RESPONSES OF DIABROTICA SPECIOSA AND CEROTOMA ARCUATA TINGOMARIANA (COLEOPTERA: CHRYSMELIDAE) TO VOLATILE ATTRACTANTS

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ABSTRACT

The relative responses of Diabrotica speciosa (Ger.) and Cerotoma arcuata tingomariana Bechyné (Coleoptera: Chrysomelidae) to traps baited with chemicals were studied. Volatile substances of Curcubita maxima Duchesne blossoms, other previously reported volatile attractants for Diabroticites and mixtures of chemicals were tested in common bean, Phaseolus vulgaris L., and soybean, Glycine max (L.) Mer., fields. Traps baited with 1,4-dimethoxybenzene caught 29.4 times more beetles than solvent controls in fields of soybeans, and 9.4 times more in common bean fields. Traps baited with VIP (veratrole + indole + phenylacetaldehyde) caught 6.5 times more beetles than solvent controls in soybean and 3.5 times more in common bean plots, whereas traps baited with TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) caught 6.7 times more beetles in soybean and 3.5 times more in common bean plots. Volatile chemicals used in this study did not attract C. a. tingomariana. In a dose-response study, captures of D. speciosa increased significantly with increasing doses of 1,4-dimethoxybenzene.

Key Words: Diabrotica speciosa, Cerotoma arcuata tingomariana, Phaseolus vulgaris, Glycine max, semiochemical, kairomone

RESUMO

As respostas relativas de Diabrotica speciosa (Ger.) e Cerotoma arcuata tingomariana Bechyné (Coleoptera: Chrysomelidae) para armadilhas com substâncias químicas foram estudadas. Substâncias voláteis de flores de Curcubita maxima Duchesne, outros atraentes voláteis, previamente reportados para diabroticíneos, e misturas de substâncias foram testadas em campos de feijão, Phaseolus vulgaris L., e soja, Glycine max (L.) Mer. Armadilhas com 1,4-dimethoxybenzene capturaram 29,4 vezes mais besouros do que a testemunha com solvente em campos de soja, e 9,4 vezes mais em feijão. Armadilhas com VIP (veratrole + indole + phenylacetaldehyde) capturaram 6,5 vezes mais besouros do que a testemunha com solvente em campo de soja, e 3,5 vezes mais em feijão, enquanto que armadilhas com TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) capturaram 6,7 vezes mais besouros em soja e 3,5 vezes mais em feijão. As substâncias não atraíram C. a. tingomariana. Em estudo de dose-resposta, as capturas de D. speciosa aumentaram significativamente com doses crescentes de 1,4-dimethoxybenzene.

Relationships between Diabroticites and plants of the family Cucurbitaceae are mediated by kairomones. The extremely bitter cucurbitacins are arrestants and feeding stimulants for Diabroticite and Aulacophorite beetles (Luperini tribe) (e.g. Chamblis & Jones 1966, Ferguson et al. 1983, Howe et al. 1976). Adult Diabrotica also are attracted to Curcubita spp. blossoms by volatile chemicals. These substances play an
important role in cucurbit selection by Diabrotica beetles. Morgan & Crumb (1928) first reported the attraction of Diabroticites to volatile chemicals when they described the attraction of *D. undecimpunctata howardi* (Barber) to cinnamaldehyde and cinnamyl alcohol baits. Snapp & Swingle (1929) attracted the same species with benzyl alcohol. *D. barberi* Smith and Lawrence and *D. cristata* (Harris) were attracted to eugenol, which also attracts the Japanese beetle, *Popillia japonica* Newman (Ladd et al. 1983, Lampman & Metcalf 1988) and to eugenol analogs (Ladd 1984). The isolation and identification of several volatile compounds from *Cucurbita* spp. blossoms (Andersen & Metcalf 1986, Andersen 1987) contributed to the knowledge of *Diabrotica* spp. and related genera in their specific responses to chemicals. A series of *Cucurbita* blossom kairomones and closely related compounds (parakairomones) attract *Diabrotica* spp. and *Acalyema* spp. (e.g. Andersen & Metcalf 1986, Lampman & Metcalf 1987, 1988, Metcalf & Lampman 1989, Lewis et al. 1990, Lance et al. 1992, Deem-Dickson & Metcalf 1995, Petroski & Hammack 1998). *Diabrotica speciosa* (Ger.) and *Cerotoma arcuata tingomariana* Bechyné were captured in traps baited with cucurbitacins (Roel & Zatarin 1989, Ventura et al. 1996) upon which these beetles feed compulsively and sequester and store 23,24-dihydrocucurbitacin D (Nishida et al. 1986, Nishida & Fukami 1990). This widespread similarity in behavioral response metabolism of cucurbitacins provides strong evidence for coevolution between these Chrysomelidae and Cucurbitaceae.

Although North American *Diabrotica* responses to volatile substances have been studied since the beginning of this century (Morgan & Crumb 1928), no reports are available on South American pests. We report here the results of field trials testing the relative attraction of *D. speciosa* and *C. a. tingomariana* to volatile kairomones and mixtures from *C. maxima* blossoms and some North American *Diabrotica* spp. parakairomones.

**Material and Methods**

Field experiments were carried out at the Universidade Estadual de Londrina School Farm, Londrina (latitude 23°19'S, longitude 51°12'W), Paraná State, Brazil. Soybean, *Glycine max* (L.) Mer., cv. Oecpar 14 (sown on December 19, 1996) and common beans, *Phaseolus vulgaris* L., cv. Iapar 59 (sown on February 26, 1997; February 10, 1998) fields (0.5-ha plots) were used as testing sites.

In 1997, beetle traps consisted of transparency film (15.2 × 27.9 cm) (3M do Brasil, Ribeirão Preto, SP, Brazil) painted with yellow gold Suvinil paint 2450-0103 (BASF S.A., São Bernardo do Campo, SP, Brazil) on the interior. Yellow traps previously were successful in capturing *D. speciosa* and *C. a. tingomariana* (Ventura et al. 1996). The film was clamped into a 15.2-cm tall cylinder and externally coated with the clear insect adhesive, Tangle Trap (Tangle Foot Co., Grand Rapids, MI, USA). In 1998, 750-ml plastic cups painted with the same paint replaced the transparency film traps. Dental wicks (40 mm long × 10 mm diameter) soaked with test chemicals were clamped (transparency film) or glued (on the bottom of the cups) to the traps. Solid chemicals were prepared as standard wt/vol. solutions in acetone. The baited traps were placed in the field at 3:00 P.M. and removed after 24 hours.

Traps with 100 μl or 100 mg of each chemical were fixed on a wooden stake above canopy height in soybeans on March 20, 1997 and 0.25 m height in common beans on March 22, 1997. Control traps received only acetone. The chemicals tested included the *C. maxima* blossom volatile substances 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene (nerolidol); benzyl alcohol; 2,3-benzopyrrole (indole); phenylacetaldehyde; 1,2-dimethoxybenzene (veratrole); 1,2,4-trimethoxybenzene; benzoicdehyde; 4-[2,6,6-tri-
methyl-1-cyclohexen-1-yl]-3-buten-2-one (β-ionone); benzyl acetone; α-ionone (Sigma Chemical Company, St Louis, MO); 1,4-dimethoxybenzene; 4-methoxyphenethyl alcohol; cinnamyl alcohol; trans-cinnamaldehyde (Aldrich Chemical Co., Milwaukee, WI) and the Diabrotica spp. parakairomone 2-methoxy-4-(2-propenyl) phenol (eugenol), and eugenol-related 4-allyl-1,2-methylenedioxybenzene (safrole) (Sigma). SIC (safrole + indole + trans-cinnamaldehyde), TIC (1,2,4-trimethoxybenzene + indole + trans-cinnamaldehyde) and VIP (veratrole + indole + phenylacetaldehyde) mixtures were used at a dosage of 100 mg or 100 µl of each single chemical per trap. Traps were returned to the laboratory where the beetles were identified to species and sexed. Sex ratio of field populations of beetles was also determined from sweep net samples taken when the traps were removed.

The responses of D. speciosa and C. a. tingomariana to a range of dosages (1, 3, 10, 30, 100 or 300 mg per trap) of the compound most attractive to D. speciosa were evaluated on April 20, 1998. A regression analysis was performed to evaluate the relationship between lure concentration and trap effectiveness.

All experiments were conducted in a four replicate randomized complete block design. Distance between traps was 5 m within a block, and 10 m between blocks. Analysis of variance (ANOVA) was performed and Tukey's studentized range test (HSD) was used to compare individual means (SAS Institute 1989) on volatile chemicals screening. Data were transformed by \log (x + 1) constant to normalize the data and reduce heterogeneity of variances. Means and standard errors of means are presented for untransformed data.

RESULTS AND DISCUSSION

Only traps baited with 1,4-dimethoxybenzene, VIP and TIC mixtures caught significantly more D. speciosa than the controls and this was true in both soybeans and common bean (Tables 1 and 2). 1,4-Dimethoxybenzene was the most attractive compound. Traps baited with the latter compound, VIP and TIC baited traps captured both males and females. The sex ratio of D. speciosa beetles determined with sweep net sampling was 1.1 (n = 100) in common beans and 1.0 (n = 100) in soybeans. The TIC mixture is a strong attractant to North American Diabrotica spp. (Lampman & Metcalf 1987, 1988, Lance et al. 1992) and Acalymma vittatum (F.) (Lewis et al. 1990). Despite its geographic isolation from the North American inhabitants, Diabrotica spp. and A. vittatum, D. speciosa was also attracted to the simplified blend of C. maxima blossoms (Tables 1 and 2).

The Diabrotica genus has been grouped in three taxonomic units; two of which contain pest species (Branson & Krysan 1981). The fucata species group in which D. speciosa is included is multivoltine, polyphagous and overwinters as adults in regions where frost seldom occurs. The virgifera species group is univoltine, oligophagous, has an egg diapause and overwinters in soil at temperatures below zero (Branson & Krysan 1981, Krysan et al. 1989). D. speciosa shows responses similar to D. u. howardi, a species also belonging to the fucata group, in its responses to VIP and 1,4-dimethoxybenzene. D. u. howardi was attracted to other benzenoid compounds (Lampman et al. 1987). In contrast, VIP was reported to be largely non-attractive to the virgifera group species (Lampman & Metcalf 1987).

D. speciosa was not attracted to single-component lures that are known to attract other species of Diabrotica in either the virgifera or fucata groups [i.e. benzyl acetone, benzaldehyde, cinnamyl alcohol, eugenol, indole, β-ionone, phenylacetaldehyde, cinnamaldehyde, and veratrole (Andersen & Metcalf 1986, Lampman et al. 1987, Lampman & Metcalf 1987, 1988, Metcalf & Lampman 1989, Lewis et al. 1990)] but
exhibited its own species-specific pattern of response. 1,4-Dimethoxybenzene is the major floral volatile component in *Curcubita maxima* Duchesne cv. True Hubbard and the 4th major one in cv. Blue Hubbard (Andersen 1987). *C. maxima* blossoms attract adults of *Diabrotica* species (Fischer et al. 1984, Andersen & Metcalf 1987). However, despite the great proportion of 1,4-dimethoxybenzene in the *C. maxima* floral odor, no records of its attractiveness to Luperini beetles have been reported. Metcalf & Metcalf (1992) reviewed the attractiveness of volatile chemicals from blossoms to Diabroticite beetles and attributed a >100-mg threshold of response by *D. barberi*, *D. cristata*, *D. u. howardii* and *D. v. virgifera*.

*D. speciosa* apparently is attracted to methoxylated or methylene-bridged analogs, with or without an allyl or propenyl moiety, possibly with or without a free phenolic group. Further investigations concerning these structure-activity aspects might be achieved.

The response by *D. speciosa* appeared to vary in magnitude (only one comparison) depending on the host (differences in soybeans versus common beans). The common bean is recognized as a very attractive crop to this beetle, mainly early in its phenological cycle (Ventura et al. 1996). Similarly, *D. virgifera virgifera* LeConte was re-

### TABLE 1. MEAN NUMBER (±SE) OF ADULTS AND THE SEX RATIO (FEMALES PER MALE) OF *D. speciosa* AND *C. a. tingomariana* CAUGHT PER YELLOW TRANSPARENCY FILM STICKY TRAPS IN A SOYBEAN CROP AFTER 24 H (MARCH 20, 1997).

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>D. speciosa</em></th>
<th><em>C. a. tingomariana</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl acetone</td>
<td>5.0 ± 1.0bc (1.5)</td>
<td>4.2 ± 2.4ab (1.4)</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>8.7 ± 3.5bc (1.3)</td>
<td>6.0 ± 1.7ab (1.0)</td>
</tr>
<tr>
<td>Benzaldehyde</td>
<td>8.2 ± 3.2bc (0.9)</td>
<td>7.2 ± 0.9ab (0.9)</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>6.5 ± 1.1bc (1.0)</td>
<td>5.0 ± 3.3ab (0.7)</td>
</tr>
<tr>
<td>1,4-Dimethoxybenzene</td>
<td>108.7 ± 25.6a (1.2)</td>
<td>5.7 ± 1.2ab (1.1)</td>
</tr>
<tr>
<td>Eugenol</td>
<td>4.7 ± 1.1bc (0.9)</td>
<td>5.7 ± 1.5ab (0.9)</td>
</tr>
<tr>
<td>Indole</td>
<td>10.2 ± 3.9bc (1.1)</td>
<td>5.5 ± 1.0ab (1.0)</td>
</tr>
<tr>
<td>α-ionone</td>
<td>2.5 ± 1.5c (1.0)</td>
<td>4.5 ± 1.0ab (0.8)</td>
</tr>
<tr>
<td>β-ionone</td>
<td>7.5 ± 2.4bc (1.3)</td>
<td>7.7 ± 2.3ab (1.3)</td>
</tr>
<tr>
<td>4-Methoxyphenethyl alcohol</td>
<td>4.5 ± 2.2bc (1.2)</td>
<td>3.5 ± 1.0ab (1.8)</td>
</tr>
<tr>
<td>Nerol</td>
<td>20.7 ± 9.4bc (1.1)</td>
<td>13.2 ± 3.6a (0.9)</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>11.2 ± 1.1bc (1.2)</td>
<td>3.7 ± 1.3ab (0.7)</td>
</tr>
<tr>
<td>Safrole</td>
<td>5.0 ± 1.2bc (1.0)</td>
<td>4.5 ± 1.0ab (0.8)</td>
</tr>
<tr>
<td>Trans-cinnamaldehyde</td>
<td>3.7 ± 1.8c (2.0)</td>
<td>6.2 ± 2.4ab (1.5)</td>
</tr>
<tr>
<td>1,2,4-Trimethoxybenzene</td>
<td>10.0 ± 4.8bc (1.7)</td>
<td>4.0 ± 1.3ab (1.3)</td>
</tr>
<tr>
<td>Veratrole</td>
<td>7.7 ± 1.9bc (1.1)</td>
<td>5.2 ± 2.8ab (0.9)</td>
</tr>
<tr>
<td>SIC3</td>
<td>12.5 ± 1.4bc (1.5)</td>
<td>2.2 ± 1.3b (1.2)</td>
</tr>
<tr>
<td>TIC4</td>
<td>24.7 ± 2.2b (1.2)</td>
<td>4.0 ± 1.5ab (1.7)</td>
</tr>
<tr>
<td>VIP5</td>
<td>24.2 ± 5.4b (1.1)</td>
<td>3.0 ± 1.5ab (1.0)</td>
</tr>
<tr>
<td>Control</td>
<td>3.7 ± 0.9c (1.5)</td>
<td>6.2 ± 1.7ab (0.7)</td>
</tr>
</tbody>
</table>

1*Means in the same column with different letter are significantly different based on Tukey’s studentized range test (P < 0.05), n = 4.
2Single and mixed chemicals are dosed at 100 mg or 100 µl of each compound per trap.
3Safrole + indole + trans-cinnamaldehyde.
41,2,4-Trimethoxybenzene + indole + trans-cinnamaldehyde.
5Veratrole + indole + trans-cinnamaldehyde.
corded as responding differently to volatile attractants according to the host plant phenology (Andersen & Metcalf 1987, Lampman et al. 1987).

*D. speciosa* is a polyphagous beetle associated with numerous plant species and plant parts, but principally leaves and flowers (Lima 1952, Krysan 1986). Further investigation of the attraction and composition of volatile chemicals in flowers of species more frequented by *D. speciosa*, especially the wild South American Cucurbitaceae, may reveal more chemicals involved in insect-host interactions. The response of this pest to 1,4-dimethoxybenzene indicates that cucurbitacin-baited traps could be improved by adding this volatile chemical. This would be useful for crops in which *D. speciosa* is a rootworm pest, such as corn, *Zea mays* L.; wheat, *Triticum aestivum* L.; and potato, *Solanum tuberosum* L.; in which growers are not able to easily and quickly assess economic thresholds.

There were no significant differences between captures of *C. a. tingomariana* in traps baited with single test compounds or mixture of compounds and the controls (Tables 1 and 2). The sex ratio of *C. a. tingomariana* beetle samples with a sweep net was 1.1 (n = 100) when collected in soybean and 1.0 (n = 100) in common bean. Although *C. a. tingomariana* feeding is strongly stimulated by cucurbitacins (Nishida et al. 1986, Nishida &

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**TABLE 2.** Mean number (±SE) of adults and the sex ratio (females per male) of *D. speciosa* and *C. a. tingomariana* caught per yellow transparency film sticky traps in common bean crop after 24 h (March 22, 1997).

<table>
<thead>
<tr>
<th>Treatment</th>
<th><em>D. speciosa</em></th>
<th><em>C. a. tingomariana</em></th>
</tr>
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<tbody>
<tr>
<td>Benzyl acetone</td>
<td>8.5 ± 3.0c</td>
<td>0.7 ± 0.2a</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>17.2 ± 3.5bc</td>
<td>2.0 ± 1.4a</td>
</tr>
<tr>
<td>Benznaldehyde</td>
<td>10.7 ± 4.2bc</td>
<td>1.2 ± 0.2a</td>
</tr>
<tr>
<td>Cinnamyl alcohol</td>
<td>15.0 ± 7.4bc</td>
<td>2.7 ± 0.5a</td>
</tr>
<tr>
<td>1,4-Dimethoxybenzene</td>
<td>77.0 ± 32.0a</td>
<td>1.0 ± 1.0a</td>
</tr>
<tr>
<td>Eugenol</td>
<td>11.7 ± 2.8bc</td>
<td>1.5 ± 0.3a</td>
</tr>
<tr>
<td>Indole</td>
<td>16.0 ± 4.9bc</td>
<td>1.0 ± 0.6a</td>
</tr>
<tr>
<td>α-ionone</td>
<td>6.7 ± 2.4c</td>
<td>0.7 ± 0.2a</td>
</tr>
<tr>
<td>β-ionone</td>
<td>8.5 ± 3.3c</td>
<td>1.5 ± 0.9a</td>
</tr>
<tr>
<td>4-Methoxyphenethyl alcohol</td>
<td>16.7 ± 3.9bc</td>
<td>3.7 ± 2.1a</td>
</tr>
<tr>
<td>Nerolidol</td>
<td>18.5 ± 2.8bc</td>
<td>4.5 ± 2.9a</td>
</tr>
<tr>
<td>Phenylacetaldehyde</td>
<td>15.5 ± 5.9bc</td>
<td>0.7 ± 0.5a</td>
</tr>
<tr>
<td>Safrole</td>
<td>9.5 ± 3.3bc</td>
<td>4.0 ± 1.3a</td>
</tr>
<tr>
<td>Trans-cinnamaldehyde</td>
<td>9.0 ± 1.1c</td>
<td>2.2 ± 1.3a</td>
</tr>
<tr>
<td>1,2,4-Trimethoxybenzene</td>
<td>11.2 ± 4.1bc</td>
<td>1.5 ± 0.9a</td>
</tr>
<tr>
<td>Veratrole</td>
<td>14.7 ± 3.6bc</td>
<td>3.7 ± 1.9a</td>
</tr>
<tr>
<td>SCI</td>
<td>17.5 ± 6.8bc</td>
<td>0.5 ± 0.3a</td>
</tr>
<tr>
<td>TIC</td>
<td>28.5 ± 13.5b</td>
<td>4.0 ± 2.0a</td>
</tr>
<tr>
<td>VIP</td>
<td>29.0 ± 13.0b</td>
<td>3.7 ± 1.9a</td>
</tr>
<tr>
<td>Control</td>
<td>8.2 ± 1.7c</td>
<td>2.7 ± 1.5a</td>
</tr>
</tbody>
</table>

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41,2,4-Trimethoxybenzene + indole + trans-cinnamaldehyde.
5Veratrole + indole + trans-cinnamaldehyde.
Fukami 1990, Ventura et al. 1996), no records of this species infesting flowers of cucurbits exist. While the polyphagous *D. speciosa* is a pollen feeder that responds to volatile substances in *Cucurbita* spp., as well as a pest of corn, beans and cucurbits (among other hosts) (Krysan 1986), the oligophagous *C. a. tingomariana* is associated with beans (larvae and adults) and Cucurbitaceae (adults). It has been suggested that Luperine species, including Diabroticite and Aulacophorite, primarily coevolved with Cucurbitaceae, and their preference for other hosts is recent (Metcalf & Lampman 1989). These beetles retain an attraction to volatile substances in *Cucurbita* spp. blossoms and a feeding stimulation by cucurbitacins. It is possible that squash-bean-corn plantings in the pre-Columbian New World influenced host plant range (Metcalf & Lampman 1989). However the non-pollen feeder *C. a. tingomariana* might have a more recent relationship with wild Cucurbitaceae because the lack of response of *C. a. tingomariana* to attractants indicates the association with cucurbitacins is not as strong as with *Diabrotica* species. Feeding by another bean leaf beetle of the *Cerotoma* genus, *C. trifurcata* Forster, is deterred by cucurbitacins (Metcalf et al. 1980). *C. a. tingomariana* must have expanded its host range to tolerate the bitter cucurbitacins. This species sequesters 23,24-dihydrocucurbitacin D, as *D. speciosa* does, after which it gains bitterness in body tissue, and strongly deters feeding by predators (Nishida & Fukami 1990). The lack of attractiveness of volatile compounds to *C. a. tingomariana* is a limitation in the improvement of lures to be used in beans and cucurbits, crops in which *D. speciosa* and *C. a. tingomariana* are simultaneous pests.

![Graph](image_url)

*Fig. 1. Relationship between mean number of adults caught ±SE per yellow plastic cup sticky traps (n = 4) of *D. speciosa* and *C. a. tingomariana* after 24 h (on 20 April 1998) and dosage of 1,4-dimethoxybenzene. The linear regression equations were \( y = 0.936242 + 47.084 \log x \) \( (r^2 = 0.74, P < 0.0001, n = 6) \) for *D. speciosa* and \( y = 3.930574 - 0.2467 \log x \) \( (r^2 = 0.0136, P < 0.5871, n = 6) \) for *C. a. tingomariana*.\)
Captures of *D. speciosa* in traps increased significantly with rising doses of 1,4-dimethoxybenzene (Fig. 1) in a dose-dependent manner. The dose-dependent response pattern would be especially advantageous to a mass trapping concept (Hoffmann et al. 1996). *D. speciosa* is a very important pest in many species of vegetables and fruits cultivated in small field areas or in greenhouses in Latin America. In such crops, traps could be used baited with 1,4-dimethoxybenzene to reduce beetle populations.

**ACKNOWLEDGMENTS**

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EXPOSURE TO MALE PHEROMONES ENHANCES ANASTREPHA SUSPENSA (DIPTERA: TEPHRITIDAE)
FEMALE RESPONSE TO MALE CALLING SONG

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ABSTRACT

Anastrepha suspensa (Loew) females are attracted to traps baited with male pheromone and/or broadcast calling song, but a high variability in female responsiveness has hindered attempts to use such attractants in practical trapping systems. Prior experience is one factor that may contribute to variability in female responses. To investigate this possibility, female responses to male calling song were compared after 38-40-h prior exposure to different combinations of live males, live females, synthetic pheromone components, and broadcast song. The broadcast song, obtained from a sexually successful male, contained a series of wing-fanning pulse trains averaging 0.31-s in duration, separated by 0.36-s quiet intervals. Within the pulse trains, the frequency rose quickly from ~125 to 148 Hz and then slowly declined to ~120 Hz. The proportions of females responding to the broadcast calling song were greatest when females were first exposed to live males or pheromone components. These proportions significantly exceeded 0% difference between the proportions under silent and broadcasting speakers. The proportion of females that responded after prior exposure to broadcast song alone was significantly higher than the proportion that responded after no prior exposure to sexual stimuli, but it did not significantly exceed 0%.

Key Words: calling song, pheromone, Anastrepha suspensa, attraction

RESUMEN

Hembras de Anastrepha suspensa Loew son atraídas a trampas con señuelo de feromonas masculinas y/o transmisión de canción de llamado, pero una alta variabilidad de reacción en la hembra ha impedido intentos para desarrollar sistemas prácticos de trampas. Un factor que puede contribuir a la variabilidad de reacción es experiencia previa a señales sexuales. Para investigar este efecto, respuestas de hembras a canción de llamado fueron comparadas después de 38 a 40-h de pre-exposición a diferentes combinaciones de machos, hembras, feromona masculina, y transmisión de canción. El estímulo de canción fue estandarizado por copias concatenadas de una grabación de 7.2-s de un macho sexualmente exitoso, y contenía trenes de pulsos de abanicos de ala promediando 0.31-s de duración, separados por intervalos de silencio de 0.36-s. Dentro de los trenes de pulso, la frecuencia subió rápidamente de ~125 a 148 Hz y después declinaron lentamente a ~120 Hz. Las proporciones de hembras que respondieron a las transmisiones de canción de llamada fueron las mayores cuando las hembras fueron pre-expuestas a machos o feromona masculina. Estas proporcio-
nes significativamente excedieron niveles nulos. Las proporciones de hembras que respondieron después de pre-exposición solo a transmisión de canción fueron significativamente mayores que las proporciones de hembras respondientes que no habían tenido pre-exposición al estímulo sexual, pero en ningún grupo las proporciones excedieron niveles nulos.

Sexual courtship in the Tephritid pest, *Anastrepha suspensa* (Loew) (‘caribfly’), involves male-produced pheromonal (Nation 1972), acoustic (Webb et al. 1976), and visual signals (Burk 1981) that are attractive to females and consequently have potential uses in trapping and monitoring programs. These signals are usually presented to females by males that aggregate in leks on larval host plants in late afternoon (Burk 1983, Norrbom & Kim 1988). Males compete for single-leaf territories on which they emit pheromone, produce a calling song generated by repeated bursts (trains) of wing-fanning pulses, and semaphore with patterned wings (e.g., Sivinski & Burk 1989, Aluja et al. 2000). When a female lands on his leaf, he approaches and mounts her if she permits, producing an intense precopulatory song while attempting to engage her genitalia (Sivinski et al. 1984). The precopulatory song is continuous rather than pulsed, and is more intense and higher in frequency than the calling song (Webb et al. 1984). As in most systems where courtship occurs in aggregations (Alexander et al. 1997), males vary considerably in their mating success.

Bioassays designed to identify the signal characteristics that distinguish sexually successful males from nonmaters have yielded ambiguous results. For example, initial laboratory studies comparing female responses to different combinations of pheromone and calling song suggested that calling song combined with pheromone was more attractive than pheromone alone (Webb 1973, Chambers 1975). In field-cage studies, however, statistically significant numbers of females were captured in traps baited with live males, pheromone alone, and broadcast calling song alone, but not in traps with a combination of broadcast song and pheromone (Webb et al. 1983).

