalism may be minimized by releasing lacewing eggs on or before the third day after receipt.

Even with the development of efficient egg release technologies (Gardner & Giles 1996, Wunderlich & Giles 1999), high post-release egg mortality due to environmental conditions (Daane & Yokota 1997) and intraguild predation (Tauber et al. 2000) reduce efficacy of egg releases as compared with larval releases. Our results indicate that for larval releases, holding eggs for four days allows a majority (approximately 70%) of total hatch to occur while limiting larval holding time to 24 hours. Waiting an additional day

**Table 1. Composition of 25 mg Samples taken from Chrysoperla rufilabris Egg Shipments.**

<table>
<thead>
<tr>
<th>Insectary 1: 10 shipments, total of 11 samples</th>
<th>Insectary 2: 10 shipments, total of 13 samples</th>
<th>Insectary 3: 4 shipments, total of 10 samples</th>
<th>ANOVA (num. df = 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Green Egg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (±SEM)</td>
<td>Mean (±SEM)</td>
<td>Mean (±SEM)</td>
<td></td>
</tr>
<tr>
<td>63.5 a (±15.4)</td>
<td>15.8 a (±15.4)</td>
<td>52.3 a (±24.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Striped Egg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>204.7 a (±20.1)</td>
<td>288.3 b (±20.1)</td>
<td>209.9 ab (±31.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Emerged Larvae</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 b (±0.2)</td>
<td>0.0 a (±0.2)</td>
<td>0.7 ab (±0.3)</td>
<td></td>
</tr>
<tr>
<td><strong>Dead Egg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>36.6 b (±5.3)</td>
<td>13.1 a (±4.9)</td>
<td>38.0 b (±5.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>307.7 a (±8.0)</td>
<td>315.4 a (±7.8)</td>
<td>301.0 a (±10.3)</td>
<td></td>
</tr>
</tbody>
</table>

Means (±SEM) listed are estimated least square means based on a nested ANOVA model with unequal sample size. Means within a row followed by the same letter did not differ statistically (Tukey-Kramer’s test, p < 0.05).
may increase hatch, but that may be countered by an accompanying increase in cannibalism (O’Neil et al. 1998).

The few reports of *C. rufilabris* egg hatch rates found in the literature are for untreated controls within studies of egg release methodologies. Our observed hatch rates of 70.9 to 73.9% after seven days fall within the 64.1% after five days reported by Gardner & Giles (1996) and the 91.2% after seven days reported by Daane & Yokota (1997), but direct comparison of these rates is difficult because specific holding conditions were not reported for each. The question should be addressed in future egg hatch studies in which effects of temperature, relative humidity and light regime are compared and related to conditions an average consumer may be able to replicate.

Whereas the development stages present in egg shipments differed slightly among the three insectaries, all contained mostly eggs that were close to emergence and few contained larvae. This is an improvement on the average percentage larvae in shipments ranging up to 52.6% reported by O’Neil et al. (1998). Although sources were not specified in that study, perhaps the wider range of larval emergence they observed was a result of including both producers and distributors. Eggs shipped through distributors may be older and therefore more likely to hatch before arrival than eggs shipped directly from the producer.

Shipments from all three insectaries contained a similar number of eggs per unit weight, indicating little difference in contamination levels. One of the insectaries, however, had less variation in shipment weight, significantly fewer damaged and desiccated eggs, and slightly more uniformity in egg developmental stage as indicated by shipment composition and hatch data. Uniform age structure could contribute to a more synchronous and predictable larval hatch, equally useful for timing of egg releases as for larval releases. These observations suggest slightly better handling techniques and precision in egg collection that perhaps could be employed in the other two insectaries.

**ACKNOWLEDGMENTS**

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EVALUATION OF EXOTIC SOLANUM SPP. (SOLANALES: SOLANACEAE)
IN FLORIDA AS HOST PLANTS FOR THE LEAF BEETLES LEPTINOTARSA DEFECTA AND L. TEXANA (COLEOPTERA: CHRYSOMELIDAE)

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ABSTRACT

Tropical soda apple, Solanum viarum Dunal, wetland nightshade, S. tampicense Dunal, and turkey berry, S. torvum Swartz, are considered three of Florida's most invasive plant species. These nonnative perennial broadleaf weeds are disrupting native plant communities in agricultural areas and natural ecosystems. The lack of natural enemies in Florida is thought to be an important factor contributing to their invasiveness. The North American leaf beetles Leptinotarsa defecta (Stål) and L. texana (Schaeffer) that attack silverleaf nightshade, Solanum elaeagnifolium Cav., a native congener of the three nonnative solanums, were evaluated for their potential as biological control agents. The suitability of tropical soda apple, wetland nightshade and turkey berry as host plants for the native Leptinotarsa beetles was studied in a quarantine laboratory using single plant and paired plant tests. Neonate larvae of L. defecta developed to the pupal stage only on their natural host plant silverleaf nightshade. Feeding damage on turkey berry and wetland nightshade was negligible and no feeding occurred on tropical soda apple. In contrast, development and reproduction of L. texana on the nonnative turkey berry were comparable with silverleaf nightshade. These results suggest the nonnative turkey berry may be included in the potential host range of the native silverleaf nightshade beetle L. texana.

Key Words: Biological control, weeds, Solanum viarum, S. tampicense, S. torvum, S. elaeagnifolium, risk assessment

RESUMEN

Solanum viarum Dunal, S. tampicense Dunal, y S. torvum Swartz se consideran como tres de las especies de plantas más invasoras en Florida. Estas maizales perennes no nativas de hoja ancha están perturbando las comunidades de plantas en áreas agrícolas y ecosistemas naturales. Se piensa que la falta de enemigos naturales en Florida es un factor importante que contribuye a su habilidad para ser invasoras. Se evaluaron los escarabajos norteamericanos, Leptinotarsa defecta (Stål) y L. texana (Schaeffer) que atacan las hojas de Solanum elaeagnifolium Cav., una planta nativa en el mismo género de los tres solanums no nativos, para determinar su potencial como agentes de control biológico. Se estudió si las plantas de Solanum viarum, S. tampicense y S. torvum podrían ser hospederos adecuados de los escarabajos nativos Leptinotarsus en el laboratorio de la cuarentena usando pruebas de plantas individuales y en pares. Se desarrollaron las larvas recién nacidas de L. defecta hasta la etapa de pupa solamente en su planta hospedera natural Solanum elaeagnifolium. El daño de alimentación en el S. torvum y S. tampicense fue insignificante y no se alimentó de Solanum viarum. Al contrario, el desarrollo y la reproducción de L. texana sobre S. torvum no nativo, fue similar con los de S. elaeagnifolium. Estos resultados sugirieron que se puede incluir S. torvum no nativo entre los hospederos potenciales del escarabajo de Solanum elaeagnifolium no nativo, L. texana.

Tropical soda apple, Solanum viarum Dunal, wetland nightshade, S. tampicense Dunal, and turkey berry, S. torvum Swartz, are perennial nonnative invasive weeds that have been identified as candidates for biological control (Cuda et al. 2002). Tropical soda apple was first discovered in Florida in 1988 (Mullahey et al. 1993, Mullahey et al. 1998), and by 1995 infested between 0.25 and 0.5 million ha of prime agricultural and nonagricultural lands (Mullahey 1996a, Mullahey et al. 1998). This invasive weed infests a variety of habitats including improved pastures,
natural areas, citrus (Citrus spp.), sugar cane (Saccharum officinarum L.), sod fields, ditch banks, and roadsides. After establishing in Florida, tropical soda apple continued to expand its range into Alabama, Georgia, Louisiana, Mississippi, North Carolina, South Carolina, Pennsylvania, Puerto Rico and Tennessee (Westbrooks & Eplee 1996, Mullahey et al. 1998). The Pennsylvania infestation has since been eradicated (Westbrooks 1998).

The foliage and stems of tropical soda apple are prickly and unpalatable to livestock. However, cattle and wildlife readily ingest the fruits and spread the seeds in their droppings. If left uncontrolled, pasture production declines and stocking rates are drastically reduced (Mullahey et al. 1993). In 1994, production losses to Florida cattle ranchers attributed to tropical soda apple infestations were estimated at US $11 million annually (Cooke 1997). Tropical soda apple also serves as a reservoir for various diseases and insect pests of solanaceous crop plants (McGovern et al. 1994a, 1994b, Medal et al. 1999). A special symposium devoted entirely to various aspects of tropical soda apple and to a lesser extent wetland nightshade’s biology, ecology, environmental effects and control strategies was held in Florida in 1996 to address these emerging weed problems (Mullahey 1996b).

Wetland nightshade is a bramble-like plant with spiny tangled stems and leaves that was first reported in Florida in 1983 (Wunderlin et al. 1993, Fox & Bryson 1998). In contrast to tropical soda apple, which dominates upland sites, regularly flooded wetlands are particularly vulnerable to invasion by wetland nightshade (Wunderlin et al. 1993, Fox & Bryson 1998). The largest infestation, approximately 60 ha, occurs in southwest Florida (Fox & Wigginton 1996, Wunderlin & Hansen 2000). The ability of wetland nightshade to form dense thickets that are difficult for other species to penetrate suggests this noxious weed has the potential to invade and alter many of the state’s wetland habitats thus impeding access to and use of water resources (Fox & Wigginton 1996, Fox & Bryson 1998).

Turkey berry is a large, prickly shrub that can attain heights of up to 3 m (Ivens et al. 1978). Turkey berry was first collected in Columbia Co., Florida, in 1899, and has been reported in at least nine counties throughout the state (Wunderlin & Hansen 2000, Cuda et al. 2002). This noxious solanum invades disturbed sites such as pastures, crop fields, roadsides, damp waste areas and forest clearings where it competes with desirable plants for moisture, light and nutrients. Although it is frequently cultivated as a yard plant in south Florida (Westbrooks & Eplee 1989), turkey berry is potentially poisonous to animals (Chadhokar 1976, Abatan et al. 1997), and possibly carcinogenic to humans (Balachandran & Si-ramarkrishnan 1995). Turkey berry has been reported as a reservoir for Alternaria solani Sorauer (Deuteromycetes: Dematiaceae), the causative agent of wilt disease in potatoes and tomatoes (Mune & Parham 1967), and is considered one of the most invasive weeds on other continents, particularly in parts of Australia and South Africa that are climatically similar to Florida (Holm et al. 1979). In the Pacific region, turkey berry was identified as a possible target for classical biological control (Waterhouse & Norris 1987). The occurrence of this plant as an invasive weed in other countries is perhaps the most compelling evidence for predicting its eventual effect on Florida’s native plant communities.

Tropical soda apple, wetland nightshade and turkey berry are currently recognized as three of Florida’s most invasive nonnative plant species (FLDACS 1999, FLEPPC 1999, Langeland 2001). Although it is unclear why these exotic solanaceous plants have become weeds, the lack of host-specific natural enemies in Florida (the introduced range) may have afforded these plants a competitive advantage over native species (Cuda et al. 2002). Tropical soda apple and wetland nightshade are native to South America (and possibly the West Indies), and Mexico, respectively (Wunderlin et al. 1993), whereas turkey berry is thought to have originated in West Africa (Ivens et al. 1978), Central or South America and the Caribbean region (Morton 1981, Waterhouse & Norris 1987), or Asia (Medal et al. 1999).

Silverleaf nightshade, Solanum elaeagnifolium Cav., is a close relative of tropical soda apple, wetland nightshade, and turkey berry that is native to the southern United States, Mexico and possibly Argentina (Goeden 1971, Boyd et al. 1983), and belongs to the same subgenus Leptospermum as the three nonnative Solanum spp. (D’Arcy 1972, Nee 1991). Silverleaf nightshade is attacked by many insect herbivores in the southwestern United States and Mexico (Goeden 1971). Two of the most damaging insects attacking silverleaf nightshade in its native range are the defoliating beetles Leptinotarsa defulta (Stål) and L. texana (Schaeffer) (Jacques 1988). Both L. defulta and L. texana were released recently in South Africa for biological control of silverleaf nightshade (Olckers et al. 1999), and their biology were summarized by Olckers et al. (1995).

Silverleaf nightshade is considered the natural host plant of L. defulta and L. texana (Goeden 1971, Neck 1983, Jacques 1988). This solanum defines the actual, realized or field host range of the beetles (Kogan & Goeden 1970, Cullen 1990, van Klinken 2000). Host range encompasses those plants on which an insect completes normal development in nature (Hanson 1983). However, the study by Olckers et al. (1995) demonstrated that under laboratory conditions these two beetles also developed and reproduced on other solanums that
do not occur in the insects’ native ranges. Similarly, Hsiao (1981) observed *L. texana* developed and reproduced to some extent on eggplant as well as three native plant species—*S. dulcamara* L., *S. carolinense* L. and *S. rostratum* Dunal. These solanaceous plants are not typically exploited by the beetles in nature but are capable of supporting some development and reproduction, and comprise what is considered the insects’ potential, physiological or fundamental host range (Kogan & Goeden 1970, Cullen 1990, van Klinken 2000). Horsenettle (*S. carolinense*) and presumably Florida horsenettle (*S. carolinense* var. *floridanum* Chapm.) are the only potential host plants of *L. texana* that are native to Florida (Wunderlin & Hansen 2000). In spite of its native status in Florida, horsenettle is listed as a troublesome weed by Hall & Vandiver (1991).

Silverleaf nightshade is adventive in Florida, occurring sporadically from the Panhandle to the Keys (Wunderlin 1982, Wunderlin & Hansen 2000). Its natural enemies *L. defecta* and *L. texana* have not spread to Florida (Jacques 1985,1988), presumably because the Gulf of Mexico is an effective barrier to insects like *L. texana* that are incapable of long range aerial dispersal (see Hoffmann et al. 1998). However, a computer model (CLIMEX) that uses various climatic factors to determine whether insects can colonize and persist in new geographic areas (Sutherst & Maywald 1985) predicted that *Leptinotarsa* beetles collected from silverleaf nightshade in the Brownsville area of south Texas could establish and persist in peninsular Florida if tropical soda apple, wetland nightshade or turkey berry were suitable host plants.

The purpose of this research was to determine whether the nonnative and invasive tropical soda apple, wetland nightshade or turkey berry are capable of supporting normal development and continuous reproduction of the North American silverleaf nightshade leaf beetles *L. defecta* and *L. texana*. If these native insects are capable of establishing ‘new associations’ with the exotic solanums (Hokkanen & Pimentel 1984), they could be introduced into Florida for biological control of these weeds after preintroduction host specificity tests demonstrated they were safe to release.

**Materials and Methods**

Collections of the silverleaf nightshade leaf beetles *L. defecta* and *L. texana* were made during the months of June-October 1997 and May 2001 in Starr County, TX, USA, by personnel affiliated with the USDA-Animal and Plant Health Inspection Service, Mission Plant Protection Center, Mission, TX. Parasitoid-free colonies of *L. defecta* and *L. texana* were maintained on potted silverleaf nightshade plants held in screen cages at the laboratory in Mission, TX. Egg masses of *L. defecta* and *L. texana* deposited on silverleaf nightshade were shipped via overnight mail to the Quarantine Laboratory, Entomology & Nematology Department, University of Florida after USDA, APHIS, PPQ issued an importation permit. A shipment of 138 eggs of *L. defecta* and 310 eggs of *L. texana* was received on 8 September 1997. The eggs were deposited in small masses on individual silverleaf nightshade leaves separated by species in petri dishes sealed with Parafilm® to prevent desiccation. The eggs were removed from the silverleaf nightshade leaves with a camel hair brush and transferred to moistened filter paper placed inside another petri dish. This procedure ensured that neonate larvae were not preconditioned by feeding on silverleaf nightshade prior to the host acceptability tests, which would bias the results of the feeding trials.

Percent survival, development time, and amount of feeding for the larval stages of both leaf beetles were measured on each test plant species. Single plant (no-choice) and paired plant (choice) host suitability tests with three replications were conducted with neonate larvae in a quarantine room maintained at a temperature of 24.0 ± 3.1°C, relative humidity of 66.8 ± 6.8% and a 16-h photophase. Leaves used in the experiments were obtained from potted plants fertilized with Peters® 20-20-20 (N: P: K) solution and maintained in a glasshouse or an outdoor shade house. In the single plant tests, five neonate larvae were transferred directly to a freshly excised leaf of each test plant. The leaf was placed inside a large covered petri dish (25.0 cm diam. by 9.0 cm depth) lined with a Seitz® filter disk (25 cm diam.). The filter disk was routinely moistened with deionized water to prevent the leaf from desiccating, and the leaf was replaced each day or every other day until the larvae pupated or died. Leaf consumption was measured by scanning the leaves photometrically before and after exposure to the larvae. The difference in leaf areas was assumed to be the amount eaten by the developing larvae. The single plant larval feeding and development tests were initiated in early September and completed in late November 1997.

Paired plant (choice) tests of the feeding preferences of *L. texana* larvae were conducted with silverleaf nightshade as the control. Four leaf disks (30 mm diam.) were punched from the base of freshly detached leaves of silverleaf nightshade and turkey berry, the test plant species that supported larval development of *L. texana* in the single plant trials (See Results). The leaf disks were positioned alternately by species and equidistantly around the perimeter of the same container used in the single plant trials. Ten neonate larvae were placed in the center of the container and allowed to select their food source when presented with a choice of silverleaf nightshade or turkey
berry leaf disks. The amount of feeding on each test plant species in the paired comparison tests was measured by the same procedure used in the single plant trials. The paired plant (choice) larval feeding trials with three replications were initiated in mid-September and were completed by the end of December 1997 when the last larva pupated or died.

On 9 May 2001, a final shipment of 72 adults of L. texana (48 males, 24 females) was received from Texas to compare the beetle’s reproductive performance on turkey berry with silverleaf nightshade, and larval feeding and development on potato tree, Solanum donianum Walpers. Potato tree is a state listed threatened species (Coile 1998), and a critical non-target plant that would be vulnerable to attack by L. texana if this insect were approved for release in Florida for biological control of turkey berry.

The beetles were equally divided among whole plants of either silverleaf nightshade or turkey berry in 3.8 liter (1 gal.) pots covered with acrylic cylinders (41 cm height × 14 cm diam.). The tops of the cylinder cages were covered with Nitex® (41 × 42 in. mesh) to prevent the beetles from escaping. Individual leaves with the egg masses intact were removed from the plants daily, and placed in standard petri dishes with moistened filter paper to incubate. When the larvae hatched, a maximum of 10 larvae was transferred to a plastic rectangular container (20 cm × 14 cm × 10 cm) provisioned with leaves of the same host plant from which they originated, and a piece of paper toweling to collect the frass produced by the developing larvae. Each plastic container also had a hardware cloth insert (16 cm × 14 cm × 5 cm) that served as a platform to keep the leaves from coming in contact with the frass at the bottom of the container. By elevating the leaves in this manner, disease problems were avoided. When the larvae stopped feeding, they were allowed to pupate in the same plastic containers filled to a depth of 5 cm with vermiculite.

New adults (F₁ generation) that emerged in the containers were sexed, and exposed to the same species of potted plant (silverleaf nightshade or turkey berry) on which they completed their development. In total, 12 cages of silverleaf nightshade and 12 of turkey berry, each containing 2 males and 1 female of L. texana, were maintained inside the quarantine room under the same environmental conditions. Survival of the F₁ females as well as the number of egg masses produced, eggs per mass, and percent larval eclosion on each test plant species were recorded.

A final single plant (no-choice) feeding and development test was conducted to determine the acceptability of potato tree as a host plant for L. texana. The experimental procedures and conditions were the same as those described above for the other single plant tests except the neonates used in this test were F₁ generation larvae of L. texana obtained from F₁ adults reared on turkey berry, the control plant in this experiment. The adult reproduction and potato tree risk assessment experiments were completed in late December 2001.

Data Analysis

The data on larval development time and leaf consumption were analyzed by ANOVA (SAS 1990). Leaf consumption means were compared with Tukey’s Studentized Range (HSD) test. Non-parametric estimates of larval survival data were analyzed using the LIFETEST procedure (SAS 1990), and were compared with chi-square. The TTEST procedure (SAS 1990) was used to compare the effect of plant species (silverleaf nightshade or turkey berry) on adult female reproductive performance, and plant species (turkey berry or potato tree) on larval feeding and development of L. texana. Data obtained on larval eclosion (%) were arcsine transformed prior to analysis.

RESULTS

Larval Feeding and Development

Single plant tests. As expected, larvae of both Leptinotarsa beetles completed development on their natural host plant silverleaf nightshade (Figs. 1 and 2). The durations of the first, second, third and fourth stadia for L. defecta on silverleaf nightshade were 3.7 ± 0.3, 3.7 ± 0.3, 3.7 ± 0.3, and 9.0 ± 1.5 days, respectively (Fig. 3). However, L. defecta was unable to develop on any of the non-native solanum species tested (Fig. 1). All larvae on turkey berry, tropical soda apple, and wetland nightshade died by day 7 and none developed to the second instar. The likelihood ratio test for homogeneity of the survival curves was significant (Chi square = 7.9413, df = 3, p < 0.05), indicating that differences in survival occurred among larvae fed the different host plant leaves.

In contrast, development of L. texana larvae on turkey berry was comparable to that on silverleaf nightshade (Figs. 2 and 4). Durations of the first, second, third and fourth stadia for L. texana reared on silverleaf nightshade were 3.0 ± 0.0, 2.0 ± 0.0, 3.0 ± 0.0, and 8.7 ± 1.9 days compared to 2.7 ± 0.3, 3.0 ± 0.0, 3.0 ± 1.0, and 9.5 ± 0.5 days for turkey berry, respectively. Host plant diets of either silverleaf nightshade or turkey berry in the single plant trials did not affect total larval development time. Likewise, the test for equality of the survival curves for L. texana reared on silverleaf nightshade or turkey berry was not significant (Chi square = 5.942, df = 4, p > 0.05), suggesting that no differences in survival could be detected on these two solanum species.
The amount of feeding observed on the four solanums by larvae of *L. defecta* and *L. texana* in the single plant feeding trials is presented in Table 1. Larvae of *L. defecta* consumed on average $64.0 \pm 9.2$ cm$^2$ of silverleaf nightshade leaf tissue, and mean survival to the pupal stage (≈ day 18) on its natural host plant was $46.7 \pm 24.0\%$ (Fig. 1). Although a small amount of feeding occurred on turkey berry and wetland nightshade, all larvae died as first instars. Furthermore, newly hatched larvae confined on tropical soda apple leaves did not feed at all and died within a few days. In contrast, larvae of *L. texana* readily accepted turkey berry leaves as a food source. Larvae ingested $104.5 \pm 26.4$ cm$^2$ of turkey berry leaf tissue compared to only $52.3 \pm 7.7$ cm$^2$ for silverleaf nightshade (Table 1). Also, larval survival on both plant species was the same for *L. texana*. Survival to the pupal stage (≈ day 18) was $40.0 \pm 23.1\%$ and $40.0 \pm 11.5\%$ for turkey berry and silverleaf nightshade, respectively (Fig. 2).

Potato tree, which is considered a threatened species in Florida, was not an acceptable host plant for *L. texana*. Although the leaves sustained some feeding damage, average leaf consumption by the larvae was significantly lower on potato tree ($17.8 \pm 17.8$ cm$^2$) compared to turkey berry ($98.06 \pm 22.33$ cm$^2$) ($t = 2.81$, df = 4, $p < 0.05$). More importantly, no larvae of *L. texana* restricted to a diet of potato tree leaves survived beyond the second instar on this high risk species whereas seven out of 15 larvae, or 47%, experienced normal development and pupation exclusively on a diet of turkey berry leaves. The amount of turkey berry leaf tissue consumed by larvae in this test was not statistically different ($t = 0.239$, df = 4, $p > 0.05$) from that observed for turkey berry in the earlier single plant test shown in Table 1.

**Paired plant tests:** Paired comparison tests were conducted only with *L. texana* because the single plant trials demonstrated this insect was capable of completing its development to the pupal stage on turkey berry in the absence of its natural host plant silverleaf nightshade. When offered a choice between leaf disks of silverleaf nightshade and turkey berry as a food source, the larvae did not exhibit a clear preference for silverleaf nightshade over turkey berry (Table 1). Although average leaf consumption on silverleaf nightshade was $76.2 \pm 6.69$ cm$^2$ compared to $40.0 \pm 12.9$ cm$^2$ for turkey berry, the observed differences were not significant ($t = 2.49$, df = 4, $p >
Survival and development of *L. texana* to the pupal stage (≈ day 18) in the choice tests were virtually identical (40.0 ± 5.8%) to that observed in the single plant trials (Fig. 2).

### Adult Female Survival and Reproduction

In total, eight out of 12 females (67%) of the F1 generation survived and reproduced on silverleaf nightshade compared to only three F1 females (25%) on turkey berry. However, the surviving females on average lived as long on turkey berry (58.0 ± 18.3 days) as they did on their natural host plant silverleaf nightshade (58.1 ± 22.4 days) (*t* = 0.00, df = 9, *p* > 0.05) (Table 2).

Adults of *L. texana* caged on potted turkey berry plants exhibited an unusual feeding behavior not observed on the silverleaf nightshade plants in this study. Beetles often completely stripped the turkey berry plants of their leaves by feeding on the petioles where they were attached to the stem. This feeding behavior resulted in complete defoliation of the turkey berry plants even at the low adult densities (1 to 3 beetles per plant) maintained in this study. Hoffmann et al. (1998) observed a similar phenomenon on silverleaf nightshade but only when *L. texana* reached high densities following its release and establishment in South Africa for biological control of this weed.

The reproductive performance of female *L. texana* on potted turkey berry plants was similar to silverleaf nightshade in this study (Table 2). The number of egg masses deposited by the surviving females on silverleaf nightshade was 18.9 ± 3.8 compared to 9.7 ± 6.7 on turkey berry, but the difference was not significant (*t* = 1.24, df = 9, *p* > 0.05). Also, the number of eggs laid in each mass by females confined to each of these test plants was similar. The number of eggs per mass averaged 22.6 ± 1.8 for silverleaf nightshade compared to 16.0 ± 3.0 for turkey berry (*t* = 1.96, df = 9, *p* > 0.05). More importantly, the viability of the eggs produced by the F1 females reared exclusively on a diet of either silverleaf nightshade or turkey berry leaves was the same. Average percent eclosion of F1 generation larvae from eggs deposited on silverleaf nightshade and turkey berry
Fig. 3. Average stadiad length (in days) of each larval instar of *Leptinotarsa deflecta* on *Solanum elaeagnifolium* (silverleaf nightshade, SLN) in single plant (no-choice) feeding tests in the laboratory.

