USE OF RADIATION TO STERILIZE TWO-SPOTTED SPIDER MITE (ACARI: TETRANYCHIDAE) EGGS USED AS A FOOD SOURCE FOR PREDATORY MITES

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ABSTRACT

One-, 2- and 3-day-old two-spotted spider mite eggs were treated with increasing doses of gamma radiation ranging from 0-280 Gy. Percent egg hatch decreased as radiation increased for each age group; however, older eggs required higher doses of radiation to prevent egg hatch than did younger eggs. Based on the regression lines for 1-, 2- and 3-day-old eggs, the best estimates of the doses of radiation that would prevent 100% of the eggs from hatching were 43.6 Gy, 55.1 Gy and in excess of 280 Gy, respectively. In general, irradiating spider mite eggs had no significant effect on their acceptability as prey by females of the predatory mite *Neoseiulus californicus* McGregor, except for 1-day-old eggs treated at 240 Gy. Female *N. californicus* consumed 50-75% fewer of these eggs than they did eggs of other treatments, in both no-choice and choice experiments.

Key Words: *Tetranychus urticae*, *Neoseiulus californicus,* biological control, rearing

RESUMEN

Huevecillos de *Tetranychus urticae* de uno, dos y tres dias de edad fueron tratados con dosis de radiación gamma entre 0 y 280 Gy. En general, el porcentaje de eclosión de los huevecillos tratados disminuyó proporcionalmente al aumento en la dosis de radiación en huevecillos de todas edades, sin embargo, los huevecillos de mayor edad requirieron mayores dosis para prevenir eclosión. Basados en las líneas de regresión obtenidas en estos experimentos, las dosis requeridas para prevenir el 100% de eclosión en huevecillos de *T. urticae* son de 43.6 Gy, 55.1 Gy y mas de 280 Gy, para huevecillos de uno, dos y tres dias de edad, respectivamente. En general, la irradiación de huevecillos no tuvo un efecto significativo en cuanto a su aceptabilidad como alimento para hembras de *Neoseiulus californicus* McGregor, exceptuando en huevecillos de un dia de edad tratados con 240 Gy. Las hembras de *N. californicus* consumieron 50-75% menos de este tratamiento, tanto en experimentos donde tuvieron opción de escoger, como en experimentos donde no tuvieron opción de escoger entre varios tratamientos como alimento.

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The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is an extremely polyphagous pest that has been reported from more than 900 host species and is described as a serious pest of at least 30 economically important agricultural and ornamental plants, including corn, cotton, cucumber, beans, tomato, eggplant, peppers and roses (Helle & Sabelis 1985a, b; Navajas 1998). Unfortunately, chemical control of this pest can be compromised because of resistance (Gould et al. 1982; Croft et al. 1984; Cranshaw & Sclar 2001). As a result, a more integrated approach utilizing biological control with predatory mites is increasingly being recommended (Hamlen & Lindquist 1981; Osborne et al. 1985; Grafton-Cardwell et al. 1997; Nicetic et al. 2001).

Biological control of spider mites has centered on the use of predatory mites in the family Phytoseiidae (Helle & Sabelis 1985a, b; Schausberger & Croft 1999). *Phytoseiulus persimilis* Athias-Henriot is the most studied species and is the primary species used to control spider mites in greenhouses (Osborne et al. 1985; UF/IFAS 2002).

Another promising predatory mite is *Neoseiulus* (=*Amblyseius*) *californicus* (Chant) (Castagnoli & Simoni 1999; Roy et al. 1999). Although less specialized than *P. persimilis*, it has been shown to provide excellent control of spider mites over a wide range of climatic and management conditions (Oatman et al. 1977; Pickett & Gilstrap 1986; McMurtry & Croft 1997). In the United States there are five species of predatory mites that are available commercially and used in biological control programs: *Galendromus* (=*Metaseiulus*) *occidentalis* (Nesbitt), *Mesoseiulus* (=*Phytoseiulus*) *longipes* (Evans), *N. californicus*, *N. fallicus* (Garman), and *P. persimilis* (UF/IFAS 2002).

Castagnoli and Simoni (1999) showed that the long-term feeding history of *N. californicus* affects its functional and numerical responses when exposed to different densities of two-spotted spider mite eggs and protonymphs. In general, *N. californicus* that were wild collected or routinely reared on spider mites performed better than those reared on pollen or dust mites (*Dermatophagoides* spp.). Because of studies such as this, commercial shipments of predatory mites usually contain spider mite eggs as a food source (Osborne et al. 1985; Penn 1999). The eggs are easy to handle and help insure that the predatory mites are in good condition when they arrive.

A problem with the use of live host material in commercial shipments of predatory mites (or other natural enemies) is the risk of introducing new pest species or strains, including chemically resistant strains, along with the natural enemy (Penn 1999). One solution to this concern that has been discussed but never tested is the use of radiation to reproductively sterilize host material prior to shipment.

Although radiation has been used extensively for many years to sterilize insects used in sterile insect release programs (IAEA 2000), there is limited published literature on the radiation biology of spider mites. Henneberry (1964) irradiated two-spotted spider mite adults and examined the effects on male and female fertility and their progeny. Feldmann (1975) studied the induction of heritable sterility factors such as translocations and inversions in the two-spotted spider mite at different doses of gamma radiation and confirmed the holokinetic nature of their chromosomes. Nothing is known about the effect of radiation on spider mite eggs.

In an effort to determine the potential for using irradiated spider mite eggs as a food source for predatory mites, studies were initiated with the following objectives: (1) determine the dose of gamma radiation that would prevent specific age groups of *T. urticae* eggs from hatching; and (2) assess the impact of egg age and exposure to gamma radiation on acceptability as a food source by the predatory mite, *N californicus*.

Materials and Methods

Experimental Material and Site

Lima bean (*Phaseolus lunatus* L.) leaves and two-spotted spider mites were obtained from the North Florida Research and Education Centers at Monticello and Quincy, FL. Bean plants were started from seeds sown in Perlite® planting medium every 2-3 days and kept in a greenhouse until they reached the four-true leaf stage. They were then transferred to a humid cement-block room, where they were infested with spider mites and kept for 7-10 days before being discarded and replaced with fresh plants.

Irradiation of spider mite eggs was conducted at the USDA-ARS Crop Pest Management and Research Unit in Tifton, Georgia, using a $Cobalt^{60}$ Gammacell 220 Irradiator® with a dose rate of approximately 20.06 Gy/min.

The experiments were carried out in the USDA-ARS Laboratory at the Florida A&M University Center for Biological Control, Tallahassee, FL. All experiments were set-up at ambient room temperature and relative humidity (approximately 21-24°C and 60-65% RH). Controlled conditions after experimental set-up were maintained using a Forma Scientific Growth Chamber® with a photoperiod of 12:12 (L:D), temperature of 28 ± 1°C, and relative humidity of 58%. Under these conditions, normal egg hatch began four days after oviposition.

Neoseiulus californicus were purchased from IPM Laboratories of New York, placed in 15 cm plastic petri dishes with fresh bean leaves, and fed spider mites *ad libitum* until needed for tests.

Dose Response

Approximately 25 gravid female two-spotted spider mites were transferred to freshly excised, young, bean leaves devoid of spider mites and other arthropods and allowed to oviposit for 18-24 h. The leaves were prevented from drying out by placing them on moist filter paper in covered 7.0 cm plastic petri dishes and kept in a growth chamber as specified above. After 24 h, the petri dishes containing the bean leaves were placed under a dissecting microscope and all motile stages of spider mites removed, leaving only newly oviposited (0-24 h old) eggs. The bean leaves were then cut into pieces containing 25 eggs and each piece placed separately in the center of a new 7.0 cm petri dish containing moist filter paper.

Petri dishes containing 1-day-old (0-24 h) eggs were transported by car (approximately 2 h) in a small cooler to Tifton, GA, and irradiated. Petri dishes containing eggs that were to be irradiated at 2- (24-48 h) and 3-days (48-72 h) of age were kept in the growth chamber for an additional 1 and 2 days, respectively, before transport to Tif-

ton. Once irradiated, petri dishes and eggs were immediately driven back to Tallahassee, FL, and placed in a growth chamber as before. Petri dishes were checked daily for egg hatch. Newly eclosed larvae were removed, and the number of larvae and remaining eggs recorded. Egg hatch in each petri dish was monitored for a period of 2 weeks from the time egg hatch began. Filter paper in the petri dishes was moistened as needed throughout the course of the experiment.

Spider mite eggs were treated with increasing doses of radiation until none of the eggs hatched. The 1-day-old eggs were treated with the following doses of radiation during June 2001: 0, 10, 20, 30, 40, and 50 Gy. Two-day-old eggs were exposed to 0-60 Gy at intervals of 10 Gy from June to September 2001, and 3-day-old eggs were exposed to 0 to 140 Gy at intervals of 10 Gy and 160 to 280 Gy at intervals of 20 Gy from June to November 2001. Four replicate petri dishes of 25 eggs were used at each dose. Dose response for this experiment was analyzed with polynomial regression analysis (Damon & Harvey 1987; SAS Institute 1994). Before analysis, proportion data were transformed with an arcsine square-root transformation. Backtransformed data are presented in regression equations.

Predation Study, No-Choice Test

Fresh spider mite eggs were obtained as previously described by placing 25-30 gravid females on clean bean leaves and allowing them to oviposit for 18-24 h. The bean leaves were cut into 2 cm square pieces containing 25 eggs. One- and 3-day-old eggs were then irradiated at 0, 40, 140 or 240 Gy. Following irradiation, the filter paper in a given petri dish was moistened to the point of standing water and a few drops of detergent were added to prevent mites from leaving the leaf disc. A single adult female *N. californicus*, which had been starved for 24 h, was added to each arena. Each female was allowed to feed for 24 h, after which the number of eggs eaten was recorded. Twenty replicates of both 1- and 3-day-old eggs at each treatment dose were conducted. The data were analyzed by ANOVA, and means separated using Duncan's Multiple Range Test (SAS Institute 1994).

Predation Study, Choice Test

Two-spotted spider mite eggs were obtained and treated as above, except that bean leaves were cut into pieces 2 cm × 1 cm containing 10 eggs. Following irradiation (0, 40, 140 and 240 Gy), one leaf strip containing 10 1-day-old eggs was fitted next to a leaf strip containing 10 3-day-old eggs that had been treated with the same dose of radiation. Petri dishes were prepared as before, and a single adult female *N. californicus* that had been starved for 24 h was added to the center of each 2 cm × 2 cm arena. Each female was allowed to feed for 24 h, and the numbers of eggs eaten were recorded. Twenty replicates at each treatment dose were conducted. Analysis of the data was done by determining the preference of 1-day-old eggs over 3-day-old eggs (=number of 1-day-old eggs consumed minus the number of 3-day-old eggs consumed) for each pairing. Data were analyzed by paired difference t-test (SAS Institute 1994).

Results

Dose Response

Within each age group percent egg hatch decreased as the dose of radiation increased (Fig. 1). Egg hatch for the three age groups showed significant curvilinear responses to radiation. For each age group, there appeared to be a threshold beyond which the magnitude of the radiation effect decreased as dose increased. Significant differences in the slopes of the regressions indicate that

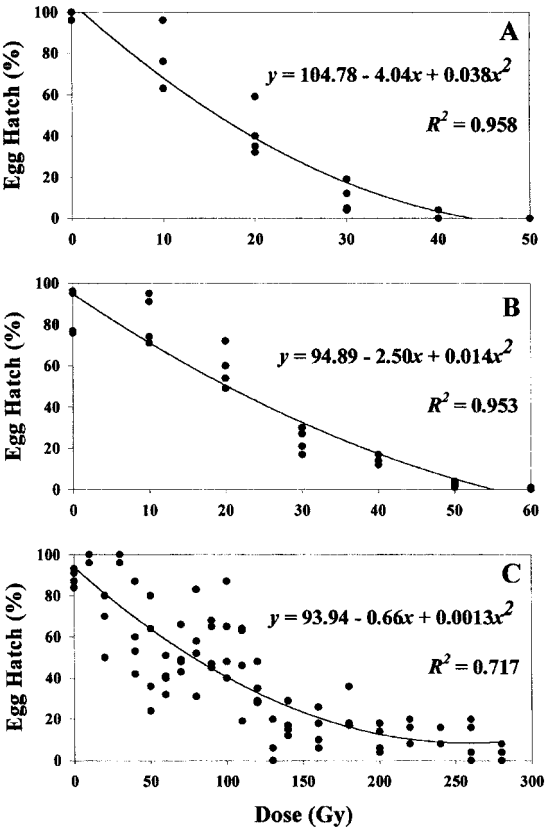

Fig. 1. Eclosion dose response curves for two-spotted spider mite eggs that were irradiated when they were (A) 1-, (B) 2-, or (C) 3-days-old with increasing doses of gamma radiation. Note different scales on *x*-axes.

one-day-old eggs were more sensitive to radiation ($y = 104.78 - 4.04x + 0.038x^2$, $R^2 = 0.958$, df = 2, 21, $n = 24$, $P = < 0.0001$) than were 2-day-old eggs ($y = 94.98 - 2.50x + 0.014x^2$, $R^2 = 0.953$, df = 2, 25, $n = 28$, $P < 0.0001$), and that 3-day-old eggs were the most resistant to radiation ($y = 93.94 - 0.66x + 0.0013x^2$, $R^2 = 0.717$, df = 2, 85, $n = 88$, $P < 0.0001$). Irradiation of 1-day-old eggs at 40 Gy resulted in a 1.0 ± 1.0% egg hatch, and at 50 Gy no eggs hatched. Irradiation of 2-day-old eggs at 40 Gy resulted in 14.0 ± 1.0% egg hatch; 50 Gy resulted in 3.0 ± 1.0% egg hatch, and at 60 Gy 0.3 ± 0.3% of the eggs hatched. Irradiation of 3-day-old eggs at 60 Gy resulted in 41.0 ± 4.0% egg hatch. Even at 280 Gy, 5.0 ± 1.9% of the eggs hatched, although none of the hatching individuals survived past the second larval instar.

Predation Study, No-Choice Test

At 40 and 240 Gy female *N. californicus* consumed a greater number of 3-day-old spider mite eggs than 1-day-old eggs (Table 1). Within an age class, irradiation of the eggs did not affect the number of eggs eaten except at the highest dose, 240 Gy, for the 1-day-old eggs.

Predation Study, Choice Test

When *N. californicus* females were given a choice between 1- and 3-day-old spider mite eggs that had been exposed to either 0, 40, 140, or 240 Gy of gamma radiation, the paired t-test indicated that there was no preference for 1- or 3-day-old eggs, except at 240 Gy, where 3-day-old eggs were preferred over 1-day-old eggs (Table 2).

Discussion

The results show that it is possible to use gamma radiation to prevent two-spotted spider mite eggs from hatching. The results also showed that irradiated eggs are still acceptable as a food source to the predatory mite *N. californicus*. As such, irradiated spider mite eggs could be used to provision shipments of predatory mites to eliminate concerns that the shipments are contaminated with reproductively viable pest material.

Gamma radiation treatment had a negative effect on egg hatch overall, with egg hatch decreasing as dose increased. One- and 2-day-old eggs were much more sensitive to radiation than were 3-day-old eggs. Based on the regression lines for 1- and 2-day-old eggs, the best estimates of the doses of radiation that would prevent 100% of the eggs from hatching are 43.6 Gy and 55.1 Gy, respectively. Although 280 Gy, the highest radiation level tested against 3-day-old two-spotted spider mite eggs, did not prevent 100% of the eggs from hatching, none of the hatching individuals survived past the second instar.

Not only were older eggs much less sensitive to radiation, but the variation in the dose response (i.e., the percentage of eggs hatching at a given dose) increased with egg age. This can be seen in the R^2 values for the regression lines, which were 0.958, 0.953 and 0.717 for 1-, 2- and 3-day-old eggs, respectively. Why older eggs were less sensitive to radiation and showed a greater variation in dose response is not known. The egg stage in the two-spotted spider mite only lasts about 4 days at 28°C, so many developmental and physiological changes are occurring during this time. Radiation dose response experiments that controlled egg age more precisely (e.g., used eggs that were laid over 1 h intervals rather than 24 h intervals) would likely show less variation. If such experiments were coupled with studies on the developmental changes occurring in two-spotted spider mite eggs, particularly between 2 and 3 days of age, they might provide insights as to why older eggs are more radio-resistant.

In general, irradiating two-spotted spider mite eggs had no significant effect on their acceptabil-

Table 1. Mean (±SD) number of spider mite eggs consumed per adult female *N. californicus* in 24 h in a no-choice test, when provided with 25 1- or 3-day-old eggs that had been exposed to 0, 40, 140 or 240 Gy of gamma radiation.

	Mean no. eggs eaten	
Dose (Gy)	1-Day-Old	3-Day-Old
0	7.7 ± 3.95 A, a[1]	9.0 ± 4.27 A, ab
40	6.3 ± 2.14 A, a	10.8 ± 4.05 B, a
140	7.6 ± 3.91 A, a	10.0 ± 5.38 A, ab
240	2.4 ± 2.34 A, b	6.7 ± 3.01 B, b

[1]Means followed by different capital letters within a row or lower case letters within a column are significantly different at $P < 0.05$ using Duncan's Multiple Range Test.

Table 2. Mean (±SD) number of spider mite eggs consumed per adult female *N. californicus* in 24 h in a choice test, when provided with 10 1-day-old and 10 3-day-old eggs that had been exposed to 0, 40, 140 or 240 Gy of gamma radiation.

	Mean no. eggs eaten	
Dose (Gy)	1-Day-Old	3-Day-Old
0	6.00 ± 2.17 a[1]	5.60 ± 2.52 a
40	4.05 ± 2.41 a	3.75 ± 1.50 a
140	3.55 ± 2.68 a	4.55 ± 2.45 a
240	2.40 ± 2.03 a	6.00 ± 3.07 b

[1]Means followed by different letters within a row are significantly different at $P > 0.05$ using a paired difference *t*-test.

ity as prey by female *N. californicus*, except for 1-day-old eggs treated at 240 Gy. Female *N. californicus* consumed 50-75% fewer of these eggs than they did eggs of other treatments, in both no-choice and choice experiments. Research has shown that phytoseiid mites determine prey acceptance primarily by contact chemoreception (Dicke et al. 1988; Vet & Dicke 1992). Our research did not attempt to determine what factors are assessed by *N. californicus* to determine prey (egg) acceptability or what biochemical or physiological effects radiation was having on spider mite eggs, other than reducing egg hatch. However, it should be noted that 240 Gy was 5-6 times the dose of radiation needed to prevent egg hatch of 1-day-old eggs. A similar drop in acceptability might have been seen with 3-day-old eggs if a dose of radiation that was 5-6 times their lethal dose had been used (280 Gy × 5 = 1,400 Gy). If this were true, one explanation for these results might be that female *N. californicus* are able to assess egg viability and prefer healthy live prey to dead prey; thus, eggs that were irradiated at a dose well in excess of the dose that would prevent them from hatching were less preferred.

The fact that irradiated spider mite eggs were still acceptable as prey to *N. californicus* was not unexpected. Non-viable lepidopteran eggs resulting from irradiated parents have been shown to be acceptable as developmental hosts for trichogrammatid egg parasitoids (Cossentine et al. 1996; Carpenter et al. 2003). Not only could irradiated spider mite eggs be used as a risk-free, high quality food source in shipments of predatory mites, but they could also be used to maintain predatory mite colonies that are free of spider mites and yet avoid the quality problems associated with rearing them on non-host foods such as pollen. Future studies should attempt to rear *N. californicus* over multiple generations on irradiated spider mite eggs and compare demographic effects (e.g., longevity, reproductive potential, etc.) with those when using other food sources or host stages.

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PERFORMANCE OF STERILE *CACTOBLASTIS CACTORUM* (LEPIDOPTERA: PYRALIDAE) FEMALES IN LURING MALES TO TRAPS

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ABSTRACT

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) is renown for its control of invasive cacti (*Opuntia* spp.). Its accidental arrival in Florida and its rapidly expanding range along the Gulf coast pose an imminent threat to native *Opuntia* spp., especially in the southwestern U.S. and Mexico. Adequate survey techniques are crucial in order to delineate the rate of spread of this invasive species. Virgin female-baited sticky traps have been effective in detecting *C. cactorum* adult males in areas where visual surveys failed to detect larval damage. However, the use of fertile females in traps placed beyond the currently infested area is discouraged because an escaped fertile female might establish a breeding population and expand the infested area. In this study we compare the attractiveness and the longevity of fertile and irradiated (sterile) females deployed as bait in traps. Traps baited with females sterilized with gamma radiation were as effective as traps baited with unirradiated (fertile) females in detecting populations of feral *C. cactorum* male moths.

Key Words: invasive species, *Opuntia*, cactus moth, survey, SIT

RESUMEN

Cactoblastis cactorum (Berg) (Lepidoptera: Pyralidae) es un insecto bien conocido por su efectividad como agente de control biológico de especies invasoras de cactus (*Opuntia* spp.). La llegada accidental de *C. cactorum* al estado de Florida y su rápida expansión a lo largo de la costa del Golfo de Méjico, representan una amenaza real para las especies nativas de *Opuntia* spp., especialmente en areas del suroeste de los Estados Unidos y Méjico. El desarrollo de técnicas adecuadas de detección es de suma importancia para poder delinear la distribución y expansión de esta especie. Trampas que utilizan hembras vírgenes como cebo atractivo han sido efectivas en la detección de machos de *C. cactorum* en areas donde no se ha detectado la presencia de esta especie por daño en plantas. Sin embargo, el uso de hembras vírgenes fértiles como cebo en trampas colocadas fuera del area de infestación no es recomendable debido a que si las hembras se escapan podrian establecer un nuevo foco de infestación. En este estudio, la atractividad y longevidad de hembras fértiles como cebo en trampas se comparó con la atractividad y longevidad de hembras irradiadas (estériles). Las trampas con hembras estériles resultaron igualmente eficaces en su habilidad de detección y captura de machos silvestres de *C. cactorum*.

Translation provided by author.

The cactus moth, *Cactoblastis cactorum* (Berg) (Lepidoptera: Pyralidae) is renown for its control of invasive cacti (*Opuntia* spp.) in Australia (Dodd 1940), and it has been cited as one of the most successful examples of biological control of weeds (Sweetman 1936). However, the accidental arrival of *C. cactorum* in Florida (Habeck & Bennett 1990; Dickel 1991), first detected in 1989, has raised concerns about its potential impact on native *Opuntia* in the southern United States and Mexico (Johnson & Stiling 1998; Zimmermann et al. 2001). Pemberton (1995) estimated that infestations of *C. cactorum* should be able to survive as far north as Charleston, South Carolina, San Antonio, Texas, and the lower altitude areas of New Mexico, Arizona and California. Current distributional information published in Hight et al. (2002) suggests that the range of *C. cactorum* is expanding by 50-75 km per year. Although specific interactions cannot be predicted at this time, establishment of *C. cactorum* in the southwestern U.S. and Mexico could have devastating effects on the landscape and biodiversity of native desert ecosystems, and on the forage and vegetable *Opuntia*

industries in these areas (Soberón et al. 2001; Zimmermann et al. 2001).

No satisfactory method of control has been identified for *C. cactorum* (Habeck & Bennett 1990; Stiling 2002). Because many of the *Opuntia* species in the U.S. are associated with sensitive ecological areas, widespread use of pesticides is not recommended (Leibee & Osborne 2001). The use of insect pathogens does not appear to hold promise, as most Lepidopteran viruses and parasitic nematodes have non-selective modes of action and could negatively impact other native Lepidoptera present in the area of infestation. In its native habitat in South America, a number of natural enemies have been found attacking *C. cactorum*, including members of the families Braconidae, Ichneumonidae, Chalcididae and Tachinidae (Habeck & Bennett 1990; Pemberton & Cordo 2001). However, most of the species are generalist parasitoids and their host range and potential non-target effects would have to be carefully scrutinized before their release would be approved in the U.S. (Pemberton & Cordo 2001). Our research is focusing on the potential application of the Sterile Insect Technique (SIT) and the phenomenon of inherited or F_1 sterility to help study and manage the spread of *C. cactorum* (Carpenter et al. 2001a, b). SIT/F_1 sterility is a species-specific pest control tactic that could be used to eradicate new or localized infestations, protect environmentally sensitive areas, or establish a barrier to prevent further geographic range expansion.

The ability to quickly detect new infestations, accurately delimit the size of an infestation (i.e., the leading edge of an expanding population), and assess population trends are of critical importance to the successful application of any strategy using SIT/F_1 sterility. Unfortunately, although females produce a pheromone that attracts males, no synthetic pheromone has been identified yet for *C. cactorum*, which makes continuous insect monitoring especially difficult. Hight et al. (2002) reported on the use of sticky traps baited with virgin female *C. cactorum* to corroborate field damage and better understand the current distribution of the species in Florida and Georgia. However, trapping beyond the leading edge of the currently infested area using fertile females is not recommended. Here, we compare the ability of laboratory-reared, virgin fertile females to attract male *C. cactorum* into sticky traps with that of females treated with a sterilizing dose of 200 Gy. The results are discussed in the context of developing an SIT program for *C. cactorum* management in the U.S. and elsewhere.