Additional ambiguities have appeared in studies comparing responses to songs that contained systematic differences in acoustic parameters. Sexually successful caribfly males produce songs generated from precisely featured bursts of wing fanning pulses (example in Fig. 1). The bursts have a mean pulse-train duration (PTD) of ~0.32 s and are separated by ~0.34-s pulse train intervals (PTI) (Sivinski & Webb 1986). The mean inter-pulse interval (IPI) is ~7.14 ms, corresponding to a frequency of ~140 Hz (Sivinski & Webb 1986). Changes in the magnitudes of one or more of these parameters often result in reduced responsiveness of females. For example, Sivinski et al. (1984) found that female caribflies failed to respond to songs recorded from conspecific males with pulse trains of typical frequency and duration (~140-Hz pulses, 0.273-s PTD) but atypically long PTI (1.115s). Female caribflies were also unresponsive to male precopulatory song and to song produced by a male Queensland fruit fly, *Bactrocera neohumeralis* Hardy. The Queensland fruit fly produces song with the correct PTI but a higher frequency (~379 Hz) and shorter PTD (~0.12 s) (Sivinski et al. 1984). Such results suggest that the songs most attractive to female caribflies are those with low frequencies, long PTDs, and short PTIs. Indeed, Burk & Webb (1983) had reported that females mate preferentially with larger males, and that larger males produced lower frequency songs with shorter PTIs than smaller males. Other studies, however, did not always find that larger males produce song with shorter PTIs (Webb et al. 1984).
Some of this ambiguity may have resulted from a lack of understanding of potentially important features of caribfly pulse trains. Historically, calling songs were described simply by their mean frequencies (e.g., Webb et al. 1983, Sivinski & Webb 1986). However, Webb et al. (1987) and others observed later that the frequency is not constant within pulse trains, but decreases toward the end of each train. The magnitudes of these frequency changes have not been characterized and their effects on female response are unknown.

Additional ambiguity may be explained by variability in the levels of female responsiveness (cf. Searcy & Andersson, 1986). Prior experience with sexual signals is one potential contributor to this variability. Caribfly males are known to change their calling patterns in the presence of females and other males (Sivinski & Webb 1986). However, the effect of prior experience on female caribfly behavior has not been investigated.

The objective of this study was to investigate the effect of prior exposure to sexual stimuli on female caribfly responsiveness to broadcast song. We conducted a series of bioassays during which we exposed virgin females to different combinations of pheromone and/or male calling song prior to experiments, and then assessed their responses to play-back of precisely characterized song recorded from a sexually successful male.

Fig. 1. Example of 2 pulse trains in a recording of male caribfly calling song, with inset showing 6 individual pulses at the start of 1st train. The horizontal axis shows time in seconds. The vertical axis shows the microphone signal on a relative scale (see text for amplification details): PTD, pulse train duration; PTI, pulse train interval; IPI, interpulse interval; horizontal axis shows time (s); vertical axis shows microphone signal in relative scale.
Insects

Caribflies used in this study were obtained as pupae from the Florida Department of Agriculture, Division of Plant Industry in Gainesville, Florida. After eclosion, adult flies were given water and a 3:1 mixture of refined cane sugar and hydrolyzed brewer’s yeast. They were maintained in a laboratory with a photoperiod of 12:12 h (L:D) at room temperature and ambient humidity. Adult flies were sorted by sex 3-4 days after eclosion, placed in cubic screen cages (30-cm per side), and the females thereafter were kept in a separate “female room”. Because most males do not signal sexually until after 5-7 d (Sivinski 1994), the sorted females were unlikely to have been exposed to significant amounts of male pheromones.

Sexually mature females (10-18 d old) were used for all experiments. They had no exposure to adult live males from time of sorting until use in an experiment unless otherwise stated.

Male Calling Song

The acoustic signals were generated by concatenating multiple copies of a 7.2-s segment of song from a sexually successful male (Fig. 2) onto an endless loop tape. Webb et al. (1983) used this same song segment to produce a continuous-loop recording that successfully captured female caribflies when it was broadcast from a trap in a field cage. The first two pulse trains of the signal are shown on smaller time scales in Figure 1.

Webb et al. (1976, 1983) measured temporal patterns and the mean frequencies and Sound Pressure Levels (SPLs) of A. suspensa calling song bursts, but frequency patterns and SPLs within bursts could not be measured using the technology then available. For this report, we analyzed the dynamic features of the song bursts using a Bruël and Kjaer (B & K) model 4145 microphone, a model 2639 preamplifier, and a model 2610 measuring amplifier (Mankin 1994). Signals were amplified 20-40 dB (where dB = 20 \log_{10}(V_{out}/V_{inp})$, and $V_{out}$, $V_{inp}$ are the amplifier output and input voltage levels, respectively) and digitized at 25 kHz using a 12-bit MetraByte (Keithley/MetraByte Inc., Taunton, MA) DAS-16G A/D converter installed in a Pentium 350-mHz microcomputer. The digitized signals were analyzed with custom-written software (Mankin 1994, Mankin et al. 1996a). The customized software located the peak of each wingbeat pulse, marked its time within the recording, and measured the inter-pulse interval (IPI, Fig. 1). A pulse train was identified as a series of unbroken pulses separated by an IPI of no more than 20 ms (approximately 3 typical pulses). A custom-written subroutine noted the order of each pulse within the train and calculated the instantaneous frequency (1/IPI). Another subroutine noted the beginning and end of each pulse train for calculations of pulse train duration (PTD) and interval (PTI).

The mean frequency within the pulse train was calculated as an average for each IPI measurement in the train, based on its order number from the beginning of the train (e.g. IPI #s 1-5 in Fig. 1). The mean frequency at the 5th IPI for example, was the average of all values of 1/IPI between the 5th and 6th pulse.

Sound Pressure Levels were calibrated as in Mankin et al. (1996b), and the speaker output was adjusted to produce 55 dB SPL (relative to 20 (Pa) mean signal level at a distance of 12 mm. This is the level used in previously successful trapping.
studies (Webb et al. 1983). Such sounds are audible to humans over distances of 2-4 m in the laboratory, but the range of detectability by female caribflies has not been measured.

Bioassay Arenas and Response Measurements

Separate exposure treatments were conducted in a laboratory and a wind tunnel. The effect of prior exposure to live males was tested by moving some of the caged virgin females into a 1.8 × 1.8 × 2.5 m “male room” containing several hundred caged males of all ages. These females were adjacent to, but physically isolated from males, and were exposed to sight, sound, and pheromone from males. After 38-40 h exposure in the “male room”, 20 females were moved to a (20 × 20 × 20 cm) screen cage in a separate room for the acoustic bioassay.

In the acoustic bioassays, 2 pairs of monaural headphones (Realistic or Archer, both including the foam pads) were placed on top of the cage, facing down. One pair was silent and the other was connected to a recorder (Realistic CTR-62 or CTR-66) playing the endless-loop tape. The signals were broadcast during the peak of the daily courtship-signaling period, 8.5 h after the start of the 12-h photophase. The females standing directly under each speaker were counted every five minutes, a total of 13 times for each 1-h replicate. The locations of silent and broadcasting speakers were alternated at least once during each trial.

Attraction to male song was measured as the mean of the 13 measurements of the difference between the proportions of females under the broadcasting and the silent speakers. The responses of females exposed in the “male room” prior to assays were
compared with the responses of females kept continuously in the “female room”. Thirteen replications were done in the “female room” tests and seven replications in the “male room” tests.

In the second experiment, females were exposed in a wind tunnel (Heath et al. 1993) to different combinations of male courtship signals for 38-40 h before the acoustic bioassay. The combinations were filtered air in a “clean tunnel”, air from the “male room”, “live males” (10 males in a cage, 1.1 m upwind), “pheromone” (exposure to putative synthetic pheromone), or “sound only” (calling song broadcast continuously from speakers on top of the cage). The putative synthetic pheromone was composed of ~5% ocimene; ~2% nonenols ((Z)-3-nonon-1-ol and (Z,Z)-3,6-nonadien-1-ol); ~10% suspensolide ((E,E)-4,8-dimethyl-3,8-decadien-10-olide); ~5% E,E-a-farnesene; ~35% ß-bisabolene; ~10% anastrephin (trans-hexahydro-trans-4,7α-dimethyl-4-vinyl-2-(3H)-benzofuranone); and ~33% epi-anastrephin (trans-hexahydro-cis-4,7α-dimethyl-4-vinyl-2-(3H)-benzofuranone), which approximated the ratio of components that are released under natural light condition in late afternoon (Heath et al. 1993). Synthetic components were formulated in glass capillaries (ocimene and nonenols) and on rubber septa (remaining components) using protocols reported previously (Weatherston et al. 1985a, 1985b, and Heath et al. 1986, respectively). The putative synthetic pheromone was formulated to release ~900 ng per h or the equivalent of release from 10 male caribflies in late afternoon (Heath et al. 1993).

The cages used for the wind tunnel treatments had solid sides (14.2 x 30 cm) and bottoms (13.3 x 30 cm) with single-screen covered circular openings (9 cm diameter) cut into the front and back pieces (13.3 x 14.2 cm). Two screen-covered circular openings were cut into the top for placement of the speakers. The foam pads were removed to prevent oviposition on the speakers in the “sound only” experiment. Attractiveness of broadcast songs after the wind tunnel treatment was measured as in the previous experiment. This experiment had 10 replications.

Behavioral Response Analysis

Nonparametric statistical analyses were used due to nonnormal frequency distributions of the raw data. Dunn's multiple comparison (based on Kruskal-Wallis rank sums) (Hollander & Wolfe, 1973) was used to compare the treatments in the second experiment.

RESULTS

Female Acoustic Attraction Response

Male caribfly calling song elicited a detectable attraction response when females were in the presence of synthetic pheromone or had been exposed to males. In the first experiment, females that had been kept in the male-room responded at significant levels to calling song, but the responses of females that had been kept in the female room were not significantly different from zero (Fig. 3). The two treatments differed in the proportion of females under broadcasting speakers (z = 2.774, p < 0.005, Mann-Whitney U-test). In the second experiment, females that had been kept in the male room, exposed to live males, or exposed to synthetic pheromone in the wind tunnel were attracted to male calling song (Fig. 4). Females that had been kept in the clean tunnel or exposed only to male calling song showed no attraction toward broadcast
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Dynamic Features of Male Calling Song

The 7.2-s segment of male calling song broadcast in these recordings (Figs. 1 and 2) consisted of 11 pulse trains with a mean frequency ± Standard Error (SE) of 140.38 ± 3.86 Hz. The pulse trains had a mean duration (PTD) of 0.31 ± 0.02 s, separated by silent intervals (PTI) of 0.36 ± 0.05 s. The frequency varied within the pulse train. On average, the train began with pulses of ~125 Hz (Fig. 5). The frequency increased rapidly to a maximum of ~148 Hz by the 5th IPI (~36 ms into the train), and then gradually declined to below 120 Hz by the 30th IPI (~225 ms into the train).

Fig. 3. Comparison of responses to calling song by females kept only with other females or kept in a room with caged males. The vertical axis shows the difference in the proportion of females at the broadcasting and the silent speakers, with positive values indicating a greater proportion of females observed under the broadcasting speakers. Medians are indicated by the larger circles and vertical lines indicate the 0-25th and 75-100th percentiles. Smaller dots (right) show the measured proportion differences. Filled median circles indicate that the median differs significantly from zero according to the sign test (p < 0.016). The numbers underneath list the number of cages (replicates) in each treatment.

songs. The “clean tunnel” and “male room” tests in the second experiment were essentially equivalent to the “female room” and the “male room” tests in the first experiment. The results of the two experiments were comparable.
DISCUSSION

The result that females exposed to male pheromone before a bioassay were more responsive to calling song than unexposed females is consistent with at least two alternative hypotheses. One is that exposure to pheromone is necessary to trigger a response to calling song. A second is that experience with the male courtship repertoire may increase the responsiveness to subsequent courtship signals. The first hypothesis is plausible because, in nature, (cf. Sivinski & Burk, 1989) females usually smell the males before they hear or see them. However, Sivinski et al. (1984) found that virgin females with no experience of male pheromone increased their levels of activity when they heard broadcast male calling song. In this study, the responses of females that had been exposed only to broadcast song were significantly greater than the responses of females that had never been exposed to any sexual stimuli. Such results suggest that acoustic attraction can occur without exposure to pheromone, but the attraction is weaker than that to pheromone.

The results here and those of Sivinski et al. (1984) are most consistent with the second hypothesis, that prior experience of the female with the male courtship repertoire increases female responses to newly encountered courtship stimuli. This effect

**Fig. 4.** Comparison of female responses to male calling song after previous exposure in a wind tunnel to clean air, caged males, putative synthetic male pheromone, or calling song. Positive values indicate that proportionally more females were observed under the broadcasting speakers. Medians are indicated by the larger circles and vertical lines indicate the 0-25th and 75-100th percentiles. Smaller dots (right) show the measured proportion differences. Filled median circles indicate that the median differed significantly from zero according to the sign test. Treatments with the same letter had medians that were not significantly different according to Dunn’s multiple comparison (experimentwise error rate = 0.09).
might be similar to one observed in wind tunnel experiments with female parasitoid attraction to host larvae (e.g. Drost et al. 1986; Eller et al. 1988; Turlings et al. 1989). If the second hypothesis is correct, however, the nonsignificant result for females that had been exposed to calling song but not pheromone suggests that exposure to pheromone has a greater effect on subsequent responsiveness.

Because the female responses to the broadcast calling song in these bioassays were too low for practical applications, the main benefit of this study is some insight for improvements in the design of fruit fly acoustic bioassays. First, female responsiveness to acoustic signals can be increased by exposure to pheromone, either before or during the acoustic testing. Second, the broadcasting of acoustic stimuli by speakers may present an inadequate stimulus to female fruit flies. The adequacy of acoustic stimuli for attraction has been a problem in many other insect bioassays as well (Searcy & Andersson 1986).

The response in this bioassay was low despite the use of song generated by a sexually successful male. The mean frequency is lower than the 149-Hz average of sexually successful males in Webb et al (1984), but a lower frequency may correlate with larger size and improved mating propensity (Burk & Webb 1983). The PTI and PTD are comparable to measurements from other sexually successful males (Webb et al. 1984). Consequently, it is not likely that the signal pattern itself was deficient but
some other stimulus feature. One potential contributor to the low female responsiveness was that the sound was produced by a speaker rather than a vibrating wing. The speaker generates a signal of larger spatial extent and lower air velocity than the vibrating wing. In addition, the vibrating wing has visual components that have not yet been demonstrated as affecting caribfly female mate choice (Aluja et al. 2000) but have been shown to affect mate choice in other Diptera (e.g., Lunau 1992). The continued development of new acoustic signal analysis and signal presentation capabilities may improve our future ability to elicit an attraction response of female fruit flies to synthetic courtship stimuli.

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PARASITOID-HOST MATCHING BETWEEN THE LITTLE DECAPITATING FLY PSEUDACTEON CURVATUS FROM LAS FLORES, ARGENTINA AND THE BLACK FIRE ANT SOLENOPSIS RICHTERI

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ABSTRACT

Matching biotypes of potential biocontrol agents to target host populations can greatly improve the effectiveness of control. This study was designed to determine if the fly Pseudacteon curvatus Borgmeier from Las Flores, Buenos Aires Province, Argentina prefers its natural host, the black fire ant, Solenopsis richteri Forel. We found that P. curvatus strongly preferred S. richteri from Argentina, S. richteri from the United States, and hybrid (S. richteri × S. invicta) fire ants from the United States when each was tested against S. invicta from the United States. The time to pupation of developing parasitoids was 10% and 21% longer in hybrid and red fire ants than in black fire ants. Parasitism rates, however, were not significantly different among these ant hosts in no-choice parasitism tests.

Key Words: Diptera, Phoridae, Hymenoptera, Formicidae, Solenopsis invicta, host preference, hybrid fire ants, biocontrol, biotype

RESUMEN

Hacer corresponder a biotipos de agentes potenciales de control biológico con poblaciones huéspedes puede, en gran medida, mejorar la eficiencia en el control. Este estudio fue diseñado para determinar si la mosca Pseudacteon curvatus Borgmeier de Las Flores, Provincia de Buenos Aires, Argentina, prefiere a su huésped natural, la “hormiga brava” negra, Solenopsis richteri Forel. Encontramos que P. curvatus prefirió marcadamente a S. richteri de Argentina, a S. richteri de los EE.UU. y a la forma híbrida (S. richteri × S. invicta) de los EE.UU. cuando fue comparada con S. invicta de los EE.UU. El período hasta pupación de los parasitoides en desarrollo fue 10% y 21% más largo en la forma híbrida y en la “hormiga brava” roja que en la “hormiga brava” negra. Las proporciones de parasitismo, sin embargo, no fueron significativamente diferentes entre los huéspedes en pruebas de parasitismo de no-elección.

Two exotic fire ants have become established in the United States, the black imported fire ant, Solenopsis richteri Forel, and the red imported fire ant, Solenopsis invicta Buren (Trager 1991). The black imported fire ant is found in northern Mississippi and Alabama where it occupies about 30,000 km² (Shoemaker et al. 1994). The red imported fire ant is found in 11 southeastern states from North Carolina through Texas and occupies about 1,100,000 km² (Callcott & Collins 1996). Between these two species is a broad band of hybridization from the Mississippi River to Atlanta, GA (Shoemaker et al. 1994). Hybrid fire ants occupy about 130,000 km². De-
spite this broad zone of hybridization, red and black imported fire ants are still considered separate species because they apparently do not hybridize in South America (Ross & Trager 1990).

*Pseudacteon curvatus* Borgmeier is a small decapitating fly from South America that parasitizes *Solenopsis* fire ant workers (Porter 1998). This species was released in several states in the spring of 2000 as a potential biocontrol agent for red and black imported fire ants in the United States (unpublished data). In South America, *P. curvatus* occurs over a very wide range from São Paulo, Brazil westward into Mato Grosso do Sul, Brazil and southward to Buenos Aires Province, Argentina (Borgmeier 1925; SDP-unpublished data). Over this range, *P. curvatus* is known to parasitize at least three species of South American fire ants: *Solenopsis saevissima* (F. Smith), *S. invicta*, and *S. richteri* (SDP-unpublished data).

Host-specificity tests in the United States (Porter 2000) demonstrated that *P. curvatus* flies from Argentina strongly prefer red imported fire ants over the native fire ants *Solenopsis geminata* (Fab.) and *Solenopsis xyloni* (McCook). This preference is not surprising because *P. curvatus* is not a natural parasite of either *S. geminata* or *S. xyloni*. Forced laboratory rearing tests showed that *S. geminata* and *S. xyloni* are both very poor hosts for *P. curvatus* (Porter 2000).

Matching parasitoid biotypes to target host populations can greatly improve the success of biocontrol programs (Van Driesche and Bellows 1996, p. 149). *P. curvatus* flies from Buenos Aires Province, Argentina normally parasitize the black fire ant *S. richteri*. The objective of this study was to determine if *P. curvatus* flies from Buenos Aires Province are better adapted to *S. richteri* (their normal host) than the red fire ant, *S. invicta* (a host in other parts of South America). Results of this study will help us decide where *P. curvatus* should be released in the United States.

**MATERIALS AND METHODS**

*P. curvatus* flies used in this study were originally collected from El Toro Ranch southeast of Las Flores, Buenos Aires Province, Argentina in March 1997 (Porter 2000). A few flies from the same location were added to the lab colony several times up to December 1998.

To examine *P. curvatus* preferences for *S. richteri*, *S. invicta*, and hybrid fire ants, 3-hour old and 1-day old flies were introduced into white plastic trays (42 × 28 × 15 cm) with screened vents and tight-fitting glass lids (described in detail by Porter 2000). We used both 3-hour and 1-day old flies to produce an age mixture similar to what might occur in the field. In the bottom of each tray, were two parallel chambers (7 × 30 × 5 cm, l × w × h) for two kinds of ants. Ants were contained in the two bottom chambers by coating the sides with Fluon® CICI, Wilmington, DE).

A small opaque inverted cup (4 cm diameter) was placed on the bottom of each of the two small parallel chambers. These cups were moved back and forth from one end of a chamber to the other with a long aspirator arm (Porter and Alonso 1999) each time most of the ants had crawled under a cup to hide. This procedure kept the ants in both sides trailing continuously from one end of a bottom chamber to the other so the flies always had an opportunity to attack workers of either type of ant.

We used 7 colonies of *S. richteri* from Las Flores, Buenos Aires Province, Argentina, 9 colonies of *S. richteri* from northeastern Mississippi (Tupelo - 4 colonies, Booneville - 3, Corinth - 1, Mayhew - 1), and 7 colonies of hybrid fire ants from around Starkville, MS (USDA Lab - 4, Mayhew - 3). The identities of *S. richteri* and hybrid fire ants from Mississippi were confirmed by gas chromatography (Vander Meer et al. 1985). For each trial *S. richteri* and hybrid fire ants were paired with similar-sized *S. invicta* workers from Gainesville, FL. Different colonies of each kind of ant were used
for each trial to assure that results were not due to differences in the attractiveness of individual colonies. Tests with S. richteri fire ants from Argentina were conducted in January 1999. Tests with hybrid fire ants and S. richteri from the United States were conducted in June 1999. Tests for all three kinds of ants were run 1-3 weeks after colonies were collected in the field. Voucher specimens have been deposited in the Florida Museum of Arthropods, Gainesville, Florida, USA.

Each test run lasted about 3 h and used 14-18 female flies with an equivalent number of males. Test ants contained 0.25 g of workers (~400) and 0.5 g of brood. The trays were inspected every 10 min and the number of female flies hovering in attack mode over each species of ant was recorded by visual count. Females considered in attack mode hovered 3-10 mm above the ants and oriented to their movements. Males of this species are not attracted to the ants.

To determine if P. curvatus flies were equally successful in parasitizing black, hybrid, and red fire ants, we conducted a series of no-choice parasitism tests. The trays used in these tests contained a single solid bottom covered with moistened plaster as described by Porter (2000). Timer motors were used to automatically raise an inverted cup in one end of each tray while lowering a cup at the other end of each tray. This caused the test ants to continuously trail back and forth between the two cups. Timer motors were set to run for 8 h a day (10:00 to 18:00 h).

We conducted 6 trials each with: S. richteri from northeastern Mississippi (Corinth - 1 colony, Booneville - 2, Tupelo - 3), hybrid fire ants from Starkville, MS (3 colonies) and Mayhew, MS (3 colonies), and S. invicta from Gainesville, FL (6 colonies). All colonies were collected in June 1999 and used 1-2 weeks after collection. Tests contained 0.5 g of workers (~800) and 1.0 g of brood. Different colonies were used for each test replicate. We used mostly the same colonies for the no-choice parasitism tests as we did for the paired preference tests.

Fifteen to sixteen female flies and an equivalent number of males were added to all no-choice trials on day 1. Tests lasted 2 days. P. curvatus adults usually only live a day or two in the attack trays; consequently, most of the flies were dead by the end of the trials. Inactive flies usually live several days longer in the lab. Longevity in the field is unknown, but it is likely to be intermediate between inactive flies and flies in the attack trays. At the end of each trial, worker ants were transferred into small boxes (20 x 2 x 5 cm) with tight-fitting vented lids. Ants were fed fresh sugar water every 3-4 days. We inspected the head capsules of dead workers for fly pupae every 1-2 days for a period of 30 days.

**Results**

When given a choice in paired tests, about 70% of the P. curvatus females preferred to attack black fire ants or hybrid fire ants over red fire ants (Fig. 1). We found highly significant differences in the number of attacking flies for each of the following pairs using paired t-tests: S. invicta versus S. richteri from Argentina (t = 4.95, d.f. = 6, P = 0.0026), S. invicta versus S. richteri from Mississippi (t = 3.48, d.f. = 8, P = 0.0083) and S. invicta versus hybrid fire ants from Mississippi (t = 7.11, d.f. = 6, P = 0.0004). However, no significant differences were found between the number of flies preferring S. richteri from Argentina, S. richteri from Mississippi, or hybrid workers (S. richteri x S. invicta) from Mississippi (all tested against S. invicta from Florida; ANOVA, F = 0.81, d.f. = 2.20, P = 0.46).

Once a fly began attacking workers in the choice tests, the average number of oviposition strikes per 15 seconds was respectively 0.67 ± 0.05, 0.87 ± 0.13, 1.15 ± 0.14, and 1.57 ± 0.26 for S. richteri workers from Argentina, S. invicta from Florida, S. richteri from the United States, and hybrid fire ants. The attack rate for hybrid fire ants
was significantly higher (Fisher’s PLSD, $P < 0.004$; 1-way ANOVA) than rates for either *S. invicta* or *S. richteri* from Argentina. Other pairwise comparisons were not statistically significant. The biological basis and importance of this pattern is not clear.

While *P. curvatus* strongly preferred black and hybrid fire ants when given a choice, significant differences were not found in the number of pupae produced in the no-choice parasitism tests (Table 1, ANOVA, $F = 0.42$, df = 2,15, $P = 0.66$). However, the mean time to pupation varied significantly among hosts (Table 1, ANOVA, $F = 12.6$, df = 2,13, $P = 0.0009$). Data were log transformed to equalize variance, two colonies were deleted (*S. invicta* - 1, *S. richteri* - 1) because they each produced less than 40 pupae. The development time to pupation was 21% longer in *S. invicta* than in *S. richteri* and 10% longer in hybrid fire ants than *S. richteri* (Table 1). The mean variability of pupation time (as measured by SD) was also significantly larger for flies developing in *S. invicta* and hybrid fire ants than in *S. richteri* (Table 1; ANOVA, $F = 5.7$, df = 2,13, $P = 0.017$).

**DISCUSSION**

*P. curvatus* from Las Flores, Argentina appears to have evolved a specialized relationship with *S. richteri*, its natural host. Specifically, these flies demonstrated a strong preference for *S. richteri* and hybrid fire ants over *S. invicta* (Fig. 1).
the source of this attraction is a qualitative trait that is not diminished in the hybrid. However, a one-on-one comparison would be necessary to determine whether the flies prefer black and hybrid fire ants equally. Preferences for specific hosts are likely based on chemical cues (Porter 1998a, b). Which cues these might be are unknown, but black, red, and hybrid fire ants exhibit distinctive differences in their cuticular hydrocarbons, venom alkaloids, and pheromones (Vander Meer et al. 1985, Obin & Vander Meer 1989, Vander Meer & Lofgren 1989). It is notable that a strong preference for *S. richteri* was maintained, even after flies had been cultured for 1-2 years (about 8-16 generations) in the lab using exclusively *S. invicta* workers as hosts. Retention of a strong preference for *S. richteri* over this period demonstrates that this preference was not quickly obscured either by behavioral experience or genetic adaptation.

*S. richteri* populations in the United States are much more likely to have originated from Argentine or Uruguayan port areas rather than landlocked Las Flores (170 km south of Buenos Aires). The fact that the percent preference for *S. richteri* workers from Las Flores, Argentina and the preference for *S. richteri* workers from northeastern Mississippi were quite similar suggests that host preferences are primarily species-level rather than population-level differences. Head-to-head comparisons of fire ant workers from a variety of locations would, of course, be necessary to fully evaluate the extent and nature of parasitoid preferences for different host ant populations.

We found that fly developmental rates increased significantly from *S. richteri* to hybrids to *S. invicta* (Table 1). This relationship is what would be expected by non-dominant genetic hybridization.

In contrast with preferences and developmental rates, rates of parasitism in no-choice laboratory tests were not clearly associated with the type of ant tested (Table 1); perhaps additional replicates would eventually show a modest effect, but this is not certain. Previous tests showed that *P. curvatus* females do not do well at parasitizing the two most common native fire ants in the United States (Porter 2000).