Fig. 4. Average stadiad length (in days) of each larval instar of *Leptinotarsa texana* on *Solanum elaeagnifolium* (silverleaf nightshade, SLN) and *Solanum torvum* (turkey berry, TBY) in single plant (no-choice) tests in the laboratory.
was 78.9 ± 6.4% versus 78.0 ± 7.1%, respectively (t = 0.08, df = 9, p > 0.05). Taken together, these data strongly suggest that *L. texana* is capable of continuous reproduction on turkey berry.

**DISCUSSION**

Risk assessment has been a cornerstone of the practice of weed biological control since its inception because of safety concerns for crop species (Strong & Pemberton 2000). Clearly, any insect introduced for the biological control of a weed must not itself become a plant pest. The rigorous screening process ensures that non-specialist insects capable of reproducing on economically important, or environmentally sensitive species that are close relatives of the target weed, are dropped from further consideration. In recent years, risk assessment has focused less on crop species and more on native plant species related to the target weed, and the ecological consequences of "environmental spillover"—when a non-target species is attacked by the insect after its introduction (Tisdell et al. 1984). The ecological risks associated with releasing an insect for weed biological control with a host range that includes non-target native species (especially those threatened with extinction) are high, and it is unlikely that the effects will be reversible once the insect is introduced (Strong 1997, Louda et al. 1997, Strong & Pemberton 2000, Louda & O’Brien 2002).

Environmental risks can be reduced by selecting weed targets for classical biological control that (a) are nonnative invasive plant species, and (b) have few native relatives in the United States that could become host plants of the introduced insects (Center et al. 1997, Strong & Pemberton 2000). From this premise, it follows that selecting the nonnative solanum species tropical soda apple, wetland nightshade and turkey berry as candidates for classical biological control raises questions about the potential effects of imported insect herbivores on the numerous nontarget cultivated and native representatives of the genus *Solanum* in North America.

The genus *Solanum* contains over 30 species that are indigenous to the United States, 27 of these occurring in the southeast (Soil Conservation Service 1982). Two native species that are especially vulnerable to attack are the potato tree in Florida (Coile 1998), and *S. pumilum* Dunal, a diminutive species once thought to be extinct yet persists in a few sites in Alabama and Georgia (C.T. Bryson, personal communication). In this study, the potato tree was found to be an unacceptable host plant for *L. texana*.

The genus and family (Solanaceae) also contain economically important crop plants closely related to tropical soda apple, wetland nightshade, and turkey berry (Bailey 1971). Species such as bell pepper (*Capsicum*), tomato (*Lycopersicon*), tobacco (*Nicotiana*), eggplant and potato (both *Solanum* spp.) contribute significantly to Florida’s economy. For example, the combined economic value for Florida’s solanaceous crop plants in 1998 was reported to be over US $920 million (FLDACS 1998).

To reduce the risk of non-target damage, insect natural enemies imported from the native range of the nonnative solanaceous plants should use only the target weeds as host plants. However, the high degree of host specificity that must be demonstrated in order to obtain federal and state approval for release of these insects in the United

### Table 1. Feeding (cm²) by larvae of *Leptinotarsa defecta* and *L. texana* on *Solanum* spp. in the laboratory.

<table>
<thead>
<tr>
<th>Test/Plant Species</th>
<th><em>L. defecta</em> Mean (±SEM)</th>
<th><em>L. texana</em> Mean (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single Plant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLN</td>
<td>64.00 (9.2) a</td>
<td>52.30 (7.7) b</td>
</tr>
<tr>
<td>TBY</td>
<td>0.02 (0.01) c</td>
<td>104.50 (26.4) a</td>
</tr>
<tr>
<td>TSA</td>
<td>0.00 (0.0) c</td>
<td>0.00 (0.0) d</td>
</tr>
<tr>
<td>WLN</td>
<td>0.17 (0.04) b</td>
<td>0.08 (0.4) c</td>
</tr>
<tr>
<td><strong>Paired Plant</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLN</td>
<td>—</td>
<td>76.20 (6.6) b</td>
</tr>
<tr>
<td>TBY</td>
<td>—</td>
<td>40.00 (12.9) b</td>
</tr>
</tbody>
</table>

1SLN = silverleaf nightshade; TBY = turkey berry; TSA = tropical soda apple; WLN = wetland nightshade.
2Amount of feeding per n = 3 groups of 5 larvae; each group of larvae exposed to only one test plant species. Values for leaf consumption were based on the number of larvae surviving in each trial.
3Amount of feeding per n = 3 groups of 10 larvae; each group of larvae exposed to both plant species simultaneously. Values for leaf consumption were based on the number of larvae surviving in each trial.
4Means followed by the same letters within columns are not statistically different (p > 0.05) according to Tukey’s Studentized Range (HSD) test.
5Not tested.
TABLE 2. LABORATORY SURVIVAL AND REPRODUCTIVE PERFORMANCE OF FEMALE Leptinotarsa texana ON SOLANUM TORVUM COMPARED TO ITS NATURAL HOST PLANT S. ELAEAGNIFOLIUM.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SLN Mean (±SEM)</th>
<th>TBY Mean (±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Longevity (days)</td>
<td>58.1 (22.4)</td>
<td>58.0 (18.3)</td>
</tr>
<tr>
<td>Egg Masses</td>
<td>18.9 (3.8)</td>
<td>9.7 (6.7)</td>
</tr>
<tr>
<td>Eggs/Mass</td>
<td>22.6 (1.8)</td>
<td>16.0 (3.0)</td>
</tr>
<tr>
<td>% Larval Eclosion</td>
<td>78.9 (6.4)</td>
<td>78.0 (7.1)</td>
</tr>
</tbody>
</table>

1Data in each category derived from 8 ovipositing females in the S. elaeagnifolium tests, and 3 females in the tests with S. torvum. Adults were obtained from neonate larvae reared through one generation on each of the test plants before the experiment was initiated.
2SLN - silverleaf nightshade, S. elaeagnifolium; TBY - turkey berry, S. torvum.
3Means within a row compared by t-test; none were statistically different (p > 0.05, df = 9).

States may be an unrealistic expectation. For example, *Leptinotarsa undecimlineata* Stål, a congener of the two leaf beetles whose host plant relationships were examined in this study, is purported to be monophagous on turkey berry in Cuba (Ballou 1928, Pospisil 1972). In reality, *L. undecimlineata* is actually oligophagous, attacking several different host plants in the genus *Solanum* (Hsiao and Hsiao 1983, Jacques 1985). This particular example is relevant not only because it concerns the same group of insects and one of the plants that were the subject of this study, but clearly illustrates that most plant-feeding insects feed on a small group of closely related plants instead of a single species (Pemberton 1996).

The risk assessment process is further complicated by the fact that herbivorous insects that are screened as candidates for weed biological control projects often exhibit expanded host ranges under confined laboratory conditions (Cullen 1990, Blossey 1995, Olckers et al. 1999). For example, several candidates for classical biological control of tropical soda apple and other solanaceous weeds usually developed in laboratory studies on eggplant, *Solanum melongena* L., potato, *Solanum tuberosum* L. and tomato, *Lycopersicon esculentum* Mill., and other solanums that were not attacked in nature (Olckers et al. 1995, Hill & Hulley 1996, Olckers 1996, 1999, Gandolfo 1997, Medal et al. 1999, 2002).

An alternative to classical biological control—the importation of natural enemies from the native range of the target weed—is to select native insects from North American congeners, and attempt to establish ‘new associations’ between these native insects and the nonnative *Solanum* spp. (Hokkanen & Pimentel 1984). This approach differs from classical biological control in that the natural enemies have not played a major role in the evolutionary history of the host plant, and are therefore considered “new associates” (Hokkanen & Pimentel 1984). In theory, insect natural enemies from closely related plant species growing in similar climates but different geographical areas from the target plant are potentially more damaging than co-evolved natural enemies. The target weed is more likely to experience greater damage by the “new associates” because it lacks the appropriate defense mechanisms to resist attack (Hokkanen & Pimentel 1984). The ‘new association’ approach for selecting plant-feeding insects as biological control agents has been critically examined and supported by some practitioners of biological control of weeds (Dennill & Moran 1989, DeLoach 1995), but has been criticized as being based on faulty data by other specialists (Goeden & Kok 1986).

Although there are risks associated with releasing an insect in Florida from a congener of the nonnative *Solanum* spp. that occurs in another geographical region of North America ecologically similar to Florida (e.g., South Texas), the risk of collateral attack on non-target species may be acceptable. The only known potential host plants for *L. texana* in Florida are eggplant and horehound. In the unlikely event that eggplant were to be attacked by *L. texana*, insecticides used for crop production in Florida would be an effective feeding deterrent (Nesheim & Vulinec 2001). Likewise, minor damage to horehound could be viewed as beneficial as this native solanum is regarded as a weed in Florida (Hall & Vandiver 1991). More importantly, the ‘new association’ approach has been attempted in the United States against Eurasian watermilfoil, *Myriophyllum spicatum* L. (Haloragaceae), (Buckingham 1994, Sheldon and Creed 1995) and more recently English cordgrass, *Spartina anglica* Lois. (Poaceae) (Wu et al. 1999) without harming native plant communities.

The results of this study indicate that the native leaf beetle *L. texana*, which attacks silverleaf nightshade, is capable of using the nonnative turkey berry as a host plant whereas none of the non-native solanums supported development in the laboratory of its congener *L. defecta*. The inclusion of turkey berry in the potential host range of *L. texana* was not entirely unexpected. Studies by Hsiao (1981) and Olckers et al. (1995) showed the
potential host ranges of *L. defecta* and *L. texana* are much broader than their actual host ranges would indicate. In these laboratory studies, both beetles exhibited limited reproduction on several native *Solanum* spp. as well as on cultivated eggplant. However, the study by Olckers et al. (1995) also showed these beetles would not attack other members of the plant family Solanaceae that are vital to Florida agriculture, including potato, tomato, or bell pepper, and would not survive on plants outside the genus *Solanum*.

The acceptance of eggplant as a host plant in laboratory tests by candidate natural enemies of solanaceous weeds appears to be the rule rather than the exception (Olckers 1996, Medal et al. 1999, 2002). Eggplant apparently is devoid of certain feeding deterrents (chemical or physical) that normally play a role in host plant selection, and often produces false positives in a laboratory setting. However, *L. texana* never has been recorded on eggplant in south Texas even though this economically important solanum is often cultivated extensively in the vicinity of its natural host silverleaf nightshade. Furthermore, eggplant crops in Florida would be chemically protected from attack by *L. texana*. Thus, the risk to eggplant from damage by *L. texana* would be low if the insect were approved for released in Florida for biological control of turkey berry.

If *L. texana* were approved for release, this “new associate” might provide substantial control of one of Florida’s most invasive solanaceous weeds. Sustained defoliation by *L. texana* could severely stress turkey berry and perhaps make it less competitive with native plants. More importantly, the ecological risks associated with the release in Florida of *L. texana* may be acceptable because of the behavior exhibited by the beetle following its introduction and establishment on silverleaf nightshade in South Africa. Hoffmann et al. (1998) reported that *L. texana* attained high densities and had well-developed wings, but was unable to fly or reluctant to do so. The beetle remained in the release area until the food supply was exhausted and only dispersed by crawling en masse to adjacent plants. Because it appears that *L. texana* is incapable of flight, the beetle could be confined to a small area during the initial release and establishment phase where appropriate mitigation procedures would be implemented if post release surveys indicated that non-target plants were vulnerable to attack.

Although *L. texana* is native to North America, and would be exempt from the rigorous screening and approval process required by the federal Technical Advisory Group on the Introduction of Weed Biological Control Agents (TAG) (Lima 1990), other nonweedy members of the genus *Solanum* that are native to Florida could be attacked. The risk to these non-target species should be thoroughly assessed and the appropriate state agencies consulted to obtain their approval before releasing *L. texana* in Florida for biological control of turkey berry.

**ACKNOWLEDGMENTS**

We thank John Capinera and Howard Frank for reviewing an earlier version of the manuscript. We also thank Lucy Treadwell for technical assistance. This project was funded by grants from the Florida Department of Environmental Protection, Bureau of Invasive Plant Management Contract No. ERP039, and the Office of the Dean for Research, UF/IFAS. Florida Agricultural Experiment Station Journal Series No. R-07585.

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The little known mymarid genus *Kalopolynema* was described by Ogloblin (1960) from two females belonging to the type species, *K. discreetans* Ogloblin. Both the holotype and the paratype were collected in the Province of Buenos Aires, Argentina, and until recently this genus has not been recorded outside that country. Our interest in studying *Kalopolynema* was sparked by the recent discovery of a specimen from Monticello, Florida, that we first thought to represent a new Nearctic genus of Mymaridae (Huber 1997), but later, when more specimens of *P. cautum* Ogloblin became available to him, the presence of subbenthal grooves on the face was added to the generic diagnosis (Ogloblin 1967).

*Kalopolynema* was not treated by Annecke and Doutt (1961) nor was it included in the key to the Nearctic genera of Mymaridae (Huber 1997), where it would key in the same couplet together with *Polynema* Haliday. To facilitate recognition of *Kalopolynema* from related genera in the New World, a key is provided that also includes *Platypolynema* and *Polynema* s. l.

Our interest in the *Polynema* group of genera in a broad sense, which corresponds roughly to the tribe Mymarini of Annecke and Doutt (1961) and where both *Kalopolynema* and *Platypolynema* belong, was further instigated by the recent discovery of a specimen from Monticello, Florida, that we first thought to represent a new mymarid genus from that group. However, after a
second female of the same species was discovered and both specimens were carefully studied, we decided that they would be better placed in Kalopolynema as a new subgenus and species, described here respectively as Floripolynema and K. (Floripolynema) mizelli. Inclusion of this taxon in Kalopolynema requires broadening of its diagnosis compared to that given by Ogloblin (1960).

Terms for morphological features are those of Gibson (1997). Measurements are given in micrometers (µm) as length or, where appropriate, as length/width. Abbreviations (codens) for depositories of specimens are as follows: CNCI, Canadian National Collection of Insects, Ottawa, Ontario, Canada; FSCA, Florida State Collection of Arthropods, Gainesville, Florida, USA; MLPA, Museo de La Plata, La Plata, Buenos Aires, Argentina; UCRC, Entomology Research Museum, University of California, Riverside, California, USA; USNM, National Museum of Natural History, Washington, D.C., USA. An abbreviation used in the text is: F = funicle segment.

**Diagnosis.** Face (Figs. 3, 7) with two converging subantennal grooves extending from toruli to margin of clypeus; prosternum "open", not closed by propleura anteriorly; mesoscutum and scutellum with cellulate sculpture; forewing (Figs. 2, 6, 9) narrow, marginal vein relatively short and with 1 (in Floripolynema subgen. nov.) or 2 (in the nominal subgenus) dorsal macrochaetae; petiole in dorsal view subquadrate, subrectangular, or cross-shaped (Fig. 10), at most 2× as long as wide, attached posteriorly to gastral tergum; female with ovipositor usually longer than body and strongly exerted beyond apex of gaster.

**Comments.** The previous diagnoses of Kalopolynema are incomplete because they failed to indicate perhaps the most important morphological feature that distinguishes Kalopolynema, including the new subgenus described below, and its sister genus Platypolynema from all other described genera belonging to the Polynema group: the presence of well-developed subantennal grooves on the face. The diagnoses by Ogloblin (1960) and Yoshimoto (1990) emphasized the peculiar shape of the petiole in Kalopolynema, which is also almost as short in Platypolynema; however, such a character is not unique among the Polynema-group genera (e.g., it is also found in the Australian Polynema quadripetiolatum Girault), although it is very rare. Kalopolynema and Platypolynema are closely related but can be separated from each other by the combination of several morphological features given in the key.

Known host associations of Kalopolynema include two species of the plant hopper genus Megamelus Fieber (Delphacidae) that reproduce on plants in or near water. The females of Kalopolynema species are equipped with a long ovipositor, apparently to be able to reach their hosts' eggs imbedded in the host plant tissue, which could be the aerenchyma in some water plants.

**KEY TO THE SUBGENERA AND SPECIES OF KALOPOLYNEMA, FEMALES**

1. Marginal vein of forewing with two dorsal macrochaetae (Figs. 2, 6); propodeum without dorsal elevation in the middle (subgenus Kalopolynema Ogloblin s. str.) .................................................. 2
   — Marginal vein of forewing with only one (the distal) dorsal macrochaeta (Fig. 9); propodeum with dorsal elevation in the middle (Floripolynema S. Triapitsyn and Berezovskiy, subgen. nov.) ................................................................. 2
     K. (Floripolynema) mizelli S. Triapitsyn and Berezovskiy, sp. nov.
2. F1 almost as long as pedicel; F4 almost as long as F1 .................................. K. (Kalopolynema) discrepans Ogloblin
   — F1 less than 0.5× length of pedicel; F4 longer than F1 .................................. 3
3. Petiole in dorsal view longer than wide .......... K. (Kalopolynema) ema (Schaff and Grissell), comb. nov.
   — Petiole in dorsal view about as long as wide ............................................ K. (Kalopolynema) poema S. Triapitsyn and Berezovskiy, sp. nov.

**SUBGENUS KALOPOLYNEMA OJGOBLIN S. STR.**

(Figs. 1-6)


Type species: Kalopolynema discrepans Ogloblin.

**Diagnosis.** Female clava with 7 or 9 longitudinal sensilla (Figs. 1, 5); sculpture on scutellum anterior to frenal line notably less pronounced than on mesoscutum and frenal area of scutellum; propodeum smooth or with a median carina, without a dorsal elevation in the middle; marginal vein of forewing with 2 dorsal macrochaetae; petiole in dorsal view subquadrate or subrectangular; female with ovipositor usually much longer than body and very strongly exerted beyond apex of gaster.
**Kalopolynema (Kalopolynema) discrepans Ogloblin**  
(Figs. 1, 2)

*Kalopolynema discrepans* Ogloblin, 1960: 6-7, figs. 5-11.  
Type locality: Tigre, Buenos Aires, Argentina.

Types. Holotype female (MLPA), examined. On slide, labeled: 1. "Kalopolynema discrepans Ogl. HOLOTOPO △ Bs. As. Tigre IV-1942, A. O."

Diagnosis. This species is easy to separate from the other two known species in this genus by characters given in the key. Other distinguishing features of *K. discrepans* females include the presence of two longitudinal sensilla on both F5 and F6 and nine longitudinal sensilla on the clava.

Male. Unknown.

**Distribution.** Known only from the type localities, Tigre and Bella Vista, in the Province of Buenos Aires, Argentina.

Host. Unknown.

Comments. The original description of this species is adequate and well-illustrated. Here we provide drawings of the antenna (Fig. 1) and forewing (Fig. 2) of *K. discrepans* because Ogloblin’s illustrations are incomplete.

**Kalopolynema (Kalopolynema) ema (Schauff and Grissell), comb. nov.**  
(Figs. 3, 4)

*Polynema ema* Schauff and Grissell, 1982: 530-533.  
Type locality: Etherton Pond, 3 mi. N. of Pomona, Jackson Co., Illinois, USA.

Types. Holotype female and numerous paratypes (USNM and CNCI), examined.


Figs. 1 and 2. *Kalopolynema (Kalopolynema) discrepans* Ogloblin, female. Fig. 1. Antenna (paratype). Fig. 2. Forewing (holotype). Scale bars = 0.1 mm.

**Diagnosis.** This species can be distinguished from the type species of the genus, *K. discrepans*, and from the newly described *K. poema* by characters given in the key. Other distinguishing characters of both *K. ema* and *K. poema* females include the absence of longitudinal sensilla on F5 and F6 and the presence of 7 longitudinal sensilla on the clava. The mesosoma of *K. ema* is very short and compact, shorter than the gaster. The propodeum in this species has a complete median carina in the male and a broken carina in the female (Illinois specimens); in the female specimens from Florida and Georgia the propodeum is either almost smooth or with a weak trace of a broken median carina only. The petiole is about 2× as long as wide; subrectangular in dorsal view and produced into a tooth ventrally (best seen in lateral view).

Here we provide an illustration of the head in frontal view (Fig. 3) to show the presence and configuration of subantennal grooves on the face. Also illustrated are the male genitalia (Fig. 4) which are very similar to those in many *Polynema* species. These illustrations supplement the figures in Schauff and Grissell (1982).

**Distribution.** As seen from “Other material examined” above, new distribution records since Schauff and Grissell (1982) are from Canada (Ontario) and USA (Florida, Georgia, Massachusetts, Virginia); it probably will be found to occur throughout the range of its host (Schauff and Grissell 1982).

**Host.** The lily (water-lily) plant hopper, *Mega melus davisi* Van Duzee (Homoptera: Delphacidae).

**Comments.** This species was introduced in 1941 from Michigan into Honolulu, Oahu Island, Hawaii, under the incorrect name *Polynema ciliata* (Say) (its nomenclatural history was discussed by Schauff and Grissell (1982)), and successfully established there on local populations of the water-lily plant hopper (Zimmerman 1948). The likelihood that *K. ema* is also able to parasitize eggs of other *Megas melus* species is very high.

**Kalopolynema (Kalopolynema) poema** S. Triapitsyn and Berezovskiy, sp. nov.

(Figs. 5, 6)

Types. Holotype female (CNCI). On card, labeled:
2. "Kalopolynema (Kalopolynema) poema* S. Triapitsyn & Berezovskiy HOLOTYPE ♀". Paratype female (CNCI) on slide, same data as the holotype except the date is 19-XI-1999.

**Description.** Female. Color. Brown except scape, pedicel, legs, and petiole light brown; distal tarsomeres slightly darker than other leg segments; eye pink.

**Head.** Width 223, round in frontal view; face with distinct, narrow subantennal grooves and with several symmetrical rows of small setae; torulus slightly above mid level of eye. Vertex
rounded, with fine sculpture, ocelli in very obtuse triangle. Mandible tridentate. Antenna (Fig. 5) shorter than body, sparsely setose except clava more densely setose. Radicle not fused with scape, the scape smooth, 3.4 x as long as wide; pedicel pear-shaped, longer than wide, much longer than F1; all funicle segments longer than wide, F1 the shortest and F2 the longest, F3 longer than F4, F5 markedly shorter than F4 and slightly shorter than F6, all funicle segments without longitudinal sensilla; clava 2.6 x as long as wide, with 7 longitudinal sensilla, all of them subapical.

**Mesosoma.** Pronotum very short, divided mediolongitudinally; pronotum, mesoscutum, axilla, and frenal area of scutellum with conspicuous cellular sculpture; mesoscutum wider than long; scutellum about as long as wide and as long as mesoscutum; scutellar sensilla close to anterior margin of scutellum, frenal line with small foveae; metanotum strap-like; propodeum smooth, without median carina.

**Wings.** Forewing (Fig. 6) 7.3 x as long as wide; venation reaching slightly less than ¼ length of wing; longest marginal cilia almost 2 x greatest width of blade; disc hyaline, more or less uniformly setose beyond venation. Hind wing disc hyaline, with setae only along margins; longest marginal cilia 6 x maximum width of blade.

**Legs.** Coxae smooth, metacoxa longer than petiole. Protibia with 5 conical sensilla.

**Metasoma.** Petiole subquadrate in dorsal view. Gaster longer than mesosoma. Ovipositor broadly rounded anteriorly, occupying more than 4/5 length of gaster, markedly exserted beyond its apex (exserted part of ovipositor about 0.6 x its total length in paratype); ovipositor/metatibia ratio 3.4:1.


**Etymology.** The new species name means “a poem” in Russian; the sole reason for choosing it is the fact that it rhymes with the name of the closely related species, *K. ema*.

Male. Unknown.

**Diagnosis.** This species is similar to *K. ema*. It differs mainly in the shape of the petiole, as indicated in the key, as well as in the shape of the marginal vein which is relatively shorter in *K. poema*. The female clava in *K. poema* is about 2.6 x as long as wide (2.1-2.2 x as long as wide in *K. ema*).

**Distribution.** Known only from the type locality in Hurlingham, Buenos Aires, Argentina.

Comments. The material of the new species was sent to John T. Huber (CNCI) by Livy Williams, III (USDA-ARS, Stoneville, Mississippi). More detailed information on the identity of the host planthopper was provided to me recently, as a personal communication, by Alejandro Sosa (South American Biological Control Laboratory, USDA-ARS, Hurlingham, Buenos Aires, Argentina) who apparently was the actual collector of the two type specimens of *K. poema*. *Megamelus scutellaris* lives on water-hyacinth, *Eichhornia crassipes* (C. Martins) Solms-Loubach, in Argentina and has been studied there as a potential biological control agent against this aquatic weed. Should *M. scutellaris* ever be considered for establishment beyond its native range, caution must be applied to avoid an inadvertent introduction of its egg parasitoid, *K. poema*.

**FLORIPOLYNEMA** S. TRIAPITSYN AND BEREZOVSKIY, SUBGEN. NOV. (Figs. 7-10)

Type species: *Kalopolynema (Floripolynema) mizelli* S. Triapitsyn and Berezovskiy, sp. nov. Monobasic.

Diagnosis. Female clava with 7 longitudinal sensilla (Fig. 8); sculpture on scutellum anterior to frenal line as pronounced as on mesoscutum and frenal area of scutellum; propodeum with an incomplete median carina and with a dorsal elevation in the middle; marginal vein of the forewing with one (the distal) dorsal macrochaeta (Fig. 9); petiole in dorsal view cross-shaped, almost subquadrate (Fig. 10); female with ovipositor almost as long as body.

Description. Female. Head in dorsal view about as wide as mesosoma, oval in lateral view.

Figs. 7. *Kalopolynema (Floripolynema) mizelli* S. Triapitsyn and Berezovskiy, sp. nov., female (paratype). Head (frontal view). Scale bars for Figs. 7-10 = 0.1 mm.

Face (Fig. 7) with narrow, distinct subantennal grooves; torulus slightly above mid level of eye, almost touching preorbital trabecula. Vertex rounded, with fine sculpture, ocelli in very obtuse triangle. Mandible tridentate.

Antenna (Fig. 8). Scapa much longer than wide; pedicel longer than wide, funicle 6-segmented, all segments more or less cylindrical; clava entire, with 7 longitudinal sensilla.

Mesosoma. Pronotum divided mediodiagonally, neck strongly wrinkled transversely, lobes of pronotal collar with fine cellulate sculpture. Mesoscutum, axilla, scutellum, and metanotum with conspicuous cellulate sculpture; mesoscutum a little longer than wide, with prominent notauli; scutellum shorter than mesoscutum, scutellar sensilla almost in the middle and far apart from each other, frenal line with small foveae; metanotum strap-like. Propodeum smooth, in dorsal view elevated posteriorly in the middle to form a ridge projecting beyond posterior margin and in lateral view forming almost a right angle (somewhat as in *Polynema (Dorypolynema) mendeli* Girault), with an incomplete median carina in distal half of propodeum; propodeal seta strong, near posterior margin; propodeal spiracle rounded.