Materials and Methods

Test Insects

Cactoblastis cactorum used in these experiments came from a laboratory colony maintained at the USDA-ARS Crop Protection and Management Research Unit in Tifton, Georgia. Larvae were reared on cladodes of *Opuntia ficus-indica* (L.) Miller inside plastic boxes that were kept at 26°C ± 1°C, a photoperiod of 14:10 (L:D), and 70% relative humidity as described by Carpenter et al. (2001b). As larvae matured, cocoons were collected every 2-3 days from the containers. Pupae were then extracted from the cocoons and sorted by gender.

Three "types" of adult virgin females were tested in the field for their ability to attract *C. cactorum* males to sticky traps: untreated control females (Ua♀), females that were treated with a reproductively sterilizing dose of radiation as pupae (Tp♀), and females that were irradiated and sterilized as adults (Ta♀). Untreated control females were obtained by placing pupae in a screen cage (30.5 by 30.5 by 30.5 cm) and allowing them to emerge at the above mentioned conditions. Sterile females that were treated as pupae were obtained by holding pupae in a 473 ml plastic container until pharate adults had formed inside the pupal skins. Mature pupae that were within 12 h of emerging were placed in individual 30 ml plastic cups and irradiated with 200 Gy of gamma radiation using a Cobalt^{60} Gammacell 220 irradiator (J.L. Shepherd & Associates, San Fernando, CA; dose rate of 15.47 Gy/min). Treated pupae were then held at the conditions described above and allowed to emerge. Sterile females that were treated as adults were obtained by placing pupae in a screen cage as above (for control females) and allowing them to emerge. Fully eclosed females were removed every 24-36 h, placed individually in 30 ml plastic cups, and irradiated with 200 Gy as described for pupae. For all groups, only adult females that were less than 48 h old were used in the trapping experiments.

Traps and Sites

Females, either untreated (Ua♀), treated as pupae (Tp♀), or treated as adults (Ta♀), were placed individually inside modified plastic film (35 mm photographic) canisters and used to bait Pherocon 1-C wing traps. The film canisters had two 2 by 2 cm screened windows cut into them and were provisioned with a small square of *O. ficus-indica*. A cotton wick was fitted through a hole cut in the top of the canisters to provide the females with moisture. Velcro® glued to the bottom of the canisters allowed the canisters to be attached to the inside tops of the wing traps, which also were fitted with Velcro® (Fig. 1). Canisters with females were transported to the field in a small cooler.

Experiments were conducted in the proximity of a salt marsh estuary on the southern banks of the Brunswick River in Glynn County, Georgia, west of U.S. Highway 17. A large area was chosen

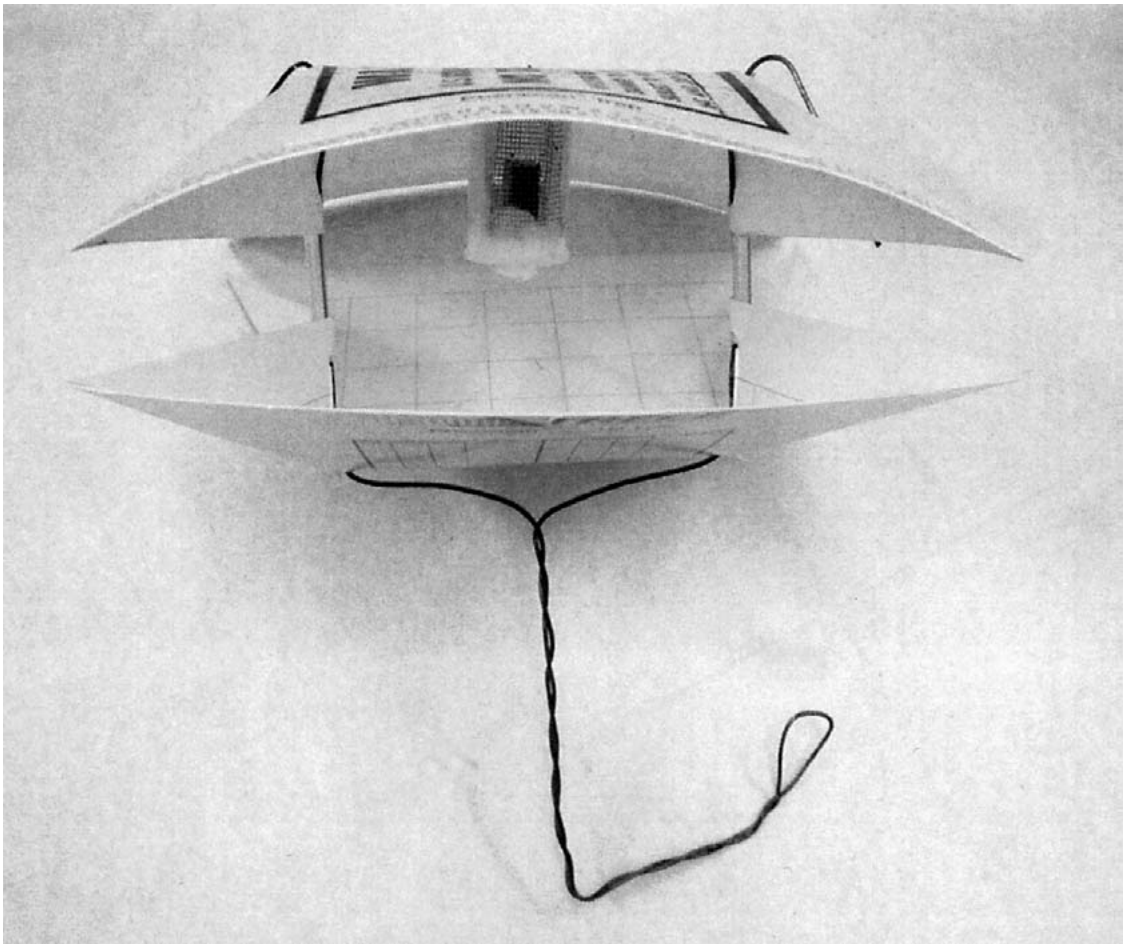

Fig. 1. Photograph of Pherocon 1-C sticky trap.

within the estuary with naturally occurring patches of *O. stricta* (Haworth) Haworth plants. Ten patches with cactus plants between 0.5-1.5 m in height were selected for the experiments. Two or three hollow metal stakes were placed in the ground at a height of approximately 0.75 m within each patch on which to attach the traps. All of the cactus patches were separated from one another by at least 10 m. The trap stakes within a patch were separated by no less than 4 m.

Overall Trap Performance

Ten Pherocon 1-C traps baited with Ua♀'s and 10 baited with Ta♀'s were deployed on 11 April 2003 and serviced every three days until 1 May 2003. Two traps, one with each type of female, were placed in each of the 10 cactus patches. During each trap servicing, the number of *C. cactorum* males captured in each trap was recorded, the traps were re-baited with canisters containing fresh Ua♀ and Ta♀, and the placement of the two treatments within each patch was alternated. The traps were serviced a total of six times.

Daily Trap Captures and Female Field Longevity

Ten untreated females (Ua♀), 10 females that had been treated with 200 Gy as mature pupae (Tp♀) and 10 females that had been treated with 200 Gy as newly emerged adults (Ta♀) were used to bait 30 Pherocon 1-C traps. Three traps, one of each type (Ua♀, Tp♀, Ta♀), were affixed to the metal stakes at random within each of the 10 cactus patches. Traps were first deployed on 19 July 2003. Cactus patches were visited every 24 h and each trap was examined to determine whether the female was alive and how many male *C. cactorum* had been captured. Traps that captured males received a new sticky bottom. Traps were then rotated clock-wise among the three trap positions within each cactus patch. Daily observations, trap servicing, and trap rotation continued until all females died.

Statistical Analysis

Trap capture data for both experiments were not normally distributed. Since $\log_{10}$ and arcsine transformations did not normalize the data, the GLM-RANK procedure was used to test for treatment effects in the daily trap capture and field longevity study, and the GLM-TTEST procedure for unequal variances was used to test for treatment effects on trap capture in the overall trap performance study (SAS 1989). The mean longevity of females used as bait in traps was analyzed using GLM and the Waller-Duncan K-ratio *t* test (SAS 1989).

Results and Discussion

In the overall trap performance trial, where traps were checked for captures every three days, we found no significant difference ($P \geq 0.2353$) between the mean (±S.D.) number of males captured in traps baited with untreated females (Ua♀ = 3.20 ± 4.24) and those baited with sterile females (Ta♀ = 3.25 ± 4.33). In the daily trap capture study, no significant differences ($P \geq 0.1039$) were seen in the overall mean (±S.D.) number of male *C. cactorum* captured in Pherocon 1-C traps baited with Ua♀ (10.7 ± 7.3), Tp♀ (7.2 ± 5.5), or Ta♀ (7.5 ± 6.7). The trend in cumulative trap captures for the traps baited with the three female types also was similar (Fig. 2). In addition, the mean (±S.D.) longevity in days for control and treated females used to bait the traps was not significantly different ($P > 0.6504$). Untreated females lived an average of 6.70 ± 0.72 d. Treated females irradiated as mature pupae or as adults lived an average of 7.56 ± 0.80 d and 7.60 ± 0.81 d, respectively.

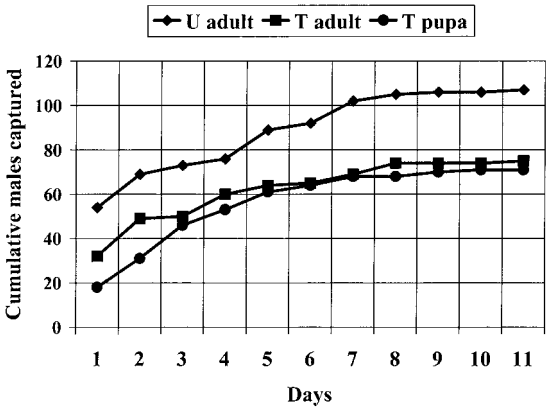

Fig. 2. Cumulative total *C. cactorum* males captured in sticky traps (n = 10 per female type) baited with *C. cactorum* females that were untreated (U adult), treated with a reproductively sterilizing dose of radiation (200 Gy) as pupae (T pupa), and treated with a reproductively sterilizing dose of radiation (200 Gy) as adults (T adult). All females were caged in the trap for the length of their life.

Hight et al. (2002) reported finding infestations of *C. cactorum* along the coast as far north as Folly Island near Charleston, South Carolina and as far west as St. George Island, Franklin County, Florida. Several previously unreported inland infestations were also identified in Orange and Osceola Counties halfway "up" the Florida peninsula. *Cactoblastis cactorum* infestations were discovered by looking for damaged cladodes exhibiting mucilage from larval entry holes or "whitened" cladodes that had fallen to the ground (Stiling 2002). Surveys are often visually based on large *Opuntia* species that are common in yards, such as *O. ficus-indica* and *O. stricta*. However, searching for damage on smaller *Opuntia* species that are common in natural areas and roadsides is difficult because they are often hidden in native vegetation. Virgin female-baited sticky traps were able to detect the presence of *C. cactorum* at a beach site with numerous low growing *O. pusilla* (Haworth) Haworth but where no larval damage was yet evident. During 2003, several additional surveys for plant damage by *C. cactorum* were conducted along the west coast of Florida (St. Joe Peninsula, Mexico Beach, Panama City, and Pensacola). Based upon sightings of larval damage in *O. ficus-indica* and *O. stricta*, the current westward limit of *C. cactorum* infestation is at Pensacola Beach on the west end of Santa Rosa Island in Escambia County (Hight et al. 2003).

The results suggest that the use of females sterilized with radiation will be just as effective as unirradiated (fertile) females in detecting populations of feral *C. cactorum* male moths. Reliable use of traps baited with irradiated females will allow for more widespread monitoring and surveying of areas beyond the current known limit of *C. cactorum* distribution without fear of establishing a new breeding population if the traps, once deployed, are vandalized or destroyed by people or wildlife. Based on our data for mean daily trap captures (Fig. 3) and female longevity, traps baited with irradiated females could be serviced once per week because females continued to attract males into the traps until they died. Although the number of males captured per living female was greatest on day 1, this does not necessarily mean that 1-d-old females are more attractive than older females. The male capture on day 1 could have been influenced by the presence of more males on that day. Additional trapping studies are ongoing to investigate the attractiveness of females at different ages, and the effectiveness of different trap densities, trap types, and trap heights.

The efficiency of the virgin female-baited traps relative to the absolute number of *C. cactorum* males present in a given area has not yet been determined. However, experiments to address this

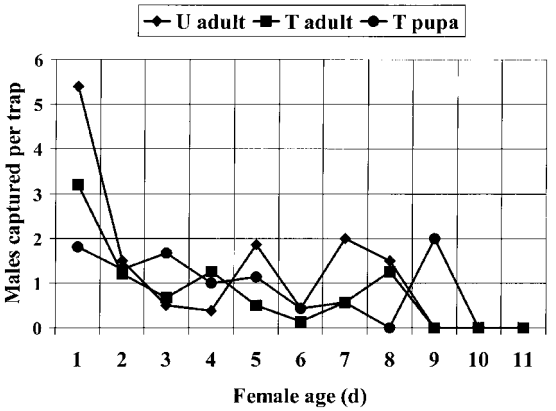

Fig. 3. Effect of female age and female treatment on the ability of *C. cactorum* females to lure male *C. cactorum* males into sticky traps (n = 10 per female type). Females were untreated (U adult), treated with a reproductively sterilizing dose of radiation (200 Gy) as pupae (T pupa), and treated with a reproductively sterilizing dose of radiation (200 Gy) as adults (T adult).

question are planned for late 2003 and 2004 in South Africa and Florida. Release-recapture studies with marked male *C. cactorum* and estimates of population density for feral *C. cactorum* will be used to determine sticky trap efficiency.

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CACTOBLASTIS CACTORUM (LEPIDOPTERA: PYRALIDAE): OBSERVATIONS OF COURTSHIP AND MATING BEHAVIORS AT TWO LOCATIONS ON THE GULF COAST OF FLORIDA

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ABSTRACT

Cactoblastis cactorum (Berg) has become an invasive pest of *Opuntia* spp. along the coastal areas of southeastern United States from the panhandle of Florida to South Carolina. Spread of this insect into cactus dominated natural areas of the United States and Mexico and into agricultural opuntia fields of Mexico is raising concerns within international governments and conservation organizations. Interest is growing in using the Sterile Insect Technique (SIT) to manage *C. cactorum* populations. Information on courtship and mating behaviors of this insect is important in the development and application of SIT. We conducted mating table studies and determined that this moth exhibits simple rather than elaborate mating behaviors and that courtship and mating take place briefly during morning twilight. Typically, females initiate calling, males respond to females, and copulation are complete before sunrise. Successfully mated females attract males within a short period (mean of 5.2 min), while unsuccessful females continue calling for about 40 minutes. Mating pairs remain in copula for a mean of 31.8 min. Generally, mated females are busy ovipositing the first few nights after mating, not exhibiting additional mating behaviors. A release of marked males revealed that males stay near the release site and can be recovered and identified for subsequent population estimate studies. This study on courtship/mating behavior is helpful to the ongoing *C. cactorum* research to develop a successful SIT program, identify the female calling pheromone, improve monitoring traps, and develop a technique to estimate adult moth population abundance.

Key Words: cactus moth, invasive pest, *Opuntia*, sterile insect technique

RESUMEN

Desde su accidental llegada al estado de Florida, *Cactoblastis cactorum* (Berg) se ha convertido en una especie invasora atacando especies de *Opuntia* a lo largo de areas costeras del sureste de los Estados Unidos desde Florida hasta Carolina del Sur. La invasión de *C. cactorum* tanto en areas naturales con predominancia de cactáceas en Estados Unidos y Méjico como en plantaciones agrícolas de *Opuntia* en Méjico estan causando gran preocupación a gobiernos internacionales y a organizaciones que se ocupan de la conservación de recursos biológicos. Sin embargo, el interés en utilizar la Técnica del Insecto Estéril (TIE) para controlar poblaciones de *C. cactorum* esta aumentando simultaneamente. Para desarrollar y aplicar la TIE de manera efectiva es importante obtener información sobre el cortejo y comportamiento de cópula de este insecto. En este estudio de campo determinamos que el comportamiento de cópula de *C. cactorum* es relativamente simple y que el apareamiento ocurre durante un periodo bastante corto, justo antes del amanecer. En general, las hembras comienzan a llamar a los machos, los machos responden y la cópula se inicia y termina antes de que salga el sol. Las hembras que logran copular típicamente llaman a los machos por corto tiempo (5.2 min), mientras que las hembras que no se aparean continuan llamando por 40 minutos. La cópula dura un tiempo promedio de 31.8 minutos. En general las hembras que copulan la primera noche se dedican a ovipositar durante la noche siguiente y no se involucran en actividades de cortejo. Realizamos una liberación de machos coloreados con polvo fluorescente que demostró que los machos permanecen en las areas donde fueron liberados y que pueden ser identificados sin problema al copular con hembras en mesas de cortejo. Nuestros resultados son útiles para el desarrollo de la TIE y asimismo para la identificación de la feromona de cópula, para mejorar el sistema de trampeo y para desarrollar un método para calcular el tamaño de la población absoluta de esta especie.

Translation provided by author

The control of invasive cacti in the genus *Opuntia* by the cactus moth, *Cactoblastis cactorum* (Berg), is often cited as the most famous example of successful classical biological control of weeds (Dodd 1940; Moran & Zimmermann 1984). However, the unintentional arrival of *C. cactorum* into Florida in 1989 (Habeck & Bennett 1990) has raised concerns for the survival of rare native *Opuntia* in the Florida Keys (Johnson & Stiling 1996). Of even greater concern is the potential westward spread of *C. cactorum* into areas of the United States and Mexico that are rich in *Opuntia* diversity (Soberón et al. 2001; Zimmermann et al. 2000; Stiling 2002). Recently, Hight et al. (2002) reported on the expanding range of *C. cactorum* in North America. By summer 2002, the moth had spread as far north as Folly Island near Charleston, South Carolina and as far west as St. George Island, Florida (Hight et al. 2002). In a 2003 survey of the western Florida panhandle, we found the new western limit of *C. cactorum* at Pensacola Beach, Florida, near the border with Alabama.

Even though the worldwide successes of *C. cactorum* as a biological control agent of weedy *Opuntia* have been carefully documented (Sweetman 1936; Dodd 1940; Pettey 1948; Julien & Griffiths 1998), little information on the insects' mating habits is available. Dodd (1940) reported that mating of *C. cactorum* in Australia took place during the early morning hours, from daylight until about 0730 hours, and that copulation was never observed at night or after 2100 hours. He also stated that adults of *C. cactorum* usually remained inactive during daylight hours and sat motionless in vegetation near their host plants. Pettey (1948) reported that *C. cactorum* mating at Uitenhage, South Africa only occurred early in the morning, during daylight of the first and second days after adult emergence. He reported that moths were active only after sunset until a little after sunrise, except in areas where temperatures were high.

The purpose of the present study was to document the courtship and mating behaviors of *C. cactorum* in Florida. In particular, we were interested in precisely documenting the field behaviors associated with mating, as we are investigating the possibility of using the Sterile Insect Technique (SIT) to manage populations of *C. cactorum* in the United States (Carpenter et al. 2001a). This technique relies on the ability of mass reared, irradiated, and released insects to effectively compete and mate with a feral population. Knowledge of the targeted species mating behavior is of crucial importance to the development and successful application of the SIT. We also are developing trapping technology that would allow more extensive and efficient surveys to be conducted for *C. cactorum*. An improved understanding of courtship and mating behaviors would be useful in improving trap design and in identifying pheromones associated with the sexual communication of this species.

Materials and Methods

Test Insects

Cactoblastis cactorum used in these experiments were reared in laboratory colonies at the USDA-ARS laboratories in Tallahassee, FL and Tifton, GA. Rearing procedures generally followed those described in Carpenter et al. (2001b). Cocoons were collected every 2-3 days from colony containers. Pupae were extracted from the cocoons and sorted by gender. Sorted pupae were placed in a screen cage (30.5 × 30.5 × 30.5 cm) or individually into 0.3 ml plastic cups with cardboard lids and allowed to emerge inside growth chambers at 26°C, a photoperiod of 14:10 (L:D), and 60% relative humidity. Virgin females (<24 h post emergence) were placed individually in small plastic cups and kept in a refrigerator (5°C) to slow their physiological ageing and activity. In the laboratory, two-thirds of one anterior wing of each female was excised with small scissors to prevent flight. Each female was returned to its plastic cup and transported to the field in an open cooler under natural light. Newly emerged males were collected and placed as a group in a 475 ml plastic container. Males were chilled, colored with fluorescent powder (Day Glo® Corp., Cleveland, OH), and transported to the field under natural light in a small cooler.

Mating Tables

Individual mating tables were similar to the ones described by McBrien & Judd (1996) with the following exceptions: the diameter of the mating arena was 17.5 cm to accommodate the larger sized female *C. cactorum*, the height of the Teflon® tape barrier was 5 cm, the Teflon® tape barrier was lightly dusted with talc, and the tables did not have roofs (Fig. 1). Communal mating tables were constructed on a base of plywood (61 × 61 × 1.5 cm) that was painted gray (Valspar American Tradition, oil based paint, light gray, #48220). A circular Teflon® tape barrier (50 cm diameter × 5 cm high) was glued to the arena and dusted with talc. Four metal legs (0.5 m high) were attached to the plywood base with metal brackets (Fig. 2).

Patches of cactus plants between 0.50-1.50 m in height were selected for placement of both individual and communal mating tables. Individual mating tables were attached to hollow metal stakes placed in the ground at a height of approximately 0.75 m and located at the edge of the cactus patches. Communal mating tables (mounted on their legs) were also placed next to cactus patches.

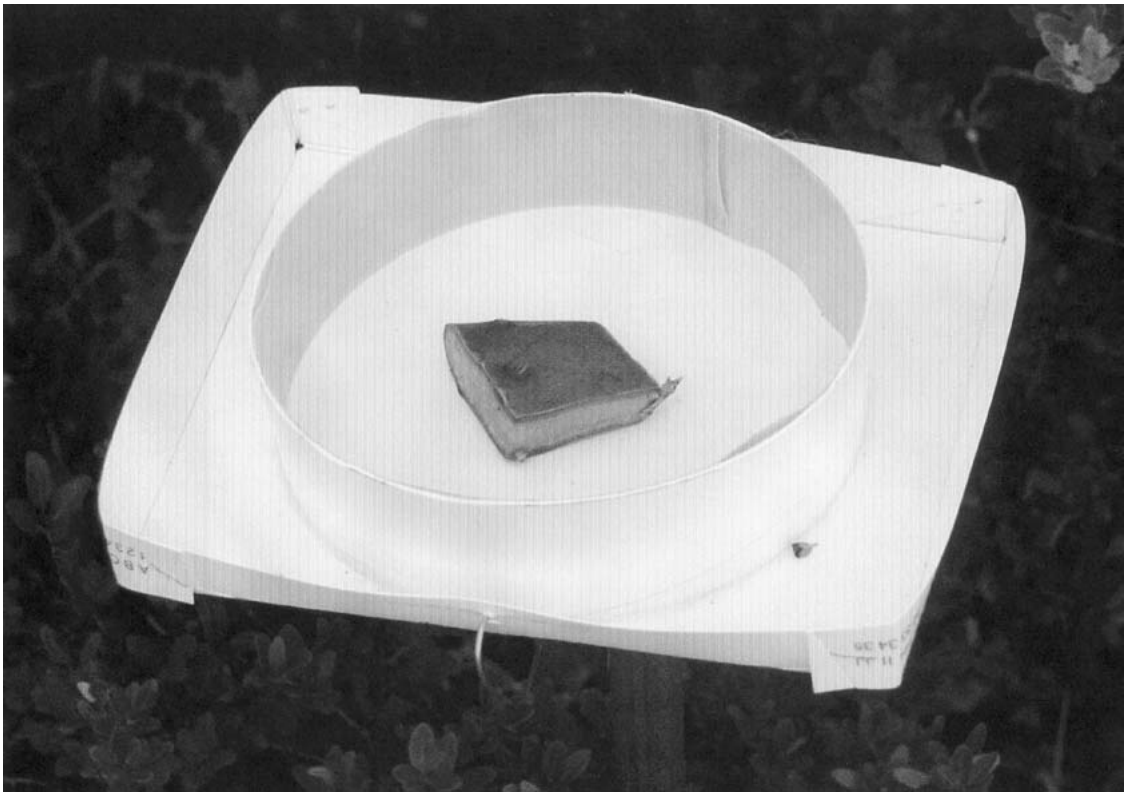

Fig. 1. Small mating table used in determining courtship and mating behaviors at St. Marks National Wildlife Refuge (7 July 2003) and Alligator Point, FL (8 July 2003), and determining precise timing of mating events at Alligator Point (15-18 July 2003).