The practical implications of this study are that *P. curvatus* flies from Las Flores, Argentina may do best if they are released onto imported black or hybrid fire ant populations in Alabama, Georgia, Mississippi, and Tennessee. Similarly, *P. curvatus* biotypes collected from regions where they normally parasitize the red fire ant *S. invicta* may be more effective in regions of the United States where this species predominates. Matching specific *P. curvatus* biotypes to their normal host would be especially

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**Table 1. Parasitism Rates of the Fly Pseudacteon Curvatus Attacking Different Kinds of Fire Ants and Mean Developmental Times of Fly Pupae in Those Species.**

<table>
<thead>
<tr>
<th>Fire Ant Species (U.S.)&lt;sup&gt;1&lt;/sup&gt;</th>
<th><em>S. richteri</em></th>
<th>Hybrid</th>
<th><em>S. invicta</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pupae Produced/Female Fly (number ± SE)</td>
<td>8.3 ± 1.7 a</td>
<td>9.4 ± 1.0 a</td>
<td>7.6 ± 1.5 a</td>
</tr>
<tr>
<td>Mean Development Time (egg to pupae, days ± SE)</td>
<td>12.9 ± 0.2 a</td>
<td>14.2 ± 0.3 b</td>
<td>15.6 ± 0.5 c</td>
</tr>
<tr>
<td>Mean Standard Deviation in Devel. Time (days ± SE)</td>
<td>2.2 ± 0.3 a</td>
<td>3.5 ± 0.5 b</td>
<td>4.2 ± 0.4 b</td>
</tr>
</tbody>
</table>

<sup>1</sup>Means within a row with different letters were significantly different (Fisher’s PLSD, P ≤ 0.05).
important if the host preferences that we observed in the lab are associated with the fly’s ability to locate potential hosts at distances of several meters or more when visual abilities are likely to be ineffective. The actual importance of matching *P. curvatus* biotypes to their normal host populations will be evaluated during field releases of this parasitoid that are currently in progress in Florida, Alabama, and Tennessee.

**ACKNOWLEDGMENTS**

Robert Vander Meer (USDA-ARS, Gainesville, FL) identified hybrid and black fire ants from Mississippi using gas chromatography. Lloyd Davis (USDA-ARS, Gainesville, FL) set up and ran many of the preference tests. Cynthia Vann, Barbara Mayfield, Laura Collins, Damali Kelly, and David Almquist (USDA-ARS, Gainesville, FL) ably assisted with various aspects of this study. Lloyd Davis, Lloyd Morrison (USDA-ARS, Gainesville, FL), and Kathy Flanders (Auburn Univ., AL) read the manuscript and provided a number of valuable suggestions.

**REFERENCES CITED**


PARASITISM OF *BEMISIA ARGENTIFOLII* ON COLLARD WITH REDUCED OR NORMAL LEAF WAX

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ABSTRACT

Collard, *Brassica oleracea* var. *acephala* L., cultivars with reduced leaf wax (i.e., glossy phenotypes) possess ovipositional antixenotic resistance to the silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae). We investigated parasitism by 2 parasitoids of *B. argentifolii* reared on 2 phenotypes of the collard cultivar ‘Green Glaze’, differing in amount of leaf wax. When *Eretmocerus* sp. (Hymenoptera: Aphelinidae) parasitoids were given a choice between parasitizing whitefly nymphs on glossy and normal-wax collard, there were no significant differences in the number of parasitized nymphs on the 2 plant phenotypes. However, 4.5 times more *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) emerged from whiteflies on glossy than on normal-wax collard. In a no-choice test, the number of *Eretmocerus* sp. emerging on glossy and normal-wax plants did not differ significantly. In a similar no-choice test, more than twice as many *E. pergandiella* emerged from whiteflies on glossy collard than on normal-wax collard. Time to 50% emergence for whiteflies and both species of parasitoids did not differ on the 2 collard types in any of the no-choice tests. We conclude that management of *B. argentifolii* populations can be improved on collard, and probably other *B. oleracea* vegetables, through the use of reduced leaf wax cultivars that have antixenotic resistance to *B. argentifolii* and have no detrimental effects, possibly even beneficial effects, on important whitefly natural enemies.

Key Words: *Brassica oleracea*, plant resistance, *Eretmocerus, Encarsia pergandiella*, parasitoid, leaf wax, tritrophic interactions

RESUMEN

Cultivos de acelga, *Brassica oleracea* var. *acephala* L., con reducción de cera foliar (por ejemplo, fenotipos glaseados) poseen resistencia antixenotica oviposicional a la mosquita blanca, *Bemisia argentifolii* Bellows & Perring (Homoptera: Aleyrodidae). Investigamos el parasitismo por 2 parasitoides de *B. argentifolii* criados en 2 fenotipos del cultivo de acelga “Glaseado Verde”, con diferencia en la cantidad de cera foliar. Cuando especies parasitoides de *Eretmocerus* (Hymenoptera: Aphelinidae) fueron da-das opción entre parasitar ninñas de mosquita blanca sobre acelga glaseada o con cera normal, no hubieron diferencias significativas en el numero de ninñas parasitadas de los 2 fenotipos de plantas. Sin embargo, emergieron 4,5 veces mas *Encarsia pergandiella* Howard (Hymenoptera: Aphelinidae) de mosquitas blancas en plantas glaseadas que en plantas con cera normal. En una prueba sin opción, el numero de especies de *Eretmocerus* emergiendo en plantas glaseadas y con cera normal no difirió significativamente. En una prueba similar sin opción, mas de 2 veces *E. pergandiella* emergieron de mosquitas blancas en acelga glaseada que en acelga de cera normal. El tiempo para 50% de surgimiento de mosquitas blancas y las dos especies de parasitoides no difirió en los 2 tipos de acelga en cualquiera de las pruebas sin opción. Concluimos que la administración de poblaciones de *B. argentifolii* puede ser mejorada en acelga, y probablemente otros vegetales de *B. oleracea*, a través del uso de cultivos con
The silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, also known as the “B” strain of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius), is a serious pest of vegetable, ornamental, and agronomic crops throughout tropical and, increasingly, temperate regions of the world. Cruciferous vegetables, such as collard, *Brassica oleracea* var. *acephala*, and broccoli, *B. oleracea* var. *italica*, are important overwintering hosts for *B. argentifolii* in the southern United States (Simmons & Elsey 1995) and are sources of infestation for spring and summer crops (Coudriet et al. 1985, Simmons & Elsey 1995). Reduction of whitefly populations in *Brassica* vegetables is desirable, not only to reduce the need for insecticides and prevent economic loss in these vegetables, but also to reduce spring whitefly populations available to infest newly planted crops.

Host plant resistance to *B. argentifolii* has been investigated in many crops. Leaf characteristics, such as trichome abundance and orientation (McCreight & Kishaba 1991, Kishaba et al. 1992, Wilson et al. 1993, Heinz & Zalom 1995, Lambert et al. 1995, McAuslane et al. 1995, McAuslane 1996), presence of glandular exudates (Liedl et al. 1995), and vascular bundle density (Cohen et al. 1996) and depth within the leaf (Chu et al. 1998, 1999), have been implicated in resistance to *B. argentifolii*. Recently, antixenotic resistance to whiteflies has been demonstrated in collard and broccoli genotypes that have reduced leaf wax (Farnham & Elsey 1995, Jackson et al. 2000). These genotypes have a glossy or shiny appearance due to their smaller wax load. Whiteflies preferred to oviposit on normal-wax genotypes; however, if offered no choice of oviposition host, whiteflies oviposited similar numbers of eggs and their progeny developed and survived equally well on glossy and normal-wax plants (Elsey & Farnham 1994, Jackson et al. 2000).

*Bemisia argentifolii* can suffer much mortality by natural enemies in many crops that are not sprayed extensively with broad-spectrum insecticides, such as peanut (McAuslane et al. 1993), organic vegetables (Stansly et al. 1997), and collard (Simmons & Jackson 2000). It is well known that plant characteristics can affect the behavior and physiology of the predators and parasitoids at the third trophic level (Price et al. 1980). For example, leaf hairs on cucumber (van Lenteren et al. 1995) and tomato (van Roermund & van Lenteren 1995) interfere with locomotion and parasitization efficiency of *Encarsia formosa* Gahan on *Trialeurodes vaporariorum* (Westwood). Hairs on soybean reduced parasitism of *B. argentifolii* by *Encarsia* and *Eretmocerus* species (McAuslane et al. 1995). Eigenbrode and colleagues (Eigenbrode et al. 1995, 1996, 1999) have demonstrated that several generalist predators control diamondback moth, *Plutella xylostella* L., more effectively on glossy cabbage cultivars and that this is due to more efficient locomotion and prey location behaviors on glossy than on normal-wax genotypes. Little is known, however, about the potential influence of leaf epidermal waxes on parasitoids of *B. argentifolii*.

The purpose of this research was to determine the potential effect of collard leaf wax on parasitoids of *B. argentifolii*. We selected one *Eretmocerus* species, a thelytokous undescibed species from Hong Kong (McAuslane & Nyugen 1996), and one *Encarsia* species, *Encarsia pergandiella* Howard, a common species native to the New World (Polaszek et al. 1992). We chose one species of each genus because of the different oviposition habits of the genera. *Encarsia* species oviposit through the nymphal host exoskeleton whereas *Eretmocerus* species insert the ovipositor between the whitefly nymph and the leaf surface. In this study, we measured parasitism by these parasitoids when presented whiteflies on normal-wax or glossy collard in no-choice and choice situations.
Materials and Methods

Plants and Insects

Seeds of ‘Green Glaze’ collard were obtained from M. W. Farnham (U.S. Vegetable Laboratory, Charleston, SC). This cultivar segregates in a 3:1 ratio for individual plants with either glossy (i.e., reduced foliar waxbloom) or normal-wax appearance (Jackson et al. 2000). Seeds were sown in a greenhouse in a soil-less medium (Metro-mix 200, Grace Sierra, Milpitas, CA). When seedlings could be distinguished as either glossy or normal-wax, they were transplanted into 12-cm-diameter pots filled with a 1:1 mixture of Metromix 200 and Metromix 500, and were fertilized with approximately 5 g of a slow-release fertilizer (14-14-14, N-P-K, Osmocote, Scotts-Sierra, Marysville, OH). Plants were used for experiments 5 to 9 weeks post-germination.

Whiteflies, *B. argentifolii*, used in experiments with *Eretmocerus* sp. were obtained from a colony reared on cotton, *Gossypium hirsutum* L., ‘DPL 90’, and collard, ‘Georgia Southern’, in a climate-controlled room (28°C, 14:10 (L:D) photoperiod, 30-50% RH). The thelytokous *Eretmocerus* sp. has been maintained on *B. argentifolii* on hibiscus, *Hibiscus rosa-sinensis* L., since it was introduced into the United States from Hong Kong in October 1992 (McAuslane & Nguyen 1996). Rearing conditions for *Eretmocerus* sp. were the same as those for *B. argentifolii*. *Bemisia argentifolii* used in experiments with *E. pergandiella* were from a colony maintained in a greenhouse on several vegetable species. The original feral adults were collected from a field of sweetpotato in Charleston Co., SC (Simmons 1994); feral adults from sweetpotato were added to the colony annually. An endemic population of *E. pergandiella* was maintained on *B. argentifolii* on several species of vegetables in a greenhouse. The parasitoids were collected at the same time as the whiteflies. The colony was occasionally supplied with cotton wicks soaked in 10% honey water.

Parasitism by *Eretmocerus* sp. in a no-choice test

Experiments with *Eretmocerus* sp. were conducted in an indoor climate-controlled room (28°C day/24°C night, 14:10 (L:D)), 30-50% RH) illuminated with high output 110-W cool-white fluorescent lights. Fifteen glossy and 15 normal-wax 6-week-old collard plants bearing 6 to 9 leaves were placed individually in plastic cylindrical cages (15 cm diameter × 30 cm high) with lids and 2 side openings screened with fine plastic mesh (94 × 94 mesh). The 2 oldest leaves were removed from each plant and then each cage was infested with 30 pairs of whiteflies. Whiteflies were removed after 72 hours. We assumed that whitefly oviposition on the 2 collard types was equal because the number of eggs laid on normal-wax and glossy collard is equal in no-choice situations (Elsey & Farnham 1994); however, we did not count whitefly eggs. Five female *Eretmocerus* were added to each cage 10 days later when whiteflies had developed to the first or second instar. Parasitoids were removed 24 hours later. When emergence began, newly-emerged whiteflies and parasitoids were aspirated from the plants and their exuvia were counted and removed from the leaves with a pin each day. This was continued until no further emergence was noted.

Parasitism by *Eretmocerus* sp. when presented a choice between glossy and normal-wax collard

Foraging behavior and plant preference was indirectly studied by allowing parasitoids a choice of glossy or normal-wax collard plants on which to forage for whitefly nymphs. Plants were 6 weeks old bearing 5 to 6 leaves. Four plants of the same phenotype were placed in a screened cage (70-mesh organdy fabric bag supported on a 60
cm $\times$ 60 cm $\times$ 60 cm plastic PVC-pipe frame) and were infested with 150 pairs of whiteflies. Whiteflies were removed 24 hours later. As in the previous experiment, we did not count whitefly eggs but assumed that there were similar numbers on glossy and normal-wax plants. The infested plants were then rearranged randomly among cages so that each screened cage contained 2 whitefly-infested glossy and 2 infested normal-wax plants. Ten cages were set up in this manner. Twelve female parasitoids were released into the center of each cage 11 days after adult whiteflies were removed when first and second instars were present. Parasitoids were not removed. Fifteen days later, leaves were cut from the plants and examined under a microscope. Whitefly exuvia and parasitized whitefly nymphs were counted on upper and lower surfaces of all leaves. Emergence of whiteflies and number of parasitized whiteflies were calculated on a per cage basis (= sum of whitefly exuvia or parasitized nymphs on 2 plants of the same genotype).

Parasitism by E. pergandiella in a no-choice test

Collard seeds were germinated in a greenhouse and then grown in an indoor temperature-controlled room under fluorescent lighting (40-W cool white and 40-W Vitalite® Duro-test® Power-Twist®). Upon reaching the 4-5 leaf stage, the plants were placed in an open greenhouse colony of B. argentifolii. Since whiteflies had a choice of ovipositing on normal-wax or glossy collard plants during the infestation procedure, normal-wax plants were exposed to whiteflies for 2 hours and glossy plants for 1 hour longer to compensate for reduced oviposition on the glossy collard. Exposure times were based on data obtained from field experiments (Jackson et al. 2000) and preliminary greenhouse studies (unpublished data). The plants were then moved from the colony and adult whiteflies were removed first by the air flow from an electrical fan and then with an aspirator. Two plants of each collard type were placed in a Plexiglass cage (45 cm wide $\times$ 45 cm long $\times$ 46 cm high) below fluorescent lamps (as described above) in a temperature-controlled room (14:10 L:D photoperiod supplying ca. 452 lux at plant height). Four cages per trial were set up. Temperature within the cages was 26-27°C. After the whiteflies developed to the second to third nymphal instar, all leaves below a single tagged target leaf (3-4 from bottom) were detached, as were any leaves younger than the targeted leaf that contained whitefly nymphs. Forty E. pergandiella (unsexed) were released into each cage. The parasitoids were retrieved with an aspirator after 24 hours. Upper and lower surfaces of the tagged leaves were checked daily for whitefly or parasitoid emergence. Any adults and exuvia observed were removed daily, and exuvial counts were recorded. This was continued until no further emergence was noted. The experiment was repeated to obtain a second trial. Emergence of whiteflies and parasitoids was calculated on a per cage basis (= sum of emergence on 2 plants of the same genotype).

Parasitism by E. pergandiella when presented a choice between glossy and normal-wax collard

Collard plants at the 5- to 6-leaf stage were infested during a 12-14 hour exposure to whiteflies in a greenhouse. The adult whiteflies were removed using an aspirator and then the plants were transferred to a temperature-controlled room. One glossy and one normal-wax plant were placed in a Plexiglass cage (45 cm wide $\times$ 45 cm long $\times$ 46 cm high) and 8 replicate cages were set up. Forty parasitoids (unsexed) were added to each cage when whiteflies had developed to the second or third nymphal instar. After 3 weeks, exuvia from which either a whitefly or a parasitoid emerged were counted.
Statistical Analyses

We compared whitefly emergence, parasitoid emergence, and number of parasitized nymphs between glossy and normal-wax collard using analysis of variance (PROC GLM; SAS Institute 1997). Because only a small percentage (<5%) of immature whiteflies develop on the top leaf surface of collard (Simmons 1994) and leaf surface does not affect development (Simmons 1999), emergence data were pooled between leaf surfaces. Data for emerged whiteflies and parasitoids or parasitized nymphs were log \((x + 0.1)\)-transformed, when necessary, to correct for variance increasing with the mean. Means shown in tables are untransformed. We compared developmental time of whiteflies and parasitoids between the 2 collard types by estimating time to 50% emergence on each plant using linear regression (PROC REG; SAS Institute 1997) and performing analysis of variance (PROC GLM) on estimated times to 50% emergence. Significant least square means were separated by the probability of a significant difference at \(= 0.05\) (PROC GLM).

RESULTS

Parasitism by *Eretmocerus* sp.

In the no-choice test, one plant which was initially classified as a normal-wax collard was in fact glossy and another normal-wax collard was destroyed during the experiment leaving 13 replicate normal-wax collard plants and 16 glossy collard replicates. Whiteflies emerged over a 15-day period beginning 21 days after the plants were infested. The time to 50% emergence of whiteflies did not differ on the 2 collard wax types \((F_{1,12} = 0.11; P = 0.75)\) and averaged 4.4 ± 0.8 days (mean ± se), with the first day of whitefly emergence being day 1. *Eretmocerus* parasitoids emerged over an 11-day period, beginning on day 13 of whitefly emergence. The time to 50% emergence of parasitoids did not differ between the 2 collard types \((F_{1,12} = 0.41; P = 0.53)\) and averaged 4.4 ± 0.3 days. The number of parasitoids emerging was not influenced by collard type \((F_{1,11} = 1.48; P = 0.25)\) nor was whitefly emergence \((F_{1,12} = 1.69; P = 0.22)\) (Table 1).

In the choice test, neither whitefly emergence \((F_{1,9} = 0.24; P = 0.63)\) nor the number of parasitized nymphs \((F_{1,8} = 0.92; P = 0.37)\) differed significantly between normal-wax and glossy collard (Table 1).

Parasitism by *E. pergandiella*

In the no-choice test, whiteflies emerged over a 13-day period in trial 1 and an 11-day period in trial 2. Time to 50% emergence was not influenced by collard type \((F_{1,7} = 0.04; P = 0.86)\), but there was a significant effect of trial \((F_{1,7} = 13.06; P = 0.009)\). Time to 50% emergence of whiteflies was 4.7 ± 0.4 days in trial 1 and 2.2 ± 0.7 days after first whitefly emergence in trial 2. *Encarsia pergandiella* emerged over a 12-day period in trial 1 and an 8-day period in trial 2, but there was no significant effect of collard type \((F_{1,4} = 0.31; P = 0.61)\) or trial \((F_{1,4} = 0.06; P = 0.82)\) on time to 50% emergence, which averaged 3.8 ± 0.5 days after the first parasitoid emerged.

Whitefly emergence was influenced by trial \((F_{1,12} = 33.91; P = 0.001)\), collard type \((F_{1,9} = 12.00; P = 0.013)\), and the interaction of trial × collard type \((F_{1,12} = 14.05; P = 0.0095)\). Significantly more whiteflies emerged from glossy collard in trial 1 than from normal-wax collard (Table 2). Emergence did not differ in trial 2. Emergence of *E. pergandiella* was significantly influenced by trial \((F_{1,12} = 5.39; P = 0.039)\) and collard type
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Table 1. Number of emerged Eretmocerus sp. parasitoids and whiteflies on glossy and normal-wax "Green Glaze" collard in no-choice and choice tests (means ± SE, range).

<table>
<thead>
<tr>
<th>Wax type</th>
<th>No. Eretmocerus emerged</th>
<th>No. whiteflies emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-choice test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossy</td>
<td>36.0 ± 6.3</td>
<td>134.8 ± 24.0</td>
</tr>
<tr>
<td></td>
<td>(0 - 74)</td>
<td>(19 - 439)</td>
</tr>
<tr>
<td>Normal-wax</td>
<td>24.2 ± 3.8</td>
<td>168.2 ± 29.5</td>
</tr>
<tr>
<td></td>
<td>(5 - 47)</td>
<td>(28 - 408)</td>
</tr>
<tr>
<td>Choice test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossy</td>
<td>88.5 ± 12.3</td>
<td>940 ± 211</td>
</tr>
<tr>
<td></td>
<td>(36 - 160)</td>
<td>(382 - 2644)</td>
</tr>
<tr>
<td>Normal-wax</td>
<td>97.9 ± 11.4</td>
<td>937 ± 206</td>
</tr>
<tr>
<td></td>
<td>(45 - 166)</td>
<td>(415 - 1565)</td>
</tr>
</tbody>
</table>

\((F_{1,12} = 11.38; P = 0.006)\), but there was no trial x collard type interaction \((F_{1,12} = 0.15; P = 0.71)\). More than twice as many parasitoids emerged from glossy plants than from normal-wax plants (an average of 35.5 ± 4.8 vs. 14.6 ± 5.0 per plant, respectively) (Table 2).

In the choice test, there was no significant difference in whitefly emergence on the 2 collard types \((F_{1,7} = 0.01; P = 0.93)\) (Table 2). However, 4 times as many \(E. pergandiella\) emerged from glossy collard than from normal-wax collard \((F_{1,6} = 23.87; P = 0.003)\).

**Discussion**

The glossy leaf-wax trait in Brassica vegetables has been associated with resistance to several important insect pests such as the cabbage aphid, Brevicoryne brassicae (L.), the imported cabbageworm, Artogeia rapae (L.), P. xylostella (Eigenbrode & Shelton 1990, Stoner 1990, Eigenbrode et al. 1991), and B. argentifolii (Elsey & Farnham 1994, Farnham & Elsey 1995, Jackson et al. 2000). In the case of \(P. xylostella\), reduced pest populations on glossy plants were due partly to the direct physical and allelochemical effects on the insect of leaf wax components (Eigenbrode et al. 1991), and partly to enhanced predation by natural enemies (Eigenbrode et al. 1995). Eigenbrode et al. (1995) stated that the importance of predation should be evaluated during development of glossy Brassica for resistance to insects.

Our study indicates that parasitism of whitefly nymphs by \(E. pergandiella\) is enhanced on glossy phenotype ‘Green Glaze’ collard compared with a normal-wax phenotype. These phenotypes are isogenic except for the single gene mutation causing glossiness, hence, we would not expect any nutritional effects of the plant acting through the host on the parasitoid. We did not find the same increase in whitefly parasitism by Eretmocerus sp. on glossy collard. However, and of more importance to regulation of whitefly populations, we saw no decrease in parasitism by Eretmocerus sp. on glossy collard.

We had expected, given the nature of the oviposition behavior of these 2 parasitoid species, that Eretmocerus sp. would be more affected, either negatively or positively, by
collard leaf wax because *Eretmocerus* sp. females must locate a suitable gap between the whitefly nymph and the leaf surface through which to insert their ovipositor. If the marginal wax laid down by whitefly nymphs adheres differently on normal-wax and glossy plants, we might expect the ability of *Eretmocerus* sp. to insert its ovipositor to be different on the 2 plant types. On the other hand, *E. pergandiella* females oviposit through the dorsum of their host and the adhesion of the host to the leaf should not influence oviposition success. The eggs of *Eretmocerus* species, in general, lie underneath the host on the leaf surface for several days before the first instar parasitoid ecloses and chews into the whitefly nymph (Foltyn & Gerling 1985, McAuslane & Nyugen 1996). The egg is presumably in contact with leaf waxes and allelochemicals and could be influenced by physical and chemical characteristics of this wax layer. *Encarsia pergandiella* immature stages are never in direct contact with the leaf surface. In our study, contrary to our expectations, we found that parasitism of whitefly by *E. pergandiella* was in fact improved on the glossy collard while parasitism success of *Eretmocerus* was unchanged. Reasons other than those proposed above must account for the different parasitism success of these two species on glossy and normal-wax collard.

Large-bodied generalist predators of diamondback moth, such as adults of *Orius insidiosus* (Say) and *Hippodamia convergens* Guerin-Meneville, and larval *Chrysoperla carnea* (Stephens) are more mobile on glossy cabbage genotypes (Eigenbrode et al. 1996) and consequently locate *P. xylostella* larvae better on glossy cabbage than on normal-wax cabbage (Eigenbrode et al. 1995). Their greater mobility is due to the fact

<table>
<thead>
<tr>
<th>Wax type</th>
<th>No. <em>E. pergandiella</em> emerged</th>
<th>No. whiteflies emerged</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>No-choice test</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Trial 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossy</td>
<td>41.5 ± 5.7a</td>
<td>711 ± 108a</td>
</tr>
<tr>
<td></td>
<td>(32 - 58)</td>
<td>(433 - 946)</td>
</tr>
<tr>
<td>Normal-wax</td>
<td>23.0 ± 7.3ab</td>
<td>266 ± 38b</td>
</tr>
<tr>
<td></td>
<td>(8 - 42)</td>
<td>(154 - 318)</td>
</tr>
<tr>
<td><strong>Trial 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossy</td>
<td>29.5 ± 7.1a</td>
<td>120 ± 13b</td>
</tr>
<tr>
<td></td>
<td>(13 - 47)</td>
<td>(96 - 150)</td>
</tr>
<tr>
<td>Normal-wax</td>
<td>6.25 ± 4.0b</td>
<td>137 ± 14b</td>
</tr>
<tr>
<td></td>
<td>(1 - 18)</td>
<td>(101 - 170)</td>
</tr>
<tr>
<td><strong>Choice test</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glossy</td>
<td>89.0 ± 19.4a</td>
<td>135.6 ± 24.8</td>
</tr>
<tr>
<td></td>
<td>(23 - 195)</td>
<td>(52 - 270)</td>
</tr>
<tr>
<td>Normal-wax</td>
<td>19.8 ± 4.4b</td>
<td>160.4 ± 47.6</td>
</tr>
<tr>
<td></td>
<td>(2 - 44)</td>
<td>(27 - 418)</td>
</tr>
</tbody>
</table>

1Mean within a column and within a test followed by different letters differ significantly (probability of a significant difference of least squares means, α = 0.05).
that they spend less time scrambling (i.e., slipping while walking), falling off the plant, and grooming off wax particles that had accumulated on their tarsi on glossy cabbage than on normal-wax cabbage (Eigenbrode et al. 1996). Much of the reason that predators can move more efficiently on glossy genotypes is because they can generate much greater adhesive force on glossy leaves than on normal-wax cabbage leaves (Eigenbrode et al. 1999). Aphelinid parasitoids fly from plant to plant when foraging for whitefly hosts and often fly within the plant, from leaf to leaf. Parasitoids do, however, walk extensively on the leaf searching for host patches. It is not known whether these very small-bodied hymenopterans suffer the same reduced traction on normal-wax plants as do larger predators. This aspect of parasitoid behavior needs to be studied more carefully.

Other possible reasons for the difference in parasitism of *Eretmocerus* and *E. pergandiella* are potential differences in whitefly nymph distribution on leaves. If whitefly oviposition behavior differs on glossy and normal-wax collard, leading to different dispersion of nymphs, this may differently affect the foraging behavior and parasitization success of these 2 parasitoid species. Finally, although we tried to perform experiments under similar environmental conditions, *Eretmocerus* and *E. pergandiella* were studied in different laboratories. It is known that water saturation and other environmental conditions can alter the composition and amount of leaf waxes (reviewed in Eigenbrode & Espelie 1995). This may have affected parasitoid behavior and/or survival.