Wings. Forewing (Fig. 9) relatively narrow; venation short, extending about 1/4 length of wing, hypochaeta reaching posterior margin, marginal vein with one (the distal) dorsal macrochaeta and one short ventral seta at apex; disc hyaline, more or less uniformly setose beyond venation; longest marginal cilia about as long as greatest width of blade. Hind wing much shorter than forewing, typical for *Polynema*-group of genera.

Legs. Tarsi 4-segmented.

Metasoma. Petiole (Fig. 10) in dorsal view cross-shaped, almost subquadrate, attached posteriorly to gastral tergum; gaster projecting forward ventrally, almost reaching base of mesocoxa (best seen in lateral view); ovipositor long, almost as long as body, markedly exserted beyond its apex.

Male. Unknown.

Etymology. An arbitrary use of the first part of the word Florida, referring to the state where the new subgenus was found, combined with the generic name *Polynema*. Gender: neuter.

**KALOPOLYNEMA (FLORIPOLYNEMA) MIZELLI** S. TRIAPITSYN AND BEREZOVSKIY, SP. NOV. (Figs. 7-10)

Description. Female. Color. Head and mesosoma black; flagellum and gaster dark brown; scape, pedicel, wing venation, petiole, ovipositor sheath and external plate of ovipositor brown; legs light brown except base of metacoxa, apical half of mesotibia, metatibia, last tarsomeres of fore- and middle legs and metatarsus darker. Eye dirty pink.

Head. Width 241, face (Fig. 7) with several symmetrical rows of small setae. Ocellar setae small, inconspicuous.

Antenna (Fig. 8) much shorter than body, sparsely setose except clava more densely setose. Radicle almost fused with scape, scape smooth, about 3 x as long as wide; pedicel slightly longer than F1; F2 longest of funicle segments, F3 much longer than following funicle segments; F4 and F6 subequal in length (F5 slightly shorter); all funicle segments without longitudinal sensilla; F6 slightly wider than preceding funicle segments; clava 2.7 x as long as wide, with 7 longitudinal sensilla, 6 of them subapical.

Mesosoma. Lobe of pronotal collar with 5 setae; axilla small, with one weak seta; scutellum about as wide as long.

Wings. Forewing (Fig. 9) 6.5 x as long as wide; marginal + stigmal vein with 4 placoid sensilla at apex; longest marginal cilia 1.15 x greatest width of blade. Hind wing disc hyaline, with a few setae in an incomplete row in distal half; longest marginal cilia 5 x maximum width of blade.

Legs. Coxae smooth, metacoxa much longer than gastral petiole. Protibia with 3 or 4 conical sensilla.

Metasoma. Petiole (Fig. 10) slightly wrinkled transversely in basal third and with a transverse
carina ventrally. Ovipositor occupying the whole length of gaster, markedly exserted beyond its apex (by about \(\frac{1}{3}\) total length of ovipositor); ovipositor/metatibia ratio 2.1:1.


Male. Unknown.

**Etymology.** The new species is named in honor of Russell F. Mizell, III, collector of one of the type specimens.

**Distribution.** Known only from the type localities in Florida.

Host. Unknown.

**Comments.** This species is one of the most beautiful of North American Mymaridae.

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**GENUS **Platypolynema** Ogoblin (Figs. 11, 12)**


**Type species:** *Platypolynema cautum* Ogoblin, by monotypy and original designation.

**Diagnosis.** Head very large and high, markedly wider than mesosoma, with well-defined subantennal grooves; prosternum anteriorly “closed” by propleura; mesoscutum smooth, much longer than scutellum; forewing (Fig. 12) long and narrow, with a constriction of blade beyond venation; marginal vein long and with 2 dorsal macrochaetae; petiole in dorsal view subquadrate; female with ovipositor very long, acutely elbowed and strongly produced forward anteriorly beneath mesosoma.

**Comments.** Yoshimoto (1990) apparently overlooked the earlier description of the female of *Platypolynema* by Ogoblin (1967) and therefore it is included only in his key to the males of the New World genera of Mymaridae.

The biology of the single known species of *Platypolynema* is unknown. Like *Kalopolynema* species, it is quite possible that it is associated with some Auchenorrhyncha on plants near water.

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Figs. 11 and 12. *Platypolynema cautum* Ogoblin, female (allotype). Fig. 11. Antenna. Fig. 12. Forewing. Scale bars = 0.1 mm.
**PLATYPOLYNEMA CAUTUM OGLOBLIN**

(Figs. 11, 12)


Type locality: Chacra “Yabebí”, San Ignacio, Misiones, Argentina.

**Types.** Holotype male (MLPA), examined; in good condition, mounted dorso-ventrally, with part of the left antenna missing. On slide, labeled: 1. “Platypolynema cautum [dictynum—crossed out, this is a manuscript name] A. Oggl. ♂ Chacra Yabebí, S. Ignacio, Mis. 11.III.1951 A. O.”. Allotype female (MLPA), examined; pedicel and flagellum of the right antenna are missing. On slide, labeled: 1. “Platypolynema cautum A. Ogloblin ♀, Misiones, 2 de Mayo 20-XI-1964”; 2. (Mostly illegible, in pencil) “Platypolynema 2 de Mayo A. O. 15.XII.1964”. We added the word “Allotype” to the first label in order to clearly mark this specimen as such.


Comments. The holotype male is labeled slightly differently on the slide from what was indicated by Ogloblin (1960): the date of the collection is 11-III-1951 instead of 12-III-1953. However, there is no doubt that this specimen is indeed the holotype as it perfectly matches Ogloblin’s illustrations. In fact, many type specimens from Ogloblin’s collection of Mymaridae are not marked as such and often the label data on the slides contradict the published information.

The original description of the holotype male of this species and the follow-up description of the allotype female are sufficient for its recognition. Here we provide drawings of the antenna (note that the clava is collapsed) (Fig. 11) as well as of the forewing (Fig. 12), taken from the allotype female of *P. cautum*.

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A NEW PARASITOID OF WHITEFLIES FROM MEXICO, WITH A KEY TO NEW WORLD SPECIES OF THE GENUS ENCARSIELLA (HYMENOPTERA: APHELINIDAE)

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ABSTRACT

A new species, Encarsiella tamaulipeca Myartseva and Coronado-Blanco sp. nov., from Mexico is described and illustrated. A new combination is proposed, Encarsiella narroi (Gomez & García) from Encarsia. A key to the species of Encarsiella (females) of the New World is given.

Key Words: Encarsiella sp. nov., distribution

RESUMEN

Se describe e ilustra una nueva especie: Encarsiella tamaulipeca Myartseva et Coronado-Blanco sp. nov. de México. Se propone una nueva combinación de Encarsiella narroi Gómez & García a Encarsiella narroi (Gómez & García). Se incluye la clave de las especies de Encarsiella (hembras) del Nuevo Mundo.
Translation provided by author.

Among the parasitic Hymenoptera, species of the family Aphelinidae (Chalcidoidea) are among the most important biological control agents of insect pests. Aphelinid species play a significant role in ecosystems as natural enemies of many homopteran hosts and have been used successfully as biological control agents in Mexico and in many parts of the world (Clausen 1978).

The genus Encarsiella Hayat (1983) belongs to the subfamily Coccophaginae sensu De Santis (1948) and Hayat (1985), tribe Pteroptricini Ashmead, that also includes the genera Encarsia Foerster, Dirphys Howard, Bardylis Howard, Coccophagoides Girault and Pteroptrix Westwood (Hayat 1998). Relationships within the family Aphelinidae have been studied by many taxonomists, but the classification of the aphelinid genera into subfamilies and tribes is still in formative stages (Hayat 1994, Yasnosh 1976, Shafee & Rizvi 1991 and Hayat 1998). Encarsiella is characterized by having an 8–segmented antenna in both sexes, the third segment of the club oblique or transverse at apex, linea calva absent, stigmal vein narrow, submarginal vein with 2-4 long setae, mesoscutum with a variable number of setae but always more than 6 and the axilla elongate and strongly projecting forward.

ENCARSIELLA IS CLOSELY TO THE GENERA DIRPHYS AND ENCARSIELLA.
THE DIFFERENCES AMONG THESE GENERA ARE SHOWN IN THE FOLLOWING KEY:

1. Axillae small and separated medially by more than the maximal length of an axilla. Mid lobe of mesoscutum with reduced number of setae arranged in bilateral symmetry. Scutellum distinctly wider than long

Encarsiella Foerster

— Axillae large and separated medially by less than the maximal length of an axilla. Mid lobe of mesoscutum with many scattered setae, not arranged in bilateral symmetry

2. Side lobes divided; sculpture of mesoscutum aciculate. Scutellar placoid sensilla closely placed, separated by about diameter of a sensillum

Dirphys Howard

— Side lobes not divided; sculpture of mesoscutum imbricate- reticulate. Scutellar placoid sensilla widely placed, separated by distance distinctly longer than diameter of a sensillum

Encarsiella Hayat
Most *Encarsiella* species are solitary endoparasitoids of whiteflies belonging to the subfamily Aleurodicinae (Homoptera, Aleyrodidae). However, *Encarsiella boswelli* (Girault) is known to attack eggs of Heteroptera (Polaszek & Hayat 1990), and an undescribed species from India was reared from nymphs of Psyllidae (Huang & Polaszek 1996).

Nine species of *Encarsiella* are known worldwide; of these, four are recorded in the New World: *E. aleurodici* (Girault), *E. magniclava* (Girault), *E. pithecura* Polaszek, and *E. noyesi* Hayat (Huang and Polaszek 1996; Martin and Polaszek 1999). The latter species is widely distributed in Central America and has been used in biological control programs of *Aleurodicus cocois* (Curtis) (Cock 1985). Some undescribed species are known from the New World. The correct identification of the parasitoids reared from pests species is essential to the success of biocontrol programs.

G. Viggiani (1986) stated new combinations for *Encarsiella aleurodici* from *Encarsia* and *Encarsiella magniclava* from Coccophagus. We propose a new combination—*Encarsia narroi* (Gómez & García), comb.n. from *Encarsia*. This species was reared from *Aleurodicus* sp. collected on *Bauhinia variegata* L. and *Hibiscus* sp. in Mexico, Coahuila State (Gómez and García 2000). The description and illustrations of this species show characteristics belonging to *Encarsiella*, especially the number of setae on the mesoscutum (42 pairs according to the authors) and the structure of the antennal club. Thus, *Encarsiella narroi* (Gómez & García), comb.n. is the fifth species of this genus known from the New World.

*Encarsiella noyesi* was described from Mexico, reared from *Aleurodicus dugesii* Cockerell in the State of Guanajuato, and from *Aleurothrixus floccosus* (Maskell) on *Citrus aurantifolia* (Christm.) Swingle, in the State of Yucatán (Polaszek and Hayat 1992). We reared *E. noyesi* from Aleurodicinae whiteflies in the State of San Luis Potosí (new record for this State), and from an aleyrodid in the State of Tamaulipas (new record for this State). In addition, a new species of *Encarsiella* was reared from an undescribed species of Aleurodicinae on *Psidium guajava* L. in the State of Tamaulipas.

The abbreviations *R* = radicle, *S* = scape, *P* = pedicel and *F* = funicle segment are used in the following description of the new species and key to the species of *Encarsiella* (females) of the New World.

**ENCARSIELLA TAMAILIPECA**

**MYARTESEVA AND CORONADO-BLANCO SP. NOV.**

(Figs. 1-3)

**Description**

**FEMALE** (Figs. 1-2). Length: 0.75-0.82 mm (N = 8 specimens on points, 2 on slides); holotype – 0.75 mm.

**Coloration**

Head black, face ferrugineous from anterior oculus to interantennal prominence and whitish below (except upper margin of mouth, hind part of cheeks and antennal scrobes). Pedicel and antennal club brown, scape (except distal half dorsally brown) and F3 whitish; F1-F2 pale brown. Metasoma and metasoma black. Legs yellowish-white, middle and hind coxae, hind femur black, middle femur and hind tibia infuscate. Wings hyaline. Sheaths of ovipositor whitish.

**Head**

Wider than high and as wide as mesosoma. Frontovertex 2× as wide as long, about 0.5× head width. Occipital margin slightly rounded and concave. Ocelli in slightly obtuse triangle; lateral ocelli close to occiptal margin, at a distance of less than diameter of an oculus, and about 2 diameters of an oculus from eye margins. Eyes about 2× longer than cheeks. Malar sulcus present. Antenna (Fig. 1) inserted immediately under lower margin of eyes, closer to mouth margin than to eye margins. Antennal segments R–F3 and club (3–jointed) with the following ratios, length/width: R-15:9, S–60:15, P–22:13, F1–18:12, F2–25:14, F3–20:15, club–67:20. Pedicel slightly longer than F1; club slightly longer than funicle and scape. F3, F2, and club joints with two longitudinal sensilla each, sensilla absent on F1. A very thin anellus is also present.

**Mesosoma**

Sculpture of dorsum with more or less hexagonal cells, sides of mesoscutum and scutellum with longitudinal cells. Mesoscutum slightly wider than long, with many setae varying in number from 54 to 64. Scutellum about 2× wider than long, with 2 pairs of long setae. Axilla with one seta, lateral lobes with three setae. Fore wing more than 2× longer than wide, marginal fringe about 0.14× wing width. Length of marginal vein equal to submarginal vein, postmarginal vein absent, stigmal vein very short. Strong setae in two rows on anterior margin form narrow bare band, interrupted near vein by a few setae (Fig. 2). Base of wing with 7-10 setae. Marginal vein with 10-13 setae, marginal fringe 2.5× maximum wing width, discal setae uniformly distributed, hind wing more than 4.5× as long as wide. Tibial spur of middle leg slightly shorter than basitarsus.

**Metasoma:** rounded at apex, about 0.67 times length of mesosoma (in dry specimens). Ovipositor exserted, its exserted part 0.5× length of gaster (in dry specimens); ovipositor longer than middle tibia (14:11), sheaths about 0.5× inner plates.
Figs. 1-3. *Encarsiella tamaulipeca*, sp. nov.: 1- antenna, female (× 200), 2- marginal part of forewing (× 280), 3- antenna, male (× 200).

Figs. 4-6. *Encarsiella noyesi* Hayat: 4- antenna, female (× 200), 5- marginal part of fore wing (× 280), 6- antenna, male (× 200).
MALE (Fig. 3). **Coloration.** Similar to female in color, but face brown; legs black, except apices of fore and middle femora, apices of fore and hind tibiae, and the apical half of mid tibia and tarsi which are whitish.

Antenna (Fig. 3) inserted at level of lower eye margin, at equal distance from margins of eye and mouth. Funicle 4–segmented, club 2–segmented. Antennal segments with the following wing ratios length/width: R–11:8, S–50:13, P–17:13, F1–31:14, F2–36:15, F3–35:15, F4–34:16, F5–35:16, F6–34:13. Pedicel slightly less than 0.5× F1; club as long as the two preceeding segments together. F1–F6 with 3 longitudinal sensilla each. Forewing with bare base. Mesoscutum wider than long; scutellum about 1.5× wider than long.

**Diagnosis.** Using the key and the revision of *Encarsiella* species of the world provided by Polaszek and Hayat (1992), *E. tamaulipeca* sp. nov. is close to *E. aleurodici* in coloration of body and antenna, and also the following morphological features: long pedicel (P > F1), and club (>F1–F3), absence of sensilla on F1, and setaceous wing base. It differs from *E. aleurodici* in the following: female with antenonnal anellus and anterior margin of fore wing with bare band; length of marginal vein equal to submarginal vein, and marginal fringe longer (0.14× maximum wing width) in *aleurodici* it is very short), ovipositor slightly longer than mid tibia (14:11); male antennal club equal to lengths of two preceeding segments.

**Material Examined.** Holotype, female: Mexico, Tamaulipas, Ciudad Victoria, ex Aleyrodidae on *Psidium guajava*, 7-8-XII-1995, E. Chouvakhina; paratypes: same data as holotype, 6 females (all on points); 27-X-1999, S. Myartseva, 1 female, 1 male, on slides.

The holotype and one paratype are deposited in the National Museum of Natural History, Washington, D.C., USA; two paratypes in the Department of Zoology, Institute of Biology, National Autonomous University of Mexico, Mexico City, D.F., Mexico; two paratypes in the Zoological Institute, Russian Academy of Sciences, St. Petersburg, Russia; one paratype point and one female and one male on slides in the Insect Museum, Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, Mexico.

**Etymology** *Encarsiella tamaulipeca* is named after the State of Tamaulipas where it was discovered.

**Encarsiella noyesi Hayat, 1983**

Material Examined.

Mexico, San Luis Potosí, Xilitla, ex Aleyrodidae, 10-XI-1999, S. Myartseva, 16 females, 17 males, MIFA (UAT); Tamaulipas, Jaumave, ex Aleyrodidae, 36 females, 2 males, 30-IV-2000 (S. Hernández-Aguilar); USA, California, Riverside, UCR Quarantine culture, A. Briones, emerged 3-IV-2000 from *Aleurodicus dugesii* Cockerell, 7 females, 1 male, orig. from Mexico, Jalisco, Guadalajara, 5-V-1997 (D. Headrick).

Morphological differences were observed among populations of *Encarsiella noyesi* reared in several regions. For example, Mexican specimens are different from those described by Hayat, who studied specimens reared from June to August from Trinidad, St. Vincent and Tobago. The Mexican specimens are smaller (female body length 0.52-0.67 mm, male 0.45-0.62 mm) and some females have only the F1 pale yellow (also observed in specimens from California). Living female specimens have a violet-bluish face and pearlish-bluish-white scutellum. Dry female specimens are lighter yellow than the Californian specimens and the basal third of the mesopleurum is black; P equal to F1; and F1 usually has longitudinal sensilla (Figs. 4-6) (also observed in specimens from California); lateral lobes with three setae; marginal fringe of forewing longer (0.14× wing width); and ovipositor exserted and slightly longer than midtibia. Male specimens have F1–F2 more pallid and pedicel length about 0.5× F1.

**Key to Females of Encarsiella Species of the New World**

1. Scutellum entirely black ................................................................. 2
   — Scutellum pallid .................................................................................. 3

2. Discal setae on forewing uniformly distributed; marginal vein longer than submarginal vein. Ovipositor as long as mid tibia .................................................................................. E. aleurodici (Girault) Distribution: Barbados, Ecuador, Trinidad (Polaszek and Hayat 1992).
   — Forewing with a long band bare of setae along anterior margin (Fig. 3); marginal vein equal to submarginal vein in length. Ovipositor 1.3× longer than mid tibia .................... E. tamaulipeca sp. nov. Distribution: Mexico.

3. Mesoscutum entirely dark. F1 less than 2× as long as wide. Club slightly less than 3× as long as wide. Forewing with 2 large setae on submarginal vein ......................................................... 4
   — Mesoscutum pale, excluding the anterior edge and notauli. F1 2.4× as long as wide. Club slightly less than 2× as long as wide. Forewing with 2 large setae and 2-4 smaller setae on submarginal vein ......................................................................................... E. magniclava (Girault)
4. Base of forewing with an infuscated area. Antennal scrobes and clypeus entirely pale; pedicel and scape entirely pale. ................................................................. E. pithecura Polaszek

— Base of forewing hyaline. Antennal scrobes and clypeus completely dark; pedicel and scape partly or entirely dark. ................................................................. 5

5. Forewing with a long band bare of setae along anterior margin and without asetose area below stigmal vein. F1 without sensillum and F2 somewhat longer than F1 and F3. .................................................. E. noyesi Hayat

— Forewing without a long band bare of setae along anterior margin and with asetose area below stigmal vein. F1 with one sensillum, F1-F3 of about equal length ............. E. narroi (Gómez & García), comb.n.

Distribution: Mexico (Gómez and García 2000).

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DESCRIPTION OF THE SEXUAL GENERATION OF CALLIRHYTIS QUERCUSCORNIGERA AND A NEW INQUILINE (HYMENOPTERA: CYNIPIDAE)

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ABSTRACT

The alternate, sexual generation of Callirhytis quercuscornigera (Osten Sacken), comb. rev. was found by experimental rearing and field observations. Descriptions of the adult, gall, biology, and host plants of the sexual generation are given. A new species of cynipid inquiline, Ceroptres cornigera Melika & Buss, reared from galls of the sexual and asexual generations of Callirhytis quercuscornigera (Osten Sacken) of the eastern United States is also described and illustrated. This is the first known species of Ceroptres to inhabit galls from alternating generations of its host cynipid. Descriptive data, diagnostic characters, distribution, and biological information are given.

Key Words: Taxonomy, morphology, inquiline, Ceroptres cornigera, horned oak gall

RESUMEN

Por observaciones de campo y cría experimental, se descubrió la generación alterna sexual de Callirhytis quercuscornigera (Osten Sacken), combinación revisada. Se describen el adulto, la agalla, el desarrollo y las plantas huesped de esta generación sexual. Se describe e ilustra también una especie nueva de cynipido inquilino, Ceroptres cornigera Melika y Buss, criada de las agallas producidas por las generaciones sexuales y asexuales de Callirhytis quercuscornigera en el este de los Estados Unidos. Esta es la primera especie de Ceroptres descrita que habita las agallas de ambas generaciones de su huesped. Se presentan datos, caracteres descriptivos, distribución e información biológica.

The greatest diversity of cynipid gall wasps (Hymenoptera: Cynipidae, Cynipini) in the world is found in the Nearctic region, especially in the United States and Mexico, with more than 600 described species. Burks (1979) listed 485 species of Cynipini in the United States, but several additional species have since been described (Melika & Abrahamson 1997a, b, 2000; Abrahamson et al. 1998a, b). However, the alternate generations are known for only a few species (Doutt 1959, 1960; Dailey & Sprenger 1973a, b; Dailey et al. 1974; Evans 1967, 1972; Lyon 1959, 1963, 1964, 1969a, b, 1970, and others).

Diagnosis. The galls of the asexual generation of Callirhytis quercuscornigera (Osten Sacken) are similar to those of C. pomiformis (Bassett), C. punctata (Osten Sacken), C. quercusclavigera (Ashmead), C. quercuspunctata (Bassett), C. quercussuttonii (Bassett), and C. seminoso (Bassett) (McCracken & Egbert 1922, Weld 1959, Lyon 1969b). However, the only other gall in which the larval chambers protrude externally is induced by C. pomiformis (McCracken & Egbert 1922), but leaf galls induced by C. quercuscornigera occur on the midveins, large lateral veins, and infrequently on petioles and tiny lateral veins (Eliason & Potter 2000).

Taxonomic comments. Osten Sacken (1862) first described the gall-maker, based on stem gall characteristics, as Cynips quercus cornigera. He later reared two specimens, a female and possibly a male, from the same gall and named the wasp Cynips cornigera based on the female (Osten Sacken 1865). Because species names based on gall descriptions before 1930 are valid, the appropriate name for this species is Callirhytis quercuscornigera (Osten Sacken 1862), comb. rev.

Description. Sexual generation. Female. Head, except mandible, scutum, and scutellum black; mesopleuron, propodeum, metasoma dark brown; antenna, mandible, and legs uniformly yellow brown. Head as broad as mesosoma, rounded, as high or very slightly higher than broad in front view; gena not broadened behind eye; malar space without sulcus, 3.5-3.7 times as short as eye height. Ocelli small, ocellar-ocular distance shorter than post-ocellar distance; distance between antennal sockets nearly equal to diameter, shorter than dis-
tance to inner margin of eye. Head finely coriaceous, except rugose lower face; clypeus separated from face by deep depression. Antenna 14-segmented (sometimes suture which indicated F12 indistinct); scape as long as pedicel, nearly twice as long as broad; F1-F4 filiform, subsequent flagellomeres broadened (Fig. 3). Mesosoma longer than high in lateral view (Fig. 1), scutum slightly broader than long in dorsal view, alutaceous, shiny, without setae, with complete notauli, median dorsal line absent or present in a form of very short triangular depression; anterior parallel and parapsidal lines absent. Mesopleuron uniformly finely coriaceous. Scutellum elongate, longer than broad in dorsal view, finely coriaceous anteriorly, with more dull sculpture posteriorly (Fig. 2); scutellar foveae with distinct carina separating them (Fig. 2). Forewing 1.4 times as long as body, pubescent, with cilia on margin, veins brown and with areolet closed; radial cell narrow, elongate, nearly 6.0 times as long as broad; cubitalis (Rs+M) joint basalis in upper ⅓ (Fig. 4). Tarsal claws without basal lobe, simple. Central portion of propodeum smooth, shiny, limited by distinct lateral longitudinal carinae, which only slightly bend outward posteriorly; side of propodeum coriaceous. Metasoma smooth, shiny, slightly longer than high in lateral view, ventral spine of hypopygium short; anterior parallel and dian dorsal line absent or present in a form of very slight swellings on small diameter branches. Twenty-two month old stem galls are smooth in texture, green-colored externally, and the internal gall tissue is pale yellow. The larval chambers, or horns, begin to push through the thin gall epidermis when galls are ~24 months old. Horns project up to 6 mm from the rounded, succulent stem galls, and harden after about a month. One asexual C. quercuscornigera larva develops at the base of the larval chamber. Horns break off several months after adults chew a circular exit hole and leave the galls at bud burst in the spring.

Asexual females exit ~33-month-old stem galls and oviposit into swelling buds (Eliason & Potter 2000). Eggs are deposited next to the midvein or large lateral veins, and are slightly embedded into the leaf tissue on the abaxial leaf surface. One larva lives in each leaf gall, but two or more galls may develop next to each other. Maximum leaf gall length (~2.0 mm) occurs by late May. Larvae completely consume the moist nutritive tissue, leaving only a thin gall layer around the final instar. Pupation occurs in May and adults exit in late May or early June.

**Ceroptres cornigera** MELIKA AND BUSS, NEW SPECIES (Figs. 5-11)

We describe a new species of inquiline, *Ceroptres cornigera*, reared from galls of the sexual and asexual generations of a cynipid gall wasp, *Callirhytis quercuscornigera*, on stem and leaf galls of pin oak, *Quercus palustris*. We also include relevant biological information potentially useful in distinguishing this species from other *Ceroptres* species.