Mating tables were set-up in the same fashion for each set of observations. A small (2 × 2 cm) section of *O. stricta* was placed in the middle of the mating arena of each individual table. One, clipped-wing, virgin female *C. cactorum* was released into each arena. In communal tables, several cladodes of fresh *O. stricta* were placed inside the arena and 7-12 clipped-wing females were placed in the center of each mating arena. Time of female deployment varied for each experiment. All times reported are in Eastern Daylight Savings Time on a 24-hour atomic clock.

Study Sites

Experiments were conducted in July 2003 at two locations in Florida along the Gulf of Mexico—St. Marks National Wildlife Refuge (N30°04', W84°10') and Alligator Point (N29°54', W84°23'). Abundant naturally occurring patches of native *Opuntia stricta* (Haworth) Haworth heavily damaged by *C. cactorum* are present at both locations. Infested *O. ficus-indica* (L.) Miller is also common among houses at Alligator Point as a planted and naturalized species. At St. Marks, the plants are located along a dike that separates the Gulf of Mexico and a salt marsh estuary. Twenty individual mating tables and two communal mating tables were established at St. Marks, each separated by no less than 10 m from one another.

At Alligator Point, plants of *O. stricta* and *O. ficus-indica* are distributed along open (un-fenced) front-yards of beach houses along the Gulf of Mexico. For the first set of observations (morning of 8 July), 12 individual mating tables and a single communal table were placed near infested cactus patches. Each table was separated from one another by no less than 3 m. For the second set of observations (mornings of 15-18 July), 15 mating tables were placed in groups of five around three heavily infested cactus patches in the same vicinity as those used for the first observations. Tables within a group were separated from each other by about 1 m.

Mating Behavior

At St. Marks, 47 marked male *C. cactorum* were released along the dike on the opposing side of mating tables. Releases were made at 1730 hours on 6 July 2003 to insure that males would

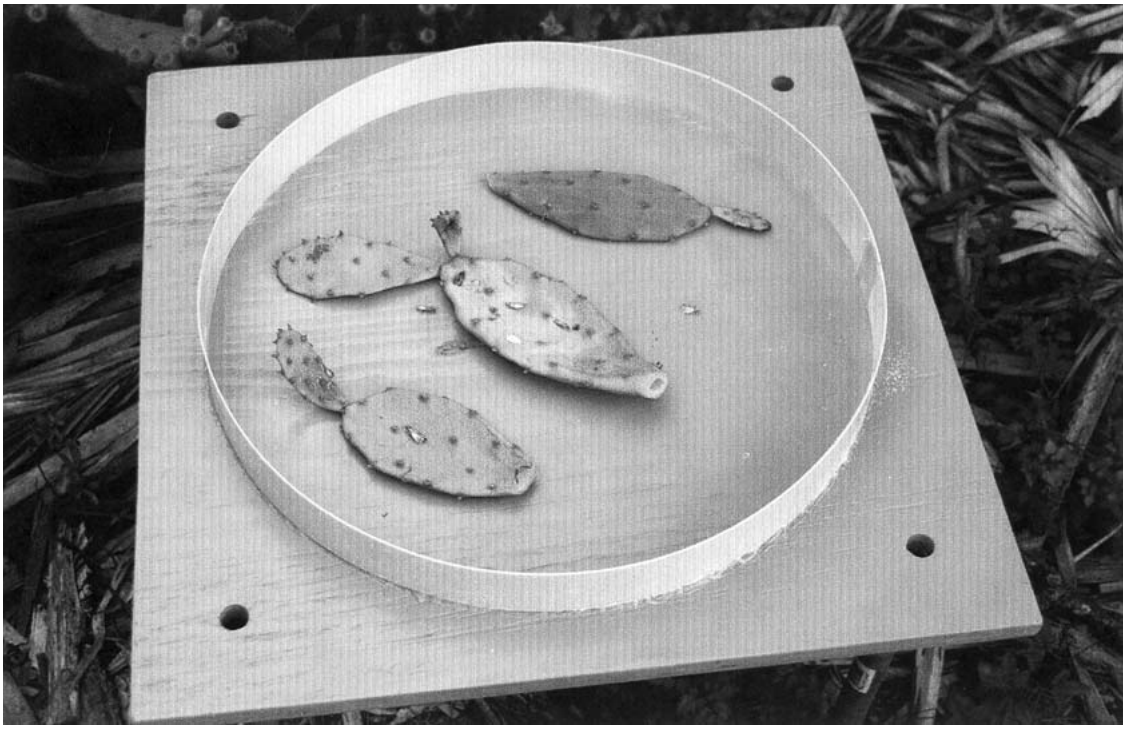

Fig. 2. Communal mating table used in determining courtship and mating behaviors at St. Marks National Wildlife Refuge (7 July 2003) and Alligator Point, FL (8 July 2003).

be present in the area. The minimum distance between male release points and the location of the mating tables was 10 m. Females were placed in individual and communal mating tables at 2000 hours and tables were observed every hour from 2100 hours until 0700 hours on 7 July 2003. Moth activity was observed using flashlights with red lenses. Moths found in copula were collected into small plastic cups and the hour noted during which each mating pair was collected. Insects were transported back to the laboratory and the type of each male (i.e., marked or wild) captured in copula was identified using ultraviolet light to detect the presence or absence of the Day Glo® dye. The total number of mating pairs recorded from individual and communal mating tables and the type of male involved in each mating (feral or released) was determined. Female mating status was confirmed by determining the presence or absence of a spermatophore in the bursa copulatrix as suggested by Ferro & Akre (1975).

After the first night of observations at St. Marks, the general timing of mating activities for *C. cactorum* was determined. Observations at Alligator Point were modified to take advantage of these findings. Forty-one marked males were released at Alligator Point at 2200 hours on 7 July 2003. Twelve females were placed in individual mating tables and 12 additional females were placed in the communal table at 2130 hours. Mating tables were checked every 5-10 minutes between 0500-0700 hours on 8 July 2003 and all mating activities observed were recorded. Pairs in copula were collected in plastic cups and taken to the laboratory where male type and female mating status were confirmed.

Precise Timing of Mating Events

Fifteen individual mating tables were set-up as described above on four consecutive nights (14 to 17 July 2003) at Alligator Point to more accurately document the duration of all events associated with *C. cactorum* mating. Newly emerged, clipped-wing virgin females were prepared each day and placed in the mating arenas between 2200-2400 hours. Observations began at 0500 hours each morning and continued uninterrupted until all mating activities ceased. The following mating behavior events were recorded: time female initiated calling posture, time female terminated calling posture, time first male responded to calling female, time last male responded to calling female, time copula was initiated, and time copula was terminated. Verification of successful copula was confirmed in the laboratory by the presence of a spermatophore in the female upon dissection. Light intensity was measured in

the early morning hours of 18 July with a HOBO data logger (Onset Computer Corp., Pocasset, MA).

Female Refractory Period

Females that successfully mated at Alligator Point during the precise timing experiments were observed on subsequent mornings to determine whether they produced an eggstick, resumed a calling posture, and/or were attractive to males. The five females that mated on the morning of 15 July 2003 were placed on individual mating tables at the same time and in the same manner as each group of 15 new females on evenings of 15-17 July 2003. Three females that mated on the morning of 16 July 2003 and six females that mated on the morning of 17 July 2003 were observed on the morning of 18 July 2003. Any eggsticks that were produced were collected, the time of oviposition noted, and the number of eggs counted.

Results

Weather conditions for each morning's observation were relatively similar. Skies were mostly clear, temperatures were 25-27°C, and relative humidity was 95-100%. Rain never occurred during our observation periods and winds were variable, differing most mornings in relation to speed and/or direction.

Mating Behavior—St. Marks National Wildlife Refuge

No mating activity was observed between 2100 hours on 6 July 2003 and 0500 hours on 7 July 2003. At each hourly observation, females were motionless and most were perched on host plant material. However, when observations were made during the 0600 hours check on 7 July 2003, 89% of the females (32 of 36) were found to be engaged in courtship/mating activities and males were observed flying around the mating tables. Twenty-one females (58%) were positioned in a typical calling posture (abdomen protruding upwards through the wings and held at an angle approximating 45°), eleven females (31%) were found in copula and four females (11%) were still inactive. Seven of the copulating females were in individual tables and four in the communal tables (three in one and one in the other). By 0625 hours, all mating pairs had disengaged from one another.

Mating tables were again visited at 0630 hours and the number of females observed in the calling position had decreased to 15 (42%). Males could still be seen flying around the area, but no additional mating pairs were formed. When tables were checked at 0700 hours only three females (8.33%) remained in the calling position and no males were observed flying in the vicinity of the tables. When *C. cactorum* pairs were examined under UV light, six males were identified as belonging to the released group while the remaining five were feral males. Dissection confirmed that all copulating females had a spermatophore in the bursa copulatrix.

Mating Behavior—Alligator Point

Even though mating tables were under almost continuous observation from 0500 hours on 8 July 2003, no courtship/mating activities were observed until 0545 hours when 6 of 22 females assumed a calling posture. Two females placed on the communal mating table became entrapped in excessive dew and were not included in the reported outcomes. In total, nine mating pairs (41% of observed females) were collected on 8 July 2003. The first mating pair was found at 0545 hours. Thereafter, mating pairs were observed at 0550 hours (2 pair), 0552 hours (1 pair), 0553 hours (2 pair), 0611 hours (1 pair), and 0612 hours (2 pairs). Females continued to call until 0647 hours. During the entire observation period, three (14%) females did not participate in courtship/mating activities. Males and females remained in copula for a short period of time. Most pairs disengaged from one another in less than 30 min (range 14-29 min). When captured pairs were examined in the laboratory, all males were determined to be feral. Dissection confirmed that eight of nine mated females retained a spermatophore in the bursa copulatrix.

Precise Timing of Mating Events

A temporal description of *C. cactorum* courtship and mating behaviors observed during the mornings of 15-18 July 2003 is summarized in Table 1. Events related to the rising sun during these mornings are also presented in Table 1. All courtship and mating activities were concentrated during a two-hour period (0528-0733 hours), beginning each day between astronomical and nautical twilight when skies had just started to lighten. Activity ended soon after sunrise. The majority of *C. cactorum* completed all measured courtship/mating events before sunrise, including initiation of female calling (100%), male response to female (100%), initiation of copula (100%), copula termination (96%), and female calling termination (77%). In fact, the mean time between the initiation of calling behavior by females and the last male seen responding to the females was only 16 min (0602-0618 hours). Light intensity measured each minute during the evening/morning of 17/18 July 2003 was negligible from 2054 to 0628 hours and did not increase until 0629 hours when the intensity was measured at 43 lum/m^2.

Initiation of calling posture by females was immediately followed by the response of males (flying around the mating tables, landing inside the mating arenas, and attempting copulation with

TABLE 1. TEMPORAL DESCRIPTION OF COURTSHIP AND MATING BEHAVIORS OF *Cactoblastis cactorum*, AND SUNRISE EVENTS AT ALLIGATOR POINT, FL, 15-18 JULY 2003. SUNRISE EVENTS CALCULATED FROM U.S. NAVAL OBSERVATORY WEBSITE <HTTP://AA.USNO.NAVY.MIL/>. TIMES ARE REPORTED IN EASTERN DAYLIGHT SAVINGS TIME ON 24-HOUR CLOCK.

Behaviors	n	Time (hours) or duration (min)	
		Range	Mean (±SD)
Initiation of ♀ calling posture	54	0528-0624	0602 (9 min)
Termination of ♀ calling posture	31	0610-0711	0644 (12 min)
Response of first ♂ to calling ♀	35	0540-0631	0603 (10 min)
Response of last ♂ to calling ♀	20	0603-0635	0618 (9 min)
Initiation of copula	25	0540-0615	0601 (10 min)
Termination of copula	23	0610-0733	0633 (16 min)
Duration of calling for ♀ that did not mate	31	12-66	40.5 (13.0)
Duration of calling for ♀ that mated	23	1-17	5.2 (4.2)
Duration of copula	23	18-113	31.8 (18.4)
Sunrise Events[1]			
Sunrise	4	0646-0648	0647 (1 min)
Civil Twilight begins	4	0619-0621	0620 (1 min)
Nautical Twilight begins	4	0547-0549	0548 (1 min)
Astronomical Twilight begins	4	0512-0514	0513 (1 min)

[1]Definitions of these events were derived from (Seidelman 1992): Sunrise = time when the Sun's upper edge of the disk is on the horizon; Civil Twilight = begins in the morning when the center of the Sun is geometrically 6 degrees below the horizon; Nautical Twilight = begins in the morning when the center of the sun is geometrically 12 degrees below the horizon; Astronomical Twilight = begins in the morning when the center of the Sun is geometrically 18 degrees below the horizon.

the females). We did not observe any elaborate courtship behavior by the male after landing next to the female nor prior to attempting copulation. Mating pairs were formed soon after the male landed next to the female. In a few instances, females moved away from the male. Females successful at attracting males remained in the calling posture for a short time (mean of 5.2 min). Unsuccessful females continued calling for 40 min. Mating pairs remained in copula for a short time period (mean of 31.8 min), however, one pair remained in copula for 113 min. Females that did not secure a mate remained in the calling posture beyond the time when males were seen flying near the mating tables.

Figure 3 displays the proportion of females (n = 57) involved in calling or mating activities over time. The time between 0606 and 0645 hours was when the highest proportion of females was observed to be in a calling posture. The period between 0601 through 0635 hours was when the greatest number of females was found to be in copula.

Female Refractory Period

Thirteen of the 14-mated females produced an average of 1.4 eggsticks/female their first night after mating. The eggsticks averaged 37 eggs/eggstick. Only one female exhibited calling behavior on its first morning after mating. This female did not attract a male and died by the next morning without producing an eggstick, although dissection revealed successful mating had occurred. On the second night after mating, only three of the eight mated females produced eggsticks; one eggstick/female averaging 28 eggs/eggstick. One female called for 71 min without attracting a male. This female had produced two eggsticks its first night after mating but did not produce an eggstick after its second calling event. Of the five females that mated on the morning of 15 July and followed a third night/morning, two females produced two eggsticks (mean of 19 eggs/eggstick), one female died, and two females were idle.

DISCUSSION

Behaviors associated with courtship and mating in Pyralidae vary from elaborate and interactive sequences to simple straightforward behaviors. For example, males attract females through acoustic signaling from song perches, such as in *Symmoracma minoralis* Snellen (Heller & Achmann 1995). Stationary males of *Galleria melonella* (L.) produce 0.5 to 1 s bursts of wing fanning and are approached by attracted females (Flint & Merkle 1983). *Ephestia elutella* (Hübner) males approach pheromone-producing females, engage in head-to-head posturing while positioning their abdominal scent structures in close proximity to the female antennae and attempt copulation from the head-to-head position (Phelan & Baker 1990). Other species of Pyralidae exhibit very simple courtship behaviors, with

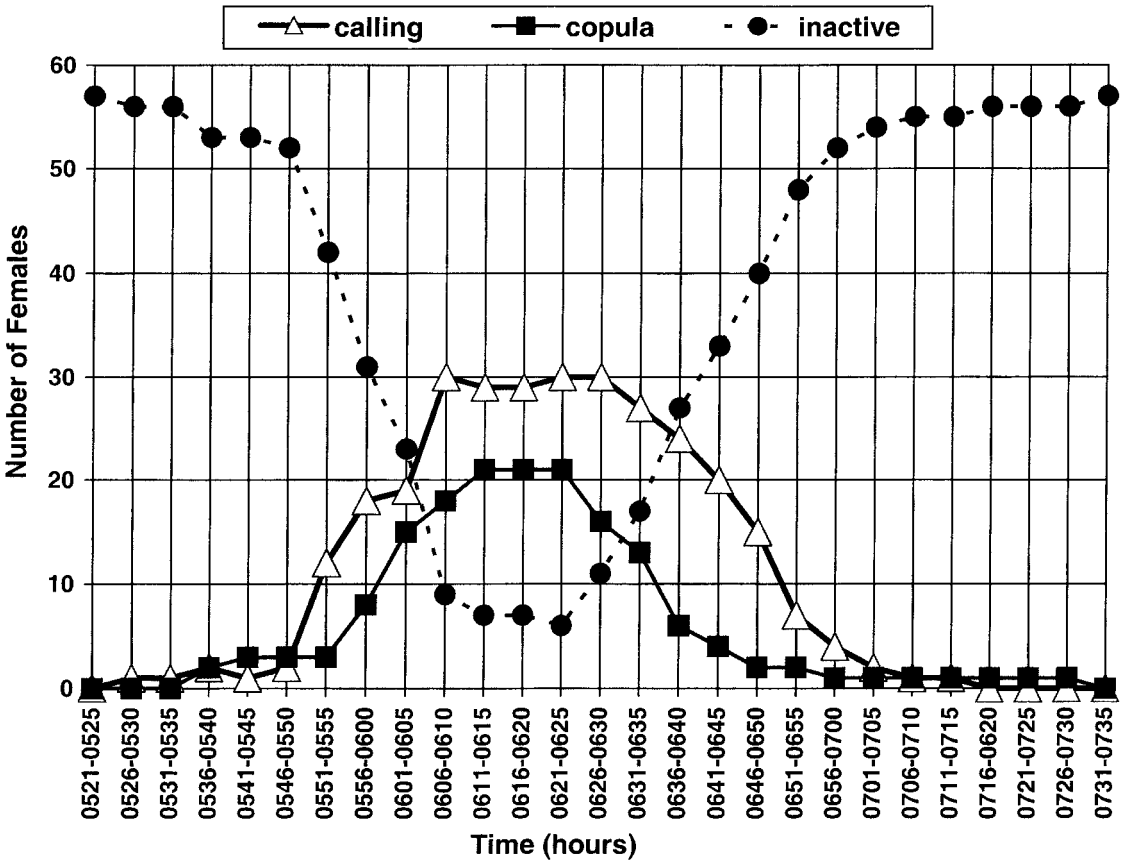

Fig. 3. Average times (in 5 min intervals) when females exhibited various courtship and mating behaviors at Alligator Point, FL during the mornings of 15-18 July 2003.

males locating pheromone-emitting females and quickly attempting copulation by lateral abdominal thrusts under the female wing without any behavioral embellishments, such as in the navel orange worm, *Amyelois transitella* (Walker) (Phelan & Baker 1990).

Our results indicate that mating behavior in *C. cactorum* closely matches the description for simple courtship behavior given by Phelan & Baker (1990). In our field studies, the initiation of calling posture by virgin female *C. cactorum* inside mating arenas was almost immediately followed by the response of males (flying around the mating tables, landing inside the mating arenas, and attempting copulation with the females) and the formation of mating pairs. The behavior sequences observed for *C. cactorum* closely match observations described for the lesser mulberry pyralid (*Glyphodes pyloalis* Walker) by Seol et al. (1986). They reported that the random flight of males continued for several tens of seconds after the females were first observed in a calling position and that males and females were observed in copula almost immediately after encountering one another.

With respect to timing of sexual activity, Wysoki et al. (1993) studied the reproductive behavior of the honeydew moth [*Cryptoblabes gnidiella* (Millière)]. They found that mating in this pyralid occurred 1-2 h before dawn (beginning at 0345 hours and ending around 0530 hours) and that duration of copulation averaged 100 min (range 70-145 min). Peak periods of sexual activity in the pyralids studied by Phelan & Baker (1990) varied in their distribution from 0-2 h subsequent to the initiation of scotophase to 2-0 h prior to the initiation of photophase. Vetter et al. (1997) reported that female carob moth (*Ectomyelois ceratoniae* Zeller) initiate calling in the fourth through seventh hour of scotophase and all calling terminates during the first hour of photophase. Carob moths mate during the fourth and eighth hours of scotophase and pairs remain in copula for an average of 2.35 ± 0.84 h. Flint & Merkle (1983) reported that Greater wax moth adults remain in copula for only a few minutes, yet, upon dissection, 82% of the female moths had sperm in the spermatheca.

Our observations on *C. cactorum* identified that no mating activity occurred during the scoto-

phase between 2100 hours and 0500 hours, 6-18 July 2003. During the 4 mornings of detailed observations, moth courtship/mating activities were restricted to a two-hour period (0528-0733 hours). A high percentage of insects initiated courtship/mating behaviors [female posturing (98%), male response (98%), and copulation (100%)] before civil twilight (0620 hours), the limit at which twilight illumination is sufficient for terrestrial objects to be clearly distinguished (Seidelman 1992). In fact, five females began calling (four of which began copula) just before nautical twilight (0548 hours), the time when general outlines of ground objects are distinguishable, but visual details are not clear (Seidelman 1992). This was about one hour before sunrise occurred. All but two matings were complete before sunrise. We conclude that the peak period of sexual activity for *C. cactorum* begins between nautical and civil twilight and ends before sunrise.

The underlying physiology responsible for the production of the *C. cactorum* male sex attractant is unknown; however, the female appears to be receiving stimuli that initiate the mating process before the beginning of nautical twilight. Astronomical twilight, the time at which the Sun begins to illuminate the sky (Seidelman 1992), occurred during our observations at 0513 ± 0.01 hours. Molecular scattering of ultraviolet radiation and imperceptible sky illumination in the high altitudes of the troposphere and stratosphere (Lee & Hernández-Andrés 2003) present at this time may be providing the stimuli for female *C. cactorum* to initiate their physiological and behavioral courtship/mating behaviors.

We saw no evidence of elaborate courtship behaviors after the male landed next to the female, nor prior to copulation. Mating pairs were formed soon after the male landed and only in a few instances did the female move away from the male. Mating pairs remained in copula for a short period of time (mean of 31.8 min) and almost 100% of the females were found to contain a spermatophore in the burse copulatrix upon dissection.

The limited observations on female refractory period revealed no subsequent matings by mated females. The two mated females that exhibited calling postures failed to attract males and did not produce eggsticks after their second calling event. The average number of eggsticks produced per female was similar to reports from Australia (Dodd 1940) and South Africa (Zimmermann et al. 2000). However, additional observations over the life of mated females are planned to conclusively determine the number of matings per female and their oviposition outcomes.

Opuntia spp. occur naturally from southern Canada to South America and form a continuous distribution across the southern U.S. from Florida through the states along the Gulf of Mexico (Benson 1982). The potential spread of *C. cactorum* to the opuntia-rich areas of the western U.S. and Mexico could have devastating effects on the landscape and biodiversity of this region. Our new discovery of *C. cactorum* on the western border of Florida intensifies the concern and shortens the time in which this insect will likely spread into the southwest. Biological control of *C. cactorum* is not a recommended pest control tactic because of the non-target concerns compiled by Pemberton & Cordo (2001). Irradiation studies have determined the dose at which *C. cactorum* males and females are 100% sterile and at which the deleterious effects of substerilizing doses inherited by the F_1 generation are minimized (Carpenter et al. 2001b). An SIT program is the most plausible approach for controlling *C. cactorum* along its leading edge to limit geographical range expansion and to eradicate isolated populations in front of the leading edge. SIT could also be used as an abatement program to protect rare and endangered *Opuntia* spp. Studies on mating behavior reported herein have advanced the development of a successful SIT program. We have demonstrated that a proportion of marked males stay near their release site and can be recovered and identified. We have determined that mating behaviors are simple and straightforward, that the majority of mating behaviors are initiated and completed before sunrise, that successful matings last, on average, 37 min (female calling plus duration of copula), and that, for the most part, females are busy ovipositing the first few nights after mating, not exhibiting additional mating behaviors. Our mating behavior study is also helpful to the ongoing *C. cactorum* research to isolate and identify the female calling pheromone. Bioassays testing the attractiveness of pheromone components and blends may need to be conducted under natural lighting with observations being made between nautical and civil twilight. Our observations will also be useful in efforts to improve traps used for monitoring, and to develop a technique to estimate adult moth population numbers.

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COMPARISON OF *SCIRTOTHRIPS PERSEAE* (THYSANOPTERA: THRIPIDAE) INFESTATION LEVELS ON AVOCADO FRUIT AND LEAVES

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ABSTRACT

Avocado fruit can be severely damaged by *Scirtothrips perseae* (Thysanoptera: Thripidae) in southern California. *Scirtothrips perseae* is found on leaves and fruit, but its prevalence on one versus the other substrate has not been documented. In this study, the occurrence and infestation levels of *S. perseae* on avocado leaves and fruit during late spring and summer were compared at three sites in Ventura and Santa Barbara Counties, California, from 1998-2000. In all sites and years, adult and larval *S. perseae* were more abundant on young leaves than on small fruit from early to mid June. After leaves matured and hardened with increasing temperatures from late June through August, overall *S. perseae* populations generally declined. However, populations became proportionally higher on fruit than on leaves compared with earlier in the season. This usually resulted in equal numbers on the two substrates and sometimes in higher numbers on fruit late in the season. The change in relative *S. perseae* abundance on leaves and fruit between pre- and post-leaf hardening indicates control efforts need to be made shortly before leaves harden and become unsuitable for *S. perseae* feeding and oviposition or shortly after the first thrips move onto fruit.