Host plant resistance and biological control have long been considered the cornerstones of pest management strategies. While generally thought to be compatible and additive in nature (Bergman & Tingey 1979), these tactics have not always been so. We have demonstrated that the nonpreference ovipositional resistance (antixenosis) in glossy collards to *B. argentifolii* is fully compatible with biological control by species in the 2 most important genera of whitefly parasitoids. Parasitism of whiteflies is at least as high, if not higher in the case of *E. pergandiella*, on a glossy collard phenotype compared with that on a normal-wax phenotype. Use of glossy collard in a management program that includes natural enemies should lead to smaller whitefly infestations in collard, leading to a reduced need for insecticide application and reduced populations infesting spring plantings of other crops.

**ACKNOWLEDGMENTS**

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**REFERENCES CITED**


RESIDUAL EFFICACY OF BLATTICIDES APPLIED TO SURFACES CONTAMINATED WITH GERMAN COCKROACH (DICTYOPTERA: BLATTELLIDAE) FECES

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ABSTRACT

Crack-and-crevice treatments were simulated in the presence and absence of German cockroach [Blattella germanica (L.)] feces to evaluate its effect on insecticide efficacy toward the German cockroach. The LT50 of German cockroaches exposed to 0.39 μg of cypermethrin/cm² (Demon EC formulation) on glass Mason jars was 26 min. The LT50 increased 2.5- and 4.5-fold when Demon EC was mixed with 123 and 184 mg of cockroach feces, respectively. The presence of German cockroach feces increased the LT50 2.5-fold in Dursban EC (chlorpyrifos) and 1.2-fold in Baygon EC (propoxur). Longevity experiments with 3 l-cyhalothrin formulations in the presence of German cockroach feces resulted in significant decreases in insecticide efficacy. Feces reduced the performance of Commodore WP (l-cyhalothrin) by 12.5, 35, 55, and 97.5% on days 0, 10, 20, and 30, respectively. Initial reductions in efficacy were observed for the Demand CS (l-cyhalothrin) and Karate (l-cyhalothrin) formulations when in the presence of German cockroach feces.

Key Words: Blattella germanica, feces, insecticide efficacy, formulation

RESUMEN

Tratamientos de grietas y hendiduras fueron simulados en la presencia y ausencia de heces fecales de la cucaracha alemana [Blattella germanica (L.)] para evaluar su efecto en eficacia del insecticida hacia la cucaracha alemana. El LT50 de cucarachas alemanas expuestas a 0.39 μg de cypermethrin/cm² (formulación Demon EC) en jarras de vidrio Masón fue 26 minutos. El LT50 incrementó 2.5 y 4.5 veces cuando se mezcló Demon EC con 123 y 184 mg de heces fecales de cucaracha, respectivamente. La presencia de heces fecales de la cucaracha alemana incremento el LT50 2.5 veces en Dursban EC (chlorpyrifos) y 1.2 veces en Baygon EC (propoxur). Experimentos de longevidad con 3 formulaciones l-cyhalothrin en presencia de heces fecales de cucaracha alemana resulto en disminuciones significativas en eficacia del insecticida. Las heces fecales redujeron el desempeño de Commodore WP (l-cyhalothrin) por 12.5, 35, 55, y 97.5% en los días 0, 10, 20, y 30, respectivamente. Reducciones iniciales en eficacia fueron observadas en formulaciones de Demand CS (l-cyhalothrin) y Karate (l-cyhalothrin) cuando en presencia de heces fecales de la cucaracha alemana.

Recent public concern over the environmental and health impacts of pesticides has discouraged the traditional use of broad spray treatments for control of German cockroach, Blattella germanica (L.). Although residual insecticide treatments are still
used against German cockroaches, the method of application is typically confined to suspected cockroach aggregation sites or harborage. This method of insecticide application is commonly known as crack-and-crevice treatment. Crack-and-crevice insecticide placement reduces the amount of toxicant required for control (Bennett et al. 1988) and minimizes insecticide exposure risks to humans and pets.

German cockroaches are gregarious (Roth & Willis 1960) often spending up to 75% of their lifetime at rest in harborage (Cornwell 1968). Within these harborage, feces accumulate and may affect the efficacy of crack-and-crevice insecticide applications. Surface type (Cornwell 1972), organic matter (Niemczyk & Krueger 1987, Kamm & Montgomery 1990), and oils (Newton & Coombes 1990) influence the performance of various residual insecticide formulations.

We simulated crack-and-crevice treatments in the presence and absence of German cockroach feces for the purpose of evaluating the effect of feces on insecticide efficacy.

MATERIALS AND METHODS

Cockroaches and Feces

Adult males (1-2 wk old) of the insecticide susceptible Orlando strain of German cockroach (Koehler & Patterson 1986) were used for all bioassays. The cockroaches were reared as described by Koehler et al. (1994).

German cockroach feces were collected from rearing containers of final instar cockroaches. Feces were separated from cast skins and other debris with a steel sieve (0.71-mm² openings) followed by fine mesh steel sieve (0.48-mm² openings) for use in subsequent experiments.

Insecticides

Emulsifiable concentrate (EC) formulations of cypermethrin (Demon EC, 25.3% [AI]; Zeneca, Wilmington, DE.), chlorpyrifos (Dursban 2E, 24.1% [AI]; Dow AgroSciences, Indianapolis, IN), \( \lambda \)-cyhalothrin (Karate, 10% [AI] Zeneca), and propoxur (Baygon 1.5 EC, 14.7% [AI], Bayer, Kansas City, MO) were used in efficacy experiments. Additionally, the wettable powder (WP) and microencapsulated formulations of \( \lambda \)-cyhalothrin (Commodore WP 10% [AI] and Demand CS 9.7% [AI], respectively) were included in the study. All formulations were diluted in water to form an emulsion or suspension and pipetted into jars for tests.

Bioassays

Time-mortality relationships (Cochran 1997) were first established for formulated cypermethrin (Demon EC) in the presence of increasing quantities of cockroach feces. Cockroach feces (0, 0.24, 0.48, or 0.72 mg per cm²) were added to Mason jars (473 ml, surface area = 256 cm²; Ball Corp., Muncie, IN). The Demon EC formulation of cypermethrin was prepared at a rate of 0.1 mg[AI]/ml by adding 40 ml of the EC to 99.96 ml of water. One ml of this solution was pipetted into each jar. An additional 3 ml of water also was added to each jar to facilitate even coating with the feces-insecticide mixture. The jars were placed on a roller on their sides and rotated continuously with a gentle stream of compressed air directed into each jar. After the jars were dry (typically 4 h) the upper 2 cm of the interior was coated with a petroleum jelly: mineral oil mixture.
(3:2) to prevent cockroaches from escaping. Ten adult male cockroaches of the Orlando strain were placed into each jar individually with featherweight forceps. The cockroaches were not anesthetized with CO$_2$. The jars were placed into an environmental chamber and held at 24.9 ± 0.4°C and 69.6 ± 3.9% RH. Control jars were treated with water and feces only. A repeated measures method was used to assess lethal time values. Cockroaches were monitored every 5 min and the number dead recorded until 80% of the cockroaches in the jar were killed. Cockroaches were considered dead or moribund if unable to right themselves within 15 sec after being flipped onto their dorsum. Treatment mortality was corrected for control mortality with Abbott’s formula (1925). The entire experiment was replicated 3 times. Mortality was analyzed with the Probit procedure (SAS Institute 1988). Significant differences were determined by nonoverlap of 95% confidence intervals.

The effect of feces on lethal time was compared among EC formulations of chlorpyrifos, cypermethrin, and propoxur. Dursban 2E, Baygon 1.5 EC and Demon EC were prepared in water at 3, 11, and 0.1 mg[AI]/ml, respectively. Mason jars were treated with 0.72 mg of feces/cm$^2$ and 1 ml of formulated insecticide as described previously. Additional water (3 ml) was added and the jars were rolled until dry. Cockroaches were added to each jar, mortality recorded, and data analyzed as described previously. The experiment was repeated three times. Data were analyzed by the Probit procedure with mortality as dependent variable (SAS 1988).

The last part of the study was to determine the effect of feces on the residual performance of 3 different formulations of λ-cyhalothrin. The wettable powder (Commodore WP), capsulated suspension (Demand CS) and emulsifiable concentrate (Karate) formulations were diluted in water at 0.1 mg[AI]/ml and 1 ml was applied to the inner walls of the jars. Each insecticide formulation was added to 2 jars, one containing 0.72 mg of feces/cm$^2$ and one not treated with feces. Ten adult male cockroaches were placed in each jar. After a 1-min exposure to the treated glass surface the cockroaches were anesthetized with CO$_2$ (15 sec), placed in an untreated glass Petri dish, and subsequently placed in another untreated plastic Petri dish (100 by 15 mm). Any feces deposited with the insects was returned to its respective jar. Cockroaches were held in an environmental chamber at 24.9 ± 0.4°C and 69.6 ± 3.9% RH. Mortality was assessed 24 h later. Jars were stored in the dark at room temperature (approximately 24°C). The bioassay was repeated at 10, 20, and 30 d after treatment by using the same jars. The experiment was replicated 5 times. Control jars were treated with water and feces. Mortality in the presence and absence of feces was compared by Student’s t-test for each time period and formulation.

RESULTS

The LT$_{50}$ of Orlando cockroaches exposed to glass Mason jars treated with 0.39 μg[AI]/cm$^2$ of Demon EC was 26 min (Table 1). This value was not significantly different from jars treated with Demon EC and 0.24 mg/cm$^2$ of German cockroach feces. However, the LT$_{50}$ increased 2.5- and 4.5-fold when Demon EC was mixed with 0.48 and 0.72 mg of feces/cm$^2$, respectively. No mortality was observed in control jars devoid of insecticide but containing feces.

Among the 3 insecticide classes used in this study, cockroach feces had the greatest impact on the efficacy of cypermethrin (Table 2). The inhibition ratio (IR = LT$_{50}$ feces contaminated jar/LT$_{50}$ clean jar) for cypermethrin was 4.4-fold. The presence of German cockroach feces increased the time to mortality 2.5-fold in Dursban 2E (chlorpyrifos) and had a small, yet statistically significant (based on nonoverlap of 95% confidence intervals), effect on the toxicity of Baygon 1.5 EC (propoxur).
Residual activity of various \( \lambda \)-cyhalothrin formulations in the presence and absence of German cockroach feces is illustrated in Figure 1. Mortality was reduced significantly among all formulations in the presence of feces. Feces reduced the performance of the WP by 12.5, 35, 55, and 97.5% on days 0, 10, 20, and 30, respectively (Fig. 1A). Initial reductions in efficacy were severe for the Demand CS and Karate formulations (Fig. 1B, C). Although Demand CS was effective on clean glass throughout the study, feces nearly eliminated its ability to kill cockroaches. Mortality on the Karate-treated surface declined sharply in the absence of feces, and no significant differences were observed between treatments with and without feces at 10, 20, and 30 d.

### DISCUSSION

Cracks and crevices in furniture, kitchen equipment, wall voids, and elsewhere in structures are primary harborage sites for the German cockroach (Cornwell 1968). These harborage sites often become heavily littered with cockroach feces (Stejskal 1997). Treatment of these areas with residual insecticides is a recommended method for cockroach control in food-handling establishments (Rust 1986, Bennett et al. 1988). Unfortunately, based on our data, fecal deposits found in these areas may significantly reduce the efficacy of some insecticides.

Decreased insecticide efficacy in the presence of German cockroach feces was anticipated based on results of previous reports. For example, organic matter in soil reduces insecticide toxicity by acting as an adsorbent (Hamaker & Thompson 1972). Similarly, activated carbon has been used to protect grass seed from herbicides (Lee 1973). German cockroach control failures in kitchens also have been associated with insecticide affinity to cooking oils (Ree 1980, Schal 1988, Rust & Reierson 1988). In addition to reducing insecticide efficacy by adsorption, microbial degradation also may affect insecticide efficacy. Feces may contain microbes that could have the capacity to metabolize the insecticide. However, preliminary experiments with autoclaved feces resulted in comparable decreases in insecticide efficacy indicating that microbial degradation was not likely to be the mechanism responsible (C. Strong, unpublished data).

Among the EC formulations of 3 insecticide classes evaluated in this study, cockroach feces were most detrimental to the performance of cypermethrin, Demon EC (Table 2). Cypermethrin-treated jars containing feces (0.72 mg/cm\(^2\)) required 4.5-fold more time to kill cockroaches than insecticide in clean jars. Chlorpyrifos, Dursban 2E, toxicity also was significantly reduced (2.5-fold) by the presence of feces. The toxicity

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### TABLE 1. THE LT\(_{50}\) VALUES (MIN) FOR ADULT MALE SUSCEPTIBLE COCKROACHES EXPOSED TO A DEMON EC (CYPERMETHRIN) TREATED MASON JAR (0.39 G/CM\(^2\)) IN THE PRESENCE OF INCREASING QUANTITIES OF GERMAN COCKROACH FECES.

<table>
<thead>
<tr>
<th>Feces (mg/cm(^2))</th>
<th>Obs(^a)</th>
<th>Slope ± SE</th>
<th>LT(_{50}) (95% CI)</th>
<th>(\chi^2)</th>
<th>IR(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>420</td>
<td>1.67 ± 0.20</td>
<td>25.55 (21.22-32.16)</td>
<td>1.98</td>
<td>1.00</td>
</tr>
<tr>
<td>0.24</td>
<td>400</td>
<td>1.15 ± 0.27</td>
<td>20.63 (10.24-28.23)</td>
<td>8.65</td>
<td>0.81</td>
</tr>
<tr>
<td>0.48</td>
<td>400</td>
<td>1.31 ± 0.27</td>
<td>63.77 (50.79-92.24)</td>
<td>9.96</td>
<td>2.50</td>
</tr>
<tr>
<td>0.72</td>
<td>810</td>
<td>1.50 ± 0.22</td>
<td>115.59 (98.18-166.65)</td>
<td>1.09</td>
<td>4.52</td>
</tr>
</tbody>
</table>

\(^a\)Total number of observations recorded until 80% mortality was achieved (repeated measure).

\(^b\)IR = inhibition ratio (LT\(_{50}\) contaminated surface/LT\(_{50}\) clean surface).
TABLE 2. THE LT₅₀ VALUES (MIN) FOR ADULT MALE SUSCEPTIBLE COCKROACHES EXPOSED TO AN INSECTICIDE FORMULATION IN THE PRESENCE OR ABSENCE OF GERMAN COCKROACH FECES (0.72 MG/CM²).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Surface</th>
<th>Obs</th>
<th>Slope ± SE</th>
<th>LT₅₀ (95% CI)</th>
<th>χ²</th>
<th>IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cypermethrin</td>
<td>clean</td>
<td>420</td>
<td>1.67 ± 0.20</td>
<td>25.55 (21.22-32.16)</td>
<td>1.98</td>
<td>1.00</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>feces</td>
<td>1080</td>
<td>1.75 ± 0.11</td>
<td>113.35 (105.03-123.28)</td>
<td>14.54</td>
<td>4.44</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>clean</td>
<td>600</td>
<td>15.24 ± 1.08</td>
<td>47.12 (46.28-47.98)</td>
<td>3.86</td>
<td>1.00</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>feces</td>
<td>810</td>
<td>13.22 ± 0.83</td>
<td>117.21 (114.82-119.48)</td>
<td>2.65</td>
<td>2.49</td>
</tr>
<tr>
<td>Propoxur</td>
<td>clean</td>
<td>500</td>
<td>11.00 ± 0.83</td>
<td>23.30 (22.63-23.97)</td>
<td>5.23</td>
<td>1.00</td>
</tr>
<tr>
<td>Propoxur</td>
<td>feces</td>
<td>500</td>
<td>10.21 ± 1.48</td>
<td>28.69 (26.38-31.30)</td>
<td>7.67</td>
<td>1.23</td>
</tr>
</tbody>
</table>

*a cypermethrin = 0.39 g[AI]/cm², chlorpyrifos = 11.7 μg[AI]/cm², propoxur = 43 μg[AI]/cm².
*b Total number of observations recorded until 80% mortality was achieved (repeated measure).
*c IR = inhibition ratio (LT₅₀ contaminated surface/LT₅₀ clean surface).
Fig. 1. Residual efficacy of 3 λ-cyhalothrin-cyhalothrin formulations (A, wettable powder; B, capsulated suspension; C, emulsifiable concentrate) applied to glass Mason jars in the presence (○) and absence (●) of German cockroach feces.
of propoxur, Baygon 1.5 EC, was least affected by the presence of feces. The effectiveness of another carbamate, bendiocarb, was unaffected by soil carbon content (Kamm & Montgomery 1990).

Dramatic losses in λ-cyhalothrin efficacy were observed in the longevity experiments. For example, although the WP and CS formulations caused 95 to 100% mortality through 30 d in the absence of feces, their efficacy was reduced to nearly 0% mortality in the presence of feces at day 30.

Cockroach feces in the home may present problems other than control failures. Human consumption of food products contaminated with cockroach feces may lead to digestive disorders (Mullins & Cochran 1973) and feces often contain bacterial and fungal pathogens (Koehler et al. 1990). Additionally, cockroach feces contain potent allergens responsible for asthma and related respiratory disorders (Brenner 1995). Therefore, cleaning cockroach feces from structures before insecticide treatment provides a 2-fold benefit—reduction of disease potential and improved insecticide efficacy.

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INTERPRETIVE SUMMARY

A commonly used insecticide application method known as crack and crevice treatment, was simulated in the laboratory to evaluate the effect of cockroach excrement found in these areas on insecticide efficacy. The presence of cockroach excrement significantly decreased the efficacy of several emulsifiable concentrate insecticides (chlorpyrifos, cypermethrin, and propoxur). The efficacy of other insecticide formulations (Wettable Powder, and Capsulated Suspension) were also decreased in the presence of cockroach excrement. These results help to explain control failures when this method of insecticide application is employed. Recommendations for improving the method are suggested.

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SAMPLING OF DIAPHORINA CITRI (HOMOPTERA: PSYLLIDAE) ON ORANGE JESSAMINE IN SOUTHERN FLORIDA

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ABSTRACT

Dispersion indices and related statistics of Asian citrus psyllid, Diaphorina citri Kuwayama, on orange jessamine [Murraya paniculata (L.) Jack] shoots in southern Florida from 1998 to 1999 were determined with 235 data sets and used to develop sampling plans. Three regression models, Taylor's power law, Iwao's patchiness regression, and \( k = c + dm \) \( \left( k = m^2 / (S^2 - m) \right) \) (where \( k \) is the parameter for the negative binomial distribution) were used to analyze the data. Taylor's power law \( (a = 0.3407 \pm 0.03, b = 1.2971 \pm 0.03, r^2 = 0.88) \) fit the data better than Iwao's model \( (a = -0.3217 \pm 0.12, \beta = 1.6979 \pm 0.06, r^2 = 0.76) \). Taylor's \( b \) and Iwao's \( \beta \) were both significantly > 1, indicating that \( D. \) citri populations were aggregated. Iwao's \( a \) was significantly < 0, indicating that the basic distribution component of \( D. \) citri was the individual insect. The slope \( d \) \( (0.7489 \pm 0.48) \) was indistinguishable from 0, indicating the existence of a common \( k \) (estimated as 1.2741). The incidence \( (P_1, \text{ proportion infested}) \) and mean density \( (m) \) relationship was developed by negative binomial distribution (NBD) basis and Nachman's model \( \ln (m) = 0.2277 + 1.2444 \ln (-\ln (P_0)) \) (where \( P_0 = \text{Proportion of uninfested sampling units in a sample} \)). The NBD was appropriate for studying \( D. \) citri distribution based on comparison of NBD basis and Nachman's models. The relationship to determine sample sizes for fixed levels of precision and fixed-precision-level stop lines for sequential sampling was also developed.

Key Words: Asian citrus psyllid, Taylor's power Law, Iwao's patchiness regression, negative binomial distribution

RESUMEN

Índices de dispersión y estadísticas relacionadas con el psila de cítrico Asiático, Diaphorina citri Kuwayama, en brotes de Murraya paniculata (L.) Jack en el Sur de Florida entre 1998 y 1999 fueron determinados con 235 conjuntos de datos y usados para el desarrollo de planes de muestreo. Tres modelos de regresión, la ley de poder Taylor, la regresión Iwao, y \( k = c + dm \) \( \left( k = m^2 / (S^2 - m) \right) \) (donde \( k \) es el parámetro para la distribución binomial negativa) fueron usados para analizar los datos. La ley de poder Taylor \( (a = 0.3407 \pm 0.12, b = 1.2971 \pm 0.03, r^2 = 0.88) \) encaja los datos mejor que el modelo de Iwao \( (a = -0.3217 \pm 0.12, \beta = 1.6979 \pm 0.06, r^2 = 0.76) \). La \( b \) de Taylor y el \( \beta \) de Iwao fueron ambos significativamente > 1, indicando que poblaciones de \( D. \) citri fueron agregadas. El \( \alpha \) fue significativamente < 0, indicando que el componente básico de distribución de \( D. \) citri era el insecto individual. La pendiente \( d \) \( (0.7489 \pm 0.48) \) fue indistinguible de 0, indicando la existencia de una \( k \) en común (estimada a 1.2741). La relación entre incidencia \( (P_1, \text{provisión infestada}) \) y densidad promedio \( (m) \) fue desarrollada a base de la distribución negativa binomial (NBD) y el modelo de Nachman \( \ln (m) = 0.2277 + 1.2444 \ln (-\ln (P_0)) \) (donde \( P_0 = \text{provisión de unidades de muestreo} \).
Citrus is one of the most important economic crops in the U.S. with about 500,000 ha in citrus orchards mostly in California, Florida, Texas, and Arizona. In Florida alone, citrus encompasses 389,857 planted hectares with a total of 107 million trees in the 33 citrus producing counties. The annual earning on citrus is estimated at $1.1 billion (Tsai 1998). Citrus greening disease or Huanglungbin is the most serious disease of citrus in the world (Aubert et al. 1996, Tsai et al. 1988). The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is the most efficient vector of citrus greening bacterium, *Liberobacter asiaticum* Jagoueix, Bove & Garnier, throughout Asia and the Far East (Catling 1970, Pande 1971, Tsai et al. 1988). The combined presence of a psyllid vector and a greening agent has been the limiting factor in citrus production in these areas (Ke et al. 1988, Tsai et al. 1988). On June 3, 1998 the Asian citrus psyllid was first found in southern Florida, with the subsequent discovery of *D. citri* in Broward, Palm Beach, Martin, Dade, St. Lucie, Hendry, and Collier Counties in a 3-month period (Halbert et al. 1998). Given high reproductive potential of this vector during favorable conditions of weather and food availability (J. H. T., unpublished data), this pest is expected to spread throughout citrus producing area in Florida in 2-3 years. It poses a serious threat to other citrus producing states in the future. Based on our observations, this pest is most abundant on orange jessamine, *Murraya paniculata* (L.) Jack (J. H. T., unpublished data), which is widely planted as hedges in the urban landscape in southern Florida. It could serve as an alternate host for maintaining psyllid populations when young citrus shoots are not available.

Data on dispersion of pest populations is an important aspect of population biology because it is a result of the interaction between individuals of the species and their habitat (Sevacherian & Stern 1972). Knowledge of this dispersion allows a better understanding of the relationship between an insect and its environment and provides basic information for interpreting spatial dynamics, designing efficient sampling programs for population estimation, and pest management (Harcourt 1961, Iwao 1970, Sevacherian & Stern 1972, Taylor 1984), and the development of population models (Croft & Hoyt 1983). Methods that are commonly used to describe dispersion of arthropod populations have been summarized by Southwood (1978). Several estimates based on sample mean ($m$) and variance ($S^2$) are used as indices for aggregation (Lloyd 1967) and the dispersion parameter $k$ for the negative binomial distribution (Southwood 1978). Moreover, these indices are often convertible from one to another. Sampling plans based on these descriptions of dispersion (Kuno 1969, Green 1970) reduce sampling effort and minimize variation of sampling precision (Hutchison et al. 1988, Kuno 1991, Trumble et al. 1989). Little is known about the dispersion of *D. citri* because of its new pest status in USA. To fill this void, we gathered data on the dispersion of *D. citri* adults on orange jessamine in southern Florida from 1998 to 1999. From this information, two incidence-density relationships, the optimal sample sizes for estimating density, and the sequential sampling plans suitable for intensive population research and pest surveys were developed.
Materials and Methods

Sampling of Citrus Psyllid Population

A field survey for sampling populations of citrus psyllids was conducted from October 1998 to May 1999 in ten orange jessamine fields in Broward County, Florida. The plants were not sprayed with insecticides during the course of the study.

For the purpose of sampling, the field in each location was divided into five areas (10 x 2 m). At weekly intervals, one shoot (about 6-10 cm long) was selected at random from each square meter area by throwing a pointed object. Thus a total of 20 shoots were selected from each of the 5 areas on each sampling date. Numbers of citrus psyllid adults per shoot were counted and recorded.

Variance-Mean Relationships

The mean density \( (m) \) per shoot and variance \( (S^2) \) were calculated for shoots in each field per sampling date and related to each other using Taylor’s power law (Taylor 1961, 1971, Taylor et al. 1978) and Iwao’s patchiness regression (Lloyd 1967, Iwao 1968, Iwao & Kuno 1971).

Taylor’s power law states that the variance \( (S^2) \) of a population is proportional to a fractional power of the arithmetic mean \( (m) \): \( S^2 = am^b \). To estimate \( a \) and \( b \), the values of \( \ln(S^2) \) were regressed against those of \( \ln(m) \) using the model

\[
\ln(S^2) = \ln(a) + b \ln(m) \quad (1)
\]

where the parameter \( a \) is a scaling factor related to sample size (Southwood 1978), the slope \( b \) is an index of aggregation which indicates a uniform, random and aggregated dispersion when \( b < 1 \), \( b =1 \), \( b >1 \), respectively.

Iwao’s patchiness regression method quantifies the relationship between Lloyd’s (1967) mean crowding index \( (m^*) \) and mean \( (m) \) by:

\[
m^* = \alpha + \beta m \quad (2)
\]

where \( m^* \) was determined as \( [m + (S^2/m -1)] \) (Lloyd 1967). The intercept \( (\alpha) \) is the index of basic contagion and the slope \( (\beta) \) is the density contagiousness coefficient interpreted in the same manner as \( b \) of Taylor’s regression.

Estimation of Incidence

The relationship between the proportion of samples with one or more animals (the incidence, \( P_i \)) and the density of animals \( (m) \) per sample unit was developed using the following two methods.

One was developed by assuming that a negative binomial distribution (NBD) with variable \( k \) would describe the distribution of psyllids on the shoots. This assumption was later tested. The NBD-based relationship was chosen because of the close relationship between NBD and Taylor’s power law (Binns 1986). Estimated \( S^2 \) was described as a function of \( m \) (Taylor 1961). With this relationship, \( k \) of the NBD can be
calculated as \[m^2 / (am^k - m)\]. The incidence is then one minus the zero term of the NBD (Wilson & Room 1983, Nyrop et al. 1989):

\[P_1 = 1 - 1/[(1 + m/k)^k] \quad (3)\]

Another \(P_1\) and \(m\) relationship was developed using the model proposed by Nachman (1984). Because this model does not use a theoretical probability distribution as a basis, it was fit to the data to check the assumed applicability of the NBD (Nyrop et al. 1989). In Nachman’s model, the proportion of sample units with no animal (\(P_0\)) is related to the mean density as:

\[P_0 = \exp(-\delta m^\gamma) \quad (4)\]

where \(\delta\) and \(\gamma\) are parameters of the model. To fit the model to the data, the data were calculated based on the sampling dates and locations. The model is linearized with the mean density regressed on \(P_0\) (Nyrop et al. 1989) as:

\[\ln(m) = A + B \ln[-\ln(P_0)] \quad (5)\]

To test the applicability of the negative binomial distribution, the proportion of sample units with no psyllids (\(P_0\)) was calculated for different means using the two incidence and mean relationships and compared by chi square test.