Diagnosis. Similar to *Ceroptres quercusarbo* (Fitch, 1859) and *C. quercustuber* (Fitch, 1859), both reared from stem-swelling like galls of *Callirhytis clavula* (Osten Sacken). In *Ceroptres cornigera* submedian pits on the pronotum are distinct and large, separated with a strong carina; the entire body is black, antenna and legs are bright
brown. In *C. quercusarbo* and *C. quercustuber* submedian pits of the pronotum are indistinct and separated by a weak, narrow carina; the mesosoma only is black, gaster brown, face and mouthparts lighter than the rest of the head; antennae and legs are yellow.
Description. Female. Head, mesosoma, and gaster uniformly black; antenna and legs bright brown. Head broader than thorax, broader than high in front view, gena very slightly broadened behind eye, indistinct striae converging toward clypeus, malar space 0.37 times eye length (Fig. 5); entire head very finely punctate, with dense short white setae on face. Antenna 12-segmented (suture between F10 and F11 invisible), filiform; F1 very slightly longer than F2, F3 equal F2, subsequent flagellomeres progressively shorter, but F10 longer than F8+F9 (Fig. 7). Mesosoma shorter than gaster (Fig. 8); pronotum with two elliptical submedian pits with smooth shiny bot-
toms, broadly separated medially. Scutum nearly as long as broad in dorsal view, finely transversely rugose, with notauli distinct in posterior ⅓; median line short, anterior parallel lines distinct in anterior ⅓ (Fig. 6). Scutellum rounded, rugose, with much rougher sculpture than scutum (Fig. 6). Mesopleuron smooth and shiny. Fore wing hyaline, pubescent, with cilia on margin; radial cell closed, 2.62.7 times as long as broad; veins pale yellow (Fig. 9). Tarsal claws with strong teeth. Metasoma nearly as long as mesosoma and head together, as high as long; terga 2 and 3 fused into one large segment occupying nearly whole of gaster, smooth, shiny, with white setae at the base; indistinct fine suture present between terga 2 and 3. Subsequent terga are finely punctate (Fig. 8). Ventral spine of hypopygium short, slender. Length 1.62.1 mm.

**Male.** Differed from female in having 15-segmented antenna, F1 slightly curved and extended proximally (Fig. 10); petiole distinct, longer than in female (Fig. 11); legs and antenna lighter. Length 1.5-1.8 mm.

**Types.** Holotype female from Lexington, Fayette Co., Kentucky, from asexual galls of *Callirhytis quercuscornigera*, collected on *Quercus palustris* (Muenchhausen), 21 April 1998, emerged 18 May 1998. Also 17 female and 18 male paratypes from the same locality, from sexual and asexual galls of *C. quercuscornigera* on *Q. palustris*. Holotype, 5 female and 5 male paratypes in the National Museum of Natural History, Smithsonian Institution, Washington, D.C. (USNM); 5 female and 5 male paratypes in the American Museum of Natural History, New York City (AMNH); 2 female and 3 male paratypes in the Department of Entomology, University of Kentucky, and 5 female and 5 male paratypes in the collection of Systematic Parasitoid Laboratory, Koszeg, Hungary.

**Material examined.** We examined two similar *Ceroptris* species from the eastern USA, *Ceroptris quercusarbos* and *C. quercustuber*, known as inquilines in stem swelling-like galls of *Callirhytis clavula*. The type material for the first species, deposited in the USNM, was destroyed with only Fitch's original label remaining without an insect on the pin. Barnes (1988) mentioned only one type locality for this species: "Salem, NY, 19.iv.1859, find several of these galls on Titus's Hill." Five females and one male from the USNM were also examined. Two females are labeled as "Accession No 6032d AD Hopkins W. Va., "Ceroptris tuber Fitch," 1 female as "N. Brunsw. NJ", "Coll. Ashmead", "Ceroptris tuber Fitch," 1 female "Portsmouth, 6.28.97," 1 female "Riley Coll., Marlat," "Ceroptris tuber Fitch," and 1 male "Toronto, Ont.; "Ceroptris tuber Fitch".

*Ceroptris quercusarbos* was described by Fitch (1859) on the basis of one male as "a small black gall-fly having all its legs and antennae of a bright pale yellow color." This is the only description of the wasp given by the author. Such a coloration is typical for nearly all *Ceroptris* species. Fitch (1859) described *Ceroptris quercustuber* as "a small black gall-fly with dull pale yellow antennae, mouth and legs, its hind shanks and its antennae towards their tips being dusky, its length 0.08 and to the tips of its wings 0.13". This species was described on the basis of both the female and male. Fitch (1859) thought that he had described the gall-inducing insects, but they were inquilines.

Other *Ceroptris* species known from the eastern USA differ strongly from this new species not only in the morphology of the adults, but also in their distribution and trophic associations. Thus, we did not compare the new species to them.

**Distribution.** Kentucky (Fayette Co., Lexington).

**Etymology.** Named from the host gall, *Callirhytis quercuscornigera*, from which it was reared.

**Biology.** Members of this species are inquilines in galls of the asexual and sexual generations of *Callirhytis quercuscornigera*, which associates with *Q. palustris* and other red oaks (Burks 1979).

*Ceroptris cornigera* is one of several inquilines and opportunistic insects living within stem galls, but it is the only inquiline of the leaf galls. It is the first known species of *Ceroptris* to inhabit galls from both alternating generations of its host cynipid. *Ceroptris cornigera* larvae develop in the succulent tissue below a *Callirhytis quercuscornigera* larval chamber in young stem galls. It is uncertain whether or not this inquiline contributes to gall-maker mortality. Twenty-four female and 17 male *Ceroptris cornigera* adults emerged from stem galls collected in spring 1998. Most galls from which *Ceroptris* adults emerged (219 adults from 86 galls reared in 1999) lacked external horns and were of small diameter (2.5 ± 0.1 cm). However, horns typically protrude when galls are 25 months old. Adults may live as many as 6 days without a water or sugar source. Mean female body length is 2.2 ± 0.1 mm, and mean ovipositor length, when dissected and uncoiled, is also 2.2 ± 0.1 mm (Eliason & Potter 2000). When adults emerge in May, female ovaries are not fully mature, and may contain >100 eggs per female.
Individual *Ceroptres cornigera* develop in each leaf gall, and kill the gall-maker larva while feeding on the succulent gall tissue. In 1998, more than 1000 leaf galls were placed in individual transparent gelatin capsules in the laboratory. Of the 713 galls from which wasps emerged, 37.3% were *Ceroptres cornigera* (138 female, 128 male). Adults may live as many as 4 days without a wa-
ter or sugar source. When adults emerge in June and July, female ovaries are not fully mature (Eliason & Potter 2001).

ACKNOWLEDGMENTS

We are obliged to D. Smith and C. Anderson for sending us the types and some Ceroptres specimens from the National Museum of Natural History, Smithsonian Institution, Washington, DC. We also thank E. Foki (Systematic Parasitoid Laboratory) for the illustrations, and M. Bechtold (Systematic Parasitoid Laboratory) for mounting and labeling the type material. J. H. Frank kindly translated the abstract. This is Journal Article No. R-09002 of the Florida Agricultural Experiment Station.

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OLD TRAPS FOR NEW WEEVILS: NEW RECORDS FOR CURCULIONIDS (COLEOPTERA: CURCULIONIDAE), BRENTIDS (COLEOPTERA: BRENTIDAE) AND ANTHRIBIDS (COLEOPTERA: ANTHRIBIDAE) FROM JEFFERSON CO., FLORIDA

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ABSTRACT

Trapping studies using three different types of traps placed in several wild and cultivated habitats in North Florida (Jefferson Co.) produced 97 different species of adult weevils in the family Curculionidae and the closely related primitive families Anthribidae, Brentidae and Ithyceridae. Sixty-one of 97 species trapped have not been reported previously from Jefferson Co. Furthermore, seven species of Curculionidae are first records for the state, with four of these being important agricultural pests (Hypera meles, H. nigrirostris, H. punctata and Sitona lineatus). In addition, some weevils taken in our traps represent undescribed species of Apteromechus, Cercopeus and Conotrachelus. Herein we include: (1) an alphabetical listing of species collected in these traps, (2) a description and illustration of the traps used, and (3) months of the year and generalized habitats where traps were placed and specimens were collected. Information on host associations is provided for many species.

Key Words: Tedders trap, Stinkbug trap, Circle trap, Florida, invasive weevils

RESUMEN

Estudios de trampeo utilizando tres diferentes tipos de trampas colocadas en áreas cultivadas y silvestres en el norte de Florida (Jefferson Co.) resultaron en la colecta de 97 diferentes especies de curculiónidos pertenecientes a la familia Curculionidae y a las familias primitivas de curculiónidos Anthribidae, Brentidae e Ithyceridae. Sesenta y una de las 97 especies capturadas constituyen primeros reportes para estas especies en el condado de Jefferson. Asimismo, siete de las especies capturadas nunca habían sido reportadas en el estado de Florida y cuatro de ellas son plagas agrícolas de suma importancia (Hypera meles, H. nigrirostris, H. punctata y Sitona lineatus). Adicionalmente, algunas de las especies capturadas representan nuevas especies pertenecientes a los géneros Apteromechus, Cercopeus y Conotrachelus. En este artículo incluimos: (1) un listado alfabetico de las especies capturadas en las trampas, (2) una descripción e ilustración de las trampas utilizadas, y (3) los meses del año y descripción de las áreas donde las trampas fueron colocadas y los especímenes colectados. También hemos incluido información sobre las plantas hospederas para muchas de las especies capturadas.

Translation provided by author.

Weevils (Coleoptera: Curculionidae) are extremely important insects as plant pests and as beneficial biological control agents for noxious weeds. More than 863 genera and 7,000 species currently are recognized in North America (O’Brien & Wibmer 1982). Native species such as the plum curculio, Conotrachelus nenuphar (Herbst), and the cosmopolitan maize weevil, Sitophilus zeamais (Motschulsky), feed on everything from agronomic and fruit crops to stored products. Thousands of foreign species, particularly from the Caribbean and southeast Asia, are potential invaders of the United States, following the pattern of such destructive exotic pests as the boll weevil, Anthonomus grandis grandis Boheman, and the recently detected Myllocerus undatus Marshall in Florida (C. W. O., unpublished data).

The Florida beetle fauna is one of the most diverse in North America, given the many tropical species entering south Florida from the West Indies (Peck & Thomas 1998). In the most recent distributional checklist for the Coleoptera of Florida (Peck & Thomas 1998) the authors indicate...
that close to 18% (or = 825 species) belong to the superfamily Curculionoidea (as defined in Alonso-Zarazaga & Lyal 1999). Some of the species can be very abundant at particular sites and times of the year and, as such, can be collected with relative ease. An example of this is the Fuller rose beetle, Naapactus cervinus (Boheman) (R. F. M., unpub-lished data). However, many Curculionoidea are cryptic and nocturnally active, and collecting them can prove difficult. As a result, exotic pest weevils entering the state may remain undetected for many years until their population builds-up to economically important levels.

We conducted a series of trapping studies over a period of nine years (1993-2001) in several wild and cultivated habitats in North Florida near the town of Monticello. Three different types of traps previously reported as effective for capturing a variety of economically important weevils (Ted-ders & Wood 1994, Mizell & Tedders 1999) were tested for their ability to capture other species. Ninety-seven different species of adult weevils, Curculionidae, and the closely related primitive families Anthribidae, Brentidae and Ithyceridae (as treated in Alonso-Zarazaga & Lyal 1999) were collected. In this paper we include: (1) a table with information on all of the species collected, (2) months of the year when specimens were collected, (3) descriptions and photographs of the traps used, and (4) information on host associations for most species. Our results are discussed in the context of the importance of visual cues for weevils and the use of traps in early detection of pest weevil introductions.

MATERIALS AND METHODS

Sites

Traps were placed in various wild and cultivated habitats on the grounds of the University of Florida, North Florida Research and Education Center in Monticello, FL, which is located in Jeff-erson County about 16 km S of the Florida-Georgia state line. Different traps or trap combinations were placed in pecan (PCN) (Carya illinoiensis) and peach (PCH) (Prunus persica) orchards and in small plantings of Japanese persimmon (PSN) ( Diospyros kaki) (Table 1). These cultivated areas are interspersed with several wet woodland sites (WD) containing tree species that are typical for North Florida, including slash pine (Pinus elliottii), loblolly pine (P. taeda), longleaf pine (P. palustris), tupelo or black gum (Nyssa sylvatica), sweet gum (Liquidambar styraciflua), water oak (Quercus nigra), red oak (Quercus rubra) and shagbark hickory (Carya ovata) (USDA 1989). In addition, other weedy, for-age and ornamental plant species were present in the surrounding habitats. Among these were mixed bahia-grass (Paspalum notatum), vetch (Vicia sativa), red clover (Trifolium pratense), rabbit-eye blueberries (Vaccinium ashei), Lan-tana camara, and Sesbania spp.

Traps. Three types of traps were tested for their ability to capture weevils. We used two ac-tive pyramidal-shaped traps (Figs. 1a and 1b) similar (equal dimensions) to those described in Tedders and Wood (1994) and Mizell and Tedders (1999). Trap bodies were manufactured from 1.3 cm thick masonite and painted black (Ace® acrylic flat latex house paint #103A105) or safety yellow (Glidden® alkyd industrial formula #4540). The screen cone and collecting cylinder components of the boll weevil trap (Anonymous 1990) were used to capture the adult weevils in the black traps, while the window screen top collection device described by Mizell and Tedders (1995) was used to capture the weevils in the yellow pyramidal trap. In this manuscript we refer to the black pyramidal trap as a Tedders trap (T in Table 1) and the yellow pyramidal trap as a Stinkbug trap (SB) (after Mizell & Tedders 1995). The third trap used was a passive Circle trap (C) (Fig. 1c), made of aluminum insect screening, similar to the one described by Mulder et al. (2000) to capture the pecan weevil and the plum curculio in Oklahoma. As above for the Tedders trap, a boll weevil trap top was modified to fit the top of the circle trap to capture adult weevils.

The total number of traps of each type varied from year to year, but at no time were fewer than 20 traps of each type present in the field. Tedders, Stinkbug and Circle traps were placed in all hab-itations except for peach orchards, which did not re-ceive Circle traps. All traps were unbaited and were serviced 1-3 times per week throughout the course of the study. Trap tops were emptied, cleaned of debris and replaced. Captured insects were preserved in 95% ethyl alcohol and brought back to the laboratory, where a representative se ries of all weevils from each trap were mounted and labeled. Taxonomic determinations to species were made by C.W.O. Trap(s), habitat(s) and month(s) of the year when each species of weevil was collected were summarized, and the informa-tion is presented in Table 1. Species for which host associations are provided in the text are indi-cated in Table 1, as are the species that have been introduced into North America.

RESULTS AND DISCUSSION

Ninety-seven species of adult Curculionidae and the closely related primitive families Anthri-bidae, Brentidae and Ithyceridae were collected in our traps from 1993-2001 (Table 1). The preponderance of species collected was advanced weevils (89 species), with only six species of Anthibidae, one species of Brentidae and the one species of Ithyceridae being captured. Table 1 indicates the species for which host associations are provided in
### Table 1. Summary of trapping results in Jefferson County, Florida where three different types of traps were evaluated for their ability to capture weevils in several wild and cultivated habitats from 1993-2001. The traps used were black Tedders traps (T), yellow Stinkbug traps (SB) and Circle traps (C). The first column indicates the family (F) to which each species belongs (Anthribidae (A), Brentidae (B), Ithyceridae (I) or Curculionidae (C)) and whether the capture represents a new record for Jefferson County (*) or for the state of Florida (**). Intro ? indicates whether or not the species is exotic to North America. An "x" in the column for Host info ? indicates whether additional host association information is provided in the text.

<table>
<thead>
<tr>
<th>F</th>
<th>Genus, species and author</th>
<th>Habitat(s) where traps were placed¹</th>
<th>Type of trap</th>
<th>Month(s) when captures occurred</th>
<th>common name (ESA 1997)²</th>
<th>Intro?</th>
<th>Host info?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Araecerus coffeae (Fabricius)</td>
<td>PCH</td>
<td>T SB C</td>
<td>V</td>
<td>(coffee bean weevil)</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>A*</td>
<td>Brachycorynus rectus (LeConte)</td>
<td>PCN, PSN</td>
<td>x x</td>
<td>VIII</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>A</td>
<td>Euparius marmoreus (Olivier)</td>
<td>PCH, PCN</td>
<td>x x x</td>
<td>I-IV, VI</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>A*</td>
<td>Goniocthes bimaculatus (Olivier)</td>
<td>WD</td>
<td>x</td>
<td>III</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>A*</td>
<td>Piesocorynus mixtus LeConte</td>
<td>WD</td>
<td>x</td>
<td>III, IV</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>A</td>
<td>Toxonotus cornutus (Say)</td>
<td>PCH</td>
<td>x</td>
<td>II, IV</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>B*</td>
<td>Arrhenodes minutus (Drury)</td>
<td>WD</td>
<td>x</td>
<td>V, XII</td>
<td>oak timberworm</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
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<td>Ithycerus noveboracensis (Forster)</td>
<td>WD, PSN</td>
<td>x</td>
<td>IV, V</td>
<td>the New York weevil</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
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<td>Acalles porosus Blatchley</td>
<td>PCH, PCN, PSN</td>
<td>x</td>
<td>IV-V, VIII</td>
<td></td>
<td>no</td>
<td>no</td>
</tr>
<tr>
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<td>PCH</td>
<td>x</td>
<td>V</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C*</td>
<td>Anthonomus quadrigibbus Say</td>
<td>PCH, WD</td>
<td>x</td>
<td>III-IV</td>
<td>apple curculio</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C*</td>
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<td>x x x</td>
<td>V-IX, XI</td>
<td></td>
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</tr>
<tr>
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<td>Apinocis sp. 1</td>
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<td>x</td>
<td>?</td>
<td></td>
<td>no</td>
<td>no</td>
</tr>
<tr>
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<td>Apermethexus ferratus (Say)</td>
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<td>III, VII, VIII</td>
<td></td>
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</tr>
<tr>
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<td>x</td>
<td>III</td>
<td></td>
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</tr>
<tr>
<td>C*</td>
<td>Atrichonotus taeniatus (Berg)</td>
<td>PCH</td>
<td>x</td>
<td>VI-IX</td>
<td>yes</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Bagous magister LeConte</td>
<td>PCH, PSN</td>
<td>x</td>
<td>II</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C**</td>
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<td>IV-VI</td>
<td>(grey persimmon weevil)</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>Baris sp. 1</td>
<td>WD</td>
<td>x</td>
<td>?</td>
<td></td>
<td>no</td>
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<tr>
<td>C*</td>
<td>Chalcodermus aeneus Boheman</td>
<td>PCH, PSN, WD</td>
<td>x x x</td>
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<td>cowpea curculio</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
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<td>Chalcodermus collaris Horn</td>
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<td>V, VIII</td>
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<tr>
<td>C</td>
<td>Cercopes new sp. # 18</td>
<td>PCH</td>
<td>x</td>
<td>II</td>
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<td>no</td>
<td>no</td>
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<td>C*</td>
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<td>x</td>
<td>II</td>
<td></td>
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<td>no</td>
</tr>
<tr>
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<td>x</td>
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<tr>
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<td>x</td>
<td>IV</td>
<td></td>
<td>no</td>
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</tr>
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<td>PCH, PSN, WD</td>
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<td>III-IV, VIII</td>
<td></td>
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</tr>
<tr>
<td>C*</td>
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<td>PCH, PCN, WD</td>
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<td>III-VI</td>
<td></td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>C</td>
<td>Conotrachelus geminatus LeConte</td>
<td>PCN</td>
<td>x</td>
<td>VI</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
</tbody>
</table>

¹PCN = pecan, PCH = peach, PSN = Japanese persimmon, WD = wet woodland.
²names in parenthesis are common names not accepted by ESA (1997).
Table 1. (Continued) Summary of trapping results in Jefferson County, Florida where three different types of traps were evaluated for their ability to capture weevils in several wild and cultivated habitats from 1993-2001. The traps used were black Tedders traps (T), yellow Stinkbug traps (SB) and Circle traps (C). The first column indicates the family (F) to which each species belongs (Anthribidae (A), Brentidae (B), Thysanidae (I) or Curculionidae (C)) and whether the capture represents a new record for Jefferson County (*) or for the state of Florida (**) . Intro? indicates whether or not the species is exotic to North America. An "X" in the column for Host info? indicates whether additional host association information is provided in the text.

<table>
<thead>
<tr>
<th>F</th>
<th>Genus, species and author</th>
<th>Habitat(s) where traps were placed1</th>
<th>Type of trap</th>
<th>Month(s) when captures occurred</th>
<th>common name (ESA 1997)2</th>
<th>Intro?</th>
<th>Host info?</th>
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<tbody>
<tr>
<td>C*</td>
<td>Conotrachelus hicorniae Schoof</td>
<td>PCH, PCN, PSN, WD</td>
<td>x x x</td>
<td>IV, VI-IX</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>Conotrachelus naso LeConte</td>
<td>PCH, PCN, PSN, WD</td>
<td>x x x</td>
<td>IV-IX, XII</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>Conotrachelus nenuphar (Herbst)</td>
<td>PCH, PCN, WD</td>
<td>x x</td>
<td>II-VIII, XI</td>
<td>plum curculio</td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C*</td>
<td>Conotrachelus posticatus Boheman</td>
<td>PCH, PCN, PSN, WD</td>
<td>x x x</td>
<td>III-XI</td>
<td></td>
<td>no</td>
<td>x</td>
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<tr>
<td>C*</td>
<td>Conotrachelus similis Boheman</td>
<td>PCH, PSN, WD</td>
<td>x x</td>
<td>VI</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C</td>
<td>Conotrachelus new sp. # 9</td>
<td>PCH</td>
<td>x x</td>
<td>VIII-IX</td>
<td></td>
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</tr>
<tr>
<td>C</td>
<td>Conotrachelus new sp. # 12</td>
<td>PCH</td>
<td>x</td>
<td>IV</td>
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<tr>
<td>C*</td>
<td>Cophes fallax (LeConte)</td>
<td>PCH, PCN, PSN, WD</td>
<td>x x x</td>
<td>II-VI, VII-X-XII</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C*</td>
<td>Cophes oblongus (LeConte)</td>
<td>PCH, PCN, PSN, WD</td>
<td>x x</td>
<td>V-VI, VIII</td>
<td></td>
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<tr>
<td>C*</td>
<td>Cophes obtentus (Herbst)</td>
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<td></td>
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<tr>
<td>C</td>
<td>Cossonus corticola Say</td>
<td>WD</td>
<td>x</td>
<td>VII</td>
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<td>no</td>
<td>x</td>
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<tr>
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<td>Cossonus impressifrons Boheman</td>
<td>WD</td>
<td>x</td>
<td>VII</td>
<td></td>
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<tr>
<td>C*</td>
<td>Cryptorhynchus fuscaetus LeConte</td>
<td>PCN, PSN, WD</td>
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<td>I, III-VI, VIII-X, XII</td>
<td></td>
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<tr>
<td>C*</td>
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<td>C*</td>
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<tr>
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<tr>
<td>C</td>
<td>Curculio caryae (Horn)</td>
<td>WD</td>
<td>x</td>
<td>IX-XI</td>
<td>pecan weevil</td>
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<tr>
<td>C*</td>
<td>Curculio fulvus Chittenden</td>
<td>PCN, PSN</td>
<td>x</td>
<td>X</td>
<td></td>
<td>no</td>
<td>x</td>
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<tr>
<td>C*</td>
<td>Curculio humeralis (Casey)</td>
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<td>x</td>
<td>IX</td>
<td></td>
<td>no</td>
<td>x</td>
</tr>
<tr>
<td>C*</td>
<td>Curculio longidens Chittenden</td>
<td>PCN</td>
<td>x</td>
<td>IX</td>
<td></td>
<td>no</td>
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<td>C*</td>
<td>Curculio pardinum (Chittenden)</td>
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<td>x</td>
<td>X</td>
<td></td>
<td>no</td>
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<tr>
<td>C*</td>
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<td>X-XII</td>
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<td>x</td>
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<td>C*</td>
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<td>Curcupedium castaneus (Roelofs)</td>
<td>PCN, PSN, WD</td>
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<td>I-XII</td>
<td>Asiatic oak weevil yes</td>
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<td>x</td>
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<td>Eubulus bisignatus (Say)</td>
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<td>x</td>
<td>III</td>
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<td>VI-VIII</td>
<td>no x</td>
<td>x</td>
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<td>Eudiaogus rosenschœldi Fahraeus</td>
<td>PCH, PSN, SES</td>
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<td>C*</td>
<td>Geraeus penicillla (Herbst)</td>
<td>PCH, WD</td>
<td>x</td>
<td>III, VI</td>
<td></td>
<td>no</td>
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</tr>
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</table>

1PCN = pecan, PCH = peach, PSN = Japanese persimmon, WD = wet woodland.
2Names in parenthesis are common names not accepted by ESA (1997).
<table>
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<tr>
<th>F</th>
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<th>Habitat(s) where traps were placed</th>
<th>Type of trap</th>
<th>Month(s) when captures occurred</th>
<th>common name (ESA 1997)</th>
<th>Intro?</th>
<th>Host info?</th>
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<td>C</td>
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<td>x</td>
<td>?</td>
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<td>no</td>
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<tr>
<td>C</td>
<td>Geraeus sp. 2 (male)</td>
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<td>x</td>
<td>?</td>
<td></td>
<td>no</td>
<td>no</td>
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<td>I-V, VII, XI</td>
<td>pales weevil</td>
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<td>x</td>
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<td>Hypera meles (Fabricius)</td>
<td>PCN</td>
<td>x</td>
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<td>C**</td>
<td>Hypera nigrirostris (Fabricius)</td>
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<td>V, VII</td>
<td>lesser clover leaf weevil</td>
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<td>x</td>
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<td>V, VIII, IX</td>
<td>alalfa weevil</td>
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<td>Listroderes apicalis Waterhouse</td>
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<td>Madarellus undulatus (Say)</td>
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<td>Myrmex floridanus (Casey)</td>
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<td>Fuller rose beetle</td>
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<td>Naupactus cervinus (Boheman)</td>
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<td>x x x</td>
<td>II-VIII, XII</td>
<td>(white-fringed beetle)</td>
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<td>PCH, PCN, PSN, WD</td>
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<td>VI-IX</td>
<td></td>
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<td>V</td>
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<td>VII</td>
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<td>VII</td>
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<td>V-VIII, X-XI</td>
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<td>x</td>
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<tr>
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<td>Pachylobius picivorus (Germar)</td>
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<td>x x</td>
<td>III-VIII</td>
<td>pitch-eating weevil</td>
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<td>Pandeleitis hilaris (Herbst)</td>
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<td>x x x</td>
<td>I-III, V-VI, VIII-XII</td>
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<td>Pheloconus cricicollis (Say)</td>
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<tr>
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<td>Phyrenus divergens (Germar)</td>
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<td>C*</td>
<td>Pissodes nemorensis Germar</td>
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<td>XII</td>
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<td>IX</td>
<td>clover root curculio</td>
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</tbody>
</table>

*PCN = pecan, PCH = peach, PSN = Japanese persimmon, WD = wet woodland.
*names in parenthesis are common names not accepted by ESA (1997).
Table 1. (CONTINUED) SUMMARY OF TRAPPING RESULTS IN JEFFERSON COUNTY, FLORIDA WHERE THREE DIFFERENT TYPES OF TRAPS WERE EVALUATED FOR THEIR ABILITY TO CAPTURE WEEVILS IN SEVERAL WILD AND CULTIVATED HABITATS FROM 1993-2001. THE TRAPS USED WERE BLACK TEDDERS TRAPS (T), YELLOW STINKBUG TRAPS (SB) AND CIRCLE TRAPS (C). THE FIRST COLUMN INDICATES THE FAMILY (F) TO WHICH EACH SPECIES BELONGS (ANTHRIBIDAE (A), BRENTIDAE (B), ITHYCERIDAE (I) OR CURCULIONIDAE (C)) AND WHETHER THE CAPTURE REPRESENTS A NEW RECORD FOR JEFFERSON COUNTY (*) OR FOR THE STATE OF FLORIDA (**). Intro? indicates whether or not the species is exotic to North America. An “X” in the column for Host info? indicates whether additional host association information is provided in the text.