Key Words: *Scirtothrips perseae* populations, avocado leaves, fruit, leaf development, fruit development

RESUMEN

La fruta del aguacate puede ser dañada severemente por *Scirtothrips perseae* (Thysanoptera: Thripidae) en el sur de California. *Scirtothrips perseae* se encuentra sobre las hojas y las frutas, pero la prevalencia de substrato sobre el otro no ha sida documentada. En este estudio, los niveles de ocurrencia e infestación de *S. perseae* en las hojas y en la fruta de aguacate durante el final de la primavera y el verano fueron comparadas en tres sitios en los condados de Ventura y de Santa Barbara, California desde 1998 hasta 2000. En todos los sitios y todos los años, los adultos y las larvas de *S. perseae* fueron más abundantes en las hojas tiernas que en las frutas pequeñas desde el principio hasta la mitad de junio. Después que las hojas maduraron y endurecieron con el aumento de la temperatura desde el final de junio hasta el final de agosto, la población de total de *S. perseae* declinó generalmente. No obstante, la población se convertió proporcionalmente más alta en las frutas que en las hojas comparada con al principio de la estación. Esto usualmente resultó en números iguales sobre los dos substratos y a veces números mas altas sobre la fruta en la época posterior de la estación. El cambio en la abundancia relativa de *S. perseae* sobre las hojas y las frutas entre el pre-endurecimento y el pos-endurecimiento de las hojas indica que las medidas de control deben ser realizadas un poco antes de cuando las hojas se endurecen y se vuelven no apropiadas para la alimentación y la oviposición de *S. perseae* o un poco después de que los trips se movieron encima de la fruta.

Scirtothrips perseae Nakahara (Thysanoptera: Thripidae) is a serious pest of avocado, *Persea americana* Miller, in southern California, USA (Hoddle et al. 1998). Discovered in 1996 in Ventura County, CA (Hoddle & Morse 1997), and described in 1997 (Nakahara 1997), *S. perseae* has been responsible for millions of dollars in damage as a result of its feeding on and scarring of avocado fruit (Hoddle et al. 1998), with losses to the California avocado industry in 2000-2001 estimated at $8.65 million (Hoddle et al. 2003). Even though our understanding of *S. perseae* biology is increasing (Hoddle et al. 2000, 2001; Hoddle 2002), little is known about the relationship between *S. perseae* populations on avocado leaves and fruit, and the factors influencing their prevalence on one versus the other substrate. Factors related to infestation on fruit are of particular interest because economic damage caused by thrips feeding primarily occurs on fruit. The feeding results in scarring and downgrading or rejection of fruit in packinghouses. To better understand *S. perseae* field ecology and to develop less insecticide-oriented pest control strategies, it is neces-

sary to investigate patterns of substrate use by *S. perseae* and to relate them to patterns of leaf and fruit development.

Feeding on leaves and fruit of various hosts by other thrips species is well documented. The citrus thrips, *Scirtothrips citri* (Moulton), feeds on leaves and fruit of citrus (Metcalf & Flint 1962). The pear thrips, *Taeniothrips inconsequens* (Uzel) (Felland et al. 1995), bean thrips, *Hercothrips fasciatus* (Pergande) (Metcalf & Flint 1962), and the flower thrips, *Frankliniella occidentalis* (Pergande) (which is well known to feed in flowers) (Salguero-Navas et al. 1991), also feed on foliage and fruit of their hosts. On avocado, the greenhouse thrips, *Heliothrips haemorrhoidalis* Bouché, feeds on mature leaves and fruit (Smith 1929; Bekey 1986).

In this study, infestation levels of *S. perseae* on avocado leaves and fruit are compared and their relationships with periods of leaf and fruit development are described. We hypothesized that *S. perseae* stays on leaves that are young, but moves onto fruit as leaves mature and harden during late spring and early summer, accounting for high populations and damage observed on fruit during this time.

Materials and Methods

Study Sites

Data were collected from three orchards that were untreated with insecticides (with one exception, see below) and that had 'Hass' avocado trees in Santa Barbara and Ventura Counties, CA, from 1998-2000. Trees were 10-18 years old and 4.6-6.2 m tall. Orchards were located in the cities of Carpinteria (Santa Barbara County), Somis, and Moorpark (both Ventura County). These sites represented coastal (cool temperature), intermediate (moderate temperature), and inland (warm temperature) (Kimball & Brooks 1959) distributions of *S. perseae*, respectively. Moorpark was treated once with an insecticide (abamectin) in June 2000.

Temperatures were determined within the three sites throughout the season using StowAway XT1 temperature loggers (Onset Computer Corp, Pocasset, MA). Loggers were hung from branches in the canopy. Data were recorded daily every 30 min.

Sampling for *S. perseae* on Leaves and Fruit

Numbers of adult and larval *S. perseae* were recorded by visually examining 100 leaves and 100 fruit on the trees at the three sites from June through August 1998 and 1999 and from June through July 2000 when fruit first appeared. Adults and larvae were large enough so that a hand lens was not required. Flowers were not examined because they last only about 24 h (Bergh 1973) and because the western flower thrips, *F. occidentalis*, is the predominant species in them, not *S. perseae* (Hoddle 1999). Younger, three-quarters expanded leaves (Hoddle 2002) were sampled, but when these were unavailable, older, hardened leaves had to be sampled. Upper and lower surfaces of leaves were examined, although the majority of thrips was found on lower surfaces. Lengths of all leaves and fruit were measured. Dates when the majority of leaves began to harden were recorded. Hardening of leaves was determined visually. The youngest leaves were mostly reddish brown; older but still young, three-quarters expanded leaves were light green and flexible. Hardened old leaves were dark green, shinier, and rigid.

In 1998, 20 leaves and 20 fruit from five trees were randomly sampled. In the case of leaves, random sampling occurred within young leaves (unless absent) and not among all leaves. Thrips were congregated on young leaves, so whether the thrips were dispersed or aggregated in the tree depended on the amount of leaf flush present. In 1999 and 2000, 10 leaves and 10 fruit from 10 trees were randomly sampled. Sampled leaves and fruit were located 1.5-2.0 m above ground, a height chosen for practicality. In all years, sampling was conducted approximately every one or two weeks.

Exceptions to the above protocol in 1998 were as follows. On 10 June in Somis, 20 leaves and 20 fruit from only three trees were sampled. In three cases, there were no fruit present in two or three out of the five trees designated for sampling. Thus, on 25 June in Somis, 20 leaves on each of two trees were matched with 20 fruit from two neighboring trees. On 11 and 29 June in Moorpark, 20 leaves on each of three trees were matched with 20 fruit from three neighboring trees.

Statistics

To compare *S. perseae* infestation levels on fruit and leaves, the Mann-Whitney U-test was conducted on data within individual dates. The hypothesis tested was that the medians of thrips populations on fruit and leaves were the same (i.e., populations had the same statistics of location [Sokal and Rohlf 1981]). Numbers on fruit and leaves from the same tree were paired and considered replicated observations (n = 5 and 10 trees/date and 10 and 20 leaves or fruit/date, except n = 3 on 10 June 1998 in Somis). When there were many or all zero data on fruit or leaves, the t-test was used to compare the paired samples after counts were transformed (square root + 1). Statgraphics Plus (1997) was used for all analyses.

Results

Temperatures

Mean weekly temperatures at Carpinteria, Somis, and Moorpark from 1998-2000 are shown in Fig. 1. Carpinteria was the coolest site, Somis was

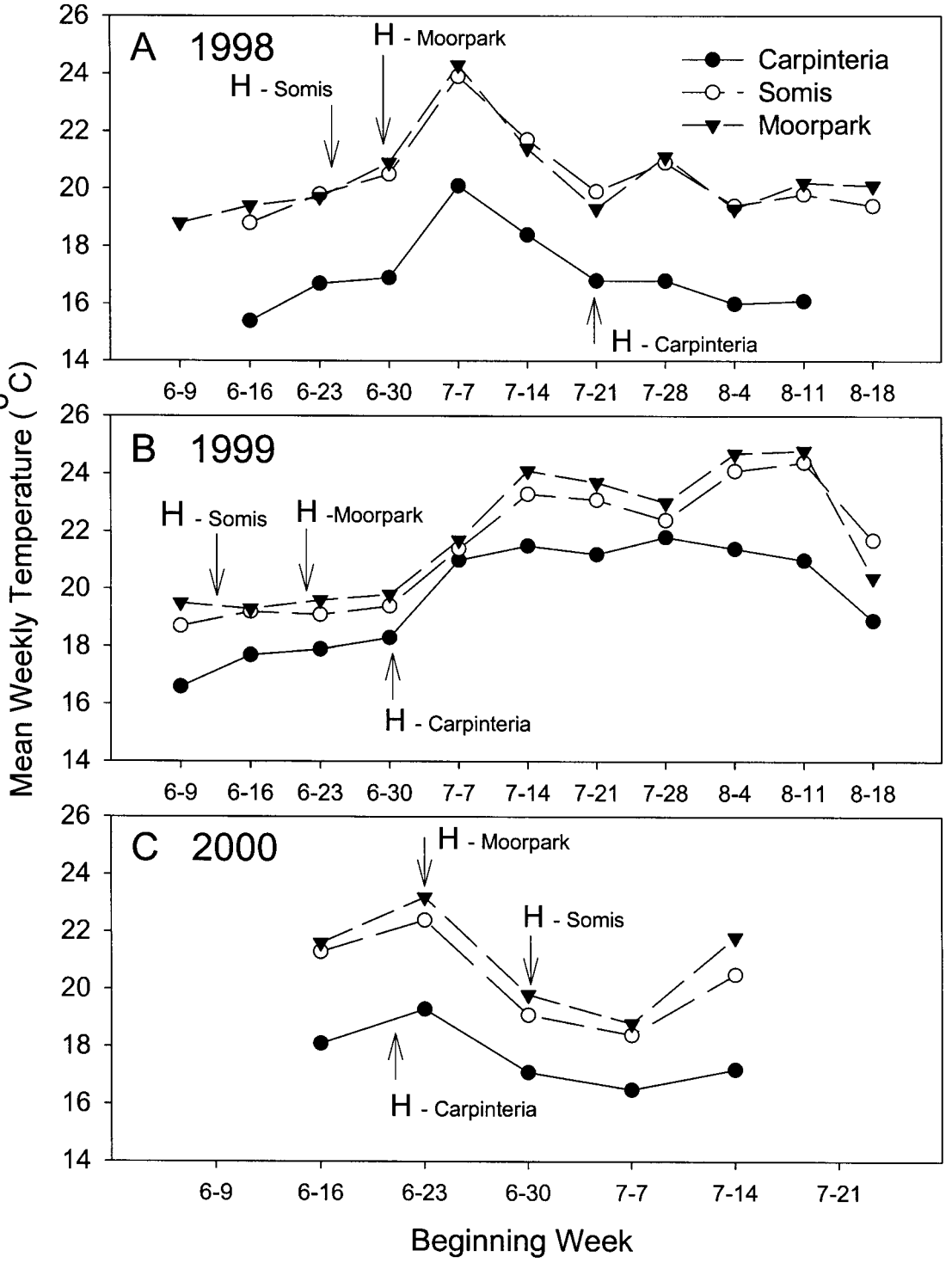

Fig. 1. Weekly temperatures in (A) 1998, (B) 1999, and (C) 2000 at Carpinteria, Somis, and Moorpark, California, study sites. H and arrows indicate sample dates when leaves began to harden.

intermediate, and Moorpark was the warmest. Gradually increasing temperatures in late June were associated with initial leaf hardening, which occurred the latest at Carpinteria, except during 2000 (Fig. 1). Mean high temperatures at Carpinteria in June, July, and August in 1998-2000 were

22.2, 24.3, and 24.6°C, respectively (no data for August 2000). At Somis, mean high temperatures were 27.7, 29.4, and 30.6°C, respectively, and at Moorpark, they were 28.9, 30.6, and 31.8°C, respectively. Sustained high temperatures in early and mid July were generally followed by overall declines in adult (Figs. 2-4) and larval (Figs. 5-7) *S. perseae* populations during late July and August.

Infestation Levels on Leaves and Fruit

In general, for all sites and years, adult *S. perseae* were found in significantly higher numbers on leaves than fruit when fruit were first seen in early June (Figs. 2-4). However, as leaves hardened from June into July, differences were usually not seen between substrates. This trend was also generally true of larvae, although larval abundance was significantly higher on fruit than on leaves after leaves hardened during late season in several cases (Figs. 5-7). Noteworthy significant site-specific patterns were as follows.

Carpinteria

At Carpinteria in 1999, a protracted period of sporadic leaf flush following initial leaf hardening coincided with higher populations of adults (Fig. 2B) and larvae (Fig. 5B) on leaves than fruit from 22 June to 10 August. This period with higher thrips numbers on leaves was longer than for the other two years.

Somis

At Somis in 2000, adult numbers were higher on leaves than on fruit for all but one sample date (Fig. 3C). However, in 1998 and 1999, larval numbers were higher on fruit than leaves on three dates in July and August of each year (Figs. 6A and 6B, respectively).

Moorpark

At Moorpark in 1999, adult numbers were higher on fruit than leaves on one date (Fig. 4B). In 1998, larval numbers were also higher on fruit than leaves on two dates in August (Fig. 7A) and in 1999 on three dates in August (Fig. 7B).

Relationships Between Infestation Levels and Leaf and Fruit Lengths

Younger leaves were approximately the same lengths from June to August. There was no correlation between adult and larval infestation levels and leaf lengths ($P > 0.05$). Thus declines in *S. perseae* abundance on leaves were not related to leaf lengths, but were associated with when leaves hardened at Somis in 1998 and 2000, Moorpark in 2000, and Carpinteria in 1998 and 1999.

Fruit lengths increased from 0.5-6 cm during June to August. Increases in *S. perseae* abundance on fruit were positively correlated with fruit lengths up to mid July, but they were not positively correlated over the season ($P > 0.05$). Thrips were first seen on 0.5-1.4 cm long fruit. Numbers were highest on 3-4 cm long fruit, but numbers declined on fruit >5 cm long (Figs. 2-7).

Discussion

The results support our hypothesis that *S. perseae* tends to stay on young leaves until they mature and harden during early summer. Despite the later onset of leaf hardening at the coolest site, the sequence of events that resulted in infestation on fruit was the same at all three sites. Growth flush was followed by simultaneous hardening of leaves and fruit appearance (and absence of flowers), which seemed responsible for *S. perseae* movement onto developing fruit. In general, these events were also associated with gradually increasing temperatures that probably caused rapid leaf development and hardening.

Our results suggest adult *S. perseae* prefer young leaves that are 15-17 cm long over small fruit that are <1.5 cm, at least as resting or feeding sites. However, larger fruit 3-4 cm long may be more preferred than smaller fruit, and perhaps more than young leaves. Within fruit, those 2.5-5.4 cm in diameter are preferred over those smaller or larger for oviposition (Hoddle 2002), and immature fruit 2.85 cm in diameter (2.87 larvae/fruit) apparently are preferred for oviposition over undersides of three-quarters expanded immature leaves (1.53 larvae/leaf) (Hoddle 2002). In our study, however, it was unclear if the higher adult and larval numbers on the 3-4 cm long fruit compared with the smaller fruit were caused by an actual preference for larger fruit or a lack of suitable leaves. In other thrips, *T. palmi* Karny and *F. occidentalis*, there is an apparent preference for foliage and flowers over fruit of cucumber (Rosenheim et al. 1990).

Leaves clearly are larval developmental sites for *S. perseae* (Hoddle & Morse 1998; Hoddle 2002). However, adults probably cannot feed on or oviposit into mature leaves and this may force them onto fruit. We have also observed larvae congregating on leaf stems when populations on leaves are high. This suggests larvae can also move from leaves to fruit after leaf resources are depleted by high feeding activity.

Interestingly, in its native subtropics, where 'Hass' avocado tree phenology is different than in the United States (Teliz 2000), *S. perseae* seems to rarely infest fruit (Phillips 2003). In Mexico, fruit appear in February during leaf flush (Phillips 2003) rather than at or near the end of leaf flush, as it usually did at our study sites. In Mexico, thrips are also found feeding on and scarring foli-

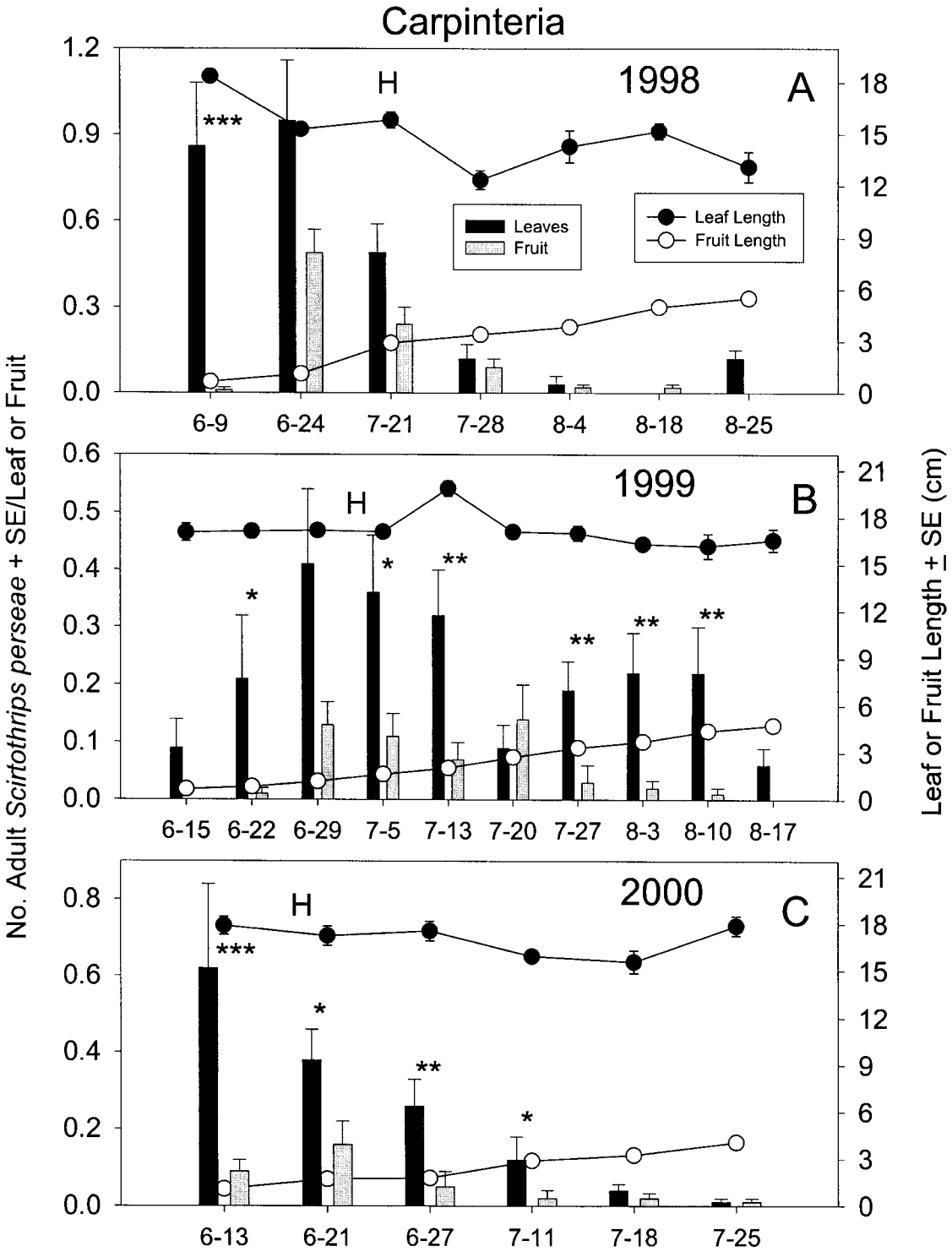

Fig. 2. Numbers of adult *S. perseae* + SE on leaves and fruit at Carpinteria, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

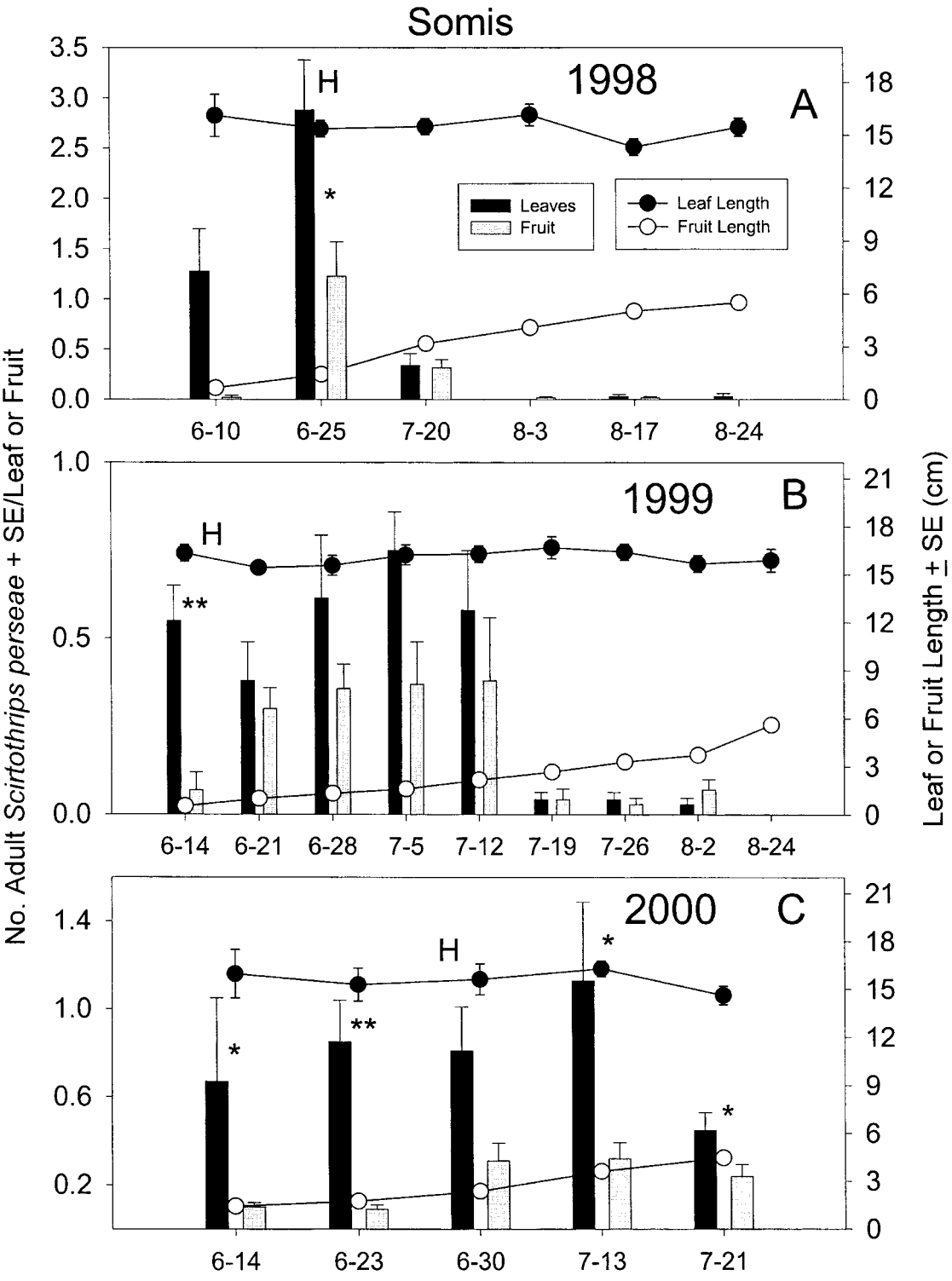

Fig. 3. Numbers of adult *S. perseae* + SE on leaves and fruit at Somis, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