Estimation of Common \(k\) for the NBD

The estimates of the dispersion parameter \(k\) for the NBD, computed as \(m^2 / (S^2 - m)\), were linearly regressed on \(m\),

\[k = c + dm \quad (6)\]

to test for the existence of a common \(k\) \((k_c)\) for each of the data sets (Southwood 1978, Feng & Nowierski 1992). A \(d\) value significantly > 0 indicates the dependence of \(k\) on mean density. The variance and mean within each area where the variance exceeded the mean were used to estimate \(k_c\) for a negative binomial distribution (Fleischer et al. 1991). Estimates of \(k_c\) were made using Elliot’s (1977) techniques, which estimates \(k_c\) by regressing \(y' = (S^2 - m)\) on \(x' = (m^2 - S^2) / n\), and \(k_c\) was defined by \(k_c = 1 / \text{slope}\). An index for spatial aggregation of arthropod populations, \(1 / k_c\), which is equal to \(m^b / m - 1\) (Southwood 1978) and is the same as Cassie’s index \(C\) (Cassie 1962), was also employed to evaluate the dispersion patterns.

The general linear model procedure (GLM) of SAS (SAS Institute 1988) was used to estimate the linear regression. Student’s t tests were used to determine if the slopes (\(b\)) of the regression lines were significantly > 1.0 (equations 1, 2) or 0 (equation 6) (Sokal & Rohlf 1981).

Sampling Plans

We determined the sample sizes for fixed levels of precision by substituting Taylor’s variance-mean relationship into the usual expression for the standard error of the mean and rearranging:

\[n = am^{b-2} / D^2 \quad (7)\]
where \( n \) is the sample size and \( D \) is the required level of precision expressed as a proportion of the mean, and \( a \) and \( b \) are the coefficients from Taylor’s power law (Pena & Duncan 1992, Walker & Allsopp 1993). We used two values of \( D \), 0.10 and 0.25; the latter allows detection of doubling or halving of sample means (Southwood 1978), whereas the former would be useful in detecting smaller changes in ecological studies (Walker & Allsopp 1993).

The number of samples after which sampling can be terminated (\( T_n \)) for a constant precision, \( D \), of the mean \( [D = (S^2 / n)^{1/2} / m] \), was determined using the equation derived by Green (1970):

\[
\log T_n = \log \left( \frac{D^2}{a} \right) + \frac{(b - 1)}{b - 2} \log n
\]  

(8)

where \( T_n \) is the stop line for sample size \( n \), \( a \) and \( b \) are from Taylor’s power law, and \( D \) is defined as above.

RESULTS AND DISCUSSION

The complete data set consisted of 235 psyllid samples from ten locations covering the period of October 1998 through March 1999. The mean density of \( D. \) citri in samples ranged from 0.1 to 8.5 adults per shoot. The highest number of psyllid adults on a shoot was 43. These 235 psyllid data sets were used for dispersion analysis.

Variance-Mean Relationships

The results of Iwao’s regression of \( m^* \) on \( m \) and Taylor’s power law analysis are listed in Table 1. Iwao’s patchiness regression described well the relationship between mean crowding and density for \( D. \) citri (Table 1, Fig. 1). The constant \( \alpha \) indicates the tendency to crowding (+ ve) or repulsion (- ve) defined as the ‘Index of Basic Contagion’ by Iwao (1970). For \( D. \) citri, the value of \( \alpha \) was < 0 \( (t = -2.546, df = 233, P < 0.05) \), indicating that for adults the basic component of the population is a single individual. Estimate of \( \beta \), the density contagiousness coefficient, was significantly > 1 \( (t = 11.27, df = 233, P < 0.001) \).

Taylor’s power law provided a highly significant relationship between variance \( (S^2) \) and mean density (Table 1, Fig. 2). Taylor’s intercept, \( \ln (a) \), was significantly > 0 \( (t = 11.57, df = 233, P < 0.001; \) Table 1). Estimate of \( b \) was significantly > 1 \( (t = 9.26, df = 233, P < 0.001) \).

<p>| Table 1. Parameter estimations of four linear models for ( D. ) citri on orange jessamine. |
|--------------------------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Model</th>
<th>Slope ± SEM(^1)</th>
<th>Intercept ± SEM(^2)</th>
<th>N</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taylor’s</td>
<td>1.2971 ± 0.03***</td>
<td>0.3407 ± 0.03***</td>
<td>235</td>
<td>0.8753</td>
</tr>
<tr>
<td>Iwao’s</td>
<td>1.6979 ± 0.06***</td>
<td>-0.3217 ± 0.12*</td>
<td>235</td>
<td>0.7637</td>
</tr>
<tr>
<td>Nachman’s</td>
<td>1.2444 ± 0.06***</td>
<td>0.2277 ± 0.04***</td>
<td>47</td>
<td>0.9189</td>
</tr>
<tr>
<td>Equation 6</td>
<td>0.7489 ± 0.48</td>
<td>1.4216 ± 0.99</td>
<td>235</td>
<td>0.0102</td>
</tr>
</tbody>
</table>

\(^1\)Table entries are significant at level of \( P < 0.05 \) (*) or 0.0001 (***)) for \( H_0: \alpha = 1 \) for Taylor’s and Iwao’s model \( (t = \text{slope-1/SE}_{\text{slope}}, df = N-1) \), and \( H_0: \alpha = 0 \) for Nachman’s model and equation 6 \( (t = \text{Slope/SE}_{\text{slope}}, df = N-1) \).

\(^2\)Table entries are significant at level of \( P < 0.05 \) (*) or 0.0001 (***)) for \( H_0: \alpha = 0 \).
Taylor's power law generally fit the data better, yielding higher value of $r^2$ (0.8753) than Iwao's model (0.7637). The aggregation indices (slopes, $b$ and $\beta$) of Taylor's power law and Iwao's patchiness regression were all significantly $> 1$ ($P < 0.05$), indicating an aggregated dispersion distribution of *D. citri* on orange jessamine (Table 1). The causes of aggregation could be attributed to either active aggregation on the part of this psyllid (such as behavior and reproductive biology), or to some heterogeneity of the environment (such as microclimate, preferred part of plant, and natural enemies) (Southwood 1978). The similar observations were also reported by Van den Berg et al. (1991) for the citrus psylla (*Trioza erytreae* Del Guercio), and Tret’yakov (1984) for the apple psylla (*Cacopsylla mali* Schmidtb). Van den Berg et al. (1991) reported that higher numbers of citrus psylla adults were apparently related to the prevailing wind direction. Catling (1970) stated the population fluctuations of psyllids were closely correlated with flushing rhythm and flush quality because eggs are laid exclusively on young flush points and nymphs develop on immature leaves. Although no data on *D. citri* is currently available for direct comparison, however, the observed values for $\beta$ and $b$ are similar to those for many moderately aggregated insects (Taylor 1961, 1971). Comparing to other aggregated Homopterans, the values of $\beta$ and $b$ for *D. citri* were lower than those for the citricola scale *Coccus pseudomag-.*
**Estimation of Incidence**

Nachman’s model gave an excellent fit to the relationship between the proportion of shoots without psyllids ($P_0$) and mean density ($m$) of *D. citri* (Table 1, Fig. 3). Using the parameter estimates (Table 1), the proportion of shoots with or without psyllids can be estimated from mean density with equation 5. For example, samples with mean densities of 0.5 and 2 psyllids per shoot correspond to $\approx 38$ and $\approx 77\%$ infested shoots, respectively. This model could be used for grove managers who wish to develop the decision-making plans when economic threshold of *D. citri* becomes available in the future.

The values of $P_0$ for various means calculated according to Nachman’s model and the NBD model are presented in Figure 4. Values of $P_0$ calculated with the NBD model
were greater than those calculated by using Nachman’s model at lower population density. On the contrary, at higher population density level, the values of $P_0$ become smaller. Generally, this deviation was small ($<0.01$) and the chi square test indicated that the two models were similar and interchangeable ($P > 0.05$).

Estimation of Common $k$ for the NBD

Figure 5 gives an overall picture on the relationship between $k$ and the mean number of psyllids from the 177 samples where the variance exceeded the mean. Regression of $k$ on the mean density per shoot using all data was not significant ($F = 2.388$, $r^2 = 0.0102$, $P = 0.1236$). Moreover, the slope of regression ($d$) was not significantly greater than 0 ($t = 1.545$, $df = 233$, $P = 0.1236$). Independence of $k$ with the mean density suggests the existence of common $k$ for the NBD of the psyllid populations. The estimates of a common $k$ was 1.2741 using Elliot’s (1977) method.

Southwood (1978) states that changes in the density of an insect often lead to changes in the distribution. However, we did not detect apparent density-dependent distribution changes in the psyllid population (Table 1, Fig. 5). Similar results were reported by Feng & Nowierski (1992) for the summer populations of Russian wheat
Fig. 4. Proportion of shoots without *D. citri* predicted from the density per shoot using NDB (dotted line) and Nachman’s models (solid line).

The relationships between mean psyllid density and required sample size for fixed precision levels of 10 and 25% are shown in Figure 6. The stop line of the fixed-precision-level of 25% of the mean for sequential sampling is presented in Figure 7. The stop line of the fixed-precision-level of 10% of the mean was not presented because of the requirement for extremely large samples from the field. Based on computer simulation, the performance of the sequential sampling procedures improved with in-
creasing psyllid density. Also because the variance-mean regression provided a good description of the data (Table 1), regression variability had only a minor effect at very low mean density.

Figures 6 and 7 show that these sampling plans required quite large sample sizes to obtain relatively precise density estimates. Although such precision in density estimates may be required for research purpose, it will probably not be of practical use in commercial citrus production.

A person sampling *D. citri* could use Figure 7 by plotting $T_n$ (accumulated adults) and $n$ (number of shoots sampled) after each sample was taken. When the plot falls above the line, sampling is stopped and the mean density ($m$) is within 25% of $m = \frac{T_n}{n}$. This sequential-count plan permits researchers and pest managers to describe the mean density more accurately than before. It may lead to a better determination of the economic threshold in the future.

Sampling small arthropods is operationally difficult and often time consuming. In this paper, we have developed a sequential sampling plan based on counts of psyllids which will be useful to anyone requiring accurate decisions based on mean numbers. As a way of easing this burden presence-absence, (binomial) sampling has been used in place of complete counts for estimating or classifying densities of

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**Fig. 5.** Scatter plots of $k$ for the NBD over mean ($m$) for *D. citri* populations on orange jessamine.
these organisms (Nyrop et al. 1989). Binomial sampling is appealing because it is often easier to determine whether one or more animals reside on a sample unit than it is to make a complete count. It is usually faster and therefore less costly on a per-sample-unit basis. In addition, there are organisms, such as psyllids, for which binomial sampling is the only feasible field-sampling method. Sequential sampling plans can result in saving up to 75% of the time compared with fixed sample size procedures having comparable error rates (Harcourt 1966). When sampling is used for decision making, it often suffices to classify a population density as opposed to obtaining an estimate. Many sequential sampling programs are based on this premise. However, due to the new pest status and vector ability of *D. citri*, further research on the biology, ecology, disease transmission and integrated management are needed.

**ACKNOWLEDGMENTS**

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Fig. 7. Sequential count plan for *D. citri* populations on orange jessamine, showing the stop line at a fixed precision level of 25%.

**REFERENCES CITED**


Davidson et al.: Whitefly Feeding System


IMPROVED ARTIFICIAL FEEDING SYSTEM FOR
REARING THE WHITEFLY BEMISIA ARGENTIFOLII
(HOMOPTERA: ALEYRODIDAE)

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ABSTRACT

The artificial rearing system for Bemisia argentifolii Bellows and Perring has been improved by the selection of an autoclavable, reusable membrane, reduction of the sucrose concentration, choice of yeast extract, use of an antifungal agent in the petri dish chamber, choice of surface sterilization agent, egg storage, and maintenance of high humidity in the chamber during the entire nymphal development. We can now produce small numbers of adult whiteflies of both sexes on these chambers, confirm-
ing the utility of these improvements. We also reared two *Bemisia tabaci* A-strain to adults and several *Trialeurodes vaporariorum* nymphs to the 3rd and 4th instar on the improved feeding system.

**Key Words:** artificial diet, *Bemisia tabaci* A-strain, *Trialeurodes vaporariorum*

**Resumen**

El sistema de cría artificial para *Bemisia argentifolii* Bellows y Perring ha sido mejorado tras la selección de una membrana reutilizable y autoclavable, reducción en concentración de sacarosa, selección de extracto de levadura, uso de un agente antihongo en la cámara de placa petri, selección de agente de esterilización de superficie, almacenaje de huevos, y mantenimiento de alta humedad en la cámara durante el completo desarrollo ninfae. Ahora podemos producir números pequeños de moscas blancas adultas de ambos sexos en estas cámaras, confirmando la utilidad de estas mejorías. También criamos dos *Bemisia tabaci* linaje-A con adultos de varias ninfas *Trialeurode vaporariorum* al 3ro y 4to instar bajo el nuevo sistema de alimentación.

We previously reported an artificial system for rearing the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring (= *B. tabaci* B-biotype), to the 3rd instar (Jancovich et al. 1997). This system used a polycarbonate chamber, a Parafilm® membrane, and a filter-sterialized diet consisting of 30% sucrose and 5% yeast extract in distilled water. Although this system has proven useful for gut function studies (E. W. D. & R. Rosell, unpublished) and for assays of potential ingested toxins (Jancovich et al. 1997; E. W. D., unpublished), it requires very careful, aseptic techniques and rarely permits development beyond the 4th instar. Assays using this system often failed due to fungal contamination. Our goals in improving the feeder system and diet were to increase egg hatch, to reduce fungal contamination, and to bring a high proportion of *B. argentifolii* nymphs to 3rd or 4th instar within 14 days, in order to use these nymphs as hosts for parasitic wasps (Davidson & Jones 1999). We also wished to investigate the use of the system for the culture of other whitefly species. We report here improvements in this system that accomplish some of these goals and also enable successful development of a proportion of *B. argentifolii* nymphs to adults.

**MATERIALS AND METHODS**

**Feeder Assembly**

Several commercially available, autoclavable filtration membranes were tested to replace the Parafilm® membrane, including MSI® TefSep (Micron Separations-Osmonics Inc., Westboro, MA), Durapore® SVLP (Millipore Inc., Bedford, MA) and Millipore® TCTP membranes with pore sizes of 0.2-10 μm. TefSep PTFE autoclavable filtration membranes, 1.0 μm, 45 mm diameter (MSI-Osmonics) were found to be most acceptable and were used in all further experiments.

The feeder assembly, which consists of a bottom chamber, membrane, and upper plate held together by binder clips (Jancovich et al. 1997), was fully assembled and autoclaved (120°C for 20 min) before filling with the diet solution. Stainless steel 20 mm electrophoresis binder clips (“Father Time Clips”, Research Products International, Mt. Prospect, IL) were used.
To inhibit fungal contamination of feeders, the interior of sterile glass or disposable plastic petri dishes was rinsed with a 0.1% solution of miconazole (Sigma, St. Louis, MO) in 95% ethanol which was then permitted to evaporate, leaving a residue of antifungal agent. High humidity was maintained by adding a damp filter paper triangle to each petri dish and by placing a sterile slide, held in place with a bent hair-clip, over the diet chamber after eggs were deposited. Sterile, filled feeders were held individually in sterile petri dishes. Assembly took place under a laminar-flow hood with a germicidal ultraviolet lamp, and all equipment was exposed to the ultraviolet light for approximately 30 min before assembly.

Egg Harvest and Treatment

*Bemisia argentifolii* eggs were harvested from cotton, collards or melon. Leaves were chosen that contained primarily darkened eggs, that were close to hatching. Leaves were dipped sequentially into a detergent solution, distilled water, a 10% household chlorine bleach solution (final concentration 0.5% sodium hypochlorite) for 2-3 min, to loosen the egg pedicel, and distilled water. In some experiments, after the 10% bleach step, leaves were dipped in a 3% solution of sodium thiosulfate to remove residual chlorine, followed by a rinse with distilled water. Eggs were removed using a WaterPik® dental device, filtered through 3 layers of organdy cloth (6-8 fibers/mm) and collected on coffee filters. Eggs were then transferred to sterile 50-ml plastic centrifuge tubes and surface sterilized using 70% ethanol (approximately 1 min) followed by either a 10% bleach solution or a 10% Roccal II® solution (final concentration 1% alkyl dimethyl butyl ammonium chloride, Sterling Drug) (2-3 min). In some experiments, a rinse of 3% sodium thiosulfate solution followed the bleach step, to neutralize residual chlorine. As Roccal is no longer commercially available, the product that has replaced Roccal, Lysol® IC (final concentration 1.1% alkyl dimethyl butyl ammonium chloride, 0.12% didecyl dimethyl ammonium chloride), was also tested, as well as 3% hydrogen peroxide. Eggs were rinsed in sterile distilled water and pipetted onto feeder membranes, then excess water was removed. Assembled feeders were held in sealed plastic boxes with an open water container, on the laboratory bench at 24-25°C and a photoperiod of 10:14 (L:D).

*Bemisia tabaci* Gennadius (A-strain) eggs were obtained from a colony maintained at the University of California, Riverside. *Trialeurodes vaporariorum* Westwood eggs were obtained from the USDA-ARS Biological Control of Insects Research Unit, Weslaco, TX. Eggs were harvested from leaves and surface sterilized using Roccal as described above.

Storage of Eggs

One cohort of *B. argentifolii* eggs washed from melon leaves was divided into 4 lots and held in water at 4°C for 0, 1, 4, or 7 days. The eggs were then surface sterilized using Roccal and placed on feeders.

Diet Modifications

Yeast extract lots manufactured by Difco (Detroit, MI) and BBL (Becton Dickinson Co., Cockeysville, MD) were compared at 5% concentration. Sucrose concentration was compared at 30% and 15%. Dietary pH was adjusted to pH 5 to 8. The antifungal agents methyl paraben and potassium sorbate (Sigma) were added to the standard diet at concentrations shown to inhibit growth in whitefly diet of fungi isolated from whitefly feeders.
Eggs were counted at day 1 after setup and nymphs were counted by instar at days 5 and 14 (+/- 1 day). Feeders were then held until day 28 to observe adult emergence. Egg sterilization procedures and modifications to the diet were evaluated based upon hatch percentage and percentage of nymphs that had achieved 3rd or 4th instar (including the “red eye” stage) by day 14, based upon total nymphs at day 5. All diet modifications were evaluated in comparison to cohorts reared on standard diet (15% or 30% sucrose, as noted, plus 5% yeast extract). Means and standard deviations were calculated using Excel® 97 SR-2 (Microsoft), and ANOVA followed by Tukey’s separation of means was performed using Systat® version 8.0. Means were compared within but not between experiments.

RESULTS AND DISCUSSION

The greatest improvement in the rearing technique has resulted from the adoption of an autoclavable, commercially available membrane to replace Parafilm®. TefSep filter membranes are composed of Teflon® (PTFE), are very thin (175 μm), and are hydrophobic. Higher hatch percentages were obtained on PTFE filter membranes than on Parafilm (data not shown). The nymphs attached and fed readily in the oval spaces between the plastic screen fibers that support the membrane. These feeding sites are equally spaced across the membrane surface and occur in parallel rows, which facilitated counting of eggs and nymphs. In preliminary trials, nymphs were unable to feed on membranes with pore sizes of 0.2 or 0.5 μm. The requirement for pores above 0.5 μm is probably related to the cross-section diameter of the stylet bundle. In adult B. argentifolii, the stylet bundle is approximately 0.3 μm at the tip and 1.8 μm in cross section closer to the head (Rosell et al. 1995). These results suggest that the nymphs insert stylets through the pores in the filter membrane, but do not puncture the membrane itself. Membranes with pore sizes of 2 μm or larger were unacceptable due to leakage. The MSI TefSep 1 μm membranes are now used in all experiments, and the ability to autoclave the entire feeder system has been a major improvement in reducing microbial contamination. These membranes are, however, significantly more expensive than Parafilm (about $2.00 each).

Ten percent bleach was previously used both to loosen egg pedicels and to surface-sterilize eggs washed from leaves (Jancovich et al. 1997). Rinses of leaves with sodium thiosulfate (Na₂S₂O₃) to neutralize chlorine led to decreased egg hatch but did not affect development (Table 1A). Rinses of bleach-treated eggs with sodium thiosulfate did not markedly improve hatch or development to 3rd or 4th instars (Table 1A). Substitution of the antibacterial-antifungal agent, Roccal, for bleach during egg surface-sterilization resulted in a higher percentage of nymphs that reached the 3rd or 4th instar by day 14 (Table 1B). Unfortunately, Roccal is no longer manufactured. The product that is sold as a replacement, Lysol IC, contains didecyl dimethyl ammonium chloride in addition to the active ingredient in Roccal, alkyl dimethyl butyl ammonium chloride. Lysol IC reduced egg hatch and development to 3rd or 4th instars when compared with Roccal (Table 1C). Hydrogen peroxide, shown to be useful in surface sterilization of leafhopper eggs (Wayandande & Fletcher 1998), led to clumping of eggs on the membrane and reduced egg hatch, but development of nymphs that became established was equivalent to that in Roccal and Clorox treatments (Table 1C).

High humidity appears to be essential to development of B. argentifolii nymphs on the artificial diet system. Placement of sterile slides over diet chambers to maintain high humidity within the chambers significantly improved development of nymphs to 3rd or 4th instars (Table 1D). Leaf osmotic potential was similarly found to influence hatch and survival of greenhouse whitefly eggs Castañé & Savé 1993). However, all water must be removed from the eggs after deposition on the membrane, as even a small amount of liquid water inhibits egg hatch.
Eggs washed from leaves, but not surface-sterilized, can be stored in water at 4°C for at least one day with no reduction in egg hatch (Fig. 1A) or development to 3rd or 4th instars (Fig. 1B). Leaves bearing eggs can also be stored at least one day under refrigeration (data not shown). These procedures facilitate setup of experiments, as egg harvest can be done at least one day in advance.

Difco and BBL yeast extract produced similar results (Table 2A, B). One lot of yeast extract from Sigma did not produce any 3rd or 4th instar nymphs by day 14 (data not shown). Difco yeast extract that had been stored at room temperature for more than 3 years was significantly less effective in maintaining whitefly growth than fresh yeast extract (Table 2A, B). Yeast extract is now purchased in small quantities and stored in a dessicator at room temperature or at –20°C.

Increasing the concentration of yeast extract in the diet from 5% to 7.5% resulted in lower egg hatch, but did not affect development to 3rd or 4th instars (Table 2B). The diet used in our original study (Jancovich et al. 1997) contained 30% sucrose, based upon analysis of phloem sap. Reduction of sucrose concentration to 15%, however, had slight or no adverse effect on nymphal development and resulted in greater egg hatch (Table 2C, D). Egg hatch was greater on TefSep membranes than on Parafilm membranes (data not shown). These results taken together suggest that water from the diet may evaporate slowly through the porous membranes, contributing to higher humidity around the eggs.

### Table 1. Effects of egg sterilization procedures, and addition of sterile slides to growth chambers on hatch percentage and nymphal development. Percentage of nymphs achieving 3rd or 4th instar by day 14 was based upon total nymphs at day 5. Standard diet (5% difco yeast extract in 15% sucrose, pH 7.0), 24-25°C (unless otherwise noted), photoperiod 10:14 (L:D). Means are compared within each experiment. Means followed by the same letter are not significantly different (ANOVA, α = 0.05).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean % egg hatch, day 5 (+/- S.D.)</th>
<th>Mean % 3-4 instars, day 14 (+/- S.D.)</th>
<th>Total nymphs, day 5 (total feeders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. sodium thiosulfate neutralization of chlorine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na$_2$S$_2$O$_3$, rinse</td>
<td>51.0 (2.6) a</td>
<td>27.6 (6.5) a</td>
<td>1349 (7)</td>
</tr>
<tr>
<td>No rinse</td>
<td>61.8 (5.3) b</td>
<td>26.4 (3.9) a</td>
<td>1084 (7)</td>
</tr>
<tr>
<td>B. egg sterilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clorox</td>
<td>61.0 (8.1) a</td>
<td>36.5 (8.1) a</td>
<td>771 (6)</td>
</tr>
<tr>
<td>Clorox + Na$_2$S$_2$O$_3$</td>
<td>56.9 (2.5) a</td>
<td>39.5 (5.2) a,b</td>
<td>633 (6)</td>
</tr>
<tr>
<td>Roccal</td>
<td>59.5 (5.2) a</td>
<td>49.0 (7.5) b</td>
<td>596 (6)</td>
</tr>
<tr>
<td>C. egg sterilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>24.3 (5.0) a</td>
<td>34.1 (3.3) a</td>
<td>333 (6)</td>
</tr>
<tr>
<td>Lysol IC</td>
<td>42.4 (5.7) b</td>
<td>27.1 (3.0) b</td>
<td>575 (6)</td>
</tr>
<tr>
<td>Roccal</td>
<td>54.7 (4.1) c</td>
<td>34.2 (4.8) a</td>
<td>924 (6)</td>
</tr>
<tr>
<td>D. addition of sterile slides to chambers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slides</td>
<td>54.2 (4.5) a</td>
<td>42.3 (5.6) a</td>
<td>1175 (7)</td>
</tr>
<tr>
<td>No slides</td>
<td>55.6 (5.1) a</td>
<td>33.6 (2.8) b</td>
<td>1327 (7)</td>
</tr>
</tbody>
</table>
Diet pH did not significantly affect egg hatch. Developmental response was variable, but pH 6.5 and 8.0 diets supported the highest percentage of nymphs to the 3rd and 4th instar (Table 2E). A pH of 5.0 failed to support development to the 3rd or 4th instar (data not shown). Salvucci et al. (1997) found that in adult whiteflies the optimum pH for ingestion of a 20% sucrose diet was between 6.5 and 7.5, in a tested range of 4.5 to 8.5.

The antifungal agent methyl paraben (p-hydroxybenzoic acid methyl ester), +/- potassium sorbate, added to the diet at concentrations effective in inhibiting fungi, was strongly inhibitory to both hatching and nymphal development (Table 2F). These agents were apparently toxic to eggs and may have acted as antibiotics against the symbiotic microorganisms that are necessary for whitefly development (Costa et al. 1997). The reduction of egg hatch due to addition of these compounds to the diet also implies that dietary components other than water may make contact with the eggs through the membranes. Eggs did not hatch when miconazole was used as an egg rinse (data not shown). However, miconazole residue in the petri dishes holding the feeders was beneficial in inhibiting fungal development in the petri dish chambers.

It is difficult to compare survivorship on artificial diet with that observed on plants, since predation, parasitism, disease, plant quality and weather conditions are not factors in mortality of artificially reared nymphs. On plants, survival from eggs to adults can range from approximately 40% to over 80% (Horowitz et al. 1984, Powell & Bellows 1992, Wagner 1995). Although production of adult whiteflies on the artificial diet was not the goal of this study, we observed emergence of adult whiteflies of both sexes by 28 days equivalent to 0.5% to 2% of the total nymphs counted at day 5. The highest percentages of *B. argentifolii* adults were produced when Roccal was used to sterilize eggs, slides were added to chambers, and sucrose was reduced to 15%.

Fig. 1. Effect of storage of eggs in water for 0, 1, 4 or 7 days at 4°C, on A) hatch rate and B) development. Percentage of nymphs achieving 3rd or 4th instar at day 14 was based upon total nymphs at day 5. Standard diet (5% Difco yeast extract in 15% sucrose, pH 7.0), 24-25°C, photoperiod 10:14 (L:D). Bars with the same letter are not significantly different (ANOVA, α= 0.05).
# Table 2. Dietary Alterations.