<table>
<thead>
<tr>
<th>F</th>
<th>Genus, species and author</th>
<th>Habitat(s) where traps were placed</th>
<th>Type of trap</th>
<th>Month(s) when captures occurred</th>
<th>common name (ESA 1997)²</th>
<th>Intro?</th>
<th>Host info?</th>
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<td><em>Sitona lineatus</em> (Linneaus)</td>
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<td>T</td>
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<td>pea leaf weevil</td>
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<td>x</td>
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<tr>
<td>C*</td>
<td><em>Sitona lineellus</em> (Bonsdorff)</td>
<td>PCN, PSN</td>
<td>x</td>
<td>VI-IX</td>
<td>yes</td>
<td>x</td>
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<tr>
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<td><em>Sitophilus oryzae</em> (Linnaeus)</td>
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<td>x</td>
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<td><em>Sitophilus zeamais</em> Motschulsky</td>
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<td>IV, VI-VII</td>
<td>maize weevil</td>
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<td>x</td>
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<td>PSN</td>
<td>x</td>
<td></td>
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<td>IX</td>
<td>no</td>
<td>x</td>
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</tr>
<tr>
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<td><em>Sphenophorus coesiros</em> Gylenhal</td>
<td>PCN, PCN, PSN, WD</td>
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<td>x</td>
<td>V, VII</td>
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<td>II-III, VI, IX</td>
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<tr>
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<td><em>Tyloderma foveolatum</em> (Say)</td>
<td>PCN, PSN</td>
<td>x</td>
<td>IV-VI</td>
<td>no</td>
<td>x</td>
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</tr>
<tr>
<td>C*</td>
<td><em>Tyloderma variegatum</em> (Horn)</td>
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<td>x</td>
<td>VI</td>
<td>no</td>
<td>x</td>
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</tr>
</tbody>
</table>

¹PCN = pecan, PCH = peach, PSN = Japanese persimmon, WD = wet woodland.
²Names in parenthesis are common names not accepted by ESA (1997).
the text (see below under First records from Florida and Host Associations). Host information was not available for 21 species of Curculionidae. Sixty-one (or almost 63%) of the species captured have not been reported previously from Jefferson Co., FL (* in Table 1) and seven species are first records for the state (** in Table 1) (see earlier list in Peck & Thomas 1998). More importantly, four of the first time records for Florida are for species that are important agricultural pests in other parts of the United States (Hypera meles, H. nigrirostris, H. punctata and Sitona lineatus). Some weevils collected in our traps represent undescribed species belonging to Apteromechus, Cercopoeus and Conotrachelus. The taxonomic descriptions of the new species will be published elsewhere.

Several economically important species captured in our traps have had a number of taxonomic name changes over the past five decades, which can cause confusion when searching the literature for pest information. We believe it is use-
ful to list some of these names here in order to aid researchers in their studies of these weevils. The anthribid weevil, *Araecerus coffeae* (Fabricius), was known as *A. fasciculatus* DeGeer until 1994, when Zimmerman published notes on the long ignored synonymy of this widespread stored product pest (Zimmerman 1994). However, there is controversy over this synonymy and, as such, the name changes may continue for this important pest species. The vegetable weevil, *Listroderes difficilis* Germain, has been called *L. costirostris* Schoenherr, which is actually a distinct Argentine species known in the United States only from California and Arizona (O'Brien & Wibmer 1982). The Fuller rose beetle, *Naupactus cervinus* (Boheman), has been introduced into many countries from its home in Argentina, including into the United States (Lanteri et al. 2002). It has been placed in and referred to as belonging to the genera *Aramigus*, *Asynonychus*, *Pantomorus* and *Naupactus*, the latter in which it is currently placed (Alonso-Zarazaga & Lyal 1999). In addition, its specific name has several junior synonyms that have been used in older literature, including *Aramigus fulleri* (Horn), *Asynonychus godmanni* (Crotch), *Pantomorus olindae* (Perkins) and *Naupactus simplex* Pascoe. Finally, *Naupactus peregrinus* (Buchanan) is another species still referred to under several different genera including *Graphognathus* and *Pantomorus*. It is important that researchers be aware of all names used for the species they investigate if they wish to make use of earlier research studies regarding life history, distribution, control options or information on natural enemies. Sources of such names are found most often in catalogs or checklists, as well as in revisions and monographs of many groups of economically important insects.

First records for Florida

1) A single adult of *Acanthoscelidius curtus* (Say) was found on 19 May 1995 in a Stinkbug trap placed in a peach orchard. We continued to have Stinkbug traps at this location, however, no other specimens of this species were collected. Blatchley and Leng (1916) report it as occurring on *Polygonum* sp. in swamps in New York. *Polygonum* spp. (Polygonaceae) are commonly known as knotweeds and grow in marshes, swamps, wet forests and ditches. *Acanthoscelidius curtus* is found in South Carolina, Kentucky, Virginia and North Carolina, as well as in the north eastern and north central United States (O'Brien & Wibmer 1982). Two other species of *Acanthoscelidius*, *A. acephalus* (Say) and *A. medicus* (Dietz), are listed as occurring in north Florida (Peck & Thomas 1998) but were not collected in our study. Most *Acanthoscelidius* species breed in evening primrose (*Oenothera* spp.) (C. W. O., unpublished data).

2) We collected specimens of *Brachystylus acutus* (Say) in Tedders and Circle traps placed in pecan and Japanese persimmon orchards. Very few specimens were obtained (8 total) and these collections occurred from April to June in 2000 and in June 2001. Blatchley and Leng (1916) call this species the gray persimmon weevil (although this is not an approved ESA common name, ESA 1997) and report that it has been collected from hickory and persimmon and swept from low herbage. They suggest that it probably is found wherever persimmon is grown. *Brachystylus acutus* is found in Georgia, Alabama, Kentucky, Mississippi, North Carolina, South Carolina, the northeastern states, and Missouri. No other species of *Brachystylus* are reported to occur in the United States (O'Brien & Wibmer 1982).

3) We collected 40 adults of *Cryptorhynchus woodruffi* Sleeper mostly from woodland habitats in Tedders and Circle traps. Two adults were captured in traps placed in pecan orchards and one in a peach orchard. The first specimen was found in May 1999 and additional collections were made in 2000 and 2001. Sleeper (1955) described the male and female of *C. woodruffi* collected at Cranberry Bog, Buckeye Lake, Ohio in June 1953, however, no information on host associations were provided for the species.

4-6) Three of the new Florida records that are important agricultural pests belong to the genus.
**Hypera**, and include *Hypera meles* (Fabricius) (the clover head weevil), *H. nigrirostris* (F.) (the lesser clover weevil) and *H. punctata* (F.) (the clover leaf weevil). These species are native to the Old World but have been reported from the southeastern United States (O’Brien & Wibmer 1982). *Hypera meles* has been reported to feed on alfalfa (= lucerne), red, zigzag and crimson clovers and black medik. *Hypera nigrirostris* feeds on alsike, white, red, crimson and zigzag clovers, alfalfa and black medik, while *H. punctata* feeds on red, crimson and white clovers, alfalfa and Jerusalem artichoke (*Helianthus tuberosus*) in Europe (Titus 1911). We collected ten *H. meles* from Tedders traps placed in a pecan orchard in September 1993. Specimens of *H. nigrirostris* were found in both Tedders and Stinkbug traps placed in pecan and peach orchards, respectively. A few specimens also were collected in Tedders traps located in forested habitats adjacent to the orchards. These weevils were collected in July 1993 and May 1995. Adults of *H. punctata* were taken from the same traps and habitats as *H. nigrirostris*, but were collected during September and November 1993, August and November of 1994 and in June 2001. The genus *Hypera* is primarily Palearctic in distribution (Puttler et al. 1973), although seventeen species are listed as occurring in North America, six of which are introduced from Europe (O’Brien & Wibmer 1982). Until now, in North America, six of which are introduced though seventeen species are listed as occurring arctic in distribution (Puttler et al. 1973), aquatic and breeds externally on leaves of (Puttler et al. 1973), and a major pest of alfalfa and other legumes the alfalfa weevil, is an immigrant from Europe Florida (Peck & Thomas 1998).

First records for Jefferson County

Fifty-four species trapped during this study are first records for Jefferson County (designated with * in Table 1). When the seven new weevil records for the state are included, the number of species new to Jefferson County increases to 61. The list includes seven species of *Conotrachelus*, six *Curculio*, three each of *Cryptorrhynchus* and *Cophes*, two each of *Chalcocodermus*, *Eudaiagogus*, *Myrmex*, *Sitona*, *Sphenophorus*, and *Tylodderma*, and one species each of *Acalles*, *Anthonomus*, *Aphrastus*, *Atrichonotus*, *Curptepistomus*, *Geraeus*, *Hypera*, *Listroderes*, *Madarellus*, *Ochyromera*, *Odontocorynus*, *Odontopus*, *Pandeleteius*, *Phelocinus*, *Pyhrdenus*, *Pissodes*, *Pseudomus*, and *Tanyneus*. Three species of Anthribidae and one each of Brentidae and Ithyceridae are also new records for Jefferson Co.

Host associations for weevils collected during this study (see above for 1st state records)

**Anthribidae. Araecerus coffeae** (F.), the coffee bean weevil, is a well-known pest of coffee beans, but larvae can develop in seeds of many kinds of plants (Zimmerman 1994). *Brachycorynus rectus* (LeConte) has been reared from *Celtis laevigata*, black locust and dead wood of sugar maple (Valentine 1998). *Euparius marmoreus* (Olivier) feeds on several species of polypore fungi (*Trametes hirsuta*, *T. versicolor*, *Megasporoporia setulosa*, *Trichaptum biforme*, *T. abietinus*, *T. sector*, *Phlebia hydnoides*, *Panis rudis* and *Pereinopora medulla-panis*) (Valentine 1998). *Goniocloea bimaculatus* (Olivier) has been collected on *Biscogniauxia sp.* fungus on winter-killed sugar maple, on *Xylaria sp.* and *Diatrype* sp. fungi and has been also been found under bark of dead oaks (Valentine 1998). Adults and larvae of *Piosocorynus mixtus* LeConte eat pyrenomycete fungi of the order Sphaeriales, families Zylariaceae and Diatrypaceae (Valentine 1998). *Toxonotus cornutus* (Say) has been found boring in *Diospyrus* sp.), and has been collected from *Prosopis* and oak seedlings (Valentine 1998).

**Brentidae. Arrenodes minutus** (Drury) occurs beneath the bark of recently felled or dying oak, poplar and beech trees (Blatchley & Leng 1916).

**Ithyceridae. Ithycerus noveboracensis** (Forster) is associated with trees from the families Betulaceae (Carpinus caroliniana, Betula populifolia), Juglandaceae (*Juglans cinerea, Carya cordiformis, C. ovata*) and Fagaceae (*Fagus grandifolia, Castanea dentata, Quercus alba, Q. macrocarpa, Q. bicolor, Q. coccinea, Q. prinus, Q. ellipsisoides & Q. borealis*). Large numbers of adults occasionally are taken from fruit trees in the family Rosaceae,
such as apple (*Malus sylvestris*), plum (*Prunus americana*) and peach (Sanborne 1981).

Curculionidae. The larvae of *Anthonomus quadrigibbus* Say feed around the core of apple, pear and haw (*Crataegus* sp.) but are rarely very injurious. They have also been collected from flowers of red haw, *Crataegus*, and hazel, shadecloth (*Amelanchier*), and various fruit trees (Blatchley & Leng 1916). *Apteromechus ferratus* (Say), as well as other species of *Apteromechus*, breed in twigs and branches of many hardwood trees and shrubs (C. W. O., unpublished data). *Atrichonotus taeniatus* (Berg) feeds on a variety of plants, with a preference for Leguminosae. Host plants for this species include lucern, subterranean clover, bean, *hibiscus*, dahlia, rose, eucalyptus, sunflower and roots of grasses (Lanteri & O’Brien 1990). *Bagous magister* LeConte is commonly collected feeding on water lily (*Nymphaea odorata*) (C. W. O., unpublished data). *Chalcodermaeus aneus* Boheman, the cowpea curculio, infests field peas, string beans, soybean, lima bean, cotton and strawberry. Several leguminous weeds, including vetch, also are hosts (Blatchley & Leng 1916).

Schoof (1942) and references cited therein, report that larvae of *Conotrachelus affinis* Boheman attack nuts of Juglandaceae (pignut, shagbark, mockernut and bitternut hickory). *Conotrachelus anaglypticus* (Say) is considered an economic pest of peach and larvae have been reported to attack cotton bolls (*Gossypium hirsutum*), and the cambium and inner bark of several fruit and shade trees (apple, pear, pignut hickory, American hornbeam, sweet birch, American chestnut, white, chestnut, and red oak, tulip tree, service berry, red maple, tupelo, flowering dogwood and sourwood), as well as columbine (*Aquilegia*), cowpea, *Japanese* plum, mulberry elm and *Crataegus*. *Conotrachelus aratus* (Germar) attack several species of Juglandaceae (pecan, bitternut, shagbark and pignut hickory), while *C. carolinensis* Schoof attacks peaches and has been collected in cotton fields. *Phecoconus cribricollis* (Say), formerly in the genus *Conotrachelus*, have been collected on *Pinus palustris*, cotton, *Ambrosia* and peach while *C. elegans* (Say) has been reported on *Pinus rigida*, and feeding on leaves and nuts of various species of Juglandaceae (pecan, mockernut and pignut hickory) as well as on plum and cotton. They have been found breeding in galls caused by *Phylloxera* on leaves of hickory and pecan. *Conotrachelus geminatus* LeConte have been bred from flowerheads of beggar-tick (*Bidens*) and giant ragweed (*Ambrosia trifida*). *Conotrachelus hircoriae* Schoof attack the nuts of pecan and peach. *Conotrachelus nase* LeConte breed in the fruit of hawthorn (*Crataegus* sp.), and in acorns of post, live, white and chestnut oaks and other species of Fagaceae, and on cotton and on dogwood. *Conotrachelus nenuphar* (Herbst), the plum curculio, has been reported from plum, cherry, peach, nectarine, apple, wild crabapple, pear and quince. In addition, huckleberry, grape, strawberry, currant, gooseberry and persimmon are listed as occasional hosts. *Conotrachelus posticatus* Boheman breeds in acorns of various species of *Quercus* (Schoof 1942). *Conotrachelus similis* Boheman breeds in the berries of wooly buckthorn (*Bumelia lanuginosa*) (Blatchley & Leng 1916).

*Cophes fallax* (LeConte) can be found when sifting the soil under beech logs, and it breeds in dead limbs of hickory and dead stems of *Cassia* (Blatchley & Leng 1916). *Cossinus corticola* Say occurs under the bark of pine and is reported to feed on pine, while *C. impressifrons* Boheman occurs under bark of butternut, sycamore, oak, chestnut and other hardwood trees (Blatchley & Leng 1916). *Cryptorrhynchus fuscatus* LeConte breeds in dead branches of hardwood trees, while *C. minutissimus* LeConte has been extracted from dead branches of pecan (C. W. O., unpublished data).

Seven species of *Curculio* were captured in our traps. Gibson (1969) reports that the pecan weevil, *Curculio caryae* (Horn) breeds in most species of the genus *Carya* (pecan, hickory). *Curculio fulvus* Chittenden has been found breeding exclusively in live oak (*Q. virginiana*). *Curculio sulcatulus* (Casey) has been found breeding in 29 species of oaks (*Quercus*), preferring the acorns of the red oak group of species, and is the only *Curculio* reported infesting *Q. mohriana*. *Curculio proboscidieus* (Fabricius) attacks 21 species of oaks, while *C. pardalis* (Chittenden) has been reported from 18, *C. longidens* Chittenden from 11 and *C. humeralis* (Casey) from 13 *Quercus* species. In addition, *C. pardalis* is the only *Curculio* found attacking *Quercus robur* and *Q. chapmani*, while *C. humeralis* is the only *Curculio* listed as attacking *Quercus margaretta* (Gibson 1969).

*Cyrtipistomus castaneus* (Roelofs), the Asiatic oak weevil, attacks many species of woody plants but seems to prefer oak and chestnut. The larval stage is a root feeder, while the adults attack the leaves. *Eubulus bisignatus* (Say) breeds in twigs and branches of many hardwood trees and shrubs (C. W. O., unpublished data). *Eubulus parochus* Herbst was reportedly beaten from elm and found under the bark of butternut. Larvae mine the inner bark and wood of weakened and decayed walnut (*Blatchley & Leng 1916*). *Eudiagogus maryae* Warner has been found feeding on and defoliating species of *Sesbania*, *Cassia* and *Daubentonia*, while *E. rosenschoeldi* Fahraeus are associated almost exclusively with three species of *Sesbania*, *S. vesicaria* (bogpod sesbania), *S. drummondii* (Drummond rattlesbox) and *S. exaltata* (hempsesbania) (Warner 1979). *Hyllobius pales* (Herbst), the pales weevil, is an important pest of new pine and Christmas tree plantations throughout eastern North America (Fettig & Salom 1998). *Listrodentes apicalis* Waterhouse is a pest of beets (*Beta*).
vulgares), sunflower (Helianthus annuus) and wheat (Triticum aestivum) (Lanteri et al. 2002). The host list for Listrodeses difficilis Germain, the vegetable weevil, includes bean, beet, burdock, cabbage, carrot, cauliflower, celery, chard, Chinese cabbage, garlic, head cabbage, kale, lettuce, mustard, mustard cabbage, onion, pepper, peanut, potato, radish, rape, spinach, tomato, turnip and sweet potato. Cultivated flowers attacked include pansy, petunia, poppy, phlox and verbena. The principal weed hosts include dandelion,mallow, milk thistle, mustard, wild aster, wild radish and wild parsnip (High 1939). Madarellus undulatus (Say) occurs on wild grape, poison ivy and Virginia creeper and bores in the latter (Blatchley & Leng 1916). Myrmex dichrous (LeConte) breeds in dead fronds of palmetto and Sabal palms (C. W. O., unpublished data), while M. florianus (Casey) has been collected from oak and Bunelia (Peck & Thomas 1998). Naupactus cervinus (Boheman), the Fuller rose beetle, is injurious to citrus trees and ornamentals such as rose and geranium (Lanteri et al. 2002). The larvae of Naupactus peregrinus (Buchanan) are serious pests of many crops and ornamental plants in the southeastern United States. Ochryromera ligustri Warner breeds in fruits of Ligustrum (C. W. O., unpublished data). Odontocoryneus salebrosus (Casey) has been swept from huckleberry and collected from Asclepias and Achillea lanulosa (Blatchley & Leng 1916). The larvae of Odontopus calceatus (Say) mine the leaves of sassafras and Liriodendron (Blatchley & Leng 1916). Pachnaeus opalus (Olivier) has been collected from crabapple and on Irish juniper (C. W. O., unpublished data). Like the pales weevil, Pachylobius picivorus (German), the pitch-eating weevil, is an important pest of new pine and Christmas tree plantations throughout eastern North America (Pettig & Salom 1998). Pandeleitus hiliaris (Herbst) has been reported from the leaves of oak, hickory and chestnut as well as from beech, smart-weed and Ceanothus (Howden 1959). Phrydenus divergens (German) occurs on black night-shade (Solanum nigrum) (Blatchley & Leng 1916). Pisidodes nemorensis (German), the Eastern pine weevil, attacks the trunks of young trees of Virginia pine, Pinus virginiana.

Sitona californicus Fahraeus has been recorded from native lupine (Lupinus polyphyllus), Ceanothus divaricata and from a variety of plants including plum, peach, crabapple, alfalfa, apple, wild sunflower, Lotus scoparius and Eleocharis macrostachya (Bright 1994). Sitona hispidulus (F.) occurs on various forage legumes, alfalfa and varieties of clover (Bright 1994). Sitona lineellus (Bonsdorff) occurs mainly on alfalfa but also can be found on vetch, several varieties of clover, peas, alsike and many garden plants (Bright 1994). The rice weevil, Sitophilus oryzae (L.), and the maize weevil, S. zeamais Motschulsky are cosmopolitan pests of wheat, maize, oats, barley, sorghum, buckwheat, rye, rice, stored cotton, table beans and cashew nuts. Adults are reported to burrow into grapes, apples, and pears (Lanteri et al. 2002). The rice weevil is so called only because rice is the food on which it was first described. Sphenophorus cariosus (Olivier) generally feeds on rushes and sedges but has proven destructive to corn. The horned or beaked rush (Rhynchospora corniculata) is the preferred host, although it occurs in several species of Cyperus and Scirpus (Vaurie 1951). Sphenophorus coelotrichs Gyllenhal has been collected from centipede grass (C.W.O., unpublished data), and adults can damage corn and are injurious to rice (Vaurie 1951). Sphenophorus inaequalis (Say) is reported from Bermuda grass (Cynodon dactylon) (Vaurie 1951). Tany meucus lacaena (Herbst) feeds on ragweed (Ambrosia artemisifolia) (C.W.O., unpublished data). Tylodera foveolatum (Say) breeds in the stems of evening primrose (Oenothera biennis) and the cut-leaved primrose (O. laciniata); it has also been reported as feeding on corn stalks and ripe strawberries (Wibmer 1981). Several life stages of T. variegatum (Horn) were collected from lizard's tail (Saururus cernuus) by Wibmer (1981).

Trap Mode of Action

Active Tedders and Stinkbug traps and passive Circle traps have been used previously to capture many economically important weevils (Sherman & Mizell 1995, Tedders & Wood 1995, Tedders et al. 1996, Stansly et al. 1997, Prokopy & Wright 1998), as well as other selected Coleoptera (Broman et al. 2002). However, we know of no earlier study where these traps have captured as many species of weevils as we report herein. Furthermore, Mizell and Tedders (1999) and Mizell et al. (2002) used baited Tedders traps to study the behavioral response to visual and olfactory cues in H. pales and Pachylobius picivorus, two pests of Christmas trees in Florida. Unlike the Tedders and Stinkbug traps, which function as silhouette mimicking structures when placed on the ground, the Circle trap is normally attached to the tree bole where it acts as a passive funnel that captures insects walking up the bole of the tree.

Raney and Eikenbarry (1968) reported that 60-80% of pecan weevil adults emerging from their pupal cells (in the soil under pecan trees) reach the tree bole by walking rather than flying. Similar behavior has been observed in many weevils that attack fruit trees or that feed on the tender new growth of their host trees (e. g., Diaprepes abbreviatus (L.) on citrus). Availability of all of these food resources (young nuts, fruits and tender foliage) is highly variable in time and space (Hunter & Lechowicz 1992). Yields in fruit and nut trees and the timing of citrus growth flushes are genetically programmed but are highly plastic, allowing
for modification in their expression through interaction with the environment. For example, pecan is an alternate bearing nut tree with cycles of high and low nut production, which vary for individual trees (Wood & McMeans 1981, Sparks 1991).

Presumably in response to the unpredictability in the nut resource, the pecan weevil has evolved a two and partial three-year life cycle, and adults tend to aggregate year after year on those trees with the highest yield of nuts. We hypothesize that the walking behavior exhibited by the pecan weevil is an adaptation that enhances the probability that emerging adults will return to the tree of their larval development, which over evolutionary time would be those trees with the highest yield of nuts. As such, we would expect the pecan weevil, as well as other weevils that use the bole as a visual cue to locate host trees after emergence, to be captured in either the silhouette mimicking traps or in traps attached to the tree trunk. Our examination of the host associations for the weevils captured during our study suggests that the preponderance of species captured are associated with tree species found in the study area. However, this hypothesis does not explain the capture of species that breed in weedy hosts or that pupate in their host plant. Nevertheless, we have demonstrated that active pyramidal (Tedders & Stinkbug) and passive Circle traps are valuable tools for capturing rare weevils and to monitor for economically important weevil immigrants.

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ABSTRACT

Thripinema khrustalevi (Chizov et al.) isolate Chile, a parasite of Frankliniella australis (Morgan), was collected from Cestrum parqui (L'Herit.) in the Aconcagua Valley of central Chile. Percent infection of males and females of F. australis by T. khrustalevi in the flowers of C. parqui was estimated biweekly for two years at three locations, including the La Campana National Park. Males were less infected than females. Numbers of F. australis adults and larvae in the flowers of C. parqui were greatest in the winter and early spring when parasitism of the adult females was low. Populations declined in late spring when parasitism was high. Parasitism remained high through the summer and fall, and populations of thrips remained very low. Highest parasitism of the females and males of F. australis was 84 and 60%, respectively.

Key Words: biocontrol, description, Frankliniella australis, insect parasitic nematode, nematode, parasite, Thripinema khrustalevi, Thysanoptera

RESUMEN

Individuos de Thripinema khrustalevi (Chizov et al.) parasitando al trips Frankliniella australis (Morgan) fueron colectados en Cestrum parqui (L'Herit.) en el valle de Aconcagua en Chile central. El porcentaje de machos y hembras de F. australis infectados por T. khrustalevi en flores de C. parqui fue estimado cada dos semanas durante dos años en tres localidades, incluido un sector del Parque Nacional La Campana. La cantidad de adultos y larvas de F. australis en flores de C. parqui fue mayor en invierno e inicio de primavera cuando el parasitismo de las hembras adultas fue bajo. Las poblaciones disminuyeron a fines de primavera cuando el parasitismo fue alto, permaneciendo así durante todo el verano y otoño, observándose poblaciones del trips muy bajas en el mismo periodo. Las tasas más altas de parasitismo de F. australis fueron de 84 y 60%, en hembras y machos, respectivamente.