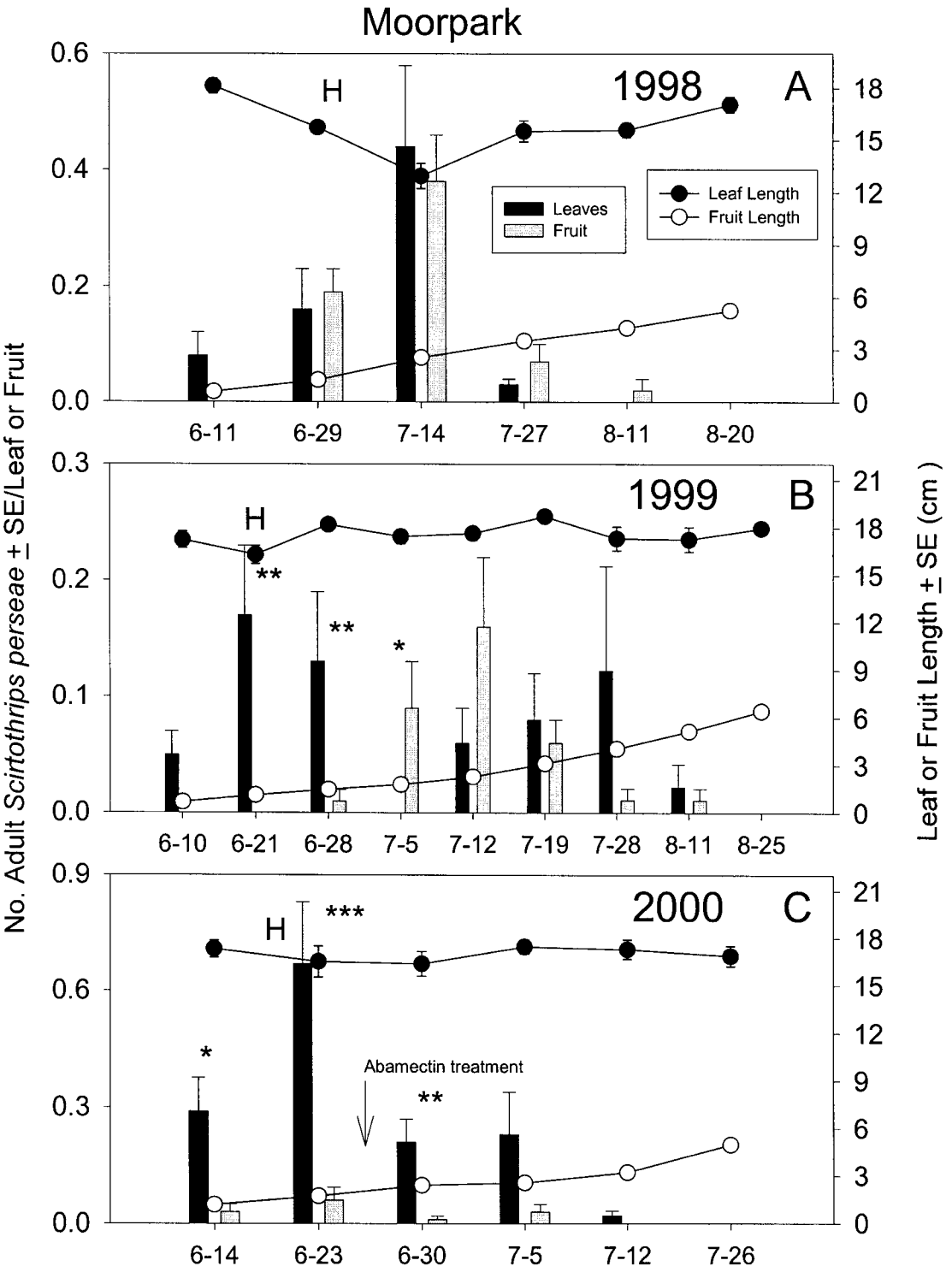

Fig. 4. Numbers of adult *S. perseae* + SE on leaves and fruit at Moorpark, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

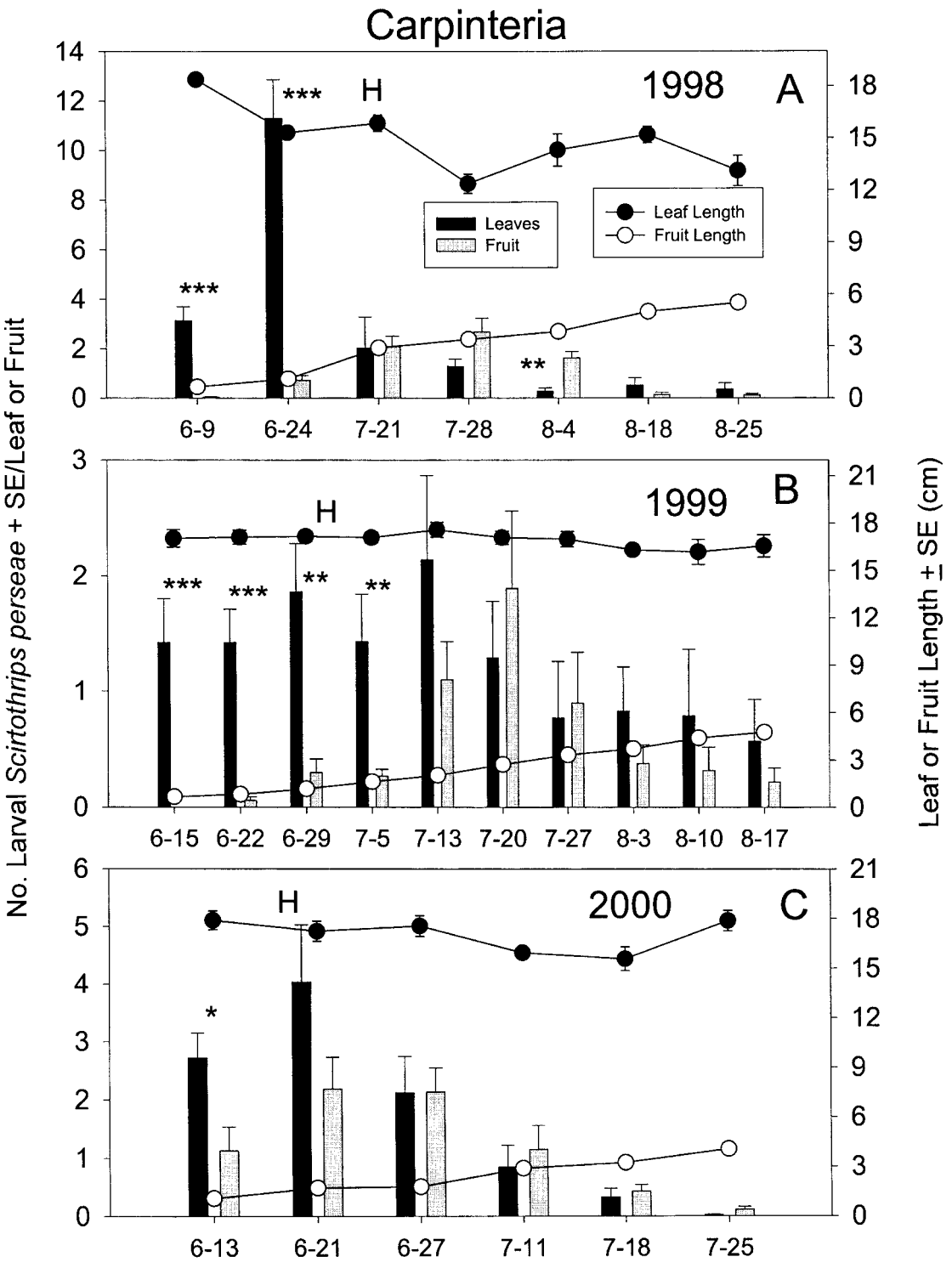

Fig. 5. Numbers of larval *S. perseae* + SE on leaves and fruit at Carpinteria, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

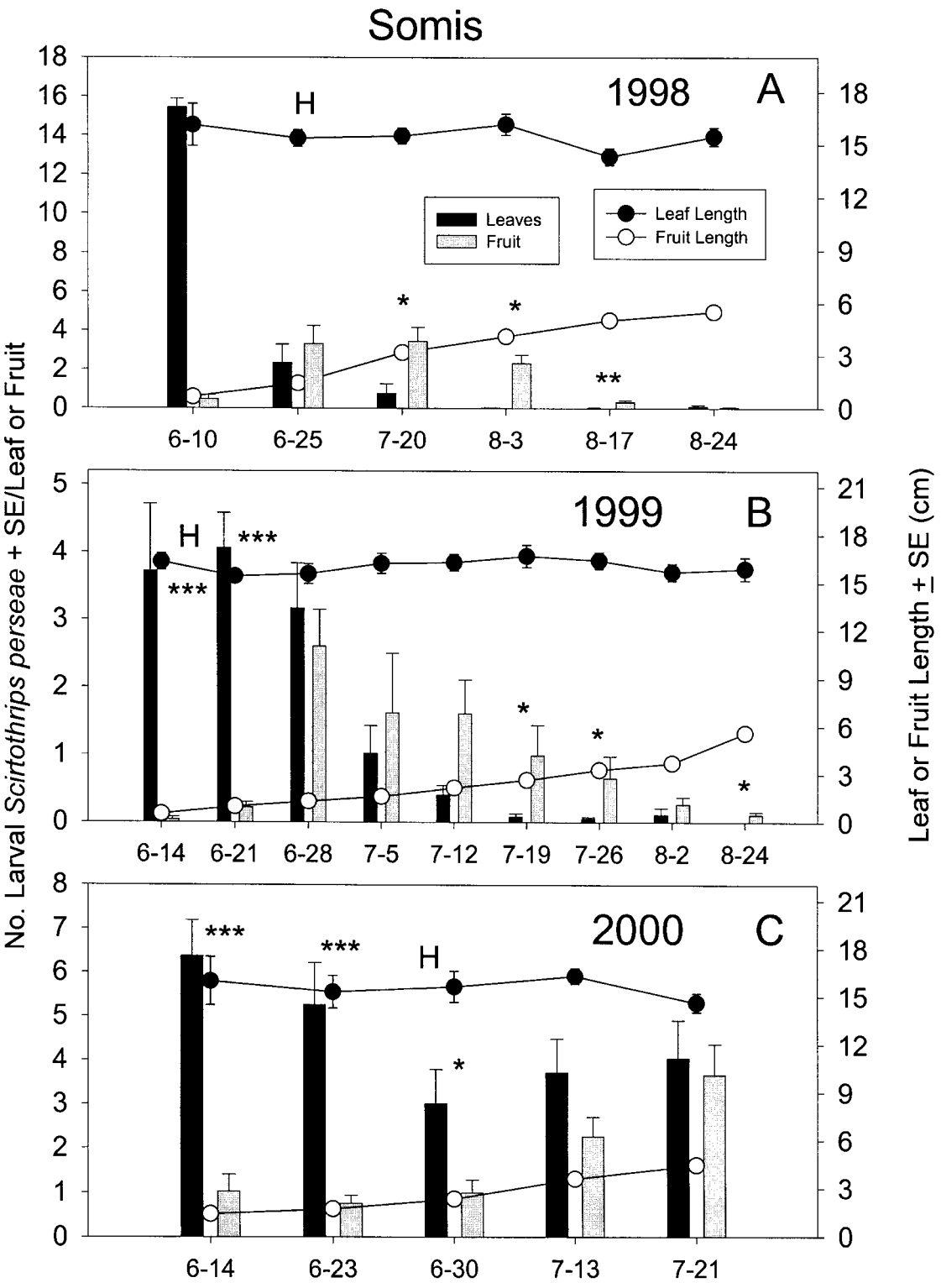

Fig. 6. Numbers of larval *S. perseae* + SE on leaves and fruit at Somis, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

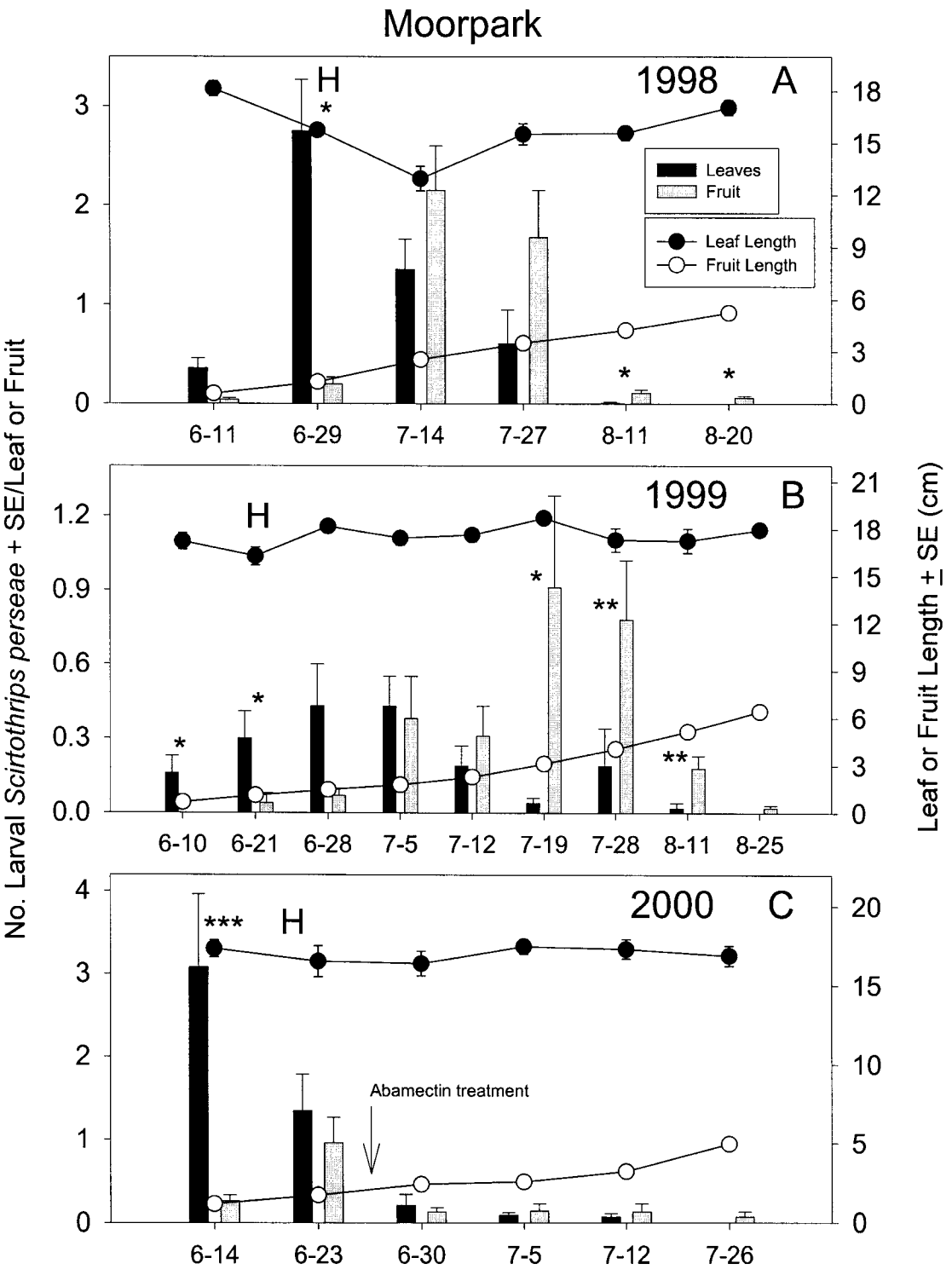

Fig. 7. Numbers of larval *S. perseae* + SE on leaves and fruit at Moorpark, California in (A) 1998, (B) 1999, and (C) 2000 related to leaf and fruit lengths. H = leaves began to harden. Asterisks indicate significance detected by the Mann-Whitney U-test or t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

age early in the season prior to fruit set. In addition, thrips populations are low by the time of fruit set, when temperatures are often well above 29°C (P. A. Phillips, unpublished). Laboratory data show that *S. perseae* develops poorly at high temperatures. When held at 30°C, 61% fewer larvae emerged from leaves than at 25°C (Hoddle & Morse 1998). Temperatures were still relatively cool and thrips populations were high at the beginning of fruit set at our three study sites. The combined evidence suggests southern California avocado growing conditions have contributed to the current pest status of *S. perseae*. It must be noted, however, that the phenology of leaf flush and hardening even within southern California varies from year to year. Thus leaves and fruit may not always be present at the same time, which may alter the patterns seen in this study.

The change in relative *S. perseae* abundance on leaves and fruit between pre- and post-leaf hardening indicates control efforts need to be made shortly before leaves harden and become unsuitable for *S. perseae* feeding and oviposition or shortly after the first thrips move into fruit. Insecticide treatments may need to be considered when a threshold of 3-5 thrips larvae per leaf is reached, as this corresponds later to 6-15% economic damage of fruit when treatments are not applied (Yee et al. 2001). Future studies need to determine more precisely how the timing of leaf growth flush in relation to fruit set can be used to predict potential movement of *S. perseae* from leaves onto fruit and to prevent scarring damage.

Acknowledgments

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ATTRACTION OF COLORED PLASTICIZED CORRUGATED BOARDS TO ADULT STABLE FLIES, *STOMOXYS CALCITRANS* (DIPTERA: MUSCIDAE)

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Abstract

The attraction of colored plasticized corrugated boards covered with adhesive to trap adult stable flies was investigated on Florida panhandle beaches. Colors consisted of blue, red, orange, and three types of white (horizontal ribbed, vertical ribbed, or opaque). Boards measured 67.3 cm (length) by 31.7 cm (height) and were placed on slotted wooden stakes, 30 m apart, along a linear transect. Fly collections were significantly ($P < 0.05$) greater on blue boards than on orange and white but there was no difference between red and blue boards. Spectral reflectance of boards peaked at 503 nm for blue, 638 nm for red, while orange and the 3 types of white boards peaked at about 630 nm. Blue boards exhibited the lowest reflective intensity when compared with the rest of the colors. Because stable flies were collected from all boards it is surmised that the boards provided leeward surfaces on which to land or remain perched in the windy beach environment. Significantly more flies were collected from the leeward side of boards compared with the windward side. Moreover, the boards may have provided vertical platforms for adult stable fly assembly, thermoregulation, and/or mating. Adhesive-treated corrugated plasticized boards may be a suitable method for luring stable flies away from human or animal hosts in recreation areas to reduce annoyance from biting pests.

Key Words: traps, management, control, behavior, biting fly

Resumen

La atracción de los adultos de la mosca de establo hacia tablas plastificadas de colores y corrugadas y cubiertas con un adhesivo para atrapar las moscas fue investigada en las playas del noroeste de la Florida. Los colores consistieron en azul, rojo, anaranjado, y tres clases de blanco (cordoncillado horizontalmente, cordoncillado verticalmente, u opaco). La tablas median 67.3 cm (de largo) por 31.7 cm (de alto) y fueron puestas sobre estacas de madera con una ranura separadas por 30 m, por un transecto lineal. El número de moscas recolectadas fueron significativamente mayores ($P < 0.05$) en las tablas en azul que en las tablas anaranjadas o blancas pero no habia una diferencia entre las tablas rojas y las azules. La reflexión espectral de las tablas fue más alta en 503 nm para la azul, 638 nm para la roja, mientras que la más alta para la anaranjada y las tres clases blancas fueron arrededor de 630 nm. Las tablas azules exhibieron la intensidad reflectiva más baja cuando fue comparada con el resto de los colores. Puesto que las mosca de establo fueron recolectadas de todas las tablas, se asume que las tablas proveen superficies sotaventos para aterrizar o quedar posadas en el ambiente airoso de la playa. El número de las moscas recolectadas del lado sotavento de las tablas fue significativamente mayor comparado con el lado barlavento. Además, las tablas pudieron proveen plataformas verticales para la congregación de los adultos de la mosca de establo, la termoregulación y/o el apareamiento. Las tablas plastificadas corrugadas tratadas con adhesivo pueden ser un método apropiado para atraer las moscas del establo fuera de los hospederos humanos y animales en áreas de recreo para reducir las picaduras de estas plagas.

Adult stable flies (*Stomoxys calcitrans* [L.]) are primarily blood-feeding pests of cattle. These flies, however, can be serious biting pests of humans when their primary animal hosts are absent. Oftentimes stable flies negatively impact the use of recreational areas (Newson 1977). Congregations of host-seeking stable flies, primarily associated with cold front passage, occur regularly on Florida's panhandle beaches from late summer through fall (King & Lenert 1936; Hogsette et al. 1987). A considerable amount of research has been conducted on the biology and management of this pest in an effort to minimize its impact on tourism (Simmons 1944; Hogsette et al. 1981; Dukes & Hallmon 1984; Hogsette et al. 1987; Jones et al. 1991).

The major control effort is targeted against adult fly populations because stable fly larval habitats are not present along the beaches. Aerially applied insecticides provide only temporary control and are constrained by prevailing weather conditions. Moreover, public concern and

environmental issues associated with area-wide application of pesticides within or near coastal ecosystems is increasing. Methods that minimize the application of pesticides while allowing individuals to manage biting pests in their immediate environment may be advantageous. This study was initiated to evaluate the attractiveness of colored corrugated plasticized boards covered with adhesive, as traps, against adult stable flies on Florida panhandle beaches.

Materials and Methods

This study was conducted on a sandy beach on the Gulf of Mexico (Panama City Beach), Bay County, Florida where stable flies often congregate after the passage of cold fronts. Corrugated plasticized boards, 67.3 cm length by 31.7 cm height (Aluma Panel, Cumming, GA), were evaluated as trapping surfaces. Plastic boards were used because they were easily obtainable and would retain their rigidity in the high humidity and winds of coastal environments. Each board was composed of two outer smooth surfaces sandwiched between an inner series of parallel grooves and ridges (referred to here as "ribbing") to strengthen the material. Red, blue, orange, and 3 types of white (i.e., horizontal ribbing or vertical ribbing, visible through the boards when held up to a bright light, and opaque no visible ribbing) were obtained from the manufacturer. These colors were chosen for testing based on previous work by Agee and Patterson (1983), Waldbillig (1968), Williams (1973), Ruff (1979), and Mihok et al. (1995). Evaluation of ribbing orientation on the white boards corresponded to work conducted by Pickens (1991) with electrocution grids. He recorded that flies were attracted to high-contrast, narrow width, multiple-edge patterns. The red, blue, and orange boards were horizontally ribbed in order to prevent buckling by winds on the beach. The ribbing was not visible through those panels.

Boards were completely covered with a thin, approximately 0.3 cm^3, film of brushable Tangletrap Insect Coating© (Tanglefoot, Grand Rapids, MI) and placed vertically about 60 cm from the ground surface in slotted 5 cm by 5 cm by 122 cm wooden stakes. Stakes were placed 30 m apart in an east to west linear transect parallel to the shoreline approximately 50 m from the water's edge. Each board faced north-south (i.e., into the wind) with the longest edge parallel with the ground.

From September 18 through November 14, during 1996 and 1998, boards were set out when stable flies were observed on the beaches, usually within 24 h after passage of a cold front with sustained northerly winds. After 24 h, the total number of flies on each side of the board was counted separately and recorded. Boards were randomly placed along the transect and used once for each 24 h collection period. The 24-hr collection period used 3 of each color and type of board for a total of 18 panels. Wind speed was monitored using an electronic anemometer (Turbo MeterF, Ben Meadows Co., Atlanta, GA) and averaged 9.3 ± 0.3 km/hr while wind direction during testing was primarily north-northwest.

Reflectance measurements of the colored boards used a USB 2000 Fiber Optic Spectrophotometer (Ocean Optics, Inc., Dunedin, FL) a UV2 UV-Vis detector, 1.2 mm lens, 25 um slit, and its bundled software that reads from 200 to 850 nm. A 12 volt tungsten bulb was attached to the top of an acrylic plastic box, 3 cm away from and angled at 35 degrees to a horizontal surface containing the flat sample (10 cm square). A fiber optic cable was set 3 cm away from the same flat sample, and angled 35 degrees to it, pointed at the center of the light spot produced by the tungsten bulb. The reference standard was black polyester cloth, and the sample measurements were subtracted from the intensities recorded for this standard from 400 to 850 nm using Excel and a laptop PC.

Statistical Analyses

The mean number of flies per color and side, per year, was transformed via $\sqrt{x} + 1$ before analysis and subjected to analysis of variance (SAS Institute 1990). No significant interaction between year and panel color was observed for data collected during both years ($F = 0.45$; df 5, 492; $P = 0.814$). Therefore, data were summarized by color. In addition, no significant interaction between year and panel surface was observed for fly collection data from leeward and windward board surfaces ($F = 0.07$; df 5, 984; $P = 0.996$). Data were pooled and compared by board surface. Mean separation of fly data from board surface, by color, used Student-Neuman-Keuls ($P < 0.05$), whereas Student's t ($P < 0.05$) was used to compare overall fly numbers on leeward and windward board surfaces (Sokal & Rohlf 1981). The overall data set consisted of 14 collection days for each year.

Results and Discussion

Significantly more stable flies were collected from blue boards compared with orange or the 3 types of white boards (Table 1). There was no difference between the number of flies on the blue or red boards. Also stable fly abundance on red, orange, and the three types of white boards did not differ from each other. Flies on white boards with horizontal or vertical ribbing were not significantly different, nor did the opacity of the board increase collections. Spectral analyses revealed that the reflectivity of blue boards peaked at 503 nm, at 638 nm for red, at 630 nm for orange and opaque, while the white horizontal and vertical ribbed boards peaked at 632 nm (Fig. 1). Blue ex-

TABLE 1. COMPARISON OF MEAN STABLE FLY (±SE) ABUNDANCE ON ADHESIVE-TREATED PLASTICIZED CORRUGATED BOARDS OF VARIOUS COLORS AND ORIENTATIONS ON A FLORIDA PANHANDLE BEACH ALONG THE GULF OF MEXICO.