“New” = stored less than 1 year; “old” = stored more than 3 years. Percentage of nymphs achieving 3rd or 4th instar at day 14 was based upon total nymphs at day 5. Ph 7.0 unless otherwise noted, 24-25°C, photoperiod 10:14 (L:D) or *28°C 14:10 (L:D). Means are compared within experiments. Means followed by the same letter are not significantly different (ANOVA, $\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Yeast extract manufacturer, %, age; sucrose %</th>
<th>Mean % egg hatch, day 5 (+/- S.D.)</th>
<th>Mean % 3-4 instars, day 14 (+/- S.D.)</th>
<th>Total nymphs, day 5 (total feeders)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. age of yeast extract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco, 5, old; 30</td>
<td>59.5 (5.4) a</td>
<td>28.3 (5.9) a</td>
<td>1327 (6)</td>
</tr>
<tr>
<td>Difco, 5, new; 30</td>
<td>61.0 (9.0) a</td>
<td>43.1 (4.9) b</td>
<td>1098 (6)</td>
</tr>
<tr>
<td>BBL, 5, new; 30</td>
<td>58.7 (4.8) a</td>
<td>39.5 (8.0) b</td>
<td>1208 (6)</td>
</tr>
<tr>
<td><strong>B. percent yeast extract</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco, 5, new; 30</td>
<td>44.1 (4.9) a</td>
<td>58.0 (9.6) a</td>
<td>388 (6)</td>
</tr>
<tr>
<td>Difco, 7.5, new; 30</td>
<td>35.4 (6.3) b</td>
<td>46.8 (6.0) a</td>
<td>208 (5)</td>
</tr>
<tr>
<td><strong>C. percent sucrose, experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco, 5, new; 30</td>
<td>27.0 (5.2) a</td>
<td>47.0 (6.3) a</td>
<td>388 (8)</td>
</tr>
<tr>
<td>Difco, 5, new; 15</td>
<td>57.6 (7.0) b</td>
<td>32.7 (6.0) b</td>
<td>1072 (10)</td>
</tr>
<tr>
<td><strong>D. percent sucrose, experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*BBL, 5, new; 30</td>
<td>40.1 (4.1) a</td>
<td>37.2 (9.1) a</td>
<td>701 (6)</td>
</tr>
<tr>
<td>*BBL, 5, new; 15</td>
<td>60.6 (12.1) b</td>
<td>32.6 (6.0) a</td>
<td>1184 (7)</td>
</tr>
<tr>
<td><strong>E. diet pH</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*BBL, 5, new; 15; pH 6.5</td>
<td>69.2 (11.8) a</td>
<td>33.8 (3.6) a,b</td>
<td>964 (7)</td>
</tr>
<tr>
<td>*BBL, 5, new; 15; pH 7.0</td>
<td>66.1 (6.6) a</td>
<td>27.0 (2.6) a</td>
<td>1157 (7)</td>
</tr>
<tr>
<td>*BBL, 5, new; 15; pH 7.5</td>
<td>57.0 (8.7) a</td>
<td>25.0 (7.0) a</td>
<td>859 (7)</td>
</tr>
<tr>
<td>*BBL, 5, new; 15; pH 8.0</td>
<td>56.0 (11.1) a</td>
<td>40.2 (11.9) b</td>
<td>772 (7)</td>
</tr>
</tbody>
</table>
TABLE 2. (CONTINUED) DIETARY ALTERATIONS. “NEW” = STORED LESS THAN 1 YEAR; “OLD” = STORED MORE THAN 3 YEARS. PERCENTAGE OF NYMPHS ACHIEVING 3RD OR 4TH INSTAR AT DAY 14 WAS BASED UPON TOTAL NYMPHS AT DAY 5. PH 7.0 UNLESS OTHERWISE NOTED, 24-25°C, PHOTOPERIOD 10:14 (L:D) OR *28°C 14:10 (L:D). MEANS ARE COMPARED WITHIN EXPERIMENTS. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (ANOVA, \( \alpha = 0.05 \)).

<table>
<thead>
<tr>
<th>Yeast extract manufacturer, %, age; sucrose %</th>
<th>Mean % egg hatch, day 5 (+/- S.D.)</th>
<th>Mean % 3-4 instars, day 14 (+/- S.D.)</th>
<th>Total nymphs, day 5 (total feeders)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. antifungal agents</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Difco, 5, new; 15</td>
<td>45.5 (4.0) a</td>
<td>39.7 (1.8) a</td>
<td>1367 (6)</td>
</tr>
<tr>
<td>Difco, 5, new; 15; 0.1% methyl paraben</td>
<td>11.9 (2.9) b</td>
<td>27.9 (6.1) b</td>
<td>300 (5)</td>
</tr>
<tr>
<td>Difco, 5, new; 15; 0.1% methyl paraben + 0.1% potassium sorbate</td>
<td>11.0 (2.2) b</td>
<td>11.4 (6.6) c</td>
<td>347 (6)</td>
</tr>
</tbody>
</table>
Twenty-six percent of *Bemisia tabaci* (A-strain) developed to 3rd or 4th instar within 14 days and two adult whiteflies emerged after 28 days on standard diet (5% Difco yeast extract, 15% sucrose) (Table 3). Although percent hatch and development for the A-strain whitefly was lower than that normally observed with *B. argentifolii*, these results suggest that the feeder system is adequate for the development of *B. tabaci* A-strain whiteflies, and may prove useful in investigating the physiological differences between these closely-related species. The greenhouse whitefly, *T. vaporariorum*, hatched and began to feed on the artificial diet system, but only a small number developed to the 3rd or 4th instar by 14 days (Table 3). Nonetheless, development of even a few *T. vaporariorum* nymphs on the *Bemisia* artificial diet suggests that this diet could provide the basis for a diet for the greenhouse whitefly. Short-term bioassay of ingested compounds against greenhouse whitefly nymphs is possible using this assay system, similar to the plant tissue culture system used by Melé et al. (1992).

ACKNOWLEDGMENTS

We are grateful to C. LaVesque, T. Perring and W. Jones for providing eggs of *B. tabaci* A-strain and *T. vaporariorum*, and to L. Lee for technical assistance. This research was supported by USDA CSREES 9702182 and a Cooperative Agreement with the USDA-ARS Biological Control of Insects Research Unit, Weslaco, TX.

REFERENCES CITED


COMPARING EFFECTS OF THREE ACARICIDES ON VARROA JACOBSONI (ACARI: VARROIDAE) AND APIS MELLIFERA (HYMENOPTERA: APIDAE) USING TWO APPLICATION TECHNIQUES

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4Colegio de Postgraduados, IFIT-Campo Córdoba, and El Colegio de la Frontera Sur. Apdo. postal 36, 30700 Tapachula, Chis. MEXICO.

ABSTRACT

Two bioassays were administered to determine the dose-lethality response of Varroa jacobsoni and the honey bee, Apis mellifera L., to amitraz, flumethrin and fluvalinate. The first bioassay method was spraying by means of the Potter-Bourgerjon’s tower. The results are expressed in mean lethal concentrations (LC50).
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ABSTRACT

Two bioassays were administered to determine the dose-lethality response of Varroa jacobsoni Oudemans and the honey bee, Apis mellifera L., to amitraz, flumethrin and fluvalinate. The first bioassay method was spraying by means of the Potter-Bourgerjon’s tower. The results are expressed in mean lethal concentrations (LC₅₀).
The second method was topical application by means of microsyringe and manual applicator. The results are expressed in mean lethal doses (LD\textsubscript{50}). Both LC\textsubscript{50} and LD\textsubscript{50} values were considerably higher in honey bees than in varroa mites, showing that a wide margin of safety exists between effective doses against mites and harmful doses for honey bees. Both methods gave similar confidence intervals; they showed a comparable sensitivity to changes in dose or concentration of pesticides.

**Key Words**: amitraz, bioassays, flumethrin, fluvalinate, honey bees, susceptibility, toxicity, varroa mites

**RESUMEN**

Se probaron dos métodos de bioensayos toxicológicos para determinar la respuesta dosis-letalidad de amitraz, flumetrina y fluvalinato sobre Varroa jacobsoni Oudemans y Apis mellifera L. El primero fue aspersión por medio de la torre de Potter-Burgerjon; sus resultados se expresan en concentraciones letales medias (CL\textsubscript{50}). El segundo fue aplicación tópica por medio de microjeringa y aplicador manual; sus resultados se expresan en dosis letales medias (DL\textsubscript{50}). Las DL\textsubscript{50} y CL\textsubscript{50} de todos los productos fueron considerablemente más altas en abejas que en ácaros, lo cual muestra que existe un amplio margen de seguridad entre dosis que son lo suficientemente tóxicas sobre los ácaros, sin llegar a ser peligrosas para las abejas. Ambos métodos de bioensayo dieron intervalos de confianza comparables y presentaron similar sensibilidad en la respuesta a los cambios de dosis y concentración aplicados.

Beekeepers in many parts of the world face severe problems because of recent introductions of a parasitic mite, Varroa jacobsoni Oudemans (Acari: Varroidae), known as varroa. Originally from tropical Asia and found on the Indian honey bee, Apis cerana Fabricius, this mite has shifted to its new host A. mellifera L. Owing to human activities, it has infested most of honey bee colonies around the world, causing severe losses.

Many control measures have been developed for varroa. Most include the use of chemicals. However, chemical control has the disadvantages of variable efficacy, increased costs, contamination of hives and hive products and the risk of target pest resistance. Varroa resistance to fluvalinate was documented for the first time in Italy (Lodesani et al. 1995) and soon in several European countries (Londzin & Sledzinski 1996, Moosbeckhofer & Trouiller 1996, Bruneau et al. 1997, Vandame et al. 1995). Elzen et al. (1999), by application of discriminating doses of fluvalinate, found indications that varroa mites from Florida and California were developing resistance to this acaricide.

Development of acaricide resistance by varroa is of concern. Chemical control necessarily involves contact of pesticides with bees and hives. When resistance occurs, doses should not be increased because of the risk of harming or killing bee hosts and increasing contamination in the hive environment and hive products (Lodesani et al. 1992). Toxicological bioassays can track changes in pesticide susceptibility of a population, by detecting changes in the calculated mean lethal concentrations or doses (LC\textsubscript{50} or LD\textsubscript{50}, respectively), compared to a maximum reference susceptibility or baseline (Georghiou 1963). Early detection of pesticide resistance is mandatory for developing a long-term strategy of chemical control, based on replacing ineffective pesticides. Bioassay methods must be sensitive to dose variations and easily repeatable, to allow comparison of lethal values (Lagunes-Tejeda & Villanueva-Jimenez 1994).

Topical application bioassays have been conducted on varroa by various researchers. Ritter & Roth (1986) determined mite susceptibility to Folbex VA (bromopropi-
late) and K79 (clorodimeformidrochloride); they found a positive correlation between lethal doses and number of previous treatments, suggesting early manifestations of resistance. Also by topical application, Abed & Ducos de Lahitte (1993) estimated LD<sub>50</sub>'s of amitraz and coumaphos.

A spraying method of application for toxicological bioassays has been proposed by Colin et al. (1994), who used the Potter-Burgerjon’s tower in testing lethality and behavioral effects of pesticides on varroa mites. This device sprays doses onto an area, simulating a field application. In this method, data are expressed in lethal concentrations (LC<sub>50</sub>) of the material surrounding the specimen; the exact quantity of pesticide contacting the specimen is unknown. Units are mg L<sup>-1</sup>, parts per million (ppm), g cm<sup>2</sup> or their equivalencies.

Study of varroa populations established in Mexico may provide useful information to other parts of the world. According to Otero-Colina & Santillan-Galicia (1996), these mites were first detected in Veracruz state in the Mexican Gulf Coast lowlands in 1992, although they probably were already present there since about 1989. Before their discovery and at least three years afterwards, they were seldom chemically treated. Thus, they have been almost free of selection pressure by pesticides for at least six years and supposedly show maximal levels of susceptibility to most acaricides.

The present study had the following objectives: a) to estimate LC<sub>50</sub> and LD<sub>50</sub> on <i>V. jacobsoni</i> and <i>A. mellifera</i> to the acaricides amitraz, flumethrin and fluvalinate, and b) to compare two toxicological bioassay methods for determining susceptibility to these pesticides of varroa mites and honey bees.

**MATERIALS AND METHODS**

All varroa specimens were obtained from a commercial apiary that had received a single treatment of fluvalinate (Apistan®, Novartis) one year before. Adult female mites were collected manually, from CO₂ anesthetized worker bees or by uncapping parasitized worker pupae. Mites were kept at 25°C and 50% R. H. and put on pupae until they were used in the tests, up to 4 hours later. Worker bees were collected from combs of healthy (non-parasitized or with low levels of infestation) European colonies (<i>Apis mellifera</i> ligustica Spinola). In order to avoid recently emerged nurse bees and to use bees of similar age, collections were made from combs without open brood (Felton et al. 1986). Adult bees were transported to the laboratory and used in bioassays about two hours later.

All acaricides were used in commercial formulations; they were amitraz (Taktic®, 12.5%, liquid, Hoechst), flumethrin (Bayticol®, 3%, emulsifying concentrate, Bayer) and fluvalinate (Fluvalin®, emulsifying concentrate 25%, Ishara). Commercial formulations were preferred as they are easily available and because they are currently in use against varroa in many countries (Arculeo et al. 1989, Benitez-Reynoso 1998, Cardenal Galvan et al. 1989). In the spraying method, the solvent was water; in topical application, the solvent was acetone.

For each pesticide, preliminary bioassays were conducted to obtain maximal dose causing 0% mortality and minimal dose causing 100% mortality. Then, logarithmic intermediate doses were applied to obtain the LC<sub>50</sub> and LD<sub>50</sub>. Four to seven intermediate doses plus extreme values were used in each replication. All dilutions were prepared immediately before being used.

**Bioassays on Mites**

When the spraying method was carried out, a Potter-Burgerjon’s tower was calibrated for applying 1.7 mg cm<sup>-2</sup> (s.d. = 0.14) of acaricide solutions, by spraying 15 mL solution at a pressure of 0.703 kg cm<sup>2</sup>, then waiting one minute for sedimentation of
droplets. A solid cone nozzle (Cat. 1/4J-SS+SU1A-SS, Spraying Systems) was used. Groups of 14 varroa females were placed in a 14 cm diameter Petri dish containing a floor of filter paper; each group was treated by an acaricide, then transferred to another Petri dish (5 cm diameter), and incubated at 32 ± 2°C, 70 ± 10% RH. To feed the mites, two or three worker pupae one to three days old were placed in each Petri dish. Pupal age was determined by their light yellow thorax, according to Jay (1953). Mortality data were taken 24 hours after the treatment.

For topical application, groups of 14 varroa females were stuck ventral side up on a microscope slide with Scotch® double sided tape; 0.1 mL of pesticide solution was then applied ventrally to each mite using a microsyringe and a microapplicator. This contrasted with the method proposed by Ritter & Roth (1986), who applied 0.2 mL solution. The slides were placed in an incubator at 25 ± 2°C and 70 ± 10% R.H. (instead of 16°C and 98% R.H., by the same authors). Mortality was recorded 24 h later. A specimen was considered dead when it did not respond to tactile stimuli. All tests comprised four replications per dose on different days; a solvent-only control was included.

Bioassays on Honey Bees

To compare results, the same bioassay methods were used on bees, with some differences owing to size, flying ability and nutritional requirements as given below. All bees were anesthetized with a stream of CO2; in the spraying test, groups of 30 workers were confined in a galvanized iron cage (15 x 20 x 25 cm, 4 mm mesh) with a filter paper floor, then sprayed in the Potter-Burgerjong® tower. In the topical application test, every bee in a group of 30 received 1 mL solution dorsally on the thorax. In both tests, after exposure to chemicals, the groups of bees (replications) were confined in 1 L plastic cages; they were supplied with solid food (candy) and water, and incubated at 25 ± 2°C and 70 ± 10% R.H. Every bioassay had three replications.

Analysis of Results

Percentages of mortality were corrected by Abbot's (1925) formula when mortality was found in the control; when mortality of one bioassay exceeded 10% of bees and 15% of mites, the results were discarded. Values of LC50 and LD50 and their confidence intervals were estimated by Probit analysis. Relative toxicity of all products was estimated in varroa and in bees, by dividing experimental lethal values by the most toxic value. Toxicity of all products was also compared on mites vs. bees, by dividing LC50 and LD50 values.

Results of aspersion and topical methods are expressed in different units and their values are not comparable. However, an attempt was made to compare these methods taking the width of confidence intervals as a measure of precision and slopes as a measure of sensitivity, the last by means of Student’s t-test (Dittrich 1962). Ease of bioassay methods was also considered.

RESULTS

Susceptibility of varroa

Spraying, LC50 and confidence intervals are shown in Table 1. Previous studies of fluvalinate LC50 levels on varroa were conducted using a residual application technique, but these results are not comparable with those of the current work, because different bioassay methods were used. Milani (1995) placed varroa specimens on flu-
valinate-impregnated paraffin and determined a LC$_{50}$ of 20 mg L$^{-1}$ for a susceptible population from Udine, whereas a resistant population from Lombardy (both in Italy) showed a LC$_{50}$ higher than 200 mg L$^{-1}$. Vandame et al. (1995), using fluvalinate-sprayed surfaces, estimated a LC$_{50}$ of 0.21 mg per mL of sedimented solution in samples from Brignoles, while specimens from Draguignan (both in France) had a LC$_{50}$ of 2.67 mg mL$^{-1}$, indicating a twelve fold resistance factor.

According to the above statements, Mexican varroa populations are considered to have maximum levels of susceptibility to most acaricides, owing to their isolation from chemically-selected strains. Thus, LC$_{50}$ values obtained in this study are proposed as baselines for testing acaricides.

Topical application. Table 1 shows LD$_{50}$ against varroa. Abed & Ducos de Lahitte (1993) estimated an amitraz LD$_{50}$ of 2.16 pg per mite, with a confidence interval of 1.46-3.2 pg. These values are close and overlap values obtained in the current work; this fact suggests comparable levels of susceptibility in both mite populations. Baseline data expressed as LD$_{50}$ are proposed now as they appear in Table 1.

**Susceptibility of Apis mellifera**

Spraying. Results are shown in Table 2. There are no published data for direct comparison with our results, since most research on bee toxicology used oral and contact bioassay methods (Oomen 1986). According to a pesticide classification of Felton et al. (1986) of toxicity to honey bees, flumethrin and fluvalinate belong to Group 1, highly toxic pesticides, with LD$_{50}$ < 1 µg/bee. Amitraz belongs to Group 2, moderately toxic, with LD$_{50}$ 1-10 µg/bee.

**TABLE 1. LC$_{50}$, LD$_{50}$ AND RANGE (CONFIDENCE INTERVALS, 95%) OF FIVE ACARICIDES AGAINST VARROA JACOBSONI, BY SPRAYING AND TOPICAL APPLICATION, RESPECTIVELY.**

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>LC$_{50}$ mg L$^{-1}$</th>
<th>Confidence int. (LV-HV)</th>
<th>HV/LV$^{2}$</th>
<th>LD$_{50}$ pg mite</th>
<th>Confidence int. (LV-HV)</th>
<th>HV/LV (LD$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>0.23</td>
<td>0.14-0.37</td>
<td>2.68</td>
<td>1.7</td>
<td>1.21-2.39</td>
<td>1.98</td>
</tr>
<tr>
<td>Flumethrin</td>
<td>875.08$^{3}$</td>
<td>201-6554</td>
<td>32.61</td>
<td>0.46</td>
<td>0.36-0.59</td>
<td>1.62</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>0.19</td>
<td>0.13 -0.29</td>
<td>2.31</td>
<td>15.42</td>
<td>9.91-24.94</td>
<td>2.52</td>
</tr>
</tbody>
</table>

$^{3}$Highest value.
$^{2}$Lowest value.

**TABLE 2. LC$_{50}$, LD$_{50}$ AND RANGE (CONFIDENCE INTERVALS, 95%) OF FIVE ACARICIDES AGAINST APIS MELLIFERA, BY SPRAYING AND TOPICAL APPLICATION, RESPECTIVELY.**

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>LC$_{50}$ µg L$^{-1}$</th>
<th>Confidence int. (LV-HV)</th>
<th>HV/LV$^{3}$</th>
<th>LD$_{50}$ µg/bee</th>
<th>Confidence int. (LV-HV)</th>
<th>HV/LV (LD$_{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>1636</td>
<td>983.79-2825</td>
<td>2.32</td>
<td>2.55</td>
<td>1.57-4.32</td>
<td>2.75</td>
</tr>
<tr>
<td>Flumethrin</td>
<td>46.87</td>
<td>21.15-95.61</td>
<td>4.52</td>
<td>0.05</td>
<td>0.03-0.09</td>
<td>3.26</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>1601</td>
<td>1429-1803</td>
<td>1.26</td>
<td>0.97</td>
<td>0.57-1.66</td>
<td>2.91</td>
</tr>
</tbody>
</table>

$^{3}$Highest value.
$^{2}$Lowest value.
Topical application. LD$_{50}$ and confidence intervals appear in Table 2; previous data were obtained by Oomen (1986) for amitraz: LD$_{50} > 16 \mu g/bee$, and by Bornek (1989) for fluvinate: LD$_{50} = 4.66 \mu g/bee$, using Mavrik; LD$_{50} = 9.12 \mu g/bee$, using Klartan. Amitraz and fluvinate LD$_{50}$ values estimated herein are lower than those obtained by both authors; however, data cannot be accurately compared because of different experimental conditions and analytical methods.

**Relative Toxicity**

Tables 3 shows relative toxicity values for all acaricides used on varroa mites and honey bees. Consistently, flumethrin was the most toxic product, while fluvinate and amitraz showed a lesser similar toxicity.

**Comparative Susceptibility**

The rates of bee LC$_{50}$ or LD$_{50}$ divided by mite LC$_{50}$ or LD$_{50}$ are presented in Table 4. These data show that all products have acaricidal, rather than insecticidal action; different toxicity ranges from 500 fold to more than one million fold. This indicates a wide safety margin between lethal levels against mites and toxic levels for honey bees.

**Comparison of bioassay methods**

As a measure of sensitivity, slopes resulting from spraying and topical application were analyzed. In most cases they attained the quality criteria proposed by Ibarra & Federici (1987) for toxicological bioassays. Table 5 shows a comparison of slopes for spraying vs. topical application (Student t test, $\alpha = 0.05$). Significantly higher slopes for spraying method occurred only in amitraz and fluvinate applied to honey bees, representing their greater sensitivity to spraying.

**DISCUSSION AND CONCLUSIONS**

Precision, as estimated by means of the confidence intervals, is shown in Tables 1 and 2. Although in several cases the quotient HV/LV exceeded the value of 2 (proposed by Ibarra & Federeci 1987, as the highest permissible limit), sufficiently accurate es-

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**TABLE 3. RELATIVE TOXICITY OF ACARICIDES ON VARROA JACOBSONI AND APIS MELIFERA.**

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>Spraying</th>
<th>Topical</th>
<th>Spraying</th>
<th>Topical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flumethrin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Amitraz</td>
<td>262.83</td>
<td>3.7</td>
<td>34.9</td>
<td>51</td>
</tr>
<tr>
<td>Fluvinate</td>
<td>217.12</td>
<td>33.52</td>
<td>34.16</td>
<td>19.4</td>
</tr>
</tbody>
</table>
Estimates of LC₅₀ and LD₅₀ were obtained in both aspersion and topical methods. An important exception is the large confidence interval shown by spraying of flumethrin on varroa; no explanation for this fact can be given.

Samples included mixed specimens obtained from adult bees and uncapped pupae, which constituted a potential source of variation (Milani & Della Vedova 1996), and no attempt was made to detect differences in susceptibility between such origins. However, obtaining female mites from a single source was a difficult task, and confidence intervals may reflect this possible variation.

The spraying method has the advantage of treating all insects or mites at the same time; sticking individual mites to a slide as well as topical application to honey bees and mites are very laborious procedures. In addition, by using the Potter-Burgerjon’s tower, the amount of applied droplets could be narrowly regulated. Thus spraying proved to be more practical for testing on varroa mites, regardless of the need to regularly calibrate the spraying nozzle.

Although both application methods can be useful, spraying showed a more sensitive response of honey bees and it is easier in both species. So we consider it the best choice.

Fluvalinate has been widely used and, as expected, mites have developed resistance to it in many localities. Reproduction of whole bioassays as well as use of their estimated LC₉₀ or LD₉₀ as a discriminant screen will aid to decide its eventual replacing in a local or regional basis. Like fluvalinate, flumethrin is a pyrethroid. Thus, a risk exists of cross-resistance, as shown by Milani (1995). Its useful life is expected to be shorter and so early detection of resistance is important. Since amitraz is not chemically related to the pyrethroids, if an efficient and environmentally acceptable acaricide containing amitraz is available to beekeepers, it could be an option for alternating with pyrethroid treatments.

### Table 4. Comparative LC₅₀ and LD₅₀ for Acaricides Used Against Varroa Jacobsoni and Apis Mellifera.

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>LC₅₀ bee/LC₅₀ varroa</th>
<th>LD₅₀ bee/LD₅₀ varroa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>7113.04</td>
<td>1.5×10⁶</td>
</tr>
<tr>
<td>Flumethrin</td>
<td>5360.81</td>
<td>1×10⁵</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>8426.31</td>
<td>6.3×10⁴</td>
</tr>
</tbody>
</table>

### Table 5. Comparison of slopes (β) for the Dose-Lethality Relationship of Spraying (S) vs. Topical (T) Tests Conducted on V. Jacobsoni and A. Mellifera, by Means of a Student T Test (α = 0.25). H₀: βₕ = βₜ.

<table>
<thead>
<tr>
<th>Acaricide</th>
<th>On Varroa βₕ</th>
<th>On bees Results of t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amitraz</td>
<td>2.03</td>
<td>=</td>
</tr>
<tr>
<td>Flumethrin</td>
<td>1.01</td>
<td>=</td>
</tr>
<tr>
<td>Fluvalinate</td>
<td>1.87</td>
<td>=</td>
</tr>
</tbody>
</table>

¹> H₀ rejected, βₕ > βₜ.
²= H₀ accepted.


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**EVALUATION OF “TRED-NOT” AGAINST HOST-SEEKING DEER FLIES (DIPTERA:TABANIDAE) IN NORTH FLORIDA**

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**ABSTRACT**

“TRED-NOT” (6.4´14.2 cm adhesive strips) affixed to the back and front of nylon mesh solid black and solid white “baseball” caps were evaluated for their ability to trap host-seeking *Chrysops celatus* Pechuman, *C. vittatus* Weidemann, and *Diachlorus ferrugatus* (F.). Trials were conducted in a commercial pine bottomland forest habitat in northwestern Florida during peak seasonal abundance of these species. No *D. ferrugatus* were captured on patches but approximately 26% of host seeking *Chrysops* (regardless of patch location, cap color or fly species) were captured compared with a standard aerial sweep net method. Significantly more deer flies were captured on patches affixed to the back of the cap compared with patches placed on the front. No statistical difference (>0.05) existed in number of flies trapped on patches when cap colors (white versus black) were compared.

**Key Words:** *Chrysops celatus*, *Chrysops vittatus*, *Diachlorus ferrugatus*, personal protection

**RESUMEN**

Parches marca “TRED-NOT” para la captura de moscas *Chrysops celatus* Pechuman (tiras adhesivas de 6,4´14,2 cm) pegadas al frente y al dorso de mallas de nylon...
EVALUATION OF “TRED-NOT™” DEERFLY PATCHES AGAINST HOST-SEEKING DEER FLIES (DIPTERA: TABANIDAE) IN NORTH FLORIDA

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Key Words: Chrysops celatus, Chrysops vittatus, Diachlorus ferrugatus, personal protection

RESUMEN

Parches marca “TRED-NOT™” para la captura de moscas Chrysops celatus Pechuman (tiras adhesivas de 6,4 × 14,2 cm) pegadas al frente y al dorso de mallas de nylon
Host-seeking deer flies can often become a serious nuisance and large pestiferous populations can certainly discourage enjoyment of outdoor activities. Repellents applied to exposed skin have not been very effective against these pests especially for extended periods of time (Anderson 1985). Insecticides applied to, or impregnated in, clothing have been reported to provide some repellency in field situations (Schreck et al. 1978 and Carlson 1996). Recently, adhesive patches (affixed to headwear) have been advertised in retail/outdoor recreational supply catalogs as an effective way to “stop biting deer flies”. This author is unaware of any published studies, conducted under Florida conditions, where such products have been evaluated. As a result, a field study to evaluate a commercially available adhesive patch against three species of host-seeking deer flies was conducted late spring, 1998.