Palabras Clave: control biológico, descripción, Frankliniella australis, nematódeo parásito de insecto, nemático, parasito, Thripinema khrustalevi, Thysanoptera

Translation provided by author.
known about the free-living stage. The female attaches to and enters the thrips host by boring through the soft intersegmental membranes (Lim et al., 2001). Loomans et al. (1997) noted that there was no record of a parasitized male thrips, but Tipping et al. (1998) noted that males of *F. fusca* collected in peanuts were frequently infected by *T. fuscum*. In laboratory experiments, male *F. occidentalis* were parasitized by *T. nicklewoodi* as readily as the females (Lim et al., 2001).

Sharga (1932) reported that infection of *A. ru-fus* by *T. aptini* in the UK was lowest in March (12.3%) and highest in July (37.5%). Lysaght (1937) reported a maximum of 64% in late August and early September, and infection levels less than 10% from November to March. Reddy et al. (1982) determined from weekly samples that percent infection of a population of *Megalurothrips* sp. by *T. reniraoi* in India ranged between 45 and 65%. Infection of *T. trehernei* by *T. khrustalevi* in Russia ranged between 10 and 50% (Chizhov et al. 1995). The highest percent infection of *F. fusca* females by *T. fuscum* in Florida observed by Tipping et al. (1998) and Funderburk et al. (2002) was 68%.

Surveys of the natural enemies of thrips in Chile were begun following the accidental introduction of *Frankliniella occidentalis* (Pergande) in 1992. Nematodes were found infecting a native species of thrips, *Frankliniella australis* (Morgan). Morphological and morphometric studies show that the nematode is closely related to *Thripinema khrustalevi*. In this paper we consider the nematode as *T. khrustalevi* isolate Chile. The nematode was first discovered infecting thrips collected in the La Campana National Park. *Cestrum parqui* (L’Herit.) (Solanaceae) is a native plant species that serves as a reproductive host for populations of *F. australis* and *F. occidentalis* in Chile. We report the infection rates of *F. australis* in the flowers of *C. parqui* from three locations in the Aconcogua Valley of Central Chile. Population densities of thrips and other natural enemies of thrips also are reported.

**MATERIALS AND METHODS**

Estimating Weekly Infection Rates

The three locations sampled were: (1) La Campana National Park near the access gate to Sector Ocoa, (2) near the city of Quillota, and (3) near the city of Llay Llay. Flowers of *C. parqui* were present on most sample dates from May 1999 to June 2001. From 15 to 20 females of *F. australis* were dissected from each of four samples of about 10 inflorescences randomly collected on each sample date at each location, and the number parasitized was determined. Densities of male thrips were always less than densities of females, and estimates of parasitism of males were not possible on some dates. Further, only 5 males were dissected from each of four samples on dates when males were present in adequate numbers. Parasitism of males was estimated on 12, 12, and 20 sample dates at the Llay Llay, Quillota, and La Campana locations, respectively. Adults of *F. occidentalis* also were dissected. Percent infection of males and females at each location transformed to arcsine square root were subjected to ANOVA and significant differences were determined using a t-test.

Thrips were dissected under a stereomicroscope at ×35 in distilled water and considered infected if any stage of the nematode was detected. The swollen females, eggs, and juvenile stages were easily detected during dissection. These stages are present in thrips within several days of infection by the infective-stage females (Lim et al., 2001). Prior to swelling, an infective-stage female in the absence of other stages of nematodes sometimes went undetected during dissection. To determine how much parasitism was being underestimated with the dissection procedures, 20 females on 9 to 12 dates depending on the location were maintained individually for 7 days and then dissected. This method provided percent infection that was consistently about 10% greater than the estimate of percent infection from females disected immediately after collection.

Estimating Densities of Insect Populations

Four samples of five open flowers were collected biweekly from October 1999 to June 2001 at each of the locations listed above. Flowers were placed in vials containing soapy water and returned to the laboratory for processing. Samples were examined under a stereoscope at x35, and the numbers of thrips and natural enemies determined.

**RESULTS AND DISCUSSION**

Adults of *F. occidentalis* collected from the flowers were never infected by *T. khrustalevi* isolate Chile. Similarly, Funderburk et al. (2002) reported that infection of *F. occidentalis* by *T. fuscum* in peanut flowers was rare. Adults and larvae of *F. occidentalis* were readily infected under laboratory conditions (unpublished data). The reason that these field populations of *F. occidentalis* were not parasitized by *T. fuscum or T. khrustalevi* is not clear. *Thripinema nicklewoodi* is an important parasite of *F. occidentalis* in the western USA (Loomans et al., 1997), and the species has potential for biological control if introduced into Chile or other geographic regions.

Adults and second instars of *F. australis* collected from the flowers of *C. parqui* were parasitized. The ovaries of the infected females were reduced and eggs absent. After entering the
Fig. 1. Mean number (±SEM) of adult and larval _F. australis_ and mean percent parasitism (±SEM) of the females of _F. australis_ from biweekly samples taken between May 1999 to June 2001 at three locations in central Chile (Region V).
thrips host, the females of the new isolate change into the characteristic swollen shape of the genus, and the ovary becomes convoluted with numerous flexures. Up to several hundred nematodes were observed in individual females of *F. australis*. All stages of the nematode were observed in infected males and females. Males and females of *T. fuscum* were observed exiting the thrips via the anus as reported by Lysaght (1937) for *T. aptini*, Nickle and Wood (1964) for *T. nicklewoodi*, and Tipping et al. (1998) for *T. fuscum*.

Open flowers of *C. parqui* were present on nearly all sample dates from May 1999 to June 2001 at the Quillota and La Campana locations (Fig. 1). Flowering was suppressed during the winter and there were several sample dates in July and August 2000 when population densities and % infection of thrips could not be estimated because of a scarcity of open flowers at these locations. Temperatures were lower at the Llay Llay location, and open flowers were not available for sampling for several months each winter. About 96% (range 95 to 97% between the locations) of the adult thrips in the flower samples were *F. australis*, with the remainder being *F. occidentalis*. No natural enemies other than *T. khrustalevi* were observed that could have contributed significantly to suppression of populations of thrips. The only predator in the flower samples was *Orius insidiosus* (Say), but only four adults and nymphs were found total in all of the samples. A total of 13 adults of *Ceranisus menes* (Walker) (Hymenoptera: Eulophidae), a larval parasite of thrips, were collected.

Numbers of *F. australis* increased greatly in the *C. parqui* flowers each year in October/early November when levels of % parasitism of the adult females was very low (Fig. 1). Adults and larvae were abundant through the remainder of each spring. Populations of adults and larvae decreased to near extinction in February, persisting in low numbers until the following spring. Parasitism of the adult females increased each January and was high at each location through the summer and fall until the beginning of winter in May/early June. Parasitism of males was almost always less than parasitism of females (data not shown). On dates when parasitism of males and females was estimated, % parasitism of males vs females averaged 9.2 (range 0 to 35) vs 15.2 (range 0 to 45), 11.3 (range 0 to 60) vs 16.8 (range 0 to 69), and 4.5 (range 0 to 35) vs 16.8 (range 0 to 42.5) at the Llay Llay, Quillota, and La Campana locations, respectively. Parasitism of the females was significantly greater at the La Campana location, according to the t-test (error df = 158, P < 0.0001) and the difference approached significance at the Llay Llay location (error df = 94, P = 0.08).

Reported cases of suppression of thrips populations by natural enemies were lacking until recently (Parrella and Lewis, 1997), and the population attributes of thrips including rapid colonization and growth were believed to possibly outstrip the capacities of natural enemies to regulate populations (Mound and Teulon, 1995). Funderburk et al. (2000) and Ramachandran et al. (2001) showed that *O. insidiosus* suppressed rapidly colonizing and developing populations of thrips in pepper. Funderburk et al. (2002) showed that *T. fuscum* was an important natural enemy affecting the population dynamics of *F. fusca* in peanut. Several other investigators reported high levels of infection during summer and fall for other species of *Thripinema* (Sharga, 1932; Lysaght, 1937; Reddy et al., 1982) which may indicate that natural suppression of thrips populations under local conditions by species of *Thripinema* is widespread. We conclude in this study that *T. khrustalevi* isolate Chile is an important natural enemy of *F. australis* in *C. parqui*.

ACKNOWLEDGMENTS

The authors thank K. B. Nguyen (Department of Entomology and Nematology, University of Florida) for the identification of the nematode *Thripinema khrustalevi*, and L. E. Mound (Division of Entomology, Commonwealth Scientific and Industrial Research Organization) for verifying the identification of *Frankliniella australis*. Stuart Reitz (USDA-ARS) and Russell Mizell (North Florida Research and Education Center, University of Florida) made comments on the manuscript. Ruby Mariana Silva Cutbill provided encouragement and permitted the sampling on her eco-tourism ranch at the La Campana National Park for their many assistance. This article is Florida Agricultural Experiment Station Journal Series number R-08739.

LITERATURE CITED


SALT AND WATER BALANCE IN HEXAGENIA LIMBATA (EPHEMEROPTERA: EPHEMERIDAE) WHEN EXPOSED TO BRACKISH WATER

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Like most aquatic insects, mayflies rarely occur in saline habitats, probably because of intolerance to elevated salt (NaCl) concentrations (“halophobic” species, sensu Gallardo-Mayenco 1994). However, some mayflies are tolerant to increased salinity. Berner & Sloan (1954) reported Callibaetis floridanus (Baetidae) occurring in brackish waters (2-10 ppt), and Tricorythus (Tricorythidae) was tolerant of salinities from 0.2 - 3.2 ppt (Goetsch & Palmer 1997). In an Oklahoma stream, Hexagenia limbata (Ephemeridae) was unaffected by saline discharge (Magdych 1984). Recently, a population of H. limbata was found in seasonally saline reaches (0-25 ppt) of the Mobile River (Chadwick & Feminella 2001).

Occurrence and survival of freshwater insects in brackish water prompt basic questions about what physiological mechanisms allow insects to persist in saline habitats. Aquatic invertebrates employ 2 physiological mechanisms of salinity adaptation, osmoregulation and cell volume regulation (reviewed by Pierce & Amende 1981, Bradley 1985). Osmoregulation allows the animal to maintain its hemolymph osmotic concentration, thus maintaining a relatively constant internal state. Alternatively, cell volume regulation occurs in animals that cannot regulate the osmotic concentration of their hemolymph. Hemolymph osmotic concentration increases with increasing ambient salinity, which results in an osmotic gradient that reduces intracellular water and causes cells to shrink. By increasing their internal osmotic pressure through synthesis of organic compounds (e.g., amino acids and sugars), cells can restore normal volume by regaining lost water. If nymphs at higher salinities regulated their cell volume, then these insects would have initially lost cellular water because of osmotic gradients, unless they regulated their hemolymph osmolality. That no wet-mass differences were detected at any time suggest that these insects regulate hemolymph osmolality rather than cell volume. However, this interpretation must be taken with caution because of unknown variation in wet-mass associated with water adhering to surfaces, especially the gills and within the air sacs.

Late instars (19-28 mm total length) were collected live from Dead Lake, a distributary of the Mobile River, SW Alabama. Nymphs were brought to the laboratory in chilled, aerated river water (0 ppt salinity), where they were transferred to tanks containing aerated river water, and held at room temperature for at least 7 d before being used in experiments.

To assess water balance, nymphs were exposed to 1 of 4 salinity treatments (0, 5, 8, 12 ppt, n = 5), with 1 nymph per treatment replicate. Initially, nymphs were blotted dry and weighed individually on a microbalance (Sartorius 1712 MP8) to the nearest 0.01 mg. Nymphs were then randomly placed into a salinity treatment, and reweighed 1, 2, 4, 8 h after the start of the experiment to quantify water loss or gain (as whole-animal change in wet mass). A Kruskal-Wallis test (α = 0.05) was used to examine relative mass change (initial—reweighed mass) across the time periods for each salinity treatment.

All nymphs survived the 8-h experiment. Individual wet mass varied from 62.16 to 164.78 mg. No difference in whole animal wet-mass change over time was found for any salinity treatment (0 ppt: F4,20 = 2.12, p = 0.1163; 5 ppt: F4,20 = 2.55, p = 0.0709; 8 ppt: F4,20 = 2.15, p = 0.1122; 12 ppt: F4,20 = 0.06, p = 0.9927). Further, no discernible trend in wet-mass change among salinity treatments was observed (Fig. 1).

If nymphs at higher salinities regulated their cell volume, then these insects would have initially lost cellular water because of osmotic gradients, unless they regulated their hemolymph osmolality. That no wet-mass differences were detected at any time suggest that these insects regulate hemolymph osmolality rather than cell volume. However, this interpretation must be taken with caution because of unknown variation in wet-mass associated with water adhering to surfaces, especially the gills and within the ali-

Fig. 1. Mean (±SE) whole animal wet-mass change for H. limbata nymphs after 8 h of exposure to 4 salinity treatments.
mentary canal. Nevertheless, in terms of mortality, survival of an 8-h exposure to elevated salinity is similar to what H. limbata would experience in tidal portions of the Mobile River. Thus, these results suggest individuals within this population can endure the osmotic stress associated with brief saline periods with little appreciable change in water balance.

A 2nd experiment was conducted to assess the effects of elevated salinity on hemolymph osmolality. Live nymphs not used in the water balance experiment were divided equally among 4 salinity levels (0, 5, 8, 12 ppt), where they were acclimated for 7 d. Hemolymph was then extracted by making a cut above the terminal filament and allowing hemolymph (~10 µL) to drip into sample vials. Osmolality was analyzed with a Wescor 5100c vapor pressure osmometer, and 1-way ANOVA was used to assess differences in osmolality among treatments (α = 0.05). A Dunnett’s t test was used to compare pairwise differences in osmolality between each salinity treatment and the 0 ppt control. Acute change in salinity from 0 to 12 ppt resulted in 100% mortality over the 7-d acclimation period, but, like the 1st experiment, there was a 100% survivorship within the other 3 treatments (0, 5, 8 ppt). Among treatments where nymphs survived, hemolymph osmolality differed significantly (F<sub>2,27</sub> = 19.74, p < 0.001). However, only the 8-ppt treatment differed significantly from the 0 ppt control. Mean osmolality increased with increasing salinity (Fig. 2), but the osmotic pressure (i.e., difference between ambient osmotic concentration and hemolymph concentration) decreased with increasing salinity.

In the treatments < 8 ppt, nymphs were able to regulate their hemolymph osmotic concentration at levels hyperosmotic to the ambient water. At 8 ppt, hemolymph was essentially isosmotic. Results of both experiments suggest that at salinities > 8 ppt nymphs lose ability to osmoregulate and begin to osmoconform, with mortality ensuing under exposure to increased salinity. The failure of freshwater organisms to survive in high-salinity environments is believed to result from an inability to increase cellular concentrations of organic compounds, such as amino acids and sugars, to match increases in hemolymph salt concentrations (Gainey & Greenberg 1977). Euryhaline insect larvae (e.g., mosquitoes) can survive up to 20 ppt, and do so by synthesizing proline, serine and trehalose (Garrett & Bradley 1987). It is possible that nymphs of H. limbata lack a similar ability, and are therefore limited to environmental salinities in which they are osmo-regulators.

**SUMMARY**

**Hexagenia limbata** nymphs were shown to regulate hemolymph osmolality at salinities < 8 ppt. Nymphs survived for extended periods (7 d) in isosmotic conditions (~8 ppt). However, individuals exposed to salinities > 8 ppt could not survive for extended periods, but they could tolerate these conditions for 8 h, a time period that approximates tidal inundation.

**REFERENCES CITED**


RESISTANCE OF BT TRANSGENIC MAIZE TO LESSER CORNSTALK BORER (LEPIDOPTERA: PYRALIDAE)

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2Embrapa Milho e Sorgo, Sete Lagoas, MG, Brazil
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The lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae), maize causes the symptom known as “dead heart” to maize. The objective of this research was to evaluate if Bt maize would protect seedlings against lesser cornstalk borer damage and whether different temperatures affect the insect-plant interaction.

Two experiments were conducted. Seeds from Pioneer Bt maize hybrids P33G27, P34R07, P34G82, P33A14, and P34D34, expressing the toxin Cry1A(b) were obtained from the market. Seeds from the hybrids Mycogen (experimental) and Garst 8539 expressing, respectively, the toxin Cry1F and Cry9C as well as the control Garst 8539 (non-Bt) were obtained from the seed producers. Lesser cornstalk borer for artificial infestation were obtained from a colony maintained by USDA-ARS-CPMRU, in Tifton, GA.

The first experiment included 7 maize hybrids tested at two plant growth stages (3 and 4 leaf) at a constant temperature of 27 ± 0.7°C and photophase of 14 h light: 10 h darkness. The experimental unit was 9 seedlings infested with 2 second/third instars per plant and replicated 3 times. One week after infestation, the experiment was evaluated by weighing and counting the number of surviving larvae and the number of undamaged plants. A damage score (0-no damage, 10-dead plant) was used.

There was a significant difference between the Bt and the non-Bt hybrids regarding the damage score and the number of dead plants (Table 1). However, there was no significant difference among the Bt hybrids regarding those variables. The degree of plant damage and number of dead plants/plot were highly correlated (r = 0.998). Fewer numbers of lesser cornstalk borer larvae survived on Bt than non-Bt maize. In addition, larvae surviving on Bt maize weighed less than those surviving on non-Bt maize. A significant reduction on the number of survivors, caused by Bt toxin, was reported to lesser cornstalk borer on transgenic sugarcane (Fitch et al. 1996).

<table>
<thead>
<tr>
<th>Maize hybrid (Bt toxin)</th>
<th>Plant damage score</th>
<th>Number of dead plants</th>
<th>Number of survivors</th>
<th>Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P34R07 (Cry1Ab)</td>
<td>0.9 ± 0.1 a</td>
<td>0.0 ± 0.0 a</td>
<td>1.7 ± 1.6</td>
<td>4.0 ± 2.1</td>
</tr>
<tr>
<td>P33G27 (Cry1Ab)</td>
<td>0.6 ± 0.2 a</td>
<td>0.3 ± 0.2 a</td>
<td>2.8 ± 2.3</td>
<td>3.9 ± 3.2</td>
</tr>
<tr>
<td>P34G82 (Cry1Ab)</td>
<td>0.7 ± 0.2 a</td>
<td>0.0 ± 0.0 a</td>
<td>1.9 ± 0.7</td>
<td>1.4 ± 0.8</td>
</tr>
<tr>
<td>P33A14 (Cry1Ab)</td>
<td>0.6 ± 0.1 a</td>
<td>0.0 ± 0.0 a</td>
<td>3.3 ± 2.0</td>
<td>2.8 ± 1.2</td>
</tr>
<tr>
<td>P34D34 (Cry1Ab)</td>
<td>0.9 ± 0.1 a</td>
<td>0.0 ± 0.0 a</td>
<td>2.4 ± 1.4</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>G8539 (Cry9C)</td>
<td>0.7 ± 0.2 a</td>
<td>0.0 ± 0.0 a</td>
<td>5.1 ± 2.7</td>
<td>6.1 ± 1.7</td>
</tr>
<tr>
<td>G8539 (Non-Bt)</td>
<td>9.4 ± 0.3 b</td>
<td>8.5 ± 0.2 b</td>
<td>1.7 ± 2.7</td>
<td>24.0 ± 5.5</td>
</tr>
<tr>
<td>F</td>
<td>481.79</td>
<td>576.40</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Coef. of variation</td>
<td>18.62%</td>
<td>8.47%</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1Data transformed to square root of (√ + 0.5) for statistical analysis. In each column, means followed by the same letter are not significantly different by Duncan Multiple Range Test (P ≤ 0.05).
The second experiment evaluated the temperature effect on Bt maize-lesser cornstalk borer interaction. Plants were maintained in growth chambers regulated at constant temperatures (20°C, 24°C, 28°C, or 32°C during the day and 20°C during the night). The experimental unit included 5 seedlings from the Bt maize hybrids Mycogen experimental (Cry1F), Pioneer P33G27 (Cry1Ab), and Garst 8539 (Cry9C), and non-Bt Garst 8539 and was replicated 3 times. The infestation method used was the same as the previous experiment and the evaluation was based on the number of dead plants.

The results showed a significant difference between transgenic and non-transgenic maize regarding the number of dead plants (Table 2). However, there was a significant interaction between temperature and genotype for this variable. The temperature affected the plant development and the amount of lesser cornstalk borer damage, being significantly lower on controls at 20°C compared with other temperatures (Table 2). Conversely, there was no significant difference among the Bt maize hybrids expressing the Cry1F, Cry1Ab or Cry9C toxin regarding temperature. Therefore, Bt transgenic maize expressing all evaluated toxins can be considered an effective approach to manage lesser cornstalk borer damage. While some larvae survived on the Bt transgenic maize, they were smaller and weighed less than the ones on the non-Bt maize and, at all temperatures tested, did not cause significant damage on Bt maize seedlings.

Bt plants have been used to control many pests, including lesser cornstalk borer. Fitch et al. (1996) found 80-100% mortality of lesser cornstalk borer on transgenic sugarcane. Singh et al. (1997) reported various levels of Bt transgenic peanut resistance to lesser cornstalk borer, ranging from complete mortality to a 66% reduction in larval weight. Bt maize controlled fall armyworm, Spodoptera frugiperda (Smith) (Lepidoptera: Noctuidae), southwestern corn borer, Diatraea grandiosella (Dyar) (Lepidoptera: Pyralidae), corn earworm and Helicoverpa zea (Boddie) (Lepidoptera: Noctuidae) (Lynch et al. 1999, Williams et al. 1998).

The authors thank Ariovaldo Luchiari/Embrapa Labex/USDA-ARS SWCRU, Frederick P. Baxendale and E. Heinrichs /UN-L and the Brazilian Agencies CAPES and CNPq.

**SUMMARY**

To determine if Bt maize seedlings are protected against lesser cornstalk borer damage, Bt hybrids at the 3 and 4 leaf stages were tested under temperatures between 20-32°C and artificial infestation. A high level of resistance was reported in all Bt maize as expressed by larval survival, larval weight, damage score, and number of surviving plants. All Bt maize protected plants against lesser cornstalk borer damage. Also, the resistance present in the Bt maize was not affected by daytime temperature.

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A NEW DISTRIBUTION AND HOST RECORD FOR GONATOCERUS TRIGUTTATUS IN FLORIDA, WITH NOTES ON ACMOPOLYNEMA SEMA (HYMENOPTERA: MYMARIDAE)

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Triapitsyn et al. (1998) reported results of the 1997 survey of egg parasitoids of the glassy-winged sharpshooter, Homalodisca coagulata (Say) (Homoptera: Cicadellidae), in northern Florida. As part of an on-going classical biological control program against H. coagulata in California, two of the authors conducted a survey of egg parasitoids of proconiine sharpshooters (Cicadellidae: Cicadellinae: Proconini) throughout Florida in August 2001 (Triapitsyn & Hoddle 2001). Among several species in the families Mymaridae and Trichogrammatidae (both Hymenoptera) that were collected (Triapitsyn & Hoddle 2001), there were two mymarid species on which the previously unknown information is given below.

All specimens resulting from this study were determined by S. V. Triapitsyn; the vouchers of parasitoid species were deposited in the Entomology Research Museum, University of California at Riverside, California (UCRC) and those of proconiine sharpshooter species were deposited in the California State Collection of Arthropods, California Department of Food and Agriculture, Sacramento, California; also examined was the collection of Mymaridae at the Florida State Collection of Arthropods in Gainesville, Florida (FSCA).

Gonatocerus triguttatus Girault, originally described from specimens reared from an egg mass of an unidentified leafhopper on orange, Citrus sinensis (L.) Osbeck, in Trinidad (Girault 1916), was reared recently in northeastern Mexico from egg masses of H. coagulata, Oncometopia clarior (Walker), and an unidentified Oncometopia species (Triapitsyn & Phillips 2000, Triapitsyn et al. 2002). Gonatocerus triguttatus is also known from eggs of H. coagulata in Texas (Jones 2001, Triapitsyn & Hoddle 2001).

In Apopka, Florida, we reared two females of G. triguttatus from an egg mass of the black-winged sharpshooter, Oncometopia nigricans (Walker), laid in a leaf of crape myrtle, Lagerstroemia indica L., in the parking lot of the University of Florida Mid-Florida Research and Education Center. From the same plant and several adjacent crape myrtle trees, we also collected an adult specimen and several nympha of O. nigricans, which is the prevalent proconiine sharpshooter species on woody plants in central and southern Florida, whereas relative abundance of H. coagulata decreases from north-central Florida southward (Timmer et al. 1982), although it is known in southern Florida as far as Homestead (Turner & Pollard 1959).

Gonatocerus triguttatus has not been known previously from Florida and O. nigricans is a new host record for this parasitoid. Before our discovery, the only known record of an egg parasitoid of O. nigricans was by Turner & Pollard (1959) who reported a Gonatocerus sp. (as Lymaenon sp.) in Plant City, Florida, from eggs of Oncometopia undata (Fabricius), that is an obvious misidentification of O. nigricans following Young (1968).

Material Examined. USA, Florida, Orange Co., Apopka, 21-VIII-2001, M. S. Hoddle and S. V. Triapitsyn, 2 females (emerged in UCR quarantine 30-VIII-2001 from an egg mass of O. nigricans on crape myrtle) [UCRC].

Notes on Acmopolynema sema Schauf. This species was described from a large series of type specimens reared from eggs of the johnsongrass sharpshooter, Homalodisca insolita (Walker), in Fort Valley, Georgia (Schauff 1981). We reared A. sema from egg masses of the same host, laid in Johnson grass, Sorghum halepense (L.) Persoon, collected at the grounds of the University of Florida Everglades Research and Education Center in Belle Glade, Florida, on 19-VIII-2001 and brought under permit into UCR quarantine. Numerous specimens of H. insolita were collected from the same plants on which the egg masses were found. Following emergence, which began within the sealed containers while we still were in Florida and continued en masse in UCR quarantine, female and male parasitoids were given time to mate and then were exposed to H. coagulata eggs laid in leaves of Euonymus japonica Thunberg on 24-VIII-2001. The first generation (n > 100), which consisted of both females and males (the sex ratio was 4.3:1, respectively), was successfully obtained on 10-IX-2001 and exposed to the host on 12-IX-2001. The second generation, which emerged on 29-IX-2001, however, consisted of males only (n > 400), thus our colony of A. sema was discontinued thereafter. We suspect that this was in response to the fact that H. coagulata is not the natural host for this parasitoid, which prefers to attack egg masses of H. insolita, a species that feeds and lays eggs on grasses (Turner &...
been obtained successfully on first generation, which included both sexes, had lost in the second, all male, generation, after the

case. A colony of Homalodisca coagulata (Homoptera: Cicadellidae), with notes on the distribution of the host. Florida Entomol. 83: 291-308.