Treatment	n	Mean no. flies (±SE)[a]
Blue	84	209.7 ± 23.4 a
Red	84	194.6 ± 27.9 ab
White (opaque)	84	125.9 ± 16.2 b
White (horizontal ribbing)	84	120.2 ± 17.2 b
White (vertical ribbing)	84	123.6 ± 17.4 b
Orange	84	125.6 ± 20.4 b
Overall board orientation		
Board facing leeward side	504	116.0 ± 7.0 A
Board facing windward side	504	33.9 ± 2.7 B

[a]Means followed by the same letter are not significantly different (>0.05) using Student-Newman-Keuls test (for lower case letters) or t-test (for upper case letters).

hibited the lowest reflectance intensity compared with the rest of the other colors.

Stable fly preference for variously colored and reflective surfaces have been reported by a variety of workers. Williams (1973) showed that translucent Alsynite panels were more effective at capturing stable flies than panels painted either red or black. Ruff (1979) found white flat panels of the same material were most attractive while horizontally corrugated gold panels were least attractive. Cilek (2002) reported that unpainted inflated beach balls (with blue, yellow, red, and white diamond-shaped panels) covered with adhesive were more attractive than solid white, black or black/white balls. Mihok (2002a) reported that cloth traps with vertical blue and black contrasting surfaces appeared to attract *Stomoxys* spp. in Kenya. Also traps composed of pure cotton dyed with phthalogen blue, that exhibited low reflectance at a peak wavelength of 466 nm, would be the ideal for attracting these species (Mihok 2002b).

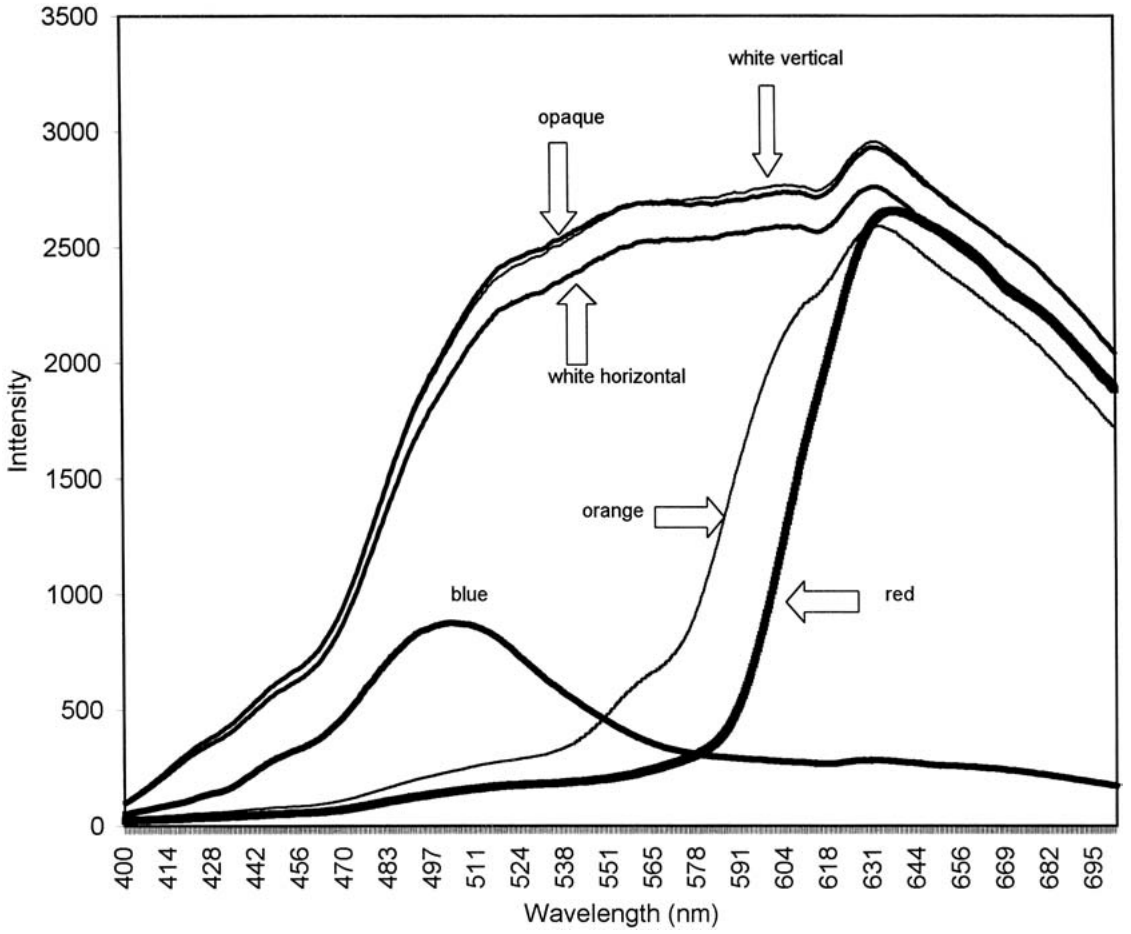

Fig. 1. Spectral reflectance curves of various colors and types of plasticized corrugated boards.

Agee and Patterson (1983) cited several authors who felt that some species of biting flies landed preferentially on low-reflective surfaces rather than on certain colors. Later, Allan et al. (1987) stated that host-seeking stable flies were attracted to low-intensity wavelengths ranging from 360 nm [UV] to 550 nm [blue-green]. This explained the reason why *S. calcitrans* collections were greatest on blue boards. It is unknown why the blue and red boards were not significantly different when collection abundance was compared. Although these two colors substantially differed in intensity and wavelength reflectance peaks, greater variation occurred between these fly collections than collections from the rest of the colored boards.

Each board probably provided leeward windbreaks that allowed flies to land and remain perched in the windy environment. In fact, stable fly abundance was significantly greater on the leeward side of boards compared with the windward side (Table 1). Broce et al. (1991) reported that stable flies often oriented, and preferably landed, on the leeward side of objects. But just as importantly, these panels may have provided vertical platforms for adult stable fly assembly, thermoregulation, and/or mating (Buschman & Patterson 1981).

It was not the intent of this study to examine stable fly trapping efficiency of the colored boards with that of a trap standard (e.g., Alsynite cylinders [Broce 1988]). However, because the Alsynite cylinder is a standard method to sample stable flies it may be of interest to compare the flies caught per cm^2 of both traps in similar habitats. Cilek (2002) reported that adhesive-treated Alsynite cylinders placed on northwest Florida beaches caught an average of 0.004 ± 0.001 flies per cm^2 while the colored boards, in this study, averaged 0.070 ± 0.004 flies per cm^2.

Adhesive-treated plasticized corrugated boards proved to be a quick and inexpensive way to trap stable flies in the coastal environs. This type of board is readily available in a variety of sizes and colors from local print and office supply sources so that the general public could fabricate this trap to reduce annoyance from host-seeking flies. Whether this trap would be useful to reduce stable flies on beach and other recreational areas, or animal facilities, warrants further investigation.

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VISUAL RESPONSES OF *LYGUS LINEOLARIS* AND *LYGOCORIS* SPP. (HEMIPTERA: MIRIDAE) ON PEACHES

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ABSTRACT

The visual response of *Lygus lineolaris* (Palisot de Beauvois) and insects in the genus *Lygocoris* to pink and white sticky traps was evaluated in a peach orchard. Pink traps significantly captured more tarnished plant bugs. For the entire season, the mean (±S.E)number of *L. lineolaris* per trap was 1.29 ± 0.064 for pink traps and 0.72 ± 0.067 for white traps. In contrast, both trap colors performed similarly in their average timing of capture and ability to track the occurrence of fruit injuries. Unlike *L. lineolaris*, few *Lygocoris* insects were captured and no difference was detected between captures from each trap color.

Keywords: Tarnished plant bug, sticky traps, catfacing insects

RESUMEN

La respuesta visual de *Lygus lineolaris (*Palisot de Beauvois) y de insectos del género *Lygocoris* hacia trampas pegajosas de color rosado y blanco fue evaluada en un huerto de durazno. Las trampas rosadas capturaron significativamente más chinches deslustrados de plantas. En la estación entera, el número promedió (± S.E.) de *L. Lineolaris* por trampa fue 1.29 ± 0.064 para las trampas y rosadas 0.72 ± 0.067 para las trampas blancas. En contraste, ambos colores de pas trampas dieron resultado similares en el promedio del tiempo de la captura y la abilidad para rastrear la ocurrencia del daño en las frutas. Al contrario de *L. lineolaris*, pocos insectos del género *Lygocoris* fueron capturados y ninguna diferencia en el número de insectos capturados en cada color de trampa fué detectada.

One of the pest problems encountered by peach growers is the complex of 'catfacing' insects which includes the tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois), *Lygocoris* spp. and several species of stink bugs. Insects in this complex can cause serious fruit injury, they are highly mobile and difficult to monitor (Hogmire 1984). The feeding injuries on fruit cause fruit deformation, scarring, water-soaked areas and gummosis (Rings 1958). The most abundant catfacing insect is *L. lineolaris* which is a serious pest of several cultivated plants, having a host range of over 120 plant species in 30 plant families found in the U.S. (Snodgrass et al. 1984). It reproduces and overwinters in weedy groundcover, hedgerows or fields adjacent to peach orchards. Adults move into the orchard in the spring. Feeding by this insect can cause blossom and fruit drop from bloom to about 30 days after bloom (Rings 1958). Other Hemiptera reported to feed on peaches include insects in the genus *Lygocoris*. *Lygocoris* spp. and the tarnished plant bug produce similar types of injury (Rings 1958). Species in this group include *Lygocoris quercalbae* (Knight) (the white oak plant bug), *Lygocoris caryae* (Knight) (the hickory plant bug) and *Lygocoris omnivagus* (Knight). These species closely resemble each other and are usually referred to as oak-hickory bugs (LeFevre 1984; Leahy 1991).

Guidelines exist on how to readily monitor *L. lineolaris* in apples using white sticky traps (Prokopy et al. 1980; Prokopy et al. 1982; Coli et al. 1985), but similar information is lacking for peach growers concerned about tarnished plant bugs and oak-hickory bugs. Thus, we investigated the visual response of tarnished plant bugs and oak-hickory bugs to two sticky trap colors. Compared to direct counts, limb jarring and net sweeps, sticky traps have been shown to be the most effective method of detecting *L. lineolaris* adults (Prokopy et al. 1982; LeFevre 1984) and *Lygocoris* spp. (LeFevre 1984). We compared pink and white sticky traps for two reasons. First, previous work in Connecticut (LeFevre 1984) and in Massachusetts (Leahy 1991) indicated that pink sticky traps may be useful in monitoring *Lygocoris* spp. Traps painted with Pink Tiara (Pittsburgh Paints Co.) gave the most consistent results as compared to other colors tested (LeFevre 1984). Second, while some extension publications state that white sticky traps could be used for *L. lineolaris* monitoring in peaches, Hogmire (1995) noted that white traps have been used without success in peach orchards. The objective of this work was to test the response of both tarnished plant bugs and *Lygocoris* bugs to the aforementioned trap colors. In addition, we collected data on the fruit injury observed throughout the season to determine how well trap catches tracked injury occurrence.

Materials and Methods

Trap Color Evaluation

The experiment was carried out in two sections of a commercial orchard. One section was located in a block of 4 year old trees and the second section in a block of 14 year old trees. Rows in the younger block were planted to the varieties 'Red Haven' and 'Harbelle Bailey'. The variety in the older block was 'Jersey Queen'. Experimental sections, which were located on the periphery of the block, did not receive insecticide applications but received only applications of sulfur as a fungicide. Twelve trees were selected for the experiments in each section. Traps were hung vertically on branches in the canopy approximately at 1.8-2 m high for the old trees and 1.5-1.6 m high for the young trees. Traps were placed at this height because traps placed higher in the tree canopy have been shown to capture more oak-hickory bugs than traps placed at a lower height (LeFevre 1984). The canopy was divided into quadrants according to NE, NW, SE, and SW orientation. One trap was placed per quadrant and the same number of pink and white traps were used for each orientation. Thus, every tree started with 2 white and 2 pink traps. Due to branch pruning early in the season, two trees retained only one trap of each color in the canopy. White traps were purchased from Gempler's (Belleville, WI) and pink traps were made by painting the same plastic substrate used in Gempler's white sticky traps (16 × 19.8 cm). The plastic rectangles were painted with Pittsburgh Paints' Pink Tiara (Pittsburgh, PA) and then covered with Tangle-Trap sticky coating (Tangle Foot Co., Grand Rapids, MI). Pink Tiara has similar spectral reflectance pattern as peach petals with a peak at 435-440 nm, lower reflectance in the yellow green range and with a second highest peak around 610nm (LeFevre 1984).

Traps were checked weekly and any *L. lineolaris* or *Lygocoris* bugs were removed and taken to the laboratory for removal of sticky material and identification. Removal of Tangle-Trap was accomplished by rinsing the specimens in BioShield citrus paint thinner (EcoDesign Co., Santa Fe, NM). Traps were cleaned of insects or were changed as needed. Traps were set out on April 21 (before petal fall) and monitoring of traps stopped well after harvest time on September 9, 1999. In addition to weekly trap inspections, the presence of stink bugs was determined through limb jarring because extremely few were being caught by the traps. This was done to assess the presence of these other 'catfacing' insects. Each week, 6 trees without traps received three limb strikes with a rubber-coated rod and a beating sheet received any dislodged insects.

Fruit Injury Inspections

At the same time the traps were inspected, damage to fruit was recorded as follows. Just after shuck-split on May 20, ten fruit per tree were randomly selected and marked by placing a wooden clothespin on the same branch as the fruit. Pins were placed far enough away from the fruit so there would be little interference to the insects. Five fruit were at a height level of 1.8-2 m and 5 others were at a lower level of 1-1.3 m in the old trees. In the young trees, fruit were selected irrespective of height since the canopy was more compact. Compass coordinates were randomly generated and used to select the fruit around the tree. These same 10 fruit per tree were inspected weekly to determine the presence of new injuries. Damage to fruit was classified according to the size of the injury as follows: pin holes, punctures, large holes and catfacing deformation. As the fruit grew, pin hole injuries usually turned to punctures and eventually became holes on the fruit. Fruit inspections stopped before harvest time on August 3.

Statistical Analysis

Data from trap captures were checked for normality and homogeneity of variances. The data for tarnished plant bug and *Lygocoris* spp. captures were transformed using the transformations $\log_{10}$ and a $\log_{10}$ (x + 1) respectively. To determine trap color effect on insect captures, trap data were analyzed using Proc GLM (SAS Institute 2000). Trap captures were classified according to trap color, orientation, tree on which the trap hung, and orchard block. The analysis took into consideration that each tree had two traps of the same color tested and trees were treated as a fixed effect to control for any differences associated with the trees. Data were aggregated over all sampling dates and the means for each tree were analyzed. Preliminary analysis showed no difference in the results from each orchard block, thus the data were pooled into one analysis.

A second analysis was performed to determine if trap color influenced the average time to insect capture. If one trap color captured more insects but was delayed in detecting them it would not be very useful. The average time of insect capture was calculated by determining when during the field season each insect was captured (e.g., the first weekly sampling corresponded to day 7 after traps were set out) and considering the total number of insects caught through the season. These data were analyzed using Proc GLM (SAS Institute 2000) and were not transformed.

Data from the various categories of fruit injuries were summed into one variable to give the total number of injuries for each fruit on a given sample day. Means were obtained for each sample date and weekly increments were calculated to determine how well trap captures tracked these increments. Fruit injury increments reflect only the new injuries appearing in any given week. In

addition, these data were used in a partial Spearman rank correlation analysis where the fruit injury observed in the tree was correlated to the trap captures (*L. lineolaris* and *Lygocoris* spp.) on that tree. The partial analysis adjusted for the two different orchards sections used.

RESULTS

Both pink and white traps performed well in capturing tarnished plant bugs during the whole season. Figure 1 shows the mean number of *L. lineolaris* captured weekly for each trap color. The two traps show the same seasonal trends but pink traps significantly captured more insects (Table 1). The mean number of tarnished plant bugs captured per pink trap was 1.29 ± 0.064 and that of white traps was 0.72 ± 0.067. In addition to trap color, trap orientation and tree on which the trap hung had significant effects on *L. lineolaris* trap captures. The traps in the NE, NW, SE and SW quadrants captured an average of 1.28 ± 0.09, 0.81 ± 0.09, 1.18 ± 0.09 and 0.74 ± 0.09 *L. lineolaris*, respectively. We also found a significant interaction between orientation and trap color. The differences in trap captures between the two colors were not as large in the NE and SW quadrants as compared with the SE and NW quadrants. However, pink traps consistently had larger mean captures of tarnished plant bug across quadrants. Trap captures of *Lygocoris* were not influenced by trap color (Table 1). Very few *Lygocoris* were captured throughout the season and this may be preventing a clear assessment of trap color effect. *Lygocoris* spp. were caught between June 3 and August 3 and the mean number captured weekly per trap was 0.05 ± 0.007 for pink traps and 0.03 ± 0.007 for white traps. With the exception of the tree effect, other sources of variation listed in Table 1 did not have a significant effect on the mean number of *Lygocoris* captured. Sticky traps captured very few stink bugs and limb-jarring sampling detected few and not until the end of the season. Also, very few *L. lineolaris* and no *Lygocoris* were captured using this sampling method.

In addition to testing the effect of trap color on the number of tarnished plant bug captures, we also examined which trap color detected insects earlier. Both trap colors had a similar average time for all *L. lineolaris* captures (F = 0.27; df = 1,64; P = 0.61). The mean in days was 77.9 ± 1.09 for pink traps and 78.6 ± 1.09 for white traps. Trap orientation also did not have a significant

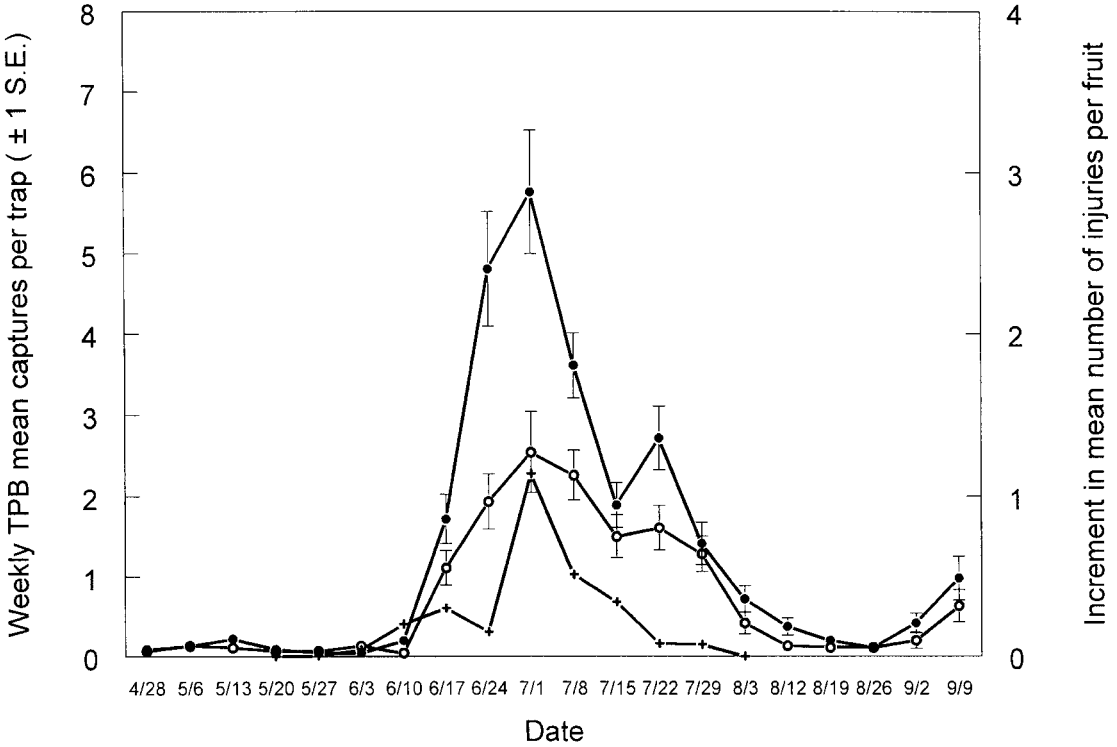

Fig. 1. Weekly trap captures of tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) as influenced by pink (—●—) or white (—○—) sticky trap color. Weekly increments in fruit injury through the season (—+—) are also shown.

TABLE 1. RESULTS OF ANOVA FOR THE EFFECTS OF TRAP COLOR, ORIENTATION AND TREE FROM WHICH THE TRAP HUNG ON THE NUMBER OF TARNISHED PLANT BUG (TPB) *Lygus lineolaris* (PALISOT DE BEAUVOIS) AND *Lygocoris* SPP. CAPTURED BY TRAPS.

		TPB		*Lygocoris* spp.	
Source of variation	df	F	P	F	P
Orientation	3,61	9.54	<0.0001	0.53	0.6637
Trap color	1,61	42.56	<0.0001	3.36	0.0715
Tree	23,61	4.04	<0.0001	1.71	0.0492
Orientation × color	3,61	3.92	0.0127	0.23	0.8760

effect on average time of captures (F = 2.01; df = 3,64; P = 0.12). Captures by both trap colors tracked very well the pattern of fruit injury occurrence through the season. Early in the season when none or few plant bugs were captured, no injuries were detected on the fruit (Fig. 1). Then, as the number of insects captured increased, the number of injuries per fruit rose as well. On July 1, when both traps showed a peak in insect captures we also observed a peak in injuries per fruit. The tree by tree correlation analysis showed that trap captures on a given tree did not correlate well to the amount of fruit injury observed on the tree. Correlation coefficients were 0.39 (P = 0.07) for white traps and 0.35 (P = 0.11) for pink traps.

DISCUSSION

The results of this project indicate that visual traps should be considered for monitoring tarnished plant bug in peach orchards. Visual traps have been shown to be an effective monitoring method for thrips (Gillepsie & Vernon 1990; Childers & Brecht 1996), flea beetles (Adams & Los 1986) and apple blotch leafminers (Green & Prokopy 1986). In apples, white sticky traps have been useful for determining if tarnished plant bug populations are sufficiently great to merit insecticide application (Prokopy et al. 1987) and for detection of other mirid species present in apple orchards (Boivin et al.1982). In addition, they are very practical because they also work well in monitoring the European apple sawfly (Owens & Prokopy 1978).

Pink Tiara was a trap color selected by LeFevre (1984) because its spectral reflection pattern closely mimicked the color of peach flower petals. Our results show that this color is highly attractive to *L. lineolaris* but it is difficult to assess why this is happening. When most insects were trapped, all of the petals were gone and only developing fruit were present. Thus, the color did not mimic any particular peach resource for the insect. Developing fruits have a spectral reflectance pattern more similar to leaves (LeFevre 1984) and fruit did not start turning pink until the end of July or August. Although no significant differences were found among several colors tested, LeFevre's (1984) work indicated that *L. lineolaris* tended to be attracted by light colors such as gloss white and yellows over dark colors such as red and black. A similar result was observed by Prokopy et al. (1979) where *L. lineolaris* were attracted to traps painted gloss white, Zn white, Zoecon Yellow and to clear plexiglass. Zn white and gloss white traps were considered super normal mimics of apple bud and blossom reflectance patterns. Because *L. lineolaris* was also captured in clear plexiglass in numbers comparable to the light colors, Prokopy et al. (1979) concluded that this insect does not specifically orient to colors mimicking those of apple structures. Nevertheless, *L. lineolaris* is exhibiting some color discrimination since they were captured more often by light color traps (Prokopy et al. 1979; LeFevre 1984) and they preferred pink over white traps. It may be possible that the pink traps captured more insects because they provided a better visual contrast against the peach foliage. The response by *Lygocoris* spp. to the two colors could not be determined because too few were caught to discern any trap color effect. Nevertheless, both trap colors were useful in detecting their presence through the season.

Although *L. lineolaris* is more attracted by the pink colored traps, white and pink traps perform similarly in other aspects. Both trap colors have similar average times of insect capture and both tracked well the timing of fruit injury. White or pink trap captures in a given tree did not correlate well with the fruit injuries observed in that tree probably due to the high vagility of *L. lineolaris* and *Lygocoris* insects. This result confirms the utility of these visual traps because there is less concern that, for example, a trap will only reflect insect activity in its host tree. This quality is desirable in common orchard situations where one trap monitors a large area. For instance, the recommended use of sticky traps to monitor tarnished plant bug in apple orchards is at least one trap per 3 acres (Coli 2003). Further evaluation of pink traps should be done in order to assess their effectiveness in integrated pest management programs for *L. lineolaris* in peach orchards.