MATERIALS AND METHODS

This study was conducted from May 27 through June 8, 1998 in Walton and Bay Counties, Florida, at a time when Chrysops vittatus Weidemann, C. celatus Pechuman and Diachlorus ferrugatus (F.) were at seasonal peak abundance as documented by Jones & Anthony (1968), Cilek and Schreiber (1996, 1999) and Cilek et al. (1994), respectively. “TRED-NOT™ DEERFLY PATCHES” (6.4 x 14.2 cm double-sided adhesive-coated fabric patches Detex, Leroy, Michigan) were used in all evaluations. Although package directions indicated that one strip be affixed to the back of a hat, or cap, comparisons were made with a strip placed in front and back to determine if location affected patch trapping effectiveness. Patches were affixed to “baseball” caps made of nylon mesh with solid foam fronts (Cobra Caps, Bangladesh). Solid-colored white and black hats were compared to determine if color influenced fly collections. Controls consisted of similar mesh caps with patches affixed in same locations as treatments but covered with a non-adhesive backing strip (used by the manufacturer to prevent adhesive strips from adhering to the packaging material). Adhesive patches were used once per test.

Evaluations were conducted in three geographically separate but similar habitats (at least 50 km apart) where only one fly species occurred. Each habitat consisted of abandoned commercial pine bottomland forests that contained a mixture of slash pine (Pinus elliottii Ex. Chapm.) magnolia (Magnolia grandiflora L.), and live oak (Quercus virginiana Mill.). Two non-continuous linear transects, each 30-m, were staked out in each location. One person walked the length of each transect back and forth (i.e. 60 m) and total number of deer flies attached to adhesive strip(s) recorded at the end of that
transect. After this, aerial net (32 cm diam) samples were then conducted by the same person in the same area. Sampling consisted of continually swinging the net in figure “8” sweeps that started at ankles and ended above the head while walking each transect (Cilek and Schreiber 1996). This method (herein referred to as a standard) was used as a “best estimate” to quantitatively compare abundance of host-seeking flies in the immediate vicinity of the sampler (i.e. control) with those captured on patches (i.e., treatment). Net collected flies were counted at the end of each transect and released. Treatments and standards were replicated twice per habitat per species on ten different dates.

Statistical Analysis

Data were transformed using $\sqrt{x+1}$ prior to analysis and subjected to ANOVA (PROC GLM, SAS Institute 1990). A Student-Newman-Keuls test was used to determine differences (<0.05) in overall mean fly abundance relative to patch (treatment) vs standard (control) collections, patch location (front vs back), hat color (white vs black), and Chrysops species (Sokal and Rohlf 1981). These data sets did not include D. ferrugatus as none were captured on adhesive patches. All mean data in tables are untransformed means.

RESULTS

Overall, significantly fewer host-seeking Chrysops ($F = 343.07$, df 1, 159; $P < 0.0001$) were collected from adhesive patches compared with the standard (Table 1). Adhesive patches captured approximately 26% of the fly population netted by the standard. Moreover, about 21% (17 out of 80) of the patches captured no flies at all.

Patches affixed to the back of caps captured significantly more flies than those affixed to the front ($F = 193.03$ df 1, 159; $P < 0.0001$) (Table 1). No significant difference was observed in number of deer flies caught on white hats versus black hats ($F = 0.81$; df 1, 159; $P = 0.37$).

Overall, significantly more C. vittatus (9.9 ± 0.9) were collected from the standard and patches compared with similar collections for C. celatus (5.4 ± 0.5) ($F = 68.98$, df 1, 159; $P < 0.0001$). This difference was probably related to location/habitat, and/or relative population size, rather than species preference. As stated earlier, no D. ferrugatus were trapped on adhesive patches regardless of cap color or patch location although they were collected in the aerial net sampling standard (mean 11.4 ± 0.5).

DISCUSSION

The effectiveness of TRED-NOT™ DEERFLY PATCHES to trap deer flies was influenced by a fly’s host-seeking behavior. Chrysops preferred the upper regions, especially the head, and were readily trapped on the patches. D. ferrugatus was not captured because this species primarily visited the lower extremities when trying to obtain a blood-meal (Fairchild and Weems 1973, McKeever and French 1997).

Adhesive patches did capture both Chrysops species. Collection differences relative to patch location (i.e. front vs back) were interesting. Attraction of host-seeking deer flies to a person walking is well known (Bram 1978) but the orientation to the back of a human’s head may result from a “downwind” plume of expired carbon dioxide. Carbon dioxide has long been recognized as a strong attractant for host-seeking Tabanidae (Bram 1978).
There appeared to be no cap color preference relative to number of *Chrysops* trapped on patches, although, it has been well documented that tabanids are generally attracted to dark objects (Bram 1978). Because the study area bordered well-known tabanid developmental habitats (i.e. bottomland swamps/marshes), color preference may have not been an important factor for short-range host seeking, when expired carbon dioxide (signalling a potential blood host) was present.

In conclusion, TRED-NOT™ DEERFLY PATCHES captured some of the *Chrysops* attracted to a person’s head but did not completely trap all these pests visiting this body region. However, the amount of personal annoyance perceived from host-seeking deerflies is often relative. Therefore any device or method, including the one evaluated here, that removed or reduced host-seeking *Chrysops* (either perceived or actual) may be of general benefit to those seeking relief from such outdoor pests.

**REFERENCES CITED**


<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Mean flies ±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Overall abundance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>adhesive patch</td>
<td>80</td>
<td>3.2 ± 0.4a</td>
</tr>
<tr>
<td>standard</td>
<td>80</td>
<td>12.1 ± 0.7b</td>
</tr>
<tr>
<td>II. Patch location</td>
<td></td>
<td></td>
</tr>
<tr>
<td>front</td>
<td>80</td>
<td>3.0 ± 0.4b</td>
</tr>
<tr>
<td>back</td>
<td>80</td>
<td>0.3 ± 0.1a</td>
</tr>
<tr>
<td>III. Cap color</td>
<td></td>
<td></td>
</tr>
<tr>
<td>white</td>
<td>80</td>
<td>1.5 ± 0.3a</td>
</tr>
<tr>
<td>black</td>
<td>80</td>
<td>1.8 ± 0.3a</td>
</tr>
</tbody>
</table>

Paired means within rows (I, II, and III) followed by different letters are significantly different (P < 0.05; SNK) after $\sqrt{x+1}$ transformation of means, untransformed means are shown in table.

FRUIT FLIES (DIPTERA: TEPHRITIDAE) INFESTING FRUITS OF THE GENUS PSIDIUM (MYRTACEAE) AND THEIR ALTITUDINAL DISTRIBUTION IN WESTERN VENEZUELA

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2 Museo de Artrópodos (MALUZ), Facultad de Agronomía, Apdo. 526, Maracaibo, Estado Zulia 4002, Venezuela

ABSTRACT

A survey of fruit flies infesting Psidium fruits was conducted in western Venezuela from June 1992 through December 1995. Of 201 fruit samples collected from 139 localities at altitudes between sea level and 2,000 m, four species of Psidium plants were found in the western region of Venezuela. These were P. guajava L. (10-1930 m), P. guineense Sw. (100-1950 m), P. caudatum McVaugh (1800-1950 m) and P. friedrichstalianum (Berg) Niedenzu (35-1700 m). Four tephritid fly species were reared: Anastrepha striata Schiner, A. fraterculus (Wiedemann), A. obliqua (Macquart), and Ceratitis capitata (Wiedemann). All four fruit fly species emerged from P. guajava. A. striata was the most common on P. guajava, P. guineense and P. friedrichstalianum, with an infestation range of 96.1%-97.0%. P. caudatum was more frequently infested by A. fraterculus (94.5% adults emergence); the plant’s distribution was restricted to highlands. Observations on the altitudinal distribution of A. striata on P. guajava showed that the highest infestation (253.9 adults/kg fruits) occurred at about 1,000 m. The infestation rate of P. guajava by A. fraterculus and A. obliqua varied with elevation. In low elevation areas (0-1,200 m), A. obliqua was found more frequently than A. fraterculus, whereas A. fraterculus was found more frequently than A. obliqua in high altitude areas (1,201-2,000 m). C. capitata was erratically encountered in this study.

Key Words: Anastrepha, Ceratitis capitata, guava, Psidium spp., altitudinal distribution
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Key Words: _Anastrepha, Ceratitis capitata, guava, Psidium_ spp., altitudinal distribution

RESUMEN

Desde junio de 1992 a diciembre de 1995 se estudiaron las moscas de las frutas (Diptera: Tephritidae) que infestan plantas del género _Psidium_ en el occidente de Ve-
nezuela. Se recolectaron un total de 201 muestras de frutas en 139 localidades comprendidas desde el nivel del mar hasta 2,000 m de altitud. Se encontraron cuatro especies de plantas del género *Psidium*: *P. guajava* L. (10-1930 m), *P. guineense* SW. (100-1950 m), *P. caudatum* Mc Vaugh (1800-1950) y *P. friedrichstalianum* (Berg) Niedenzu (35-1700 m). Se lograron criar cuatro especies de moscas de la Familia Tephritidae: *Anastrepha striata* Schiner, *A. fraterculus* (Weidemann), *A. obliqua* (Macquart) y *Ceratitis capitata* (Weidemann). De *P. guajava* emergieron las 4 especies de moscas de las frutas encontradas en el presente estudio. *A. striata* resultó ser la mosca más común en *P. guayaba*, *P. guineense* y *P. friedrichstalianum* encontrándose infestaciones comprendidas entre 96.1%-97.0%. *P. caudatum* fue encontrada como la planta hospedera preferida por *A. fraterculus* con un 94.5%. Además, su distribución está restringida a tierras altas. La distribución altitudinal de *A. striata* muestra que la mayor infestación en frutos de *P. guayava* ocurre alrededor de los 1,000 m de altitud (253.9 adultos/Kg de frutas). La infestación relativa de *A. fraterculus* y *A. obliqua* en *P. guavaja* varía con la altitud. En tierras bajas (0-1,200 m), *A. obliqua* se encontró como la especie predominante sobre *A. fraterculus*. En cambio, en tierras altas (1,201-2,000 m), *A. fraterculus* fue la especie predominante sobre *A. obliqua*. La distribución geográfica y altitudinal de *C. capitata* fue muy errática.

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The Genus *Anastrepha* is endemic to the Americas and is restricted to tropical and subtropical environments. Its range extends from the southernmost part of the United States (Rio Grande Valley of Texas and southern Florida) to South America, with the exception of the southern parts of Argentina and Chile. Fruit flies of the genus *Anastrepha* compose one of the largest and most economically important insect groups in the tropics and subtropics due to their damage to cultivated fruits. This group comprises more than 190 identified species but hosts are known for less than half (Norrbom & Kim 1988).

In Venezuela there are four economically important *Anastrepha* species: the South American fruit fly, *A. fraterculus* (Wiedemann), the West Indian fruit fly, *A. obliqua* (Macquart), the guava fruit fly, *A. striata* Schiner, and the zapote fruit fly, *A. serpentina* (Wiedemann).

In the 1980’s, cultivation of guava, *Psidium guajava*, in the northern region of Zulia State was expanded. By 1992, in the lake Maracaibo plain, about 4,000 ha of guava orchards were in production (Araujo et al. 1997). Several species of fruit flies of the family Tephritidae, especially *A. striata* and *C. capitata*, are very important from a quarantine point of view when fruit export is the objective.

*P. guajava* is found from sea level to 1,930 m in commercial orchards, backyards of houses, roadsides, pasture lands, and forests throughout western Venezuela. The work described in this paper was done to obtain basic information about the altitudinal distribution of different fruit flies infesting cultivated or wild fruits belonging to the family Myrtaceae, genus *Psidium*, which can be used in the economic management of these fruit flies.

**MATERIALS AND METHODS**

From June 1992 to December 1995, 201 *Psidium* spp. fruit samples were collected whenever available from sea level to 2,000 m elevation in 139 localities in the western Venezuelan states of Falcón, Mérida, Táchira, Trujillo, and Zulia comprising an area of 117,700 sq-km. The Northern and Southern borders of the study area are delimited
with latitudes 11°45’N and 7°32’N respectively, while Eastern and Western borders are delimited with longitudes 68°30’W and 72°40’W respectively.

Fruit samples were collected from four species of Psidium: P. guajava L. (common guava) from sea level to 1,930 m, P. guineense Sw. (mountain guava) from 100-1,950 m, P. caudatum McVaugh (jumangue) from 1,800-2,000 m, and P. friedrichsthalianum [Berg] Ndz. (cas or sour guava) from 35-1,700 m altitude. The total number of fruits collected from each Psidium spp. host plant comprised 7,015, 255, 3,816 and 59 fruits from P. guajava, P. guineense, P. caudatum and P. friedrichsthalianum respectively.

Following the technique described by Katiyar et al. (1995), fruit samples were incubated and processed in the laboratory. Mature fruits were picked from sample trees as well as from the ground and were placed in open top wooden boxes (30 x 20 x 10 cm). A sheet of plastic screening (about 4 mm/mesh) had been fitted about 2 cm from the bottom of each box. The wooden boxes containing fruit samples were placed in plastic rearing containers (35 x 24 x 13 cm). The tops of the rearing containers were fitted with a fine-screened window (15 x 8 cm) for aeration. Each container had a layer of moist sawdust about 2 cm deep at the bottom as a pupation media for the larvae. The fruit samples were taken to the laboratory in this manner. In the laboratory, samples were removed from rearing containers. The fruits were counted, weighed, and put back in the containers. Every 2-3 days the sawdust was sieved, and recovered larvae and pupae were placed in 500 cc plastic cups containing a thin layer (2-3 cm) of moist saw dust. Each container with pupae was placed inside an adult emergence cage to recover fruit flies and parasitoid adults. The emerged adults were preserved in 70% ethyl alcohol. Rearing was carried out in the laboratory at 26 ± 3°C and 60 ± 10% RH.

The climatic condition of the study area is characterized by a rainy season from April to November, followed by a dry period from December to March. The rainfall in Western Venezuela varies widely from one place to another. This variation can be observed in lowland areas (0-1,000 m) as well as in highland areas (1,001-2,000 m). During rainy season the temperature is slightly higher compared with the dry season. Table 1 presents climatic data (temperature, rainfall and RH) from 17 meteorological stations located between 5 and 2,200 m elevation in the study area.

Samples of fruit fly adults were identified by A. L. Norrbom, Systematic Entomology Laboratory, USDA, PSI, ARS, Washington, D.C.

RESULTS AND DISCUSSION

A total of 11,145 fruits (261.5 kg) were collected from four Psidium species. A total of 30,530 pupae and 20,970 adults of both sexes belonging to four species of tephritids were reared from these fruits.

Table 2 summarizes the relative abundance of each tephritid species infesting the four Psidium host plants (based on adult emergence/kg fruits). P. guajava was infested by all four tephritid species found, P. guineense by three species (A. striata, A. fraterculus and A. obliqua), P. caudatum by two species (A. fraterculus and A. striata) and P. friedrichsthalianum by two species (A. striata and A. obliqua). The results show that A. striata was the most common fruit fly pest of Psidium species in the western region of Venezuela. Based on total adult emergence from all four Psidium spp., the proportion of each species consisted of A. striata (73.9%), A. fraterculus (24.2%) and A. obliqua (1.9%).

The results also show that A. striata was the most common fruit fly in three Psidium spp. (guajava, guineense, and friedrichsthalianum) with an infestation range of 96.1%-97.0% (based on number of adults emerged/kg fruit). Whereas A. fraterculus
TABLE 1. ANNUAL CLIMATIC DATA\(^1\) (TEMPERATURE, RAINFALL AND RELATIVE HUMIDITY) IN WESTERN VENEZUELA.

<table>
<thead>
<tr>
<th>Weather station</th>
<th>State</th>
<th>Data period (years)</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Altitude (m)</th>
<th>Annual temperature (°C) Mean</th>
<th>Min</th>
<th>Max</th>
<th>Annual rainfall (mm) Mean</th>
<th>Min</th>
<th>Max</th>
<th>RH (%) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sta. Barbara</td>
<td>Zulia</td>
<td>67-98</td>
<td>08°58'</td>
<td>71°53'</td>
<td>5</td>
<td>28.4</td>
<td>23.9</td>
<td>33.2</td>
<td>1297</td>
<td>768</td>
<td>1813</td>
<td>83</td>
</tr>
<tr>
<td>Coro</td>
<td>Falcón</td>
<td>82-92</td>
<td>11°25'</td>
<td>69°41'</td>
<td>16</td>
<td>27.8</td>
<td>25.0</td>
<td>32.7</td>
<td>362</td>
<td>189</td>
<td>613</td>
<td>75</td>
</tr>
<tr>
<td>Maracaibo</td>
<td>Zulia</td>
<td>81-98</td>
<td>10°41'</td>
<td>71°38'</td>
<td>45</td>
<td>29.2</td>
<td>24.9</td>
<td>33.4</td>
<td>484</td>
<td>234</td>
<td>688</td>
<td>75</td>
</tr>
<tr>
<td>El Isiro</td>
<td>Falcón</td>
<td>82-92</td>
<td>11°18'</td>
<td>69°37'</td>
<td>72</td>
<td>29.0</td>
<td>24.7</td>
<td>34.5</td>
<td>570</td>
<td>258</td>
<td>926</td>
<td>68</td>
</tr>
<tr>
<td>Machiques</td>
<td>Zulia</td>
<td>50-98</td>
<td>10°03'</td>
<td>72°33'</td>
<td>99</td>
<td>28.2</td>
<td>22.7</td>
<td>33.6</td>
<td>1500</td>
<td>1011</td>
<td>2196</td>
<td>66</td>
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<tr>
<td>Sto. Domingo</td>
<td>Táchira</td>
<td>82-92</td>
<td>07°35'</td>
<td>72°04'</td>
<td>327</td>
<td>23.7</td>
<td>20.4</td>
<td>29.2</td>
<td>2854</td>
<td>2496</td>
<td>3572</td>
<td>84</td>
</tr>
<tr>
<td>San Antonio</td>
<td>Táchira</td>
<td>85-95</td>
<td>07°51'</td>
<td>72°27'</td>
<td>377</td>
<td>25.9</td>
<td>21.9</td>
<td>31.4</td>
<td>712</td>
<td>312</td>
<td>1168</td>
<td>72</td>
</tr>
<tr>
<td>Valera</td>
<td>Trujillo</td>
<td>86-96</td>
<td>09°21'</td>
<td>70°37'</td>
<td>582</td>
<td>24.4</td>
<td>20.1</td>
<td>29.7</td>
<td>1071</td>
<td>743</td>
<td>1380</td>
<td>81</td>
</tr>
<tr>
<td>Colón</td>
<td>Táchira</td>
<td>85-95</td>
<td>08°02'</td>
<td>72°15'</td>
<td>760</td>
<td>22.3</td>
<td>19.3</td>
<td>25.7</td>
<td>1513</td>
<td>996</td>
<td>1937</td>
<td>79</td>
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<tr>
<td>Churuguara</td>
<td>Falcón</td>
<td>74-84</td>
<td>10°48'</td>
<td>69°30'</td>
<td>920</td>
<td>22.7</td>
<td>16.8</td>
<td>27.1</td>
<td>729</td>
<td>466</td>
<td>1328</td>
<td>NA</td>
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<tr>
<td>Tovar</td>
<td>Mérida</td>
<td>69-90</td>
<td>08°20'</td>
<td>71°44'</td>
<td>952</td>
<td>21.9</td>
<td>17.7</td>
<td>26.7</td>
<td>1069</td>
<td>550</td>
<td>1622</td>
<td>73</td>
</tr>
<tr>
<td>La Grita</td>
<td>Táchira</td>
<td>74-84</td>
<td>08°08'</td>
<td>71°59'</td>
<td>1270</td>
<td>21.4</td>
<td>15.6</td>
<td>26.5</td>
<td>795</td>
<td>467</td>
<td>1911</td>
<td>82</td>
</tr>
<tr>
<td>Mérida</td>
<td>Mérida</td>
<td>51-98</td>
<td>08°36'</td>
<td>71°11'</td>
<td>1479</td>
<td>19.1</td>
<td>15.3</td>
<td>24.7</td>
<td>1732</td>
<td>1159</td>
<td>2302</td>
<td>80</td>
</tr>
<tr>
<td>San Giusto</td>
<td>Trujillo</td>
<td>80-90</td>
<td>09°17'</td>
<td>70°12'</td>
<td>1499</td>
<td>18.0</td>
<td>13.0</td>
<td>24.4</td>
<td>1848</td>
<td>1335</td>
<td>2574</td>
<td>81</td>
</tr>
<tr>
<td>Boconó</td>
<td>Trujillo</td>
<td>91-91</td>
<td>09°16'</td>
<td>70°13'</td>
<td>1560</td>
<td>18.0</td>
<td>11.6</td>
<td>23.9</td>
<td>1570</td>
<td>1437</td>
<td>1742</td>
<td>75</td>
</tr>
<tr>
<td>Sto. Domingo</td>
<td>Mérida</td>
<td>78-83</td>
<td>08°52'</td>
<td>70°40'</td>
<td>2155</td>
<td>15.6</td>
<td>10.5</td>
<td>20.4</td>
<td>1233</td>
<td>700</td>
<td>1780</td>
<td>NA</td>
</tr>
<tr>
<td>Betania</td>
<td>Táchira</td>
<td>84-94</td>
<td>07°28'</td>
<td>72°26'</td>
<td>2210</td>
<td>15.1</td>
<td>9.6</td>
<td>20.5</td>
<td>1035</td>
<td>763</td>
<td>1368</td>
<td>NA</td>
</tr>
</tbody>
</table>

\(^1\)Provided by Ministerio del Ambiente y de los Recursos Naturales Renovables de Venezuela (MARNR).
NA, not available.
was the predominant species in *P. caudatum* (the emerged adults were 94.5% *A. fraterculus* and 5.5% *A. striata*). *P. caudatum* is a native wild *Psidium* species found at relatively high elevation (1,500-2,000 m) and has a small fruit, mean weight of 1.8 g. In general *A. striata* was the dominant fruit fly in *Psidium* species except *P. caudatum* in the western part of Venezuela. *A. striata* is also reported as the major pest of *P. guajava* in several other Latin American countries. In Costa Rica, 97.8% of fruit samples were reported infested by *A. striata* (Jiron & Hedström 1988), and in Ecuador *A. striata* emerged from 70.8% of fruit samples examined (Hedström 1987).

Figure 1A shows the relative intensity of infestation by *A. striata* in *P. guajava* at different altitudes (8 strata). Results demonstrate that the most prevalent distribution for *A. striata* infestations occur between 500 to 1,500 m and the highest infestations occur at about 1,000 m.

Figure 1B shows the altitudinal distribution of *A. fraterculus* and *A. obliqua*. The results show that at low altitudes (0-1,200 m), *A. obliqua* was more prevalent than *A. fraterculus*, whereas at higher altitudes (1,201-2,000 m), *A. fraterculus* was more prevalent than *A. obliqua*. Similar results, which indicate that *A. obliqua* prefers lowland zones and *A. fraterculus* prefers higher elevation areas, have been found in other studies (Celedonio-Hurtado et al. 1995, Eskafi & Cunningham 1987, Hedström 1987).

*C. capitata* was reared only from seven fruit samples of *P. guajava* collected between 50-1650 m. The distribution of these fruit samples were four (Zulia state) at 0-250 m and one each from 250-500 m (Trujillo state), 1250-1500 m (Táchira state), and 1500-1750 m (Mérida state) elevation ranges. The altitudinal distribution and presence of this fruit fly was erratic and the infestation rate was very low (1.4 adults/Kg).

**ACKNOWLEDGMENTS**

We thank Alan L. Norrbom (Systematic Entomology Laboratory, USDA, PSI, ARS, Washington, D.C.), Daniel S. Moreno (Subtropical Agricultural Research Laboratory, Crop Quality and Fruit Insects Research, USDA, ARS, Weslaco, TX), and Andrew F. Neilid, 116 Crosslet Wale Greenwich, London SE10 8DL for their comments and suggestions in the review of earlier draft of the manuscript. We also thank 3 anonymous reviewers for critically reviewing and improving this manuscript. This study was funded by Consejo de Desarrollo Científico y Humanístico de La Universidad del Zulia (CONDES), through project 1912-96 “Programa Museo de Artrópodos”.

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**TABLE 2. RATE OF FRUIT INFESTATION OF PSIDIUM SPECIES BY ANASTREPHA SPP. IN THE WESTERN REGION OF VENEZUELA, 1992-1995.**

<table>
<thead>
<tr>
<th>Host fruit</th>
<th>Adult emergence/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. striata</td>
</tr>
<tr>
<td><em>P. guayaba</em>¹</td>
<td>110.6</td>
</tr>
<tr>
<td><em>P. guineense</em>²</td>
<td>91.2</td>
</tr>
<tr>
<td><em>P. caudatum</em>³</td>
<td>0.8</td>
</tr>
<tr>
<td><em>P. friedrichstalianum</em>⁴</td>
<td>29.8</td>
</tr>
</tbody>
</table>

¹Indicates (n = 185 samples and 249.2 Kg).
²Indicates (n = 5 samples and 3.1 Kg).
³Indicates (n = 7 samples and 6.9 Kg).
⁴Indicates (n = 4 samples and 2.3 Kg).
REFERENCES CITED


Fig. 1. Infestation of Psidium guajava by three tephritid species in western Venezuela, 1992-1995. (A) by A. striata. (B) by A. obliqua and A. fraterculus.


FIRST RECORD OF CUTEREBRA SP. (DIPTERA: CUTEREBRIDAE) INFECTION IN OTOTYLOMYS PHYLLOTIS (RODENTIA: MURIDAE).

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Maggots of the fly genus Cuterebra (bot flies) are cutaneous tissue parasites of wild species of rodents and lagomorphs. The latter are their natural hosts, although other accidental or aberrant hosts as cats, dogs, deer, cattle, mules, skunks and even humans are known to harbour Cuterebra larvae (Sabrosky 1986). The distribution of the 72 known species is restricted to the New World; 36 have neartic distribution, 36 neotropical and four overlap their geographic distribution (Catts 1982). Most of these species appear to be host specific to the rodent species infected or to a group of closely related species.

The genus Cuterebra has been reported in six genera of rodents (Microtus, Neotoma, Peromyscus, Sciurus, Tamias and Thomomys) (Catts 1982, Sabrosky 1986). The former three are murids with distribution in the Americas (Wilson & Reeder 1993). From January of 1997 through May of 1998, a population study of wild rats was carried in a dry tropical forest in the locality of Hobonil (20°00'58"N, 89°01'13"W) in the Mexican state of Yucatan (Southeast Mexico). Monthly trapping samples were carried during five days in an area of 1200 m². Rats were live-trapped using Sherman traps.

The rats were examined for bot infection. Detection of infection was done visually and by palpation and infection signs and reports ranged from cyst location to larvae extraction. From an overall sample of 427 rats of Heteromys gaumeri (269 individuals/62.99% of overall population), Ototylomys phyllotis (122/28.57%), Oryzomys melanotis (27/6.32%), Peromyscus yucatanicus (7/1.64%) and Sigmodon hispidus (2/0.46%), 16 rats were founded to be infected with larvae of Cuterebra sp. All the Cuterebra larvae or infection signs were founded in Ototylomys phyllotis. This is the first record of Cuterebra infection in the genus Ototylomys, the big-eared climbing rats.