A NOVEL AERIAL-INTERCEPTION TRAP FOR ARTHROPOD SAMPLING

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Although there are many field techniques to estimate the abundance and diversity of arthropods in terrestrial ecosystems, all have significant limitations (Chapman and Kinghorn 1955, Southwood 1978, Masner and Goulet 1981, Canaday 1987, Atkinson et al. 1988, Chénier and Philogène 1989, McEwen 1997, Dobony and Edwards 2001). In particular, methods for sampling arthropods moving through the air usually have major drawbacks, such as high cost per unit or difficulty of installation and sample retrieval. When I wished to assess the affect of fire on the arthropod community in Florida scrub at the Archbold Biological Station in Highlands Co., I also was faced with the problems of replicate sampling at several different locations and dealing with severe thunderstorms accompanied by high wind and torrential rainfall. Here I describe a novel trap that solves some of the aforementioned problems.

The trap consisted of an array of four transparent, recycled 2-liter polycarbonate beverage bottles, each having a 17 cm wide × 13 cm high strip in its side removed to allow the entry of arthropods. When viewed from the side, the area of the opening in each bottle was 10.5 × 13 cm. Hence, the effective surface area of the trap with four bottles was 550 cm$^2$. The intact bottom of each bottle served as a reservoir for ~200 ml of collecting fluid, such as soapy water. The bottles were suspended by their caps that were bolted in a 2 × 2 array centered on the underside of a 20 × 30 cm piece of 1.3 cm (0.5 in) thick exterior grade plywood (Fig. 1). This conformation stabilized the bottles in windy conditions. The wooden platform provided, along with the top portion of the bottles, shelter from precipitation. Each trap was mounted on two 1.3 cm (0.5 in) diameter × 2.5 m steel reinforcing rods (commonly called “rebar”) placed vertically to a depth of 30-40 cm in the soil. After the tops of the rods were slipped through the two 1.6 cm (5/8 in) holes near the ends of a platform (Fig. 1), the trap was lowered to the desired position 0.5, 1.0, or 1.5 m above ground and held in place by medium binder clips attached to the rods beneath the platform. For additional stability in high winds, a second binder clip could be attached to a rod just above the plywood platform.

The cost of each trap, including the two rods, was $1-2. Furthermore, I found that I could assemble and install a dozen traps at 10 m intervals along a transect in the scrub in 2-3 h.

Arthropods were removed from the four reservoirs in each trap by aspirating the fluid with a conventional meat baster and rapidly filtering it through a tea strainer, allowing the filtrate to return to a reservoir. The filtered arthropods were emptied into a plastic cup, then the cup was capped and taken to the lab where its contents were placed in 70% isopropyl alcohol for preservation until the arthropods were identified. When evaluating the traps under field conditions, I found that I spent less than 1 h processing the catch of a dozen traps, which meant that I could effectively operate a dozen traps simultaneously by myself in burned and unburned scrub even when they needed to be serviced daily.

To evaluate the overall efficiency of this trap design, I determined the diversity of arthropods caught when a dozen traps were operated for six days in a row at four month intervals (June and October 2001 and February 2002) in scrub after it was burned on February 12, 2001 by an intense wildfire. As a control, another dozen traps were operated simultaneously in nearby scrub that had recovered from a burn in July 1998. The specimens (N = 1609) were identified to order (Table 1). (A more detailed analysis will be forthcoming.) Four orders (Diptera, Hymenoptera, Coleoptera, and Homoptera) accounted for 86.5% of all arthropods collected. This result is identical to the results reported by Dobony and Edwards (2001) when evaluating their new flight-intercept trap that was operated on the ground in forests in West Virginia during May-July 1998. But the cost of their trap, constructed entirely of acrylic Plexi-
glas®, was about four times more than mine and it was susceptible to high winds. My traps withstood winds gusting to 70 km/h during a few storms and all retained the collecting fluid in the bottoms of the bottles. In addition, even though two storms each deposited ~5 cm of rain, the reservoirs in the traps did not fill completely, so trapped specimens were not lost.

To evaluate the efficiency of the traps, I divided the number of arthropods collected (1609) by the effective surface area of each trap (550 cm²) times the number of trap-days they were operated (432), which yielded a value of 0.007 arthropods/cm²/trap-day. This value was intermediate between the values reported by Canaday (1987) and by Chapman and Kinghorn (1955) using standard “window” traps in northern forests (0.002 and 0.030 arthropods/cm²/trap-day, respectively). Hence, the new trap appears to be about as effective as more traditional models.

<table>
<thead>
<tr>
<th>Order</th>
<th>% Total capture</th>
<th>Order</th>
<th>% Total capture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Araneae</td>
<td>5.3</td>
<td>Hymenoptera</td>
<td>18.0</td>
</tr>
<tr>
<td>Blattaria</td>
<td>&lt;0.5</td>
<td>Lepidoptera</td>
<td>2.9</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>10.7</td>
<td>Neuroptera</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Collembola</td>
<td>&lt;0.5</td>
<td>Orthoptera</td>
<td>0.6</td>
</tr>
<tr>
<td>Diptera</td>
<td>47.9</td>
<td>Psocoptera</td>
<td>1.7</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>1.6</td>
<td>Thysanoptera</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Homoptera</td>
<td>9.9</td>
<td>Trichoptera</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**TABLE 1**. **PERCENT OF TOTAL ARTHROPODS CAPTURED (N = 1609) IN FLORIDA SCRUB, CLASSIFIED TO ORDER. AERIAL INTERCEPT TRAPS (N = 24) WERE OPERATED FOR 6 NIGHTS EACH IN JUNE 2001, OCTOBER 2001, AND FEBRUARY 2002 AT THE ARCHBOLD BIOLOGICAL STATION, HIGHLANDS CO., FL.**

**SUMMARY**

I designed an inexpensive flight-interception trap consisting of a platform of plywood from which four clear, polycarbonate beverage bottles were suspended so that arthropods moving horizontally could enter a large opening in the side of each and fall into collecting fluid in the bottom. The platform was mounted on two pieces of vertical rebar placed into the soil and it was adjusted to be 0.5, 1.0, or 1.5 m above ground. When used in Florida scrub, I captured arthropods belonging to 14 orders at a rate comparable to those reported for “window” traps used in forests.

**REFERENCES CITED**


The Neotropical ant *Anochetus mayri* Emery (Fig. 1), a member of the subfamily Ponerinae, was first found in Florida in 1987 (Deyrup et al. 2000). This species is distinguished from somewhat similar Florida *Odontomachus* species by double-pointed petiolar scale (single point in *Odontomachus* spp.) and small size: maximum head + body length 4 mm (minimum head + body length of *Odontomachus* spp. 9 mm). The 1987 record, based on a single dealate queen from Homestead (Dade Co.), was supplemented by two specimens from south Miami (Dade Co.) in 1991. During the last decade I failed in several attempts to find additional specimens. The original site in Homestead, a pine stand near a tropical plant nursery, was obliterated by Hurricane Andrew in 1992. I had begun to wonder whether *A. mayri* still occurred in Florida.

An ant survey at the Pine Jog Environmental Learning Center in West Palm Beach (Palm Beach Co.) shows that, as of January, 2002, *A. mayri* is thriving, at least in one site. Collections were made from deep litter at the bases of pines and oaks. Litter samples, each about 1 liter, were brought back to the Archbold Biological Station and extracted with Berlese funnels. Out of the 36 samples collected, 27 produced at least one specimen of *A. mayri*. This species occurred together with the following ant species (number in parentheses is the number of co-occurrences with *A. mayri*; asterisk (*) denotes an exotic species): *Brachymyrmex depilis* (Emery) (3), *Cyphomyrmex minutus* Mayr (1), *Hypoponera opacior* (Forel) (2), *Odontomachus brunneus* (Patton) (1), *Paratrechina guatemalensis* (Forel)* (6), *Pheidole floridana* Emery (1), *P. moerens* Wheeler* (10), *Pyramica eggersi* (Emery)* (11), *Solenopsis abdita* Thompson (14), *S. invicta* Buren* (1), *S. tennesseensis* M. R. Smith (10), *Strumigenys emmae* (Emery)* (2), *S. rogeri* Emery* (6), *Wasmannia auropunctata* (Roger)* (8). *Anochetus mayri* clearly fits in well with the de-

**Fig. 1.** *Anochetus mayri* Emery, worker: A: lateral habitus view; B: frontal view of head; C: frontal view of petiolar scale.
plorably rich fauna of exotic ants in south Florida; half the species and more than half the individual associations are fellow exotics.

The trajectory of *A. mayri* in Florida ecosystems is impossible to predict at this point. It is widespread in the Caribbean and in Central and South America, but, in my limited experience, it is not a dominant species. It cannot be considered a major threat to native species in Florida as long as the Florida population is confined to urban and suburban habitats that are already somewhat disturbed and perfused with many other exotics. *Anochetus mayri* is a predaceous species that is not likely to become an economic pest. Although it is possibly capable of stinging humans, it is not aggressive or strongly defensive; live individuals picked out of the leaf litter ran about in my hand without attempting to sting. Its subterranean habitat further reduces its chances of interacting with humans. There is probably little chance of eradicating *A. mayri* from Florida, as it is a cryptic subterranean species with at least one large established population. It would be reasonable to periodically monitor areas of southeast Florida to determine whether this, or other exotic ant species, are undergoing explosive expansion. Although *A. mayri* is an unwelcome exotic species, it is also an interesting species whose biology is poorly known. One reason for publishing this note is to inform North American myrmecologists of a convenient population of *A. mayri* for scientific study.

**SUMMARY**

A robust population of the exotic Neotropical ant *Anochetus mayri* Emery has been found in Palm Beach Co., Florida. The accompanying illustration should serve to distinguish this species from other ants found in the U.S. It has not yet been found in undisturbed habitats in Florida and seems to have little potential for becoming an economic pest.

**REFERENCES CITED**

EXPLORATION IN BELIZE FOR PARASITOIDS ATTACKING EGGS OF CITRUS WEEVILS, AND AN EVALUATION OF PEDIOBUS IRREGULARIS AND HORISMENUS BENNETTI (HYMENOPTERA: EULOPHIDAE) AS POTENTIAL BIOLOGICAL CONTROL AGENTS OF DIAPREPS ABBREVIATUS AND PACHNAEUS LITUS (COLEOPTERA: CURCULIONIDAE)

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During July 16-23, 2001, Belize was explored for parasitoids attacking eggs of citrus weevils. The trip, organized by coauthors Peña and Hall and funded by a grant from the Florida Citrus Production Research Advisory Council (981-42E), was made in hopes of finding parasitoid species that could be introduced into Florida to boost biological control of the Diaprepes root weevil, Diaprepes abbreviatus (L.), a major pest of citrus (Citrus spp.) and ornamental plants in Florida (Hall et al. 2001). No parasitoids are known to attack eggs of D. abbreviatus in Florida (Hall et al. 2001). Attempts in Florida to establish egg parasitoids that attack D. abbreviatus in Guadeloupe (Ceratogramma etiennei Delvare, Hymenoptera: Trichogrammatidae), Puerto Rico (Quadrastichus haitiensis Gahan) and the Dominican Republic (Aprostocetus vaquitarum (Wolcott)) (Hymenoptera; Eulophidae) have been made (e.g., see Hall et al. 2001, Peña et al. 2000).

Exploration in Belize, conducted by coauthors Hall and Eger, was confined to the Stan Creek District in the southern half of Belize where most of the country’s citrus is grown. Five days were spent exploring five citrus groves near the town of Dangriga and one day exploring four groves near the city of Belmopan. The groves surveyed near Dangriga included Mullin’s River grove (grapefruit, C. paradisi Macf.) 4 to 5 km west to southwest of Dangriga; Buckshell’s grove (grapefruit, C. paradisi Macf.) 4 to 5 km west to southwest of Dangriga; two sweet orange groves 8 to 10 km southwest of Dangriga; and Bowman’s grove (sweet oranges) to the south of Dangriga. The groves surveyed near Belmopan included the Werrie Head Resort Inn grove (sweet oranges) around 5 km to the west of the city.

Three weevil species were collected from citrus trees during the trip: Exophthalmus vitticollis Champion, E. lunaris Champion and Tanymecus confusus (Say) (identifications by coauthor O’Brien). The Diaprepes root weevil was not observed and was not known to occur in Belize. A total of 178 adult weevils thought to be E. vitticollis were collected during the trip; 138 of these were examined by O’Brien and confirmed as E. vitticollis (voucher specimens deposited with the Florida State Collection of Arthropods (FSCA) in Gainesville and with coauthor O’Brien’s personal collection). Adult E. vitticollis were common at most collection sites and abundant at some. We encountered only two adult E. lunaris (Mullin’s River grove) and six adult T. confusus (Buckshell’s grove), and whether these species utilized citrus as a host was not known. E. vitticollis was previously known to be associated with citrus in Belize (Schauff 1987). The economic importance of citrus weevils in Belize was unclear, but it generally appeared that most growers did not consider weevils to be significant pests. Trees from which weevils were collected varied in apparent health and productivity, and it was possible that some trees that did not appear healthy were suffering from attack by weevil larvae.

The majority of time spent at each citrus location was devoted to searching citrus leaves for weevil egg masses. Specifically, we looked for weevil egg masses glued between two juxtaposing leaves (Hall et al. 2001). A total of 168 egg masses were found; these ranged from freshly deposited to possibly several weeks old. Leaves with eggs were carefully removed from trees and placed into plastic containers with ventilation screens. Each evening after collecting, leaves with eggs were moved from the containers and trimmed with scissors, leaving a minimum of leaf tissue around each egg mass. The masses were then placed into shell vials (1 dram) and plugged with a small wad
of tissue paper. Of the 168 egg masses collected, 121 were placed individually into shell vials while the remaining were placed 3 to 5 at a time into vials. The shell vials were then placed into a compartmentalized plastic box with snap lid. A moistened paper towel was laid on top of the plastic box, and the box and towel were slipped into a ziplock bag. Shell vials with egg masses were added to the plastic boxes each night. Observations after several days of collecting indicated that the humidity level inside the boxes was excessive (some leaf tissue showed beginning signs of mold); thereafter no moistened paper towels were placed inside the ziplock bags. Three such ziplock bags containing eggs in vials inside a plastic box were transported from Belize to Miami inside a small cooler with a blue ice pack. In Miami, the material was transferred to coauthor Peña and then into quarantine at the University of Florida Quarantine Center at Homestead. The importation into Florida was covered by USDA-APHIS PPQ Form 526 permit 37969.

In addition to egg masses of *E. vitticollis*, dead adult weevils infected by an entomopathogenic fungus were collected and returned to Florida. The diseased cadavers were collected on July 19 in an orange grove located about 10 km southwest of Dangriga. The dead weevils observed were widely distributed among larger numbers of live adults within the orange grove. All diseased weevils had died clutching a twig, a typical behavioral response of many insect hosts to invasion by a fungal pathogen. Diseased hosts had stalk-like structures protruding from the intersegmental areas of the body, typical of clavae formed by a Cordyceps (Samson et al. 1988). Each clava had a pinkish red head at the distal end. Until further mycological studies are completed at the ultrastructural level, the species will remain unknown. While in Belize, several diseased weevils were macerated in water and the mixture was sprayed onto new flushes of leaves, a common food source of adult citrus weevils. The treated leaves were then placed in a ventilated plastic box with about 20 adult weevils. After 3 days exposure under unknown ambient conditions, weevil behavior and mortality were assessed. None of the weevils exhibited abnormal behavior and none died. It was unknown, however, whether infective spores were in the original preparation or if environmental conditions were sufficient for infection to occur.

Two parasitoid species were recovered under quarantine from weevil eggs collected in Belize: *Pediobius irregularis* Kerrich and *Horismenus bennetti* Schauff (Hymenoptera: Eulophidae) (identifications by coauthor Evans) (voucher specimens placed with FSCA in Gainesville). *P. irregularis* had been identified previously as a parasitoid of *E. vitticollis* eggs in Belize (Schauff 1987), and *H. bennetti* had been identified previously as a suspect hyperparasitoid associated with egg parasitoids of citrus weevils in the Caribbean and West Indies (Schauff 1987) and known to occur in Belize (Etienne & Delvare 1991). A total of 280 individual adult parasitoids emerged from the material under quarantine; of 122 parasitoids formally inspected by coauthor Evans, 83 were *P. irregularis* and 39 were *H. bennetti*. Greater numbers of these parasitoids were obtained from material collected at Buckshell’s grapefruit grove than from any other collection site. Few parasitoids were recovered from Bowman’s grove, and none were recovered from material collected at the Weirrie Head Resort Inn grove. *P. irregularis* was the only parasitoid obtained from material collected at Mullin’s River grove, although relatively few weevil eggs were collected from this site. Observations under quarantine indicated 87% of the weevil egg masses from Belize were parasitized. This percentage may not have been a good indicator of actual percent parasitism levels in the field, as it is possible that parasitized egg masses are easier to find than non-parasitized eggs (i.e., the adhesive associated with an egg mass between two juxtaposing leaves may be less apt to come apart if an egg mass is parasitized, increasing the chances of finding a parasitized mass). However, general observations indicated *E. vitticollis* was subjected during July to at least moderate levels of parasitism in some locations such as Buckshell’s grapefruit grove.

The potential of *P. irregularis* and *H. bennetti* as candidate parasitoids for *D. abbreviatus* and also for the citrus root weevil, *Pachnaeus litus* (Germ.) (another weevil pest of citrus in Florida) was evaluated under quarantine by coauthors Peña and Duncan. As adult parasitoids emerged from the Belize weevil eggs, some were placed into cages (clear plastic boxes, 0.03 m$^3$) along with weevil egg masses (one to three days old) which had been oviposited onto citrus leaves, leaves of green buttonwood (*Conocarpus erectus* L.), leaves of pigmy palm (*Phoenix roebelini* O’Brien) or wax paper (some or all of these substrates as noted). Honey and water were provided in these cages as a food source for the adult parasitoids. With respect to the parent generation of *P. irregularis*, a ratio of 3 females to 1 male was observed. No parasitism of *D. abbreviatus* or *P. litus* eggs occurred by either endoparasitoid species during exposures made July 24-31 (Table 1). Low levels of parasitism by *P. irregularis* of both *D. abbreviatus* and *P. litus* eggs occurred during exposures made July 31-August 10, but the F$_1$, adult parasitoids recovered failed to parasitize the eggs of either weevil species (Table 2). The sex ratio of the F$_1$ parasitoids was not determined, but both sexes were produced from each weevil species. The F$_1$ parasitoids survived for a maximum of 17 days. No obvious predation by adult parasitoids on weevil eggs was observed, but this possibility was not investi-
### Table 1. Parasitism Outcomes in Cages Containing Egg Masses of *Diaprepes abbreviatus* and *Pachnaeus litus* Exposed to Adult *Horismenus bennetti* and/or *Pediobius irregularis*.

<table>
<thead>
<tr>
<th>Weevil species and oviposition substrate</th>
<th>Exposed to both parasitoid species together 7/24-7/31</th>
<th>Exposed to adult <em>H. bennetti</em> 7/31-8/13</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total # of egg masses provided</td>
<td>F₁ adult parasitoids produced</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on citrus leaves</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on buttonwood leaves</td>
<td>196</td>
<td>0</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on pigmy palm leaves</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on wax paper</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on citrus leaves</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on buttonwood leaves</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on pigmy palm leaves</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on wax paper</td>
<td>11</td>
<td>0</td>
</tr>
</tbody>
</table>

* A mix of *H. bennetti* and *P. irregularis* adults (202 total) introduced during 7/24-7/31.
* 70 *H. bennetti* adults introduced during 7/31-8/13.

### Table 2. Parasitism Outcomes in Cages Containing Egg Masses of *Diaprepes abbreviatus* and *Pachnaeus litus* Exposed to Adult *Pediobius irregularis*, F₀ adults from Belize and F₁ adults obtained in quarantine.

<table>
<thead>
<tr>
<th>Weevil species and oviposition substrate</th>
<th>Exposed to adult <em>P. irregularis</em> 7/31-8/10</th>
<th>Exposed to F₁ adult <em>P. irregularis</em> 8/24-8/31</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total # of egg masses provided</td>
<td>F₁ adult parasitoids produced</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on citrus leaves</td>
<td>9</td>
<td>8 (from 1 egg mass)</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on buttonwood leaves</td>
<td>259</td>
<td>5 (from 2 egg masses)</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on pigmy palm leaves</td>
<td>116</td>
<td>0</td>
</tr>
<tr>
<td><em>D. abbreviatus</em> eggs on wax paper</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on citrus leaves</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on buttonwood leaves</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on pigmy palm leaves</td>
<td>8</td>
<td>16 (from 3 egg masses)</td>
</tr>
<tr>
<td><em>P. litus</em> eggs on wax paper</td>
<td>0</td>
<td>—</td>
</tr>
</tbody>
</table>

* 174 *P. irregularis* adults introduced during 7/31-8/10.
* 31 *P. irregularis* adults introduced during 8/24-8/31.
gated because doing so would have jeopardized parasitoid recovery.

The research indicated *P. irregularis* held little promise as a candidate for a biological control program for either *D. abbreviatus* or *P. litus* and that *H. bennetti* held no promise at all. *H. bennetti* was not confirmed as a hyperparasitoid of *P. irregularis*, but this remained probable.

We thank the Belize Agricultural Health Authority (BAHA) for graciously hosting the trip, helping to obtain the required Belizean collection and exportation permits, for providing transportation and for collection assistance. Additional transportation and collecting assistance was kindly provided by the Citrus Research and Education Institute (CREI). We especially thank the following individuals for their support during this project: Orlando Sosa (BAHA), who served as our primary contact in Belize, and Francisco Gutierrez (CREI). We are grateful to Dr. Michael Tewes (BAHA) and Dr. Steven Williams (CREI) and their respective agencies for helping make this project possible. This research was partially supported by a grant from the Florida Citrus Producers Advisory Council (FCPRAC). Florida Agricultural Experiment Station Journal Series No. R-08996.

**SUMMARY**

Two parasitoid species, *Pediobius irregularis* and *Horismenus bennetti*, were recovered from weevil eggs collected in citrus in Belize. *Exophthalmus vitticollis* was the principal weevil species observed in citrus during the trip. Under quarantine in Florida, *P. irregularis* parasitized eggs of the Florida citrus weevils *Diaprepes abbreviatus* and *Pachnaeus litus*, but few F₁ adults were recovered and no F₂ adults were produced. The research indicated *P. irregularis* held little promise as a candidate for a biological control program for either *D. abbreviatus* or *P. litus* and that *H. bennetti* held no promise at all.

**REFERENCES CITED**


BOOK REVIEWS


Tiger Beetles is a synthesis of the current knowledge about a popular family of beetles, the Cicindelidae, known as tiger beetles. The authors have compiled taxonomic and ecological information, providing detailed discussions of most aspects of these topics. The book is divided into three major sections, part 1 is titled Taxonomic Diversity: Tiger Beetles in Space and Time. Part 2 is titled Ecological Diversity: Tiger Beetles in Their Environment, and Part 3 is titled Interaction of Ecological and Taxonomic Diversity. Numerous photographs (color and black and white) and drawings illustrate exotic and native North American species, adults and larvae. After their comprehensive discussion the authors present 2 appendices, one detailing how to observe and collect tiger beetles, the second presenting brief summaries of the natural history of the major tiger beetle genera of the world. Each genus discussed here is also illustrated. A lengthy list of cited references should bring the reader up to date on the most recent publications dealing with all aspects of tiger beetles.

In the introduction the authors caution that some readers may find sections too technical and recommend skipping these, to come back later for additional study. In several instances a distinction is made between professional researchers and amateur researchers and the material that may suit each of these groups. The content of many of the chapters is admittedly very technical in nature, and if one is not schooled in cladistics and molecular biology, may prove too technical to follow. I personally found several sections difficult to comprehend, and wonder if other readers will encounter the same difficulty. However, having said this, current research is indeed moving in the direction of using molecular studies to reinforce or contradict traditional classification of tiger beetles.

Chapter 1 discussed the merits of tiger beetles as research organisms. A brief history of tiger beetles studies is presented. After defining the characteristics of an ideal model test organism, the authors discuss why tiger beetles meet the requirements remarkably well. The authors conclude their discussion, stating “Molecular phylogeny, function of acute hearing, spatial modeling, and physiology of vision fields are but a few examples of areas of research made possible or greatly enhanced by researchers who recognized the value of tiger beetles as model organisms.”

Chapter 2 is a detailed discussion of what constitutes a tiger beetle. Adults and larvae are described, and detailed illustrations of external and internal anatomy illustrate the unique characters that separate tiger beetles from other beetles. In a section titled Body Parts the authors illustrate key characters used in the identification of tiger beetles. A detailed discussion of the digestive tract follows, as well as a discussion of vision in these beetles. The reproductive tract of male and female tiger beetles is presented, and the chapter concludes with a discussion of larval traits and behavior.

Chapter 3 details an in-depth overview of the classification and evolutionary schemes that have been applied to tiger beetles. Then the authors delve into the application of cladistic analysis to the classification of tiger beetles, and here is where some readers may be left behind due to the technical nature of the discussion. A list of terms used in phylogenetic construction with their definitions may help some readers, but I found the list to be difficult to apply to their discussion, and I feel too much prior knowledge of cladistics by the reader is assumed by the authors. Terms like “bootstrapping” and “jackknifing” are used without explanations. Numerous other cladistic techniques are mentioned without explanations. This section will prove too technical for many readers.

Cladistic procedures are followed by a discussion of the evolution of diversity among tiger beetles, specifically morphological diversity, color patterns, and finally phyletic diversity.

Chapter 4 deals with the species concept as applied to tiger beetles, and uses DNA sequencing examples to illustrate how morphological differences may not reflect the same relationships as molecular studies. This chapter also contains 29 color plates of various tiger beetle adults and larvae, and figures of subspecies and their ranges. The photography is striking in quality, and many species figured have not been illustrated before in such detail.

Chapter 5 discusses the genetic system of tiger beetles. Tiger beetles differ from almost all other beetles in possessing multiple sex chromosomes. I found this chapter to be of such technical nature that I would personally skip over this and come back later if the need should arise to learn about the genetics of tiger beetles.