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MANIPULATION OF FEMALE PARASITOID AGE ENHANCES LABORATORY CULTURE OF *LYSIPHLEBUS TESTACEIPES* (HYMENOPTERA: APHIDIIDAE) REARED ON *TOXOPTERA CITRICIDA* (HOMOPTERA: APHIDIDAE)

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ABSTRACT

Cultures of the endoparasitoid *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae) on the brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), previously have been reported to be difficult to establish. In this study, *L. testaceipes* colonies were initiated from parasitized brown citrus aphids obtained from field-collected citrus foliage in Florida and successfully maintained for >25 generations in the laboratory. To enhance colony rearing methods, several aspects of the parasitoid's biology were examined. An evaluation of foraging by single or multiple females determined that the presence of multiple females did not influence mean progeny yield per female. However, the mean number of progeny produced by mature (25-49 and 49-73 h) *L. testaceipes* females was higher than that produced by younger (1-25 h) females over a 24-h period. In all three parasitoid age classes, each reared on second-, third- or fourth-instar aphid hosts, significantly more mummies containing *L. testaceipes* formed on a paper coffee filter covering the soil surface compared to the number of mummies formed on citrus foliage. Mummy formation off foliage has not been reported for this aphid-parasitoid complex in citrus. Mated females of *L. testaceipes* with access to honey and water and without access to aphids or honeydew lived longer than females that had access to aphid hosts and honeydew. These data provide novel findings on the biology of *L. testaceipes* when parasitizing the brown citrus citrus, particularly on mummification sites, and allowed us to develop a protocol for routine large-scale rearing of *L. testaceipes* on brown citrus aphids on citrus.

Key Words: *Lysiphlebus testaceipes*, *Toxoptera citricida*, laboratory cultures, host instar, citrus

RESUMEN

Las crias de el endoparasitoide *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae) sobre el áfido de cítricos de color café, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), previamente han sido reportada dificiles de establecer. En este estudio, colonias de *L. testaceipes* fueron iniciadas de áfidos de cítricos de color café parasitados obtenidos del follaje de cítricos recolectado en el campo y exitosamente mantenidos por >25 generaciones en el laboratorio. Para mejorar los métodos de cria de la colonia, varios aspectos de la biologia del parasitoide fueron examinados. Una evaluación del forraje de hembras individuales o hembras multiples determinó que la presencia de hembras multiples no tuvo influencia sobre el promedio de progenie por hembra. Sin embargo, el número promedio de progenie producido por hembras de *L. testaceipes* maduras (25-49 y 49-73 h) fue más alto de lo que fué producido por hembras mas jovenes (1-25 h) en un período de 24-h. En todas las tres clases de edad del parasitoide, cada cria en áfidos hospederos en el segundo-, tercero- o cuatro-estadio, significativamente más momias contenian *L. testaceipes* formado sobre un papel filtro de café que cubria la superficie del suelo compararada con el número formado sobre el follaje de cítricos. La formación de las momias no puestas sobre el follaje no ha sido reportado para este complejo de áfido-parasitoide. Hembras de *L. testaceipes* apareadas con acceso a miel y agua y sin acceso a los áfidos o a la miel del rocío vivieron mas tiempo que las hembras con acceso a los áfidos hospederos o a la miel del rocío. Estos datos proveen descubrimientos nuevos sobre la biología de *L. testaceipes* en la parasitización del áfido de cítricos de color café, particularmente en los sitios de momificación, y nos permitieron desarrollar un protocolo para la cria rutinaria en gran escala de *L. testaceipes* sobre el áfido de cítricos de color café en los cítricos.

The brown citrus aphid, *Toxoptera citricida* Kirkaldy (Homoptera: Aphididae), currently occurs on citrus throughout Florida where it is an efficient vector of citrus tristeza virus (CTV) (Costa & Grant 1951). In a classical biological control program for the brown citrus aphid, the endoparasitoid *Lipolexis oregmae* (Gahan) (= *scutellaris* Mackauer, Miller et al. 2002) (Hymenoptera: Aphidiidae) was imported from Guam and released in citrus groves throughout Florida

(Hoy & Nuygen 2000). Another aphid parasitoid, *Lysiphlebus testaceipes* Cresson (Hymenoptera: Aphidiidae), already occurs in Florida and also has been recorded parasitizing brown citrus aphids (Michaud 1999; Yokomi & Tang 1996).

Existing accounts on the biology of *L. testaceipes* on brown citrus aphid are scarce; Carver (1984), Yokomi & Tang (1996), Michaud & Browning (1999) and Persad & Hoy (2003) have dealt specifically with this aphid-parasitoid complex. The biology of *L. testaceipes* on other aphid species is better known and include: Schuster & Starks (1975), Stary et al. 1988, Stadler & Volkl (1991), Volkl & Stadler (1991), Grasswitz & Paine (1992), Vansteenis (1994), Stechmann et al. (1996), Pike et al. (1997), Fernandes et al. (1997, 1998), Elliot et al. (1999), Rodrigues et al. (2001), Rodrigues & Bueno (2001), Gonazales et al. (2002), Tang et al. (2002).

Previous studies were conducted to determine whether competition with *L. testaceipes* would affect establishment of *L. oregmae* in Florida (Persad & Hoy 2003). The intra- and interspecific interactions of both parasitoids on the brown citrus aphid were investigated in the laboratory and the data obtained suggest that *L. testaceipes* would not exclude *L. oregmae* during interspecific interactions and so may not affect its establishment in Florida. To conduct these competition studies, cultures of *L. oregmae* were maintained on brown citrus aphids on potted citrus in the laboratory using the method of Hill (2002), Walker (2002) and Hill & Hoy (2003). However, no protocol for rearing *L. testaceipes* on brown citrus aphids on citrus existed, so several attempts were made to initiate cultures from field-collected parasitoids.

Some researchers have reported that *L. testaceipes* is not easily cultured in the laboratory on brown citrus aphids. Carver (1984) was unsuccessful in rearing *L. testaceipes* on this host in the laboratory in Australia and considered oviposition by *L. testaceipes* in brown citrus aphids as an 'egg trap' because parasitism rates were high but adult emergence was low. Michaud & Browning (1999) failed to establish colonies of *L. testaceipes* on brown citrus aphid in Puerto Rico, even when parasitoids were used that were derived from brown citrus aphid populations exhibiting high rates of emergence of *L. testaceipes* adults.

This study describes the initiation and continued propagation of *L. testaceipes* colonies on brown citrus aphid in the laboratory for >25 generations after initial failures to establish thriving colonies. In an effort to understand the initial failures and to standardize a laboratory rearing system, several evaluations were conducted. To determine the nutrient requirements of adult *L. testaceipes*, survivorship of adult parasitoids with and without nutrients and the longevity of newly emerged females when allowed access to aphids and honeydew was evaluated. To resolve whether competing *L. testaceipes* females affected progeny yield in cages, the mean number of progeny produced per female in colonies initiated from single females versus yield when the aphids were exposed to multiple (6) females, was evaluated. The relationships between female parasitoid age, mating status and aphid host stage on mummy location and progeny production also were investigated.

Materials and Methods

Initiation of Laboratory Cultures of *L. testaceipes* on Brown Citrus Aphid on Potted Citrus

Brown citrus aphids were collected from young citrus foliage obtained from citrus groves in 15 counties in Florida between August and December 2001. Approximately 350 *L. testaceipes* adults were collected from field populations and used to initiate cultures. Care was taken to ensure that the collected aphids consisted solely of brown citrus aphids using the guidelines provided by Halbert & Brown (1996). Some of the leaves had mummified brown citrus aphids, indicating the presence of parasitoids. All collected foliage was held between crumpled sheets of absorbent paper in air-inflated plastic bags in the laboratory at 22-24°C, 55-65% RH and 16:8 h light:dark cycle. Condensation in each bag was wiped off twice daily. Under these conditions, citrus foliage could be maintained for 8 to 10 days, allowing parasitized aphids that were not yet mummified to be held sufficiently long to obtain adult *L. testaceipes*. This holding system allowed greater numbers of adult *L. testaceipes* to be collected than is obtained if only mummies are sampled.

Adult parasitoids that emerged were examined under the stereomicroscope. Apart from two species of hyperparasitoids, *L. testaceipes* was identified, using the guidelines of Evans & Stange (1997), as the only primary parasitoid emerging from field-collected brown citrus aphids. Emergence of *L. testaceipes* occurred mostly in the morning and adults were collected at 800, 1200 h daily by aspirator into 6 × 1.5 cm plastic vials. Groups of up to 20 parasitoids that emerged on the same day were held together in similar vials. Honey-saturated paper strips and moistened cotton was supplied within each vial and these were supplied whenever parasitoids were stored.

Mating pairs always were observed within 1 h of introducing parasitoids into the vials. Six presumably mated females were introduced into a 60 × 60 × 60 cm mesh (size 40/ mm^2) cage which contained six potted citrus plants; each plant was infested with 200 to 250 brown citrus aphids of mixed instars. Water was provided on a moist cotton pad on the cage top and honey strips were attached to the upper corners of each cage. Adult *L. testaceipes* progeny emerging 9 to 10 d later were collected by aspirator and the cycle was re-

peated. In addition, parasitoid cultures were initiated with single *L. testaceipes* females in cages containing individual potted plants. Progeny from both culture systems were released into a 35 × 35 × 35 cm plexiglass cage to mix before re-introduction to cultures in an effort to preserve their genetic diversity.

The identity of *L. testaceipes* from our cultures was confirmed by Peter Stary, Institute of Entomology, Academy of Sciences, Czech Republic. Specimens of *L. testaceipes* were deposited at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, as voucher specimens 2002- 1742- 901. Cultures were routinely screened by extracting random samples upon emergence and observing these specimens under the dissecting microscope to ensure that only *L. testaceipes* were present in our rearing cages. All parasitoid adults used to start cultures were screened to confirm identity and establish sex ratios before introduction into cages with aphid-infested citrus plants.

The use of recently emerged female parasitoids (24 h or less) to initiate colonies resulted in similar numbers of progeny as that of the *L. testaceipes* parents and hence colonies did not increase in size. It was observed that when older (24- to 30-h-old) females were used, more progeny were obtained. To obtain mature females, all newly emerged *L. testaceipes* of both sexes (approx. ♂:♀ ratio of 1:1.5, and 25 to 30 individuals) were stored in 6 × 1.5 cm plastic vials for 24 to 30 h before allowing them access to aphids.

Inspections of mummies located on citrus foliage in these laboratory colonies revealed that most were still intact, with no emergence holes. Surprisingly, the numbers of mummies on foliage with exit holes were considerably lower than the numbers of adults obtained in the cages. Because *L. oregmae* was found to produce mummies on the soil surface when reared on brown citrus aphid (Hill 2002; Walker 2003; Hill & Hoy 2003) we examined the cages containing *L. testaceipes* and mummies containing *L. testaceipes* were noticed on the soil surface. To confirm that the excess *L. testaceipes* adults were coming from mummies on the soil surface, paper coffee filters were placed around the base of the potted plants. Parasitized aphids were observed walking or falling down to the base of the plant 5 to 6 d after adult *L. testaceipes* had been introduced into the cages and the mummies formed were sometimes firmly attached to the coffee filters. To quantify and confirm these results, and to develop a suitable rearing system for *L. testaceipes* on the brown citrus aphid, we conducted the following experiments.

Effect of Nutrients on Survival (days) of *L. testaceipes* Adults

Survival in days of *L. testaceipes* reared on brown citrus aphid was unknown, so adults were held with or without nutrients and survivorship was determined. Mummies of brown citrus aphids containing *L. testaceipes* were collected from both citrus foliage and the coffee filter on the soil surface and placed individually in gelatin capsules (size 00). Emerging adult *L. testaceipes* were allowed to mate and were placed singly in 6 × 1.5 cm plastic vials, which contained a piece of fluted paper. Parasitoids were either offered no nutrients, water in moist cotton, pure honey on paper strips (0.75 × 2.5 cm) or both water and honey strips. Fifteen parasitoids of each sex were examined in each treatment.

To determine the effect of oviposition on longevity, 15 mated *L. testaceipes* females were housed in individual vials. These were allowed access to an excess of aphids (approx. 100 of mixed stages) on citrus foliage. The foliage was inserted into each vial and replaced every 12 h so that these female parasitoids had access to aphid honeydew, honey strips and water. Observations were made daily and a record of mortality kept. Comparisons of survival times of parasitoids that were not allowed to oviposit were made by ANOVA and LSD using Statview ver. 5.0 (SAS Institute 1999).

Effects of Using Young and Mature *L. testaceipes* Females on Total Parasitoid Progeny Production in Single vs Multiple (6) Parasitoid Culture Systems

Because Shekar (1956) had reported that *L. testaceipes* reared on *Aphis gossypii* Glover produced maximum progeny per day when mature (3 d), we compared total offspring produced by young and mature *L. testaceipes* reared on brown citrus aphid. Also, we compared total progeny produced per female in two culture systems to determine if competition/ interference during host seeking by multiple *L. testaceipes* females occurred.

Brown citrus aphid mummies containing *L. testaceipes* were collected from both coffee filters and citrus foliage from colony cages and stored individually in size 00 gelatin capsules in the laboratory. Ten female *L. testaceipes* were allowed to mate with one-d-old males upon emergence and a single female was introduced into each of 10 mesh (size 40/ mm^2) (60 × 60 × 60 cm) cages within one h of emergence (young females). Mating occurred readily and generally lasted from 40 to 80 sec. Each cage contained six potted citrus plants each infested with 200 to 250 brown citrus aphids of mixed stages. Ten mated *L. testaceipes* females also were initially kept in vials for 24 h (mature females, 25-h-old) in the laboratory before introducing them individually into each of ten similarly prepared mesh cages. For evaluations of multiple females, mated young (1-h-old) *L. testaceipes* females were introduced into each of ten mesh cages in groups of six and this was repeated using mature (25-h-old) females. Both young and mature parasitoids were provided with

honey and water and were allowed to remain in the cage until death.

Mean total progeny obtained from cages in which single parasitoid females were introduced was compared to the mean produced by each female in a cage with 6 females and comparisons also were made between total progeny produced by young females and mature females in both culture systems (1 vs 6). These data were arcsine transformed and analyzed by ANOVA, using Statview ver. 5.0 (SAS Institute 1999) at the 5% significance level.

Effect of Age and Mating Status of *L. testaceipes* Females Over a 24-H Period on Mummy Location and Adult Parasitoid Emergence

To resolve the effects of female parasitoid age and mating status on mummy location and progeny, the following experiment was conducted. The trial was conducted for 24 h because the survivorship data indicated that ovipositing females only lived for an average of 1.4 d. Plastic wrap was placed around the base of a potted flushing citrus plant (24 cm tall) and taped around the base of the plant stem to form a barrier to aphids migrating down the stem toward the soil. A circular paper coffee filter was slit and placed on top of the wrap and secured in place with 3M® Scotch tape. Forty alate brown citrus aphids were placed using a damp sable hair-brush (size 000) onto young leaves of a potted citrus plant and left for 24 h. Alates were removed and the first-instar (L1) aphids present were allowed to molt to the third instar (L3); this stage was used to standardize possible variation in progeny production because of aphid size. Excess L3 aphids were removed to leave 100 L3 aphids on the plant, which was then covered with a plexiglass cylinder (13 cm diameter and 45 cm tall) with mesh tops and side windows. Plants prepared in this way did not require water for the duration of the experiments.

On emergence (usually between 900 and 1100 h), 10 female parasitoids were randomly collected and allowed to mate with one-day males (because younger males did not mate as readily) in 4 × 1 cm glass vials. After mating (<1 h), a single *L. testaceipes* female was introduced onto each of 10 plants. Each plant was pre-infested with 100 L3 *T. citricida* and the parasitoid was left on the plant for 24 h (1-25 h age class). Citrus infested with L3 brown citrus aphids yielded mummies which were located on both foliage and the coffee filter by day 6 after introducing *L. testaceipes*. Mummies were collected from both locations, labeled as to source and held individually in size 00 gelatin capsules until emergence.

Ten *L. testaceipes* females were held individually in vials for 24 h and allowed to mate (as described above); each was then introduced individually into cages containing citrus with L3 *T. citricida* and left for 24 h (25-49 h age class). Ten *L. testaceipes* females also were similarly treated and allowed to mate, but held for 48 h before introduction into cages containing plants for 24 h (49-73 h age class). All female *L. testaceipes* were introduced into the test cylinders between 1000 and 1200 h. Ten replicates of each of the three parasitoid age groups were evaluated for mummy location and total adult *L. testaceipes* progeny.

The experiment was repeated using virgin *L. testaceipes* females for each of the age classes (1-25, 25-49 and 49-73 h). Trials in which the parasitoid had died or could not be found after 24 h in all experiments were discarded. Mummies found on foliage and on the paper coffee filter (soil) were counted and transferred on the tip of a dampened hairbrush individually to gel capsules. The number of mummies and the percentage adult eclosion from both locations were recorded for each age group. Data were arc-sine transformed before analysis using the Students t-test (SAS Institute 1999).

Effect of *T. citricida* Host Instar on *L. testaceipes* Mummy Location and Percentage Adult Emergence

To resolve the effects of host instar on mummy location and adult emergence, we kept the age of females constant and tested all four instars of brown citrus aphid. Each of 10 potted citrus plants was infested with 100 L1, L2, L3 or L4 *T. citricida* by allowing the L1 to molt to the desired stage. A mated *L. testaceipes* female that was 24 to 30 h old was then introduced into each of 10 potted citrus plants containing each host instar and covered with a plexiglass cylinder. The plants were left in the laboratory for 24 h, after which the parasitoid was located and removed. Trials in which the parasitoid died or could not be found were not used in further analyses. The number of *L. testaceipes* mummies and adults emerging at each location (foliage versus soil surface) were recorded for each aphid stage tested and percentage adult emergence was determined. Data were analyzed as described in the preceding section.

Results and Discussion

Effect of Nutrients on Survival (days) of *L. testaceipes* Adults

Adults of both sexes lived significantly ($P < 0.05$) longer when given both water and honey strips (Table 1). The data suggest that *L. testaceipes* needs both free water and an energy source for optimal survival. Mated females (data not shown and treated separately), when provided with water and honey and allowed constant access to aphids, lived a mean (±SD) of 1.4 (±1.3) d (N = 15), which was comparatively shorter than mated females that were not allowed to oviposit (mean ± SD of 3.7 (±2.7) days (N = 15). Some fe-

TABLE 1. MEAN (±S.D.) SURVIVAL (DAYS) OF NEWLY EMERGED AND MATED *L. testaceipes* ADULTS.*

Treatment	Males	Females
No water or honey	1.0 ± 0.8 c[1]	1.2 ± 1.3 b
Water only	1.6 ± 1.1 b	1.8 ± 1.4 b
Honey only	0.9 ± 0.7 c	1.4 ± 0.5 b
Water and honey	2.8 ± 2.6 a	3.7 ± 2.7 a

*At 22-24°C, 55-65% RH and16:8 h photoperiod. [1]Means followed by the same letters within a column are not significantly (P > 0.05) different by ANOVA and LSD.

males died while still attempting to oviposit and the urge for newly emerged adults to oviposit till death may have contributed to the failure of our initial colonies.

Effects of Using Young and Mature *L. testaceipes* Females on Total Parasitoid Progeny Production in Single vs Multiple (6) Parasitoid Culture Systems

Young females produced equal numbers (F = 0.02, df = 19, P = 0.88, n = 10, ANOVA) of total progeny whether introduced into cages as single females (Mean ± SD = 6.5 ± 3.6) or groups of six (4.7 ± 3.8). Total *L. testaceipes* progeny produced per mature female in which single (27.4 ± 12.8) or multiple (31.3 ± 14.7) females were introduced were not significantly different (F = 1.08, df = 19, P = 0.31, n = 10, ANOVA). These data suggest that competition/interference during host seeking by multiple *L. testaceipes* females may not have a significant effect on progeny yield when aphids are abundant.

However, younger *L. testaceipes* females (1-25 h after emergence) produced significantly fewer (6.5 ± 3.6) progeny compared to mature females (27.4 ± 12.8) (F = 53.8, df = 19, P = 0.0001, n = 10, ANOVA) in single-female cultures. Likewise, in multiple-female cultures, young females also produced significantly fewer progeny (4.7 ± 3.8) per female when compared to mature females (31.3 ± 14.7) (F = 54, df = 19, P < 0.0001, n = 10, ANOVA). This deficit in total progeny production by younger females may have been a contributing factor to the low yields in our initial cultures when females were allowed access to aphids immediately upon emergence. It is common, when rearing short-lived aphid parasitoids, to introduce newly emerged females into cages as soon as possible in order to optimize their reproductive potential (Hill 2002; Walker 2002; Hill & Hoy 2003), but when rearing *L. testaceipes* on the brown citrus aphid this is counter productive. Weisser (1994) observed that older *Lysiphlebus cardui* Marshall (Hymenoptera: Aphidiidae) females produced significantly more progeny than younger females when reared on *Aphis fabae* Scopoli (Homoptera: Aphididae); he attributed this to increased patch residence time by older females.

Effect of Age and Mating Status of *L. testaceipes* Females Over a 24-H Period on Mummy Location and Adult Parasitoid Emergence

Significantly (P < 0.05) more mummies containing *L. testaceipes* were located on the paper coffee filter located on the soil surface and significantly (P < 0.05) more adults emerged from those mummies whether mated or unmated females were used (Table 2). Mated *L. testaceipes* females produced more adult progeny if exposed to hosts when they were 25-49 or 49-73 h old than the females in 1-25 h age class. Virgin and mated females produced the maximum number of progeny if they were in the 25-49 h age interval. Shekar (1956) observed maximum oviposition in *L. testaceipes* reared on *A. gossypii* on day 3, when para-

TABLE 2. NUMBER OF MUMMIES AND PERCENTAGE OF *L. testaceipes* ADULTS PRODUCED BY MATED AND VIRGIN FEMALES IN THREE AGE CLASSES* IN A 24 H PERIOD ON L3 BROWN CITRUS APHIDS.

	Mean ± S.D. number of mummies on			Mean ± S.D. percentage adults eclosing from		
	Foliage	Coffee filter	P value	Foliage	Coffee filter	P value
Mated						
1-25**	2.3 ± 1.8 b	19.4 ± 5.7 a[1]	<0.0001	20.5 ± 20.1 b	68.5 ± 18.8 a	0.0012
25-49	6.1 ± 2.5 b	37.6 ± 15.9 a	0.0001	3.3 ± 20.9 b	76.3 ± 11.3 a	<0.0001
49-73	6.9 ± 4.5 b	28.6 ± 14.7 a	0.0020	25.1 ± 22.8 b	73.3 ± 22.3 a	0.0056
Virgin						
1-25	1.1 ± 1.0 b	9.4 ± 3.6 a	0.0007	6.4 ± 5.2 b	55.5 ± 15.0 a	<0.0001
25-49	1.2 ± 1.1 b	19.8 ± 6.4 a	0.0002	8.1 ± 6.9 b	75.8 ± 17.4 a	<0.0001
49-73	2.5 ± 1.5 b	8.6 ± 4.3 a	0.0003	5.5 ± 6.8 b	59.0 ± 17.2 a	<0.0001

*When 100 L3 brown citrus aphids are exposed to parasitoids at 22-24°C, 55-65% RH and 16:8 h photoperiod.
**Three holding intervals (h) used after adult emergence to produce the three age classes.
[1]Means ± S.D followed by the same letters in a row are not significantly different by Students t-test (SAS Institute 1999).

sitoids were allowed access to aphids for one-h periods on 3 consecutive days. This suggests that mature females of *L. testaceipes* also may produce more progeny when utilizing other aphid hosts.

Mated *L. testaceipes* females produced significantly more mummies and progeny than virgin females in all three age classes (Table 2, F = 13.54, df = 59, P = 0.03, n = 10 ANOVA). Although virgins of some parasitoid females may produce fewer progeny compared to mated females, in other parasitoid species the reverse may occur, or progeny yield may not differ (Godfray 1994). Michaud (1994) reported that virgin and mated females of *L. testaceipes* had similar parasitism rates on *Aphis fabae* Linneaus, while Shekar (1956) recorded that virgin females of *L. testaceipes* took from 2 to >30 times longer to begin oviposition in *Aphis gossypi* and had reduced fecundity. These combined reports suggest that oviposition behavior in *L. testaceipes* may be influenced by aphid species.

Effect of *T. citricida* Host Instar on *L. testaceipes* Mummy Location, Percentage Adult Emergence

There was no significant (P > 0.05) difference in the number of mummies containing *L. testaceipes* located on foliage and on the coffee filter when first instar (L1) brown citrus aphids were parasitized by *L. testaceipes* (Table 3). However, significantly (P < 0.05) more mummies formed on the coffee filter than on foliage for all other *T. citricida* instars tested (L2, L3 and L4) (Table 3).