Nine Cuterebra sp larvae were collected (one of second instar and eight of third instar). The larvae were found in cutaneous cysts close to the genitals and anus in twelve rats; close to the forelegs in two rats, and in both once. In four of the reported rats, the detection was merely done by palpation and observation of the cyst.

The incidence of infection in the population of Ototylomys phyllotis was 13.11%. Thirteen of the infected rats were adult males (86.67%) and three adult females (13.33%). Excepting for two rats (male and female), that had two larvae, all had only one each. From the rats where myiasis was detected for the first time, eight were re-captured without showing any re-infection signs and one was re-infected.

The first infected rat was observed in February of 1997, five during October and September, four in January of 1998, and six during February and March (Fig. 1).

Five larvae were cultured and three adult female flies emerged, two larvae were preserved in alcohol 75%. Specimens could only been identified to genus and are deposited at the Coleccion Entomologica Regional, Universidad Autonoma de Yucatan. Although the specimens were identified as Cuterebra according to Sabrosky (1986),
more taxonomical work is needed. The internal arrangement of *Cuterebra* stills under discussion because some authors (Sabrosky, 1986) have decided to recognize at least two genera within the genus (the Neartic *Cuterebra* and the Neotropical *Metacuterebra*), while others have synonymized most of the genus-group names under *Cuterebra* (Chillcott, 1965; Guimarães, 1967).

We would like to thank Hugo Delfín for his comments to this manuscript.

**SUMMARY**

This work is the first report of infection caused by *Cuterebra* on rodents in the genus *Ototylomys*. In a wild population of *Ototylomys phyllotis*, 16 of 122 mice were infected. Nearly 87% of infected individuals were adult males, and displayed cysts around the genital organs and anus, and on the arms. Of those animals showing first-time miasis, eight recaptured individuals did not show signs of re-infection and one individual was re-infected.

**REFERENCES CITED**


GUT CONTENT ANALYSIS OF THREE SPECIES OF SAC SPIDERS BY ELECTROPHORESIS

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Predation by spiders under field conditions is inherently difficult to study; however, the existence of specific predation wounds produced by three species of sac spiders on citrus leafminer (CLM), Phylloncistis citrella Stainton, could make it possible to quantify their predation. One drawback in this system is that one of the predation marks made by the sac spiders on CLM larvae is somewhat similar to the feeding mark made by Pnigalio minio (Walker), an eulophid ectoparasitoid of CLM. A way to overcome this predation assessment problem is through analysis of predator gut contents. Preliminary observations on the analysis of the prey remnants inside the gut of the spiders are presented and discussed. The main purpose of this study is to develop a method to detect prey remnants in the gut of spiders, which will lead to better assessment of the efficiency of spiders on CLM control in nurseries and orchards.

The prey remnants inside the gut of the three species of sac spiders, Chiracanthium inclusum Hentz, Hibana velox (Becker), and Trachelas volutus (Gertsch), were detected by polyacrylamide gel electrophoresis (PAGE). This method is based on the detection of prey enzymes in homogenates of the predator after PAGE and staining for esterase activity (Van Der Geest & Overmeer 1985, Murray & Solomon 1978, Solomon et al. 1985). Esterase was selected as the indicator protein because its detection employs an enzymic reaction with substrate yielding a stain with a high extinction coefficient as shown from the previous study on analysing diets of invertebrate predators by electrophoresis (Murray & Solomon 1978) which allow detection of very small quantities of enzyme by staining for extended periods.

Spiderlings of C. inclusum, H. velox, and T. volutus were obtained from laboratory cultures. Spiders fed with an artificial diet were individually reared in laboratory glass vials (15 mm diameter × 60 mm long). The artificial diet consisted of a mixture of soybean liquid, homogenized milk, and egg yolk (Amalin et al. 1999). Spiders fed with CLM larvae were reared individually in plastic petri dishes (10 cm diameter × 1 cm high). Samples of CLM larvae were gathered from field collections. Homogenates were obtained from fourth-nymphal spiderlings fed for 2 days with a total of 5 second larval instars of CLM, spiders fed with artificial diet, and second larval instars of CLM. Spiders were placed in a Perspex® plate and squashed individually with a glass rod in 5-10 μl of maceration fluid (1X TBE buffer [0.09 M Tris-borate + 0.002 M EDTA] with 0.2% Triton X-100 and 10% sucrose). A similar maceration procedure was used for CLM larvae except that the numbers of larvae varied from 1, 2, 5, to 10 CLM in different homogenate samples. This range of larval densities was used in order to determine the difference in the intensity of esterase bands with varying numbers of CLM larvae. For each sample, 20 μl homogenate was dispensed with the aid of a loading tip to the sample holders that were positioned on top of the gel.

Electrophoresis was carried out as described by Murray & Solomon (1978) and by Solomon et al. (1985) with some modifications. Polyacrylamide slab gels with a total gradient concentration of 5-28% and a cross-link gradient of 2.5-6.2% (Margolis &
were prepared between two glass plates using a gel gradient maker. A 1X TBE buffer pH 8.3, to which 0.2% w/v Triton X-100 was added, was used as the gel buffer. The running buffer was also 1X TBE without Triton X-100. The samples were run to endpoint for 20 h at 200 volts.

After electrophoresis, gels were incubated in a medium containing 30 mM 1-naphthyl acetate and 0.2% Fast Blue RR Salt in 0.2 M phosphate buffer, pH 6.0, in order to stain proteins with esterase activity. The gel separated from the glass plate was submerged in the medium and kept inside a dark container and shaken on a shaker (Gio Gyrotory®) at a speed of 28 RPM for 24 hours or until the bands appeared. Conclusions about the identity of prey remnants inside the gut of predators were drawn by visual comparison of esterase patterns of the artificially fed spiders and CLM-fed spiders.

In many cases with other predators, the specific esterase activity patterns allow the identification of prey remnants inside the predator gut (Van Der Geest & Overmeer 1985). After electrophoresis and staining for esterase activity, the CLM larvae and prepupae showed only one esterase band (Figs. 1 and 2). There was a difference in the intensity of the esterase bands on the different numbers of CLM included in each macerated sample. The intensity increased as the number of individuals per sample increased (Figs. 1 and 2). This difference in intensity could possibly be used to quantify the number of prey consumed by the predator. No esterase was obtained from the *C. inclusum* and *T. volutus* fed with artificial medium (Fig. 1, lanes 3 to 5); however, *H. velox* that fed on the artificial medium gave one esterase band with a higher molecular weight than the CLM esterase (Fig. 2, lanes 2 to 4). The esterase obtained from *C. inclusum* fed with CLM larvae in the laboratory was somewhat similar to the esterase of CLM (Fig. 1, lane 2). Similarly, a single esterase band was obtained from *H. velox* that fed on CLM, and this band also appears to be identical to the CLM esterase (Fig. 2, lane 9). The result of this experiment is similar to that of Murray & Solomon (1978) on the single esterase pattern of *Panonychus ulmi* (Koch), the European red spider mite, and differs from the results obtained by Dicke & DeJong (1988) who obtained several esterases also on *P. ulmi*. During the current test, the CLM esterase appears to have been expressed only in spiders that fed on CLM except for *T. volutus*. No esterase activity was obtained from *T. volutus* that fed on CLM larvae. This could be attributed to the retention time of the CLM esterase in the gut of *T. volutus*. Probably, the lifetime of CLM esterase in the gut of *T. volutus* is shorter compared to that of *C. inclusum* and *H. velox*. It is then worthwhile investigating the retention time of CLM esterase in the gut of the three species of sac spiders.

![Fig. 1. Esterase patterns of CLM, C. inclusum, and T. volutus.](image-url)
More electrophoresis runs should be done including field-collected individuals. However, using field-collected spiders, a difficulty may arise in the identification of prey because spiders in the field are generally feeding on multiple prey species. Moreover, not all esterases of a prey may be found in the gut of the spider. Certain esterases may be localized in tissues that are not ingested, complicating the identification of the prey for field-collected specimens. Therefore, it is necessary to co-electrophorese all suspected prey species with the spiders on the same gel. The use of other enzymes (i.e. fumarate hydratase, glucose-6 dehydrogenase, hexokinase, and other enzymes) must also be considered in order to look for a more stable enzyme that will be used for gut content analysis.

ACKNOWLEDGMENTS

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SUMMARY

Gut content analysis using polyacrylamide gel electrophoresis (PAGE) was performed on three sac spider species. Results from the electrophoresis showed that *H. velox* fed on artificial medium gave one esterase band; whereas, no esterase was obtained from the *C. inclusum* and *T. volutus* fed on artificial medium. The esterase obtained from *C. inclusum* and *H. velox* fed with citrus leafminer (CLM) larvae in the laboratory seems to be similar to the CLM esterase. No esterase activity was obtained from *T. volutus* that fed on CLM larvae. The preliminary result of the gut content analysis using PAGE showed the potential of this method in studying the predatory spider - CLM relationship in lime orchards.

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A SURVEY OF PARASITOIDS OF TRIALEURODES VAPORARIORUM AND BEMISIA TABACI (HOMOPTERA: ALEYRODIDAE) IN EASTERN GUATEMALA

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GREGORY A. EVANS 2
ROBERT MCSORLEY 1

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Trialeurodes vaporariorum (Westwood), the greenhouse whitefly, and Bemisia tabaci (Gennadius), the sweetpotato whitefly, are serious economic pests of agricultural, horticultural, and ornamental crops throughout warm regions of the world (Byrne et al. 1990, Brown 1994). Both species also affect glasshouse production of plants in temperate regions (Byrne et al. 1990). In the tropics, T. vaporariorum is more common above elevations of 500 m, and B. tabaci tends to be the predominant species below 500 m (Caballero 1994). Whitefly nymphs are sessile and susceptible to parasitism (Gerling 1990). Trialeurodes vaporariorum has been successfully managed in glasshouse systems with parasitoids (primarily Encarsia formosa Gahan, Hymenoptera: Aphelinidae) (Vet et al. 1980). Efforts to reduce populations of B. tabaci with both introduced and native natural enemies are ongoing (Roltsch & Pickett 1995, Hoelmer 1996, Goolsby & Ciomperlik 1999). There is very little information available on whitefly parasitoids from Guatemala. A preliminary survey was carried out during April-May 1998 in eastern Guatemala to determine which whitefly parasitoid species were present. The survey was carried out at the end of the dry season, when whitefly populations, and presumably populations of whitefly parasitoids, are at their highest levels. Parasitized whitefly nymphs were collected from three areas: the Salamá Valley (approx. 1000 meters above sea level [masl]), Sanarate (approx. 850 masl), and the Motagua Valley (230-340 masl). Preliminary observations indicated that T. vaporariorum is the predominant whitefly species on horticultural crops in the Salamá Valley and in the Sanarate area, and B. tabaci is the predominant species in the Motagua Valley.

Material was collected in the Salamá Valley from the Instituto de Ciencia y Tecnología Agrícolas (ICTA) field station in San Jerónimo (15°03'40"N, 90°15'00"W) and at the farms of René Santos and Margarito Cordova. In the Sanarate area, material...
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was collected from Finca Monte Grande (14°47'02"N, 90°12'15"W), Finca El Comun, and the farm of Francisco del Cid. In the Motagua Valley, material was collected from Usumatlán (14°56'45"N, 90°W), San Augustin, and the banks of the Rio Hato where it crosses beneath the Atlantic highway. San Jerónimo is about 30 km north of Sanarate. Usumatlán is about 50 km southeast of San Jerónimo and about 50 km northeast of Sanarate.

Parasitized whitefly nymphs were collected from common bean (*Phaseolus vulgaris* L.), cucumber (*Cucumis sativus* L.), guisquïl (*Sechium edule* Schwartz., a cucurbit), squash (*Cucurbita pepo* L.), tomato (*Lycopersicon esculentum* Mill.) and watermelon (*Citrullus vulgaris* Schrad.). Cucumber and tomato were the only plant species collected from each of the three general areas. Plants were examined in the field, and leaves which appeared to have high numbers of late-instar and parasitized nymphs were placed in unwaxed cylindrical 0.95-liter cardboard cartons (Fonda Group Inc., Union, NJ, USA) for parasitoid emergence. After 4 wk, dead parasitoid adults were removed from the containers and placed on cotton wool in gel capsules. These were then mailed to the Division of Plant Industry and Consumer Services in Gainesville, FL, for identification. The dried host plant material was placed in plastic bags and mailed to Dr. Andrew Jensen, formerly of the United States Department of Agriculture in Beltsville, MD, who identified the whitefly species from nymphs on the dried leaves.

*Trialeurodes vaporariorum* was the only whitefly species found in material collected from the Salamá Valley and Sanarate, and *B. tabaci* was the only whitefly species identified from material collected in the Motagua Valley (Table 1). The parasitoid species recovered consisted of *Encarsia pergandiella* Howard, *Eretmocerus* sp. (Hymenoptera: Aphelinidae), and *Signophora aleyrodis* Ashmead (Hymenoptera: Signaphoridae), a hyperparasitoid (Table 1). In the Salamá Valley, all but one of the 1150 parasitoid adults collected were *E. pergandiella*. One *Eretmocerus* sp. was collected from cucumber in that area. *Encarsia pergandiella* predominated in the material from Sanarate, although *Eretmocerus* sp. was present in higher numbers than in the Salamá Valley (Table 1). The ratio of *E. pergandiella* to *Eretmocerus* from the Sanarate area was 158 to 8 (20:1). *Eretmocerus* was present in higher numbers than *E. pergandiella* in material collected from the Motagua Valley. The ratio of *E. pergandiella* to *Eretmocerus* was 409 to 555 (1:1.4). One *S. aleyrodis* female was recovered from cucumber in the Motagua Valley. It is unclear from this study if the shift in parasitoid ratio was due to changes in elevation, changes in whitefly host, or a combination of both.

There are apparently two distinct color forms of *E. pergandiella*. The light form is entirely yellowish in color and the dark form has dark brown areas on the mesoscutum, axillae, and gaster. Light and dark individuals were collected from *T. vaporariorum* on cucumber, guisquïl, squash, and tomato in the Salamá Valley, and from *B. tabaci* on cucumber and tomato in the Motagua Valley.

The significance of these dark and light forms is unclear. Laudonia and Viggiani (1993) found that *Encarsia partenopea* Masi produced light color individuals at temperatures around 30 C, and darker individuals at 15 C. In Guatemala, populations of both the dark and light form emerged from the same whitefly species collected on the same plant at the same time. This suggests that the observed color variation in *E. pergandiella* females is not induced by either differences in host, host plant, relative humidity, or temperature. Perhaps closer examination of these morphologically similar forms occurring sympatrically may reveal the existence of two distinct species.

Collections of whitefly parasitoids from tomato in the Salamá Valley from Nov.-Dec. 1998 indicate that parasitoid diversity may be greater in the rainy season than in the dry season (Smith 1999). The current study indicates that there are important regional differences in the species composition of whitefly parasitoids as well.
<table>
<thead>
<tr>
<th>Altitude</th>
<th>Area</th>
<th>Collection</th>
<th>Date</th>
<th>Whitefly species</th>
<th>Parasitoid spp.</th>
<th>Parasitoid Number</th>
<th>Host plant</th>
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*Trialeurodes vaporariorum* and *Bemisia tabaci* were not identified from material collected at low and higher elevations, respectively. However, each species is known to be present at low densities outside of its optimal range (Caballero 1994, Smith 1999).
<table>
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<tr>
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SUMMARY

Primary parasitoids collected from *T. vaporariorum* and *B. tabaci* on a variety of horticultural crops in eastern Guatemala consisted of *Encarsia pergandiella* and *Eretmocerus* sp.

ACKNOWLEDGMENTS

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POLYMORPHIC LARVAL RETREATS IN THE NET-SPINNING CADDISFLY MACROSTEMUM CAROLINA (TRICHOPTERA: HYDROPSYCHIDAE): FORM AND PUTATIVE FUNCTION

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Larval net-spinning caddisflies of the genus Macrostemum Kolenati (Trichoptera: Hydropsychidae) construct their catchnets within protective retreats. This genus is composed of 88 species and is distributed worldwide (Morse 1999). However, the retreat architecture has only been described for three North American species (Wallace & Sherberger 1974, 1975, Wallace 1975) and one South American species (Sattler 1963). The described retreats share a general, rather elaborate architecture (Fig. 1a) with the following characteristics: (i) two chambers, one housing the catchnet and one housing the insect, and (ii) the water entrance hole is at the end of a silken or sand grain tube that leads into the two-chamber area. Here we report an alternate retreat design constructed by some Macrostemum carolina (Banks) individuals in the Savannah River, Georgia and South Carolina.

Macrostemum carolina is widely distributed throughout the southeastern United States, and has been recorded west to Texas (Moulton & Stewart 1997) and north to New York (Ross 1944). In coastal plain streams with shifting sand streambeds, M. carolina primarily inhabits submerged snags (i.e. fallen trees or branches), gouging the base of their retreats out of the wood and covering the top of the structure with silk. In their original description, Wallace & Sherberger (1974) noted that some M. carolina individuals in the Apalachicola River construct a second, slightly different retreat than the one described above. This alternate retreat lacks a silken tube and simply has the entrance hole open into the chamber area (Fig. 1b) (some Macrostemum zebatum (Hagen) individuals construct a similar, alternate retreat (see Wallace 1975)). In the Savannah River, M. carolina individuals construct the two retreats described above as well as a third type with yet a different entrance hole configuration. The entrance hole of this third retreat also lacks a silken tube and instead has a ~180° silken backstop, with the other ~180° essentially flush with the top of the retreat (Fig. 1c). These backstops vary in size, from 3-8 mm in height, though some of this variation is positively correlated with instar (G. R. P., personal observation). Macrostemum carolina is common in the Savannah River (Cudney & Wallace 1980), and each retreat morph is regularly encountered. Individuals of a single morph are often clustered on snags, although the “flush” phenotype is generally the most prevalent (G. R. P., personal observation).

Although these three retreat morphs are discrete behaviors (though see below), the individuals in the Savannah River represent a single, panmictic population (Plague et al., in press). Therefore, retreat construction in M. carolina is either: (i) phenotypically plastic, with environmental cues influencing retreat design (e.g. Emlen 1994), (ii) genetically polymorphic, with alternative alleles at a retreat gene (or genes) controlling the design (e.g. Hori 1993), or (iii) partially heritable, i.e., a combination of genetic and plastic control (e.g. Roff 1986). Whichever is the case, natural selection probably plays a role in maintaining the alternative phenotypes (Hartl & Clark 1997, Futuyma 1998). The adaptive value of each design is likely related to the maintenance of adequate water flow through the retreat, and specifically the net. Therefore, each morph may be adapted to a particular microhabitat on the snag. For
example, “flush” retreats may be located primarily on upstream snag locations and therefore receive direct water flow into the retreat; “backstop” retreats may be located on the tops and bottoms of snags (relative to water flow), with the backstop helping to
divert water into the retreat; and “tube” retreats may occur on the downstream side of snags, reaching over the top or bottom of the snag to face into the current. Unfortunately, because snags are generally flexible and often 50 cm or more under water, assessing a retreat’s exact in situ location and orientation is often difficult. Also, water flow over a snag is undoubtedly extremely complex (Hart et al. 1996), thereby exposing different microhabitats on the snag to similar water flow regimes. Therefore, microflow location, and not simply microhabitat location, is likely a more important selective force in maintaining these alternative morphs.

Sattler & Kracht (1963) and Wallace & Sherberger (1975) proposed that the tube retreats in *Macrostemum ulmeri* (Banks) and *Macrostemum transversum* (Walker), respectively, function as Pitot tubes (L-shaped open tubes used to measure fluid velocity), essentially pulling more water (and therefore more food) through the retreat than would flow through passively. This pulling action results from equalizing the pressure differential between the vertical entrance hole (relatively high pressure) and the horizontal exit hole (relatively low pressure). The tube and backstop retreats of *M. carolina* may similarly function as Pitot tubes. If so, these two retreat designs may represent a phenotypic continuum within a single behavior, with the primary fitness differences between them being correlated with the amount of time (which equates to lost feeding time and increased exposure to predators) and energy expended to construct each design; the tube retreat type is presumably more costly on both counts. Therefore, *M. carolina* may actually exhibit only two discrete retreat morphologies: (i) flush entrance hole and (ii) structured entrance hole, with the latter expressing a range of phenotypes.

We thank Gabe Novak for an insightful discussion about the physics of net-spinner retreats, Juanita Blocker and Angela Lindell for helping produce Figure 1, and two anonymous reviewers for improving the manuscript. Manuscript preparation was supported by Financial Assistance Award Number DE-FC09-96SR18546 from the U.S. Department of Energy to the University of Georgia Research Foundation.

**SUMMARY**

In the Savannah River, larval *Macrostemum carolina* caddisflies make three different retreats, each with a distinct water entrance hole: (i) flush with the top of the retreat, (ii) at the end of a silken tube, and (iii) with a ~180° silken backstop. Herein we describe the “backstop” retreat (the others have been described previously), and discuss possible selective advantages of each retreat phenotype.

**REFERENCES CITED**


Erratum

Erratum


The statement “Florida Experiment Station Journal Series Number R-07782” was inadvertently left out of the Acknowledgements.

I am an enthusiast about ladybird beetles. When forthcoming publication of Robert Gordon’s monumental taxonomic review of the ladybird beetles of America north of Mexico (Gordon 1985) was announced, I joined the New York Entomological Society to obtain a copy. It allowed me, for the first time, to identify specimens reliably. Dixon’s book, in contrast, gives insight into the behavior of ladybird beetles and into the interdependence of their life history parameters, based upon analysis of quantitative data. The two works together (Gordon 1985 and Dixon 2000) give the entomologist reader a sound basis for identifying and understanding ladybird beetles in America north of Mexico. Neither of the two works is intended for the general public.

The book contains 10 chapters, an epilogue, references, and two indices: one taxonomic, and the other on behavior, ecology, structure and physiology. Where chapters in some other books have an introductory abstract, each chapter in this book has a conclusion, not labelled as such, but printed in a sans-serif font; this conclusion is highly worthwhile.

Chapter 1 (Introduction) briefly documents the author’s contention that there is a need to evaluate the considerable body of work that now exists on coccinellid behavior. Chapter 2 (Basic biology and structure) deals with the phylogeny of the coccinellid subfamilies, life cycle, external and internal structure, development, survival, reproduction, overwintering, and defense. Here the coccinellid groups that feed on mites, aleyrodids, psyllids, fungi, and higher plants are mentioned briefly, allowing the rest of the book to concentrate on contrasts between the aphidophagous species (about 67%) and coccidophagous species (about 17%).

Chapter 3 (Body size) deals with all the correlates and implications of body size, and Chapter 4 (Slow-fast continuum in life history parameters) does much the same for speeds of movement, development time, and fecundity. Chapters 5 (Foraging behavior), 6 (Cannibalism), 7 (Theory of predator-prey interactions), and 8 (Intraguild predation) are the core of the book, addressing the subjects that the ecologist would expect to find in a book about Coccinellidae. Cannibalism, far from being disastrous for a population, might be viewed as a means of harvesting prey; there is a parallel here with the killing behavior shown by larval Toxorhynchites (Diptera: Culicidae) to excess prey.

Chapter 9 (Biological control) begins with the objectives of biological control, reminds us that use of Rodolia cardinalis (Coccinellidae) was the formative successful example of biological control in the USA, and then opens up a discussion of the ongoing controversies within biological control. These are: conflicts of interest, and the relationship of biological control to faunal and floral conservation. The chapter points out first that there is yet no proof that use of coccinellids in biological control has had a negative effect on non-target faunas, despite much suspicion to the contrary, and second that use of coccinellids has been used successfully to protect native plants against attacks of adventive (“non-native”) pests. The chapter continues with other aspects of biological control using coccinellids (augmentative and cultural control), and then addresses integrated pest management. Chapter 10 is a too-brief summation, pointing out, however, that coccidophagous ladybirds develop, reproduce, and age more slowly than do aphidophagous ladybirds.
Quality control in this book is good. The designer of the cover got away with a typographical error in the caption of the illustration of the back cover, but the author may have had no control over this. Remarkably, I found no typographical errors within the text. I reject the spelling “predaceous” used in this book, on authority of the Oxford English Dictionary (which spells the word “predacious” and explains why), and I question use of the word data in some places as singular (“data is”) and in some places as plural (“data are”). The illustrations (all in black and white) are good and the price is reasonable for this wealth of thoughtful information.

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References Cited


MENDEZ, E. 2000. Insectos y otros artrópodos de importancia médica y veterinaria. Privately published in Panama. vii + 341 p. ISBN 1-57504-023-9. Paperback. $30.00 + $5.00 postage and packing by registered airmail from Dr. E. Méndez, Apartado postal 870317, Zona 7, Panamá, REPUBLIC OF PANAMA.

This book is unique. Most books on “medical entomology” and “veterinary entomology” weight the space devoted to arthropod groups by the relative importance of the diseases transmitted to humans, livestock, and pet vertebrate animals by those arthropods. They are written from the viewpoint of training a medical or veterinary practitioner. Many of them thus fail to mention, or give little attention to, the arthropods that bite and sting or are otherwise venomous and cause problems for the health of humans, livestock, and pet animals. They emphasize the major vectors of disease (mosquitoes, ticks, triatomine bugs, fleas, and phlebotomite sandflies) and they almost ignore non-triatomine bugs, other families of flies, blister and other beetles, urticating caterpillars, poisonous spiders, millipedes, centipedes, scorpions, wasps, ants, bees, and pentastomids. This book is instead a natural history of the arthropod groups that have any implication for “medical” and “veterinary” entomology as well as some of their relatives. It is written from the entomological, rather than medico-veterinary, perspective. From the medico-veterinary perspective, the diseases transmitted by arthropods are what matters, and diagnosis and treatment of the diseases are the stock-in-trade, so that knowledge about the arthropods is a minor part of diagnosis (and may fail), although it necessarily is a major part of prevention (and this is where the typical medico-veterinary training may be inadequate). From the entomological perspective, the appropriate training of a “compleat medical entomologist” begins with general entomology, progresses to the identification, behavior, natural history, and control methods for all arthropods having any implication for the health and welfare of vertebrate animals (including humans), and finally concentrates on arthropods that transmit diseases and imparts knowledge of the diseases and their ethiology. This book’s author presents his information from the latter viewpoint.

An argument for this latter viewpoint is a recent medical diagnosis of an affliction to the hand of the wife of a friend. The initial medical diagnosis was that she had been
bitten by a recluse spider (*Loxosceles* sp.). Fortunately, she obtained a second opinion which was of a blood clot plus shingles. If the initial examining physician had been better informed about the distribution of recluse spiders, through a book similar to this one, the initial erroneous diagnosis could have been avoided, appropriate treatment could have commenced earlier, and the patient would have been in less danger of gangrene!

This book is written from its author’s experience of arthropods in Panama because he was for many years an employee of the Gorgas Memorial Hospital. It includes also examples from other continents. It is written in Spanish. The habitus illustrations are the author’s own work, and they are very well executed. This book deserves wide circulation in Latin America, where it is highly relevant. There is, unfortunately, no comparable book in English: entomologists, physicians and veterinarians have to use one of the existing books on medical or veterinary entomology, few of which give much coverage of non-disease-transmitting arthropods, and these professionals are therefore left floundering for want of adequate information. The September 2000 issue of *Florida Entomologist* has reviews of books on “medical entomology”, none of them comparable to this one. I am not aware of comparable books on “veterinary entomology.”

This book is completed by a glossary, references cited, and an index. It has some typographical errors. Some of the information, such as claims that *Amblyopinus* and other genera of Amblyopinina (Coleoptera: Staphylinidae) are ectoparasites of rodents (p. 192), is no longer considered correct.

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