Chapter 6 concludes part 1 with a detailed discussion of biogeography as applied to tiger beetles. This chapter makes for fascinating reading and is one that every person interested in studying tiger beetles should read.

Section 2, Chapters 7-11, deals with ecological diversity and tiger beetles in their environment.
Chapter 7 deals with tiger beetles in their natural habitats and influence of environmental changes. Chapter 8 deals with mate selection and reproduction. Chapter 9 discusses enemy avoidance and antipredator strategies. Chapter 10 analyses competition for resources, foraging behavior and species radiation. Finally, Chapter 11 discusses conservation and economics as applied to tiger beetles. The authors state that the use of tiger beetles in conservation has begun to make them important in the fight for future environmental preservation.

Section 3, Chapter 12, is a discussion of the need for future studies and the use of tiger beetles to document the general decline of biological diversity. Tiger beetles and their distribution patterns are useful for studying biological diversity. This is further reinforced by the need for studying the many named subspecies and how they reflect patterns of diversity.

The authors are to be commended for bringing together such varied topics and presenting them in a logical manner. I will certainly buy this book, for I find it a useful reference to several topics of which I did not have prior knowledge, and for the color photographs of exotic species of tiger beetles that I have not seen before. In spite of the highly technical sections throughout the book I feel that most tiger beetle enthusiasts will find this a useful reference book to add to their collection of tiger beetle books.

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A PERSONAL ACCOUNT OF DEVELOPING THE STERILE INSECT TECHNIQUE TO ERADICATE THE SCREWWORM FROM CURACAO, FLORIDA AND THE SOUTHEASTERN UNITED STATES

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ABSTRACT

The history is recounted of developing the sterile insect technique to eradicate the screwworm, Cochliomyia hominivorax (Coquerel), from the Caribbean island of Curacao, Florida and the southeastern U.S. Observations of screwworm biology and challenges faced in conducting these eradication projects are described by the author who worked on all aspects of the research and field operations. Eradication was first demonstrated on Curacao, essentially a 170 mi² outdoor laboratory. The population dynamics of the wild screwworm was determined and overflooding ratios and dispersal patterns essential for population suppression were defined. Eradication was achieved with minimal resources by attacking the pest during the time of year when it is least abundant. In Florida, eradication was greatly facilitated by an unusually cold winter that reduce the range and density of the target population. Eradication could not be attained easily by only suppressing the reproduction of adult screwworms. The larval population also had to be reduced to increase the ratio of sterile to wild males. It was essential to control the immature stages by diligent inspection and treatment of animal wounds. Leadership of the cattle producers was critical not only for securing program resources from the clientele, state legislatures and U.S. Congress, but also for gaining the cooperation of virtually all livestock owners. Additionally, leadership was required to acquire adequate research, extension and public information resources. Lessons learned from this work were corroborated repeatedly as the screwworm eradication program moved into the Southwest, Mexico and the Central America.

Key Words: Screwworm, Cochliomyia hominivorax, sterile insect technique, eradication, pest management, Curacao

RESUMEN

Se hace un recuento de el primer programa de erradicación del gusano barrenador del ganado en Curacao y luego en Norteamerica y Centroamerica, con especial enfasis en las actividades realizadas en Curacao y Florida desde 1951 hasta 1957. El autor de este artículo, quien trabajó como científico investigador en todos los aspectos relacionados con investigaciones de laboratorio, campo y como coordinador de operaciones, da a conocer los pormenores de los problemas biológicos y de operación de este programa, así como también de sus soluciones, las cuales no se han sido discutidas en ninguna de las publicaciones administrativas de estos programas del USDA.

El area de 170 millas cuadradas del territorio de Curacao sirvió como laboratorio para desarrollar el programa de técnicas de insectos esteriles. Esto permitió que se realizara una determinación cuantitativa de la dinamica poblacional de la poblacion salvaje del insecto, y del area adecuada para liberar proporciones elevadas de la poblacion esteril los cuales ayudaron a establecer patrones de dispersión esenciales para la supresion de la poblacion. Efectuar liberaciones de insectos esteriles en la epoca del año cuando la poblacion natural esta en declive, fue un factor esencial para lograr la erradicacion en un programa que contaba con recursos minimos.

Tres resultados muy importantes fueron encontrados a través de los programas realizados en Florida. El primer resultado demostró que la erradicación se facilita enormemente al realizar liberaciones cuando hay cambios climaticos severos los cuales reducen el rango y densidad de la poblacion del gusano barrenador. Segundo, la erradicacion no puede ser lograda unicamente por el hecho de liberar insectos sexualmente esteriles, sino que es esencial que los ganaderos controlen oportunamente los estados inmaduros del gusano, efectuando un monitoreo y un tratamiento constante de las heridas causadas por el gusano. Tercero, el liderazgo que toma la clientela de los productores es esencialmente importante para asegurar que hayan recursos que provengan de los ganaderos, la Legislatura estatal y del Congreso de la Nación. Estas lecciones fueron corroboradas repetidamente cuando el programa tuvo que realizarse en el suroeste de los Estados Unidos de America, en Mexico y en los países centroamericanos.

Translation provided by author.
The screwworm, Cochliomyia hominivorax (Coquerel), is an obligatory parasite of living warm-blooded animals, including man. The female oviposits on any lacerated or bloody area potentially caused by fighting, barbed wire scratches, castration, dehorning, branding, or ticks, and on body openings with fetid odors. The tender navels of newborn animals are particularly attractive. Unless infested wounds are treated with an insecticide, flies will continue to oviposit on the host until it is nearly dead. The screwworm has a life cycle of about 21 days during periods of warm weather (Laake et al. 1936).

Prior to eradication, its annual overwintering periods of warm weather (Laake et al. 1936). Screwworm has a life cycle of about 21 days during periods of warm weather (Laake et al. 1936). Prior to eradication, its annual overwintering area in the U.S. varied with the severity of the winter. The average overwintering zone was 50,000 mi² in Florida and about 150,000 mi² in the southwest (Texas- 70,000; New Mexico-1,000; Arizona-35,000; and California-46,000) (Laake et al. 1936; Scruggs 1975; Meyer and Simpson 1996). It survived as far north as Oklahoma in the Southwest and South Carolina in the Southeast during mild winters. Fortunately, a severe winter in 1957-1958 eliminated screwworms above a line extending from Tampa to Vero Beach, Florida. This weather fortuitously enabled use of the sterile insect technique (SIT) to eradicate screwworms from Florida and the Southeast in about one year.

1Part of the Pioneer Lecture presented at the annual meeting of the Florida Entomological Society, Daytona Beach, Florida, August 4, 1997.

SIT involves mass rearing harmful insects, sexually sterilizing them usually with gamma radiation after adult somatic cells have developed and releasing them so that sterile males will compete with wild males for mates. The radiation dosage must be sufficient to induce sexual sterility in both sexes. Wild females mated to sterile males oviposit normally and the embryos initiate development but die before hatching.

PRE-CURAÇAO

E. F. Knipling first conceptualized the use of SIT to suppress screwworm flies on an areawide basis in 1937 (Knipling 1955). He visualized the sustained release of large numbers of sexually sterile males into a wild population to eliminate reproduction and lead to eradication. His idea was not considered seriously at the time by his superiors in the U.S. Department of Agriculture (USDA), Bureau of Entomology and Plant Quarantine. However, the it remained firmly in his mind and was discussed repeatedly with his close colleagues, Drs. A.W. Lindquist and R. C. Bushland. During World War II, Knipling and Bushland worked on projects to protect the armed forces from arthropods and arthropod-borne diseases. After the war, as Knipling rose rapidly through the Bureau’s ranks, he was able to secure limited funds to conduct research on his idea.

Bushland had already fulfilled one of the requirements of SIT by developing an artificial diet for rearing the screwworm (Melvin & Bushland 1936, 1940). The diet consisted of lean ground beef, blood, water and 0.2% formaldehyde to deter decomposition. Screwworms had been reared previously by infesting rabbits or baby calves, a very nasty and cruel procedure. Bushland evaluated various chemicals to induce sexual sterility but none was effective. However, H. J. Muller reported in 1950 that exposure of Drosophila melanogaster Meigen to high doses of x-rays induced dominant lethal mutations in the germ cells (Muller 1950). These mutations in the sperm of irradiated males, mate with untreated females, prevented the development of embryos and thereby caused sexual sterility. A. W. Lindquist read this popular article aimed at swaying public opinion against atmospheric tests of atomic bombs and showed it to Knipling. Knipling corresponded with Muller, who expressed confidence that ionizing radiation would induce sterility in the screwworm. With Knipling’s encouragement, Bushland investigated the effects of x-rays on screwworm sexual behavior and reproduction (Bushland 1952). Subsequently, he and D E. Hopkins induced sterility in screwworms exposed as late pupae or adults to x-rays or gamma rays from Colbalt 60 (Bushland & Hopkins 1951, 1953). They showed in laboratory cage tests that the capacity of sterile screwworm males to compete with untreated males in mating with untreated females was acceptable at dosages required to sterilize both males and females. Since the screwworm female mates only once, all egg masses oviposited by a wild female mated with a sterile male are non-viable.

To assist in evaluating the performance of irradiated sterile males in the field, I was reassigned from the USDA Grasshopper Control Division, to the USDA’s Insects Affecting Man & Animals Laboratory at Kerrville, Texas and later to a sub-laboratory at Orlando, Florida. Bushland had previously attempted to evaluate the field performance of irradiated screwworm males on the shoals of the Texas coast near Austwell. However, no egg masses were oviposited on wounded sentinel goats, apparently because the released flies were blown to the mainland by the strong prevailing winds. Subsequently, during the winter of 1951-1952, efforts were made to evaluate the competitiveness of sterile relative to lab-reared males in mating with released females on Sanibel Island near Ft. Meyers, Florida. It was assumed that native screwworm flies would be scarce and produce few egg masses because no livestock were on the island. However, native screwworms were found to be as numerous on the island as on the mainland and to infest feral cats, opossums, and...
rabbits. Evaluation of the releases showed that lab-reared flies performed well in nature, since a high ratio of sterile to fertile egg masses was collected from the wounds of sentinel animals. During the following winter, 1952-1953, when only sterile males were released at a rate of 100 per mi², egg mass sterility temporarily reached 100% within the first eight weeks. However, during the 12th week a fertile egg mass was found. Since Sanibel is only two miles from the mainland, a fraction of the parasite's flight range, we realized that eradication could not be sustained because of screwworm fly migration.

The use of sterile males to achieve eradication had to be evaluated on a fully isolated island, small due to our very limited budget. Vieques, nine miles from Puerto Rico, met these requirements but it was a U.S. Navy bombing range. Fortunately, B. A. Bitter, a Dutch agricultural officer, focused our attention on Curacao, Netherlands Antilles, forty miles north of Venezuela. Bitter wrote to the USDA Entomology Research Division for recommendations on protecting dairy and grazing animals from the screwworm. At Knipling's behest, I was dispatched to Curacao in July 1953 to investigate its suitability for our test. It had a serious screwworm problem dating back at least ninety years. About 25,000 goats, 5,000 sheep, 300 deer and numerous rabbits populated the island. Due to the screwworm, many of the nanny goats had only one kid or none. Dairy cattle and horses were not considered significant screwworm hosts because of close surveillance and care by the owners.

CURACAO

The eradication test began on March 17, 1954. Initially, egg mass data were collected from screwworm-infested goats maintained in ten pens distributed throughout the island. Additional pens were established later. During the first two weeks prior to the release of sterile flies, 288 egg masses were collected. All of them were fertile, indicating that unmated females did not oviposit and the egg collection and incubation techniques were adequate. For the first six weeks, 200 irradiated flies of both sexes were released per mi² and weekly egg masses collections increased from 121 to 277. An average of only 15% of these egg masses was sterile, so the release rate was insufficient to suppress the pest population. Based on this result, we compared two release rates, 200 per mi² on one half and 800 per mi² on the other half of the island. During the 3rd week of this test, the higher rate resulted in 53% and the lower 28% sterility.

Beginning on August 9, 1954, the entire island was treated weekly with about 800 sterile flies per mi², although there were some fluctuations in the supply of sterile flies from the rearing facility at Orlando. During the next four weeks, egg masses averaged only 4.6 per pen and sterility increased from 69% to 79%, reaching 100% by the 7th week. When sterility reached 100% and almost no egg masses were collected, Wes New and I experienced great suspense each evening as we tabulated the egg mass data. I wired the results daily to Lindquist who, along with Knipling, eagerly awaited the reports. The last two small sterile egg masses were collected after four weeks of zero collections. These were 2nd or 3rd ovipositions by long-lived screwworm females, verified by several eggs in each mass that developed to the spined stage characteristic of the sterilizing dosage delivered to released males. Only small single, sterile egg masses were collected during the 13th and 14th weeks; however, releases were continued through the 22nd week to assure eradication.

These remarkable results were in close agreement with projections made with Knipling's model (Knipling 1959, 1979). Eradication was achieved by increasing the release rate while the native screwworm population was in a natural seasonal decline. Interestingly, Bushland was concerned that we would eradicate the screwworm from Curacao before gaining detailed scientific information about the pest's population dynamics and other factors contributing to the success or failure of SIT. Knipling and Lindquist were confident that the technique was scientifically valid but they thought the project might fail because of operational difficulties. I felt we had at least a 50% chance of succeeding.

When eradication seemed imminent, I asked the local authorities to build a security pen completely enclosed with fly proof screen. Goats to be used as sentinel animals were infested with screwworms in this enclosure and held for three or four days. Infested wounds emit an odor produced by bacterial action that makes them much more attractive to screwworms than un-infested wounds. Before the goats were removed from this secure compound and transported to the ten or more pens used to collect egg mass data, all larvae were removed from the wounds and killed with benzol. To increase surveillance during the final weeks of the test, additional pens were established in the lower southern part of the island. Although a few sterile egg masses were collected during the first two weeks, the number quickly dropped to zero.

Procedures were immediately established to prevent re-infestation of the island. Through the news media, radio and print, we requested that livestock owners report any screwworm larval infestations. Nine cases were investigated from mid-October to mid-December and all were identified as Callitroga macellaria (Fabricius), the secondary screwworm fly, a scavenger of minor importance. However, in 1971, seventeen years after...
eradication, screwworms were rediscovered on Curacao. This reintroduction probably came from infested livestock shipped from South America to be slaughtered at the abattoir. These screwworms were eradicated by October 25, 1977 by first using the screwworm adult suppression system (SWASS), a lure and insecticide combination. It was applied for ten weeks and suppressed the population by 65-85% before releasing sterile males to achieve eradication (Coppedge et al. 1978).

The screwworm colony was less than ideal. An old strain from Texas was used because the overworked crew at Orlando, led by Jack Graham, Don Hopkins, and Frank Dudley, did not have time to colonize a strain from Curacao. Additionally, we discovered that the first 3rd of the flies to emerge in a batch were predominantly females and the last 3rd were mostly males. The early part of each weekly batch was irradiated and sent to Curacao, while the pupae latest to emerge were reserved for egg production. As a result, the colony cages contained a high proportion of males. Males are highly aggressive sexually, so females die prematurely and produce few eggs when they are greatly outnumbered by males (Baumhover 1965).

In 1954, sterile flies were released from a single engine, World War II training plane. Later, off duty pilots of the Royal Dutch Airline, KLM, flew the screwworm plane but, surprisingly, some became airsick and others eventually had different priorities for their time. Fortunately I was able to enlist the services of Peter Mijss, an adventurous former British Royal Air Force pilot. He was ready on a moments notice but we had to detour from the screwworm flight lanes to photograph incoming oil tankers, so his partner could later board the ships to sell photos to the crew.

We are deeply indebted to the Curacao Administration for helping us to conduct this historic experiment. Bitter was assigned to the project full time and helped immeasurably by procuring goats, securing the cooperation of landowners, solving technical problems, assisting with routine fly releases, and collecting egg masses. The local government also furnished a caretaker who fed and watered our goats. KLM gave priority to our fly shipments from Orlando and assured that they always arrived on time, even during the Christmas Holiday.

**FLORIDA 2000 MI² TEST**

I reported the success on Curacao at the 1956 Florida Livestock Association meeting in Sarasota. My unofficial view was that SIT could be used to rid Florida and the Southeast of the screwworm (Scruggs 1975). Apparently, my report caused the Florida Livestock Board to insist that the USDA draft a proposal for a Florida and Southeastern U.S Screwworm Eradication Program. The proposed program would require an enormous, two-year “all-out” effort to produce, irradiate and release about 50 million sterile flies per week throughout 50,000 mi². By contrast, the Curacao project required less than 200,000 flies weekly and involved only 170 mi², less than 1% as large as required in the southeastern U.S. As an initial step, SIT was tested in a 2,000 mi² area southeast of Orlando, bordering the Atlantic Coast (Baumhover 1958; Baumhover et al. 1959; Graham & Dudley 1959). This was a cooperative effort of the Florida Livestock Board, and the USDA Animal Disease Eradication Division and Entomology Research Division.

Because of foul odors associated with the larvae, a temporary rearing facility was constructed near Bithlo, an uninhabited area 20 miles east of Orlando. Several carloads of temporary building sections were sent from Beltsville, Maryland and used by the research team to construct the facility.

It was disconcerting to me and other professional scientists to be removed from exciting, on-going research projects and required to work as construction laborers. However, because adequate funds were rarely available, this was standard practice during the early years of research, development and implementation of the screwworm SIT program. Only after successful eradication of the screwworm from Florida and the Southeast, were adequate funds (ca. $500,000) appropriated annually for research to support the program in Texas, the southwestern U.S., and northern Mexico.

Beginning May 2, 1957, two million sterile flies were released weekly, at a rate of 1,000 flies per mi², against a dense wild population that was infesting 80-100% of newborn calves (Meadows 1985). Despite the severity of this screwworm outbreak, the number of egg masses in the release area declined from 575 per week to only 17 during the 16th week ending August 24, 1957. Egg mass sterility had risen to about 70% by August 10 and there also was a decline in egg masses at check pens south and west of the release zone. If egg mass sterility under these circumstances had reached only 50%, the test would have been considered a success (Knipling 1985). However, egg masses remained numerous in the north and, since the test area was not isolated, eradication could not be achieved. Trends in the egg mass collections mimicked those on Curacao, so the test was ultimately considered successful and some livestock owners, government officials, and Florida legislators were convinced that eradication was feasible in Florida.

**ERADICATION IN FLORIDA AND SOUTHEASTERN UNITED STATES**

The dreaded screwworm fly was not present in Florida prior to 1933, having been introduced into
southern Georgia with infested cattle from the drought-stricken Southwest. It spread southward to Florida, infested the entire state within three years, and caused catastrophic losses (Bruce and Sheely 1944). An estimated 75,000 screwworm cases occurred in south Georgia during 1933, and Florida had 1,300,000 in 1934. Expensive state and USDA control programs aimed at treating infested animals reduced the cases to only 48,737 during 1936 and the mortality of animals declined from 12% during 1934 to only 0.71%. This type of control is essential to screwworm eradication by means of SIT, particularly when conditions are ideal for an increase in the pest population. This was demonstrated unequivocally in attempting to eradicate the persistent infestation in Broward and adjoining counties.

Florida livestock owners were particularly anxious to be rid of the screwworm but, despite the success of the 2,000 mi² test, the USDA insisted on more research. Consequently, the print media criticized the USDA “for dragging its feet” and a conference was arranged with Governor Collins of Florida. Knipling told him that two additional years of research would save the program $2 million a year. Collins replied, “why wait two years to save $2 million when losses are $10 million per year?” Some cattlemen estimated annual losses at $20-40 million (Knipling 1959). Governor Collins’ response deeply affected Knipling’s philosophy regarding the importance of areawide insect control. Florida livestock owners, under the leadership of Okeechobee rancher J. O. Pierce, soon convinced their legislature to appropriate $3 million as their share of a proposed $6 million screwworm eradication program.

The operational program was initiated in 1957-1958 during the coldest winter ever recorded in the Southeast. Screwworms were killed southward to a line extending from Tampa to Vero Beach. To take advantage of this weather, in December 1957 USDA officials rapidly expanded the research facilities at Bithlo to produce a maximum of 13 million flies per week for use in preventing re-infestation of Georgia and the northern half of Florida (Bushland 1960). From January 18 to April 1, 1958, weekly production averaged only three million flies that were released at a rate of 200 per mi² primarily in a band across the peninsula between Gainesville and Orlando. Subsequently, production increased to 14 million flies per week that were released at the same rate across the northern half of Florida and north to Savannah, Georgia to combat scattered outbreaks north of the overwintering line. Sterile flies were also released as far north as Montgomery County, Alabama and south to Miami.

Unfortunately, quarantine lines had not been established in Florida and along the Mississippi River from screwworm infested areas in the southwest and southern Florida into screwworm free areas in the southeast and northern Florida. With adequate quarantines, sterile flies from the Bithlo facility alone might have eradicated screwworms from the much-reduced overwintering zone. Due to the new infestations, however, an additional large, expensive mass rearing facility was required. A highly talented USDA engineer, C. N. Husman, designed the facility at a World War II airbase seven miles east of Sebring (Baumhover et al. 1966). Husman frequently consulted the research team to determine optimum holding and handling conditions for the insects, and we urged him to enlarge the requested facilities. He surreptitiously, but wisely, increased production capacity by an essential 50%. After the program began, as many as 80 million flies were produced weekly to cover 85,000 mi² during peak activity.

Screwworms were only a minor problem throughout the southern half of Florida during the entire program, except for Broward, Palm Beach and Miami-Dade counties. The temperature and rainfall in these counties supported screwworm development and survival throughout the year but the pests were most abundant during the winter when most of the calves were born. Only 19 confirmed and 16 additional cases were recorded in these counties from January through August 1958, 35 weeks with a weekly average of 1.0. However, during September 1958 the average increased to 4.75 and peaked at 33.0 between mid December 1958 and mid January 1959 (Table 1). The percentage of infested wounds peaked at 5.86% during this same period. However, by February 19, 1959 the last infestation was recorded as a mass of 40 eggs, some fertile, taken from a 1-day-old calf. An additional 23 sterile egg masses were collected before March 13, 1959: four of these contained six eggs or less and two had malformed eggs, indicating they were oviposited by released females. Similar data were obtained from Palm Beach and Miami-Dade counties. Palm Beach had 32,434 wounds inspected with only 35 cases (0.15%); Miami-Dade had only 3,321 wounds with 30 cases (0.90%). Although egg mass sterility was 74.5-76.3% for eight weeks from November 23, 1958 to January 17, 1959, time for almost three generations, a substantial downturn in cases did not occur until mid February 1959, after weekly release rates had been increased from 400 to 10,600 sterile flies per mi².

There were many possible causes for the screwworm outbreak in the Broward County. The sterile flies could have been of poor quality (Sharman 1960) and there was considerable complacency among the cattlemen. Some ranchers decided prematurely that eradication had been achieved and no longer treated infested calves, allowing larvae to leave wounds, pupate and later emerge as fertile flies. Calf navels accounted for 415 out of 491 (84.5%) screwworm collections made from November 17, 1958 to March 13, 1959,
**Table 1. Average number of wounds observed and screwworm cases, number of egg masses collected and inspectors, and release rates each week during the screwworm outbreak in Broward County, 8/30/58 to 2/28/59.**

<table>
<thead>
<tr>
<th>Dates</th>
<th>Wounds</th>
<th>Cases</th>
<th>% Wounds Infested</th>
<th>Total Egg Masses</th>
<th>No. Sterile Egg Masses</th>
<th>% Sterile Egg Masses</th>
<th>Inspectors</th>
<th>Rates</th>
</tr>
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<td>8/30-9/27/58</td>
<td>689</td>
<td>4.75</td>
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<td>635</td>
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<td>1.26</td>
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<td>41</td>
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<tr>
<td>12/21-1/17/59</td>
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<td>100</td>
<td>76.3</td>
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<td>1/18-2/14/59</td>
<td>384</td>
<td>9.75</td>
<td>2.54</td>
<td>44^</td>
<td>39</td>
<td>88.6</td>
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<td>2/15-2/28/59</td>
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</table>

^All periods except the last are 4 weeks.
^Includes both confirmed and reported cases.
^Includes collections only from 1/18-2/6/59 when released females began ovipositing sterile eggs due to anoxia.
^Number of inspectors collecting samples of larvae from wounds. As many as 15 inspectors were present during the final phases to increase surveillance and spray infested herds.
^Number of sterile males released per mi² per week.
^Last evidence of native screwworm activity, a fertile egg mass from the navel of a 1-day-old calf.
including both egg masses and larvae. More than 36% of the wounds had mature 3rd instar larvae ready to pupate. One cattleman inherited another ranch and unsuccessfully attempted to maintain screwworm control without employing enough laborers. As a result, he was one to two weeks late in treating his newborn calves. A livestock inspector advised another rancher that many animals in his herd were infested yet the rancher delayed treating them for two weeks. Another rancher simply did not treat infested animals. A wildlife refuge within the problem area contained feral hogs that are notoriously susceptible to screwworm infestation because of fighting and udder wounds created by suckling pigs. Feral hogs were also implicated in persistent screwworm populations in Hardee, Desoto, and Lee counties. Finally, the supervisor of airplane release operations unilateral decided to not disperse flies over metropolitan areas until screwworms were out of control.

On June 18, 1959, a confirmed screwworm sample was received from a ranch within ten miles of the Sebring mass rearing facility. The true origin of the specimens was never determined. Screwworms may have escaped from a load of 100 Texas cattle transported by train and held on a ranch near the screwworm facility several weeks before the infestation was reported. Alternatively, fertile screwworms may have escaped from the facility, either accidentally or through sabotage by an employee as a means of extending a well paying job. Ranchers have been known to retain screwworm samples for later use to “test surveillance by program personnel”, or to obtain free CO-RAL to treat a herd for various other insect pests and ticks. Finally, the rancher may have inadvertently delayed submission of the sample.

In conclusion, early development of SIT through screwworm eradication in Curacao, Florida and the southeastern U.S. demonstrated the importance of close collaboration among scientists, industry leaders and government officials at all levels. Progress depended on acceptance of new ideas and willingness to take reasonable risks. Florida had 865 confirmed screwworm cases during the eradication period and, of these, 31 occurred more than 50 miles above the overwintering line (Baumhover 1966). Additionally, 1901 cases were reported but not confirmed of which 1,711 (90%) were probably screwworms (Knippling & Rainwater 1937). Thus, the total number of cases probably was about 2,575, or 0.08% of a possible 3 million cases that would have occurred without releasing sterile screwworm flies. Eradication of the screwworm from the southeastern U.S. was declared in November 1959, as this extremely creative, low risk, and cost effective eradication program was completed successfully.

**REFERENCES CITED**