The percentage of adult *L. testaceipes* emerging from mummies located on the coffee filter was significantly (P < 0.05) higher than on the foliage for all instars of brown citrus aphids tested (Table 3). Mean percentage of *L. testaceipes* female progeny that emerged from L1 and L4 hosts on foliage was not significantly different to that observed on the coffee filter; however, significantly more females emerged from mummies of L2 and L3 aphid hosts on the coffee filter than on the foliage (Table 3). Generally, more female parasitoids than males are produced from larger aphid hosts (Godfray 1994) and our data are consistent with this because L4 hosts produced more females than males whether they formed on the foliage or on the coffee filter. However, the observation that mummies on foliage originating from L2 and L3 aphid hosts produce a male-biased sex ratio (66-70%) while mummies on the coffee filter from these same-sized hosts produce female-biased sex ratio (27-38% males) is interesting and needs further evaluation.

The mean number of mummies containing *L. testaceipes* that occurred on the foliage was not significantly different from that observed on the coffee filter when L1 hosts were parasitized (Table 3). However, L2, L3 and L4 hosts produced significantly more mummies on the coffee filter than on foliage (Table 3). Dissection of uneclosed mum-

TABLE 3. MEAN NUMBER OF MUMMIES, MEAN PERCENTAGE *L. TESTACEIPES* ADULTS AND MEAN PERCENTAGE FEMALES PRODUCED BY MATURE *L. TESTACEIPES* FEMALES ON DIFFERENT INSTARS OF BROWN CITRUS APHID.

Host stage	Mean ± S.D. number of mummies/♀			Mean ± S.D. percentage adults/♀			Mean ± S.D. percentage females/♀		
	Foliage	Coffee filter	P	Foliage	Coffee filter	P	Foliage	Coffee filter	P
L1	21.4 ± 3.4 a	25.3 ± 10.3 a[1]	0.2345	15.4 ± 8.6 b	43.5 ± 28.9 a	0.0258	36.4 ± 8.9 a	35.4 ± 8.8 a	0.3144
L2	13.5 ± 5.3 b	38.1 ± 14.7 a	0.0021	25.4 ± 19.9 b	88.3 ± 9.1 a	<0.0001	30.2 ± 11.0 b	62.3 ± 16.9 a	0.0012
L3	4.2 ± 3.3 b	43.2 ± 11.0 a	<0.0001	27.0 ± 24.3 b	83.8 ± 7.0 a	0.0002	44.4 ± 14.9 b	72.9 ± 18.2 a	0.0031
L4	3.1 ± 2.3 b	29.8 ± 6.4 a	<0.0001	17.9 ± 21.1 b	94.7 ± 4.9 a	0.0001	62.5 ± 21.2 a	63.8 ± 15.2 a	0.5124

mies from citrus foliage in 0.8% saline under a dissecting microscope revealed many dead late-instar larvae, prepupae, or pupae of *L. testaceipes*. Stary (1989) termed this phenomenon 'incomplete parasitization'. In our study, mummies on the foliage produced higher rates of incomplete parasitization (73-85%) compared to that observed from mummies on the coffee filter (5-56%). Factors which cause more L1 hosts to produce mummies on foliage and mummies that exist on foliage to have greater rates of incomplete parasitism and a male-biased sex ratio are unknown.

Mummy location in *Lipolexis oregmae* also has been studied in the laboratory (Hill 2002;, Walker 2002; Hill & Hoy; 2003). *Lipolexis oregmae* mummifies on (or in) the soil and mummy location is independent of brown citrus aphid instar. Mummification on (or in, because coffee filters would prevent movement of aphids into the soil) the soil, however, has not been described previously for *L. testaceipes* reared on brown citrus aphid on citrus in Florida. The mechanism controlling movement of parasitized aphids to areas where there is greater chance of predation or fungal infections is unknown (Godfray 1994). Chow and Mackauer (1999) reported that the percentage of mummification off the plant of the aphid *Acyrthosiphon pisum* (Harris) when parasitized by *Ephedrus californicus* (Baker) varied with aphid density. However, the location of brown citrus aphid mummies containing *L. testaceipes* does not appear to be dependent on aphid host density. When brown citrus aphids, in densities of 40 or 200, were parasitized by single *L. testaceipes* females in laboratory trials (data not shown) a mean (± SD) percentage of 60.2 (13.2) and 71.3 (17.4), respectively, were produced on the coffee filter ($P = 0.28$, $n = 10$, Students t-test).

Mummy location also was investigated in a citrus grove adjacent to the Department of Entomology and Nematology, University of Florida, Gainesville, in fall 2001 and spring and summer of 2002. Young citrus foliage was infested with 200-250 brown citrus aphids of mixed stages and were covered with mesh sleeves. When a single female *L. testaceipes* was allowed access to these aphids, mummies were formed off the foliage in significantly higher quantities than on the foliage. Potted citrus plants in mesh cages also were placed in the grove and produced similar results (Persad & Hoy, unpublished data). This suggests that movement of brown citrus aphids containing *L. testaceipes* to the soil is not restricted to laboratory colonies. Thus, these results indicate that analysis of parasitism by *L. testaceipes* of brown citrus aphid in the field in Florida should not be limited to examining the mummies occurring on foliage.

In field evaluations in Puerto Rico, Yokomi & Tang (1996) concluded *L. testaceipes* is an ineffective parasitoid of the brown citrus aphid because they observed an emergence rate of ca. 4.0% from mummies located on field-collected citrus foliage. Michaud (1999) also commented on the low occurrence of emergence holes in mummies located on citrus terminals and observed that, despite the ubiquitous presence of *L. testaceipes*, rates of parasitism were generally too low to affect brown citrus aphid populations. Despite these negative evaluations of *L. testaceipes* as a parasitoid of the brown citrus aphid, our data suggest parasitism by *L. testaceipes* may be more extensive in citrus in Florida than previously recognized.

Rearing Protocol

The data in Tables 1 and 3 indicate that younger *L. testaceipes* females produce fewer progeny and often die shortly after being allowed constant exposure to aphids. In contrast, female parasitoids produced more progeny if their exposure to aphids was delayed for at least 24 h; it is unknown whether this effect is behavioral or physiological. This information is, however, crucial to the following guidelines for initiating and maintaining cultures of *L. testaceipes* on the brown citrus aphid:

Hold newly emerged adult parasitoids in vials with access to water and honey for 24 h before they are allowed access to aphids. Six potted citrus plants (prepared as described above), each infested with 200-250 brown citrus aphids of mixed instars, will yield 32 to 150 adult *L. testaceipes* (Mean ± SD = 85 ± 61, n = 17 culture cages) when 6 mated mature (24-30-h-old) females are allowed to parasitize their hosts until death. This protocol was used in summer 2002 to initiate 5 separate *L. testaceipes* cultures from field-collected citrus foliage infested with brown citrus aphid. Successful and expanding cultures resulted in all cases and populations increased within one generation using this protocol, indicating that no genetic selection of this parasitoid was needed to propagate it on brown citrus aphid.

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LASIODERMA SERRICORNE (COLEOPTERA: ANOBIIDAE): SPATIAL RELATIONSHIP BETWEEN TRAP CATCH AND DISTANCE FROM AN INFESTED PRODUCT

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ABSTRACT

The cigarette beetle, *Lasioderma serricorne* (Fabricius), was selected as a representative stored-product beetle to test the validity of contour mapping of trap catch for pest monitoring in warehouses and retail stores. Three experiments, each replicated 5 times, were conducted in a 3.2×9.0-m aluminum shed. Each experiment involved releasing beetles at a single point and recording the numbers captured after 6, 24, and 48 h in each of 14 baited pitfall traps distributed over the floor of the shed. The experiments differed only with respect to the point of release. Beetles were released passively from rearing boxes placed at one of 3 release points, and consecutive contour maps of trap catch tracked their dispersal from each point. As the beetles dispersed and total trap catch increased, the outlying traps captured increasingly more insects, but cumulative trap catch remained highest near the release points. The rate of capture was highest immediately after release and declined with time, rapidly at first and then more slowly until it became nearly constant. The cumulative numbers captured by any trap after 6, 24, and 48 h decreased exponentially with distance from the point of release. The observed spatial patterns of trap catch relative to sources of infestation and the inverse relationship of trap catch to distance from a source support the validity of contour mapping as a means of monitoring stored-product insects and locating foci of infestation.

Key Words: pest monitoring, trapping, spatial analysis, stored-product insects, cigarette beetle

RESUMEN

El escarabajo del cigarillo, *Lasioderma serricorne* (Fabricius), fue seleccionado como un representante de un escarabajo de productos almacenados para probar la validez de un mapa de contorno de los especimenes capturados en trampas para un monitoreo de los almacenes y tiendas comerciales. Se realizaron tres experimentos, cada uno con 5 réplicas, en un cobertizo de lauminio de 3.2×9.0-m. Cada experimento envolvia liberar los escarabajos en un solo punto y registrar en número capturados despué de 6, 24, y 48 h en cada una de las 14 trampas con cebo de caida "pitfall" distribuidas sobre en piso del cobertizo. Los experimentos variaron solamente con respeto del punto de la liberación.Los escarabajos fueron liberados en una manera pasiva de las cajas de cria puestos en uno de los tres puntos de liberación, y su dispersión fue rastreada usando mapas contornos consecutivos de los especimenes capturados en cada punto. Mientras que los escarabajos se disparsaron y el número total de los especimenes capturado aumentó, las trampas remotas capturaron progresivamente más insectos, pero el número acumulativo de los especimenes capturados en las trampas cerca de los puntos donde fueron liberados permaneció en más alto. La razón de la captura fue la más alto inmediatamente después de la liberación y bajó con el tiempo, rapidamente al principio y luego más despacio hasta que quedó casi constante. El número acumulativo de escarabajos capturados en cualquier trampa después de 6, 24, y 48 h bajó exponencialmente según la distancia del punto de liberación. Los patrons espaciales observados de los escarabajos capturados en las trampas en relacción de las fuentes de infestación y la relación inversa de los escarabajos capturados a la distancia de la fuente apoya la validéz de loos mapas contornos como un medio papa hacer un monitoreo de insectos de productos almacenados y localizar los focos de infestación

The cigarette beetle, *Lasioderma serricorne* (Fabricius), is arguably the most ubiquitous of all stored-product insects. It occurs throughout the tropical and subtropical regions of the world, and although it is restricted by low temperature and humidity, it occurs commonly in warm buildings throughout the temperate regions. It breeds on a wide variety of commodities, including both plant and animal materials (Howe 1957; LeCato 1978; Ashworth 1993), and is one of several beetle pests that commonly infest warehouses and retail stores (Arbogast et al. 2000, 2002).

Regular monitoring for insect pests in buildings, such as rice, flour and provender mills,

warehouses, and retail stores, has assumed greater importance as more emphasis is placed on integrated pest management. A combination of trapping and spatial analysis of trap catch by contour mapping has shown considerable promise as a reliable and practical method of monitoring (Brenner et al. 1998; Arbogast 2001; Subramanyam et al. 2002; Fields & White 2002) and has already gained some acceptance by the pest control industry. The value of the method lies in its ability to locate as well as detect infestation and in the utility of contour maps for documentation and communication. The maps provide graphic, easily understood evidence of insect infestation and the effectiveness of control intervention. They are thus of considerable value in communicating insect problems to managers and to maintenance, sanitation, and pest control personnel.

The method rests on the tacit assumption that there is a relationship between trap catch (number captured by a trap in a specified period of time) and proximity to a source of infestation. Although the results of studies in commercial warehouses, processing plants, and retail stores (Rees 1999; Arbogast et al. 2000, 2001, 2002; Campbell et al. 2002) have supported this assumption, experimentation in a less complex environment is needed to verify its validity and to determine the quantitative nature of the relationship. The present paper reports the results of such experimentation, using *L. serricorne* as a representative stored-product beetle.

Materials and Methods

Laboratory cultures of *L. serricorne* were established with adults collected from ground cumin in a Gainesville, FL household in December 1999. The insects were reared at 27 ± 1°C and 60 ± 5% RH with a 12-h photoperiod on a diet of whole wheat flour (10 parts), white cornmeal (10 parts), and brewers' yeast (1.5 parts). Each culture was contained in a 0.95-l mason jar capped with filter paper over screen.

Experiments were conducted in an aluminum shed (about 3.2 × 9.0 m) between June and October 2001. The walls and ceiling of the shed were covered with sheet rock over styrofoam insulation to moderate temperature changes, and the wooden floor, which was elevated about 0.25 m above a concrete slab supporting the shed, was covered with asphalt floor tile. Fourteen pitfall traps (Dome Traps, Trécé, Inc., Salinas, CA) baited with cigarette beetle pheromone lures and a food attractant oil provided with the traps were positioned on the floor as illustrated in Figs. 1-3. No heating or air conditioning was used to regulate temperature. Air temperature at floor level was monitored with HOBO temperature loggers (HO-001-02, Onset Computer Corp., Bourne, MA) placed at the trap sites and set to record temperature at 1-h intervals.

We conducted three experiments, all of which involved releasing beetles and monitoring trap catch over a 48-h period. The experiments differed only with respect to the point of release, which was either at the center of the shed (Fig. 1), near the southwest corner (Fig. 2), or near the northeast corner (Fig. 3). Each experiment was replicated 5 times. For each replicate (48-h trapping run), 2000 newly emerged adults were collected from cultures (46-47 d old) and divided equally between 2 plastic boxes (19 × 14 × 9.5 cm), each containing a shallow layer (about 300 ml) of the rearing diet. The boxes were placed side by side on the floor of the shed at one of the release points, and the lids were removed. All releases were made between 10:00 and 11:00 am, and the number of beetles in each trap was recorded after 6, 24 and 48 h. The beetles remaining in the boxes after 48 h were counted and the number remaining was subtracted from 2000 to obtain the number that had dispersed. The shed was disinfested between replicates by removing the plastic boxes and vacuuming all beetles from the walls, floor and ceiling. Also, the traps were emptied, cleaned, and provided with fresh oil, but pheromone lures were replaced only between experiments. The HOBOs were launched at the beginning of each replicate, and temperature data were downloaded at the end.

The numbers of beetles captured at each trap site were averaged over the five replicates of each experiment, and contours of mean trap catch were drawn for 6, 24 and 48 h using Surfer 8 (Golden Software, Inc., Golden, CO) (Figs. 1-3). The Multiquadric function (Radial Basis Functions) was used as the interpolation algorithm with default values of the function parameters R^2 (smoothing) and h (anisotropy). Radial Basis Functions comprise a group of interpolation methods that attempt to honor data points (that is, they are exact interpolators). The Multiquadric method is considered by many to be the best in ability to fit a data set and to produce a smooth surface (Krajewski & Gibbs 1996; Golden Software 2002), and with most small data sets (<250 observations), it produces a good representation of the data (Golden Software 1999).

Variation in rate of capture with time following release was examined by determining the cumulative number of beetles captured in each replicate 6, 24, and 48 h after release. The totals were averaged over the 15 replicates, and the regression of mean cumulative total on hours following release was plotted and analyzed (Fig. 4). The influence of distance from a source of infestation on numbers of insects captured was examined by combining the results of the three experiments, calculating mean trap catch for each distance, and plotting and analyzing the regression of mean trap catch on distance 6, 24, and 48 h after release (Fig. 5). The number of observa-

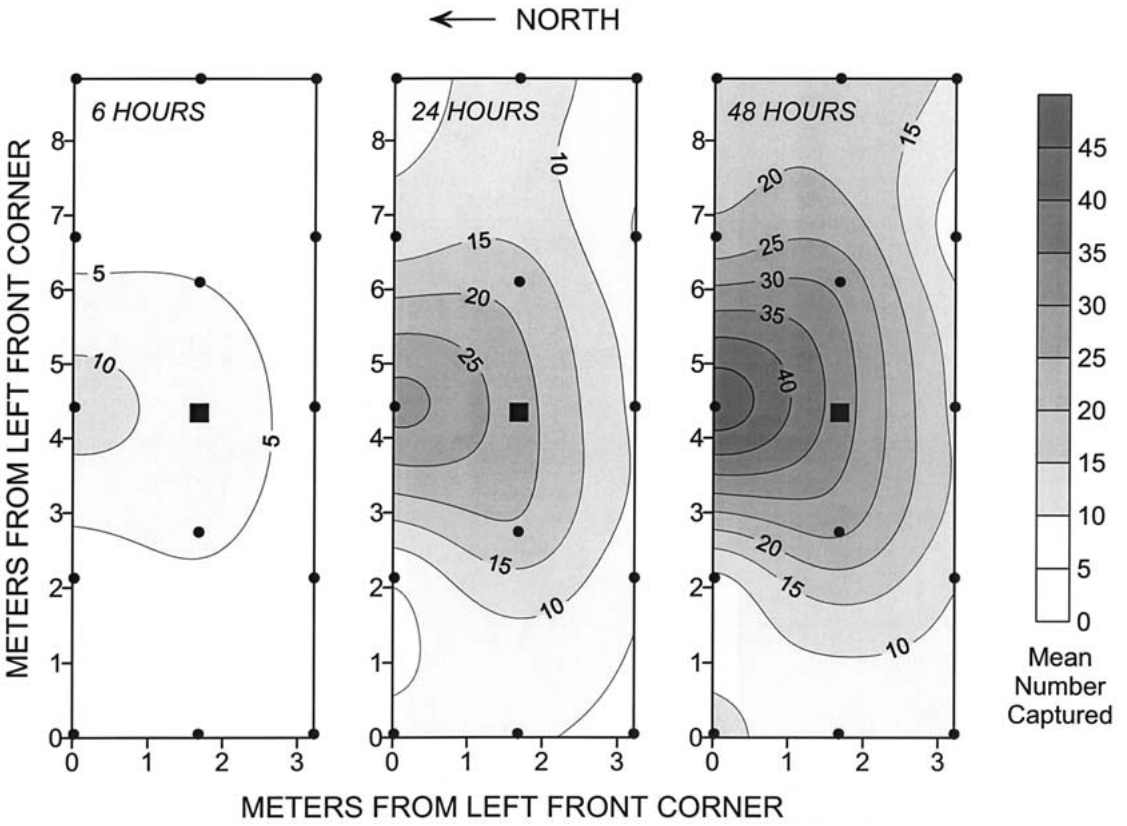

Fig. 1. Contour maps illustrating the changing spatial distribution of *Lasioderma serricorne*, as indicated by trap catch 6, 24, and 48 h after the beetles were released at the center of the shed. The release point is indicated by a solid square and trap positions by solid circles. The contours indicate mean numbers captured (5 replicates).

tions contributing to each mean ranged from 5 to 20. SigmaPlot 2001 (SPSS, Chicago, IL) was used for all regression analyses.

The mean, minimum, and maximum temperatures at each trap position were determined for each experiment, and isothermal maps were drawn to portray spatial variation in temperature over the floor of the shed. Isothermal analysis was done with Surfer 8 as already described for contour analysis of trap catch.

Hourly temperature records were used to calculate hourly means, minima, and maxima, which were then plotted against time of day to illustrate overall diurnal variation during the 15 trapping runs (Fig. 6A). The maximum temperature difference (temperature range = maximum - minimum) among trap sites was determined for each hour of each replicate and used to calculate the mean, minimum, and maximum hourly ranges for the 15 replicates combined. These ranges were then plotted against time of day to illustrate spatial variation and diurnal changes in spatial variation (Fig. 6B).

Mean, minimum, and maximum temperatures, calculated for each replicate (14 trap sites × 48 hours = 672 temperature records), were used to examine the association of temperature with the number of beetles leaving the boxes (dispersing) and with the total number that were captured. Spearman's rank order correlation coefficient (R_s) was calculated for the pooled data (15 replicates) using SigmaStat 2.03 (SPSS Science, Inc., Chicago, IL). Correlation analysis was chosen because none of the variables were fixed at a constant level, and all contained sampling variability. The nonparametric Spearman rank order correlation was used, because we could not assume bivariate normality and common variance.

Results and Discussion

Contours of trap catch 6, 24, and 48 h after release (Figs. 1-3) tracked the dispersal of beetles from the release point (source of infestation). Intuitively, we would expect the probability of capturing an insect at a fixed point in time to

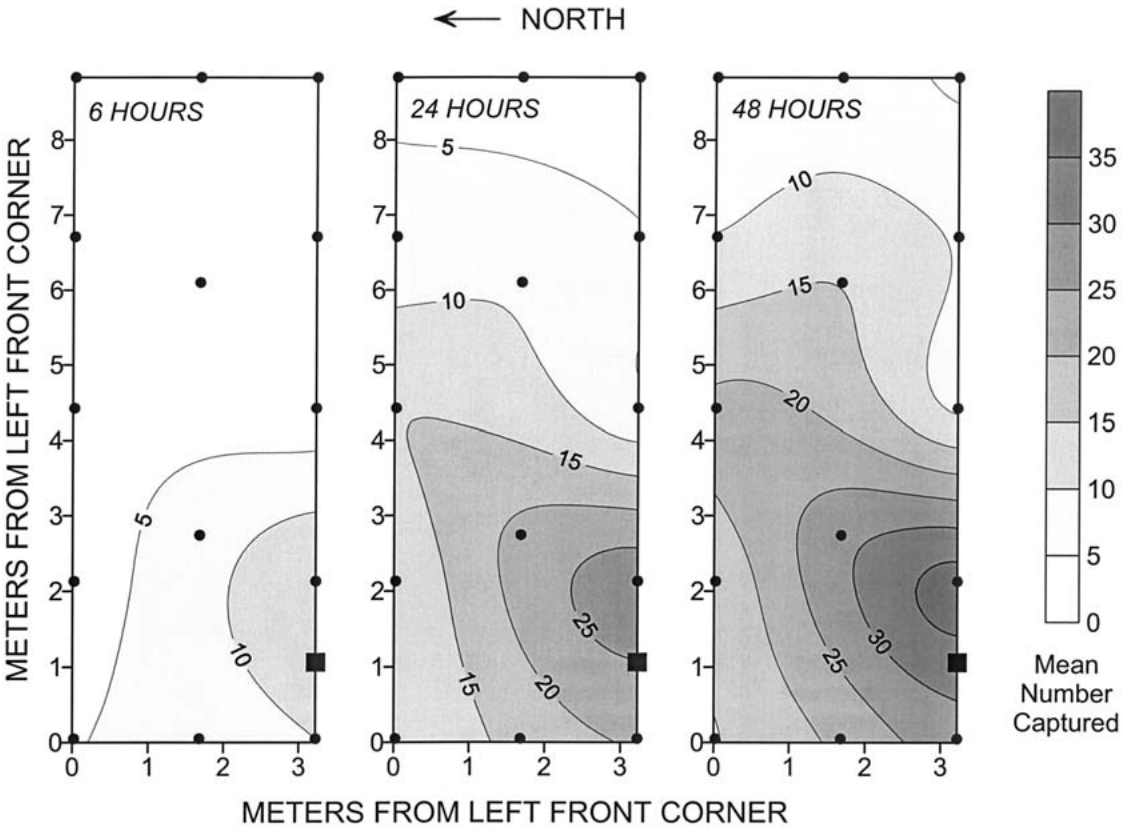

Fig. 2. Contour maps illustrating the changing spatial distribution of *Lasioderma serricorne*, as indicated by trap catch 6, 24, and 48 h after the beetles were released near the southwest corner of the shed. The release point is indicated by a solid square and trap positions by solid circles. The contours indicate mean numbers captured (5 replicates).

increase with proximity to a source of infestation. We would also expect the probability of capture at a fixed distance from a source to increase with time. The temporal changes in contour pattern observed with all three release points were consistent with these expectations. As the beetles dispersed and total trap catch increased, the outlying traps captured more insects, but cumulative trap catch remained highest near the release point. This pattern of change in consecutive contour maps simulates temporal changes in contour pattern that have been observed in retail stores with infested products (Arbogast et al. 2000). Infested products in stores often harbor continuously breeding populations of insects that provide a more or less constant source of dispersing insects. Placing traps in an infested store is analogous to releasing insects in our shed experiments after the traps were already in place, but time is measured from trap placement rather than from insect release. We have observed that sources (foci) of infestation in stores are first detected by the closest traps; the number of insects captured and the area in which the captures occur increase steadily over time, so that the contour pattern surrounding a focus intensifies and spreads outward (Arbogast et al. 2000).

We expected the beetles to disperse equally in all directions unless their freedom of movement was constrained by the walls of the shed, but this did not happen. When the beetles were released at the center of the shed, movement was predominately toward the midpoint of the north wall (Fig. 1). The same bias in dispersal pattern was evident when the beetles were released near the southwest corner (Fig. 2). Although this directional bias in dispersal from the release point shows that factors other than proximity to a focus of infestation can influence trap catch, it did not render trapping and contour analysis ineffective as a means of locating these foci. In both cases, and also when the beetles were released near the northeast corner (Fig. 3), the greatest number of beetles captured after 6, 24, or 48 h were captured by the traps nearest the release point. Although real world situations are more complex, so that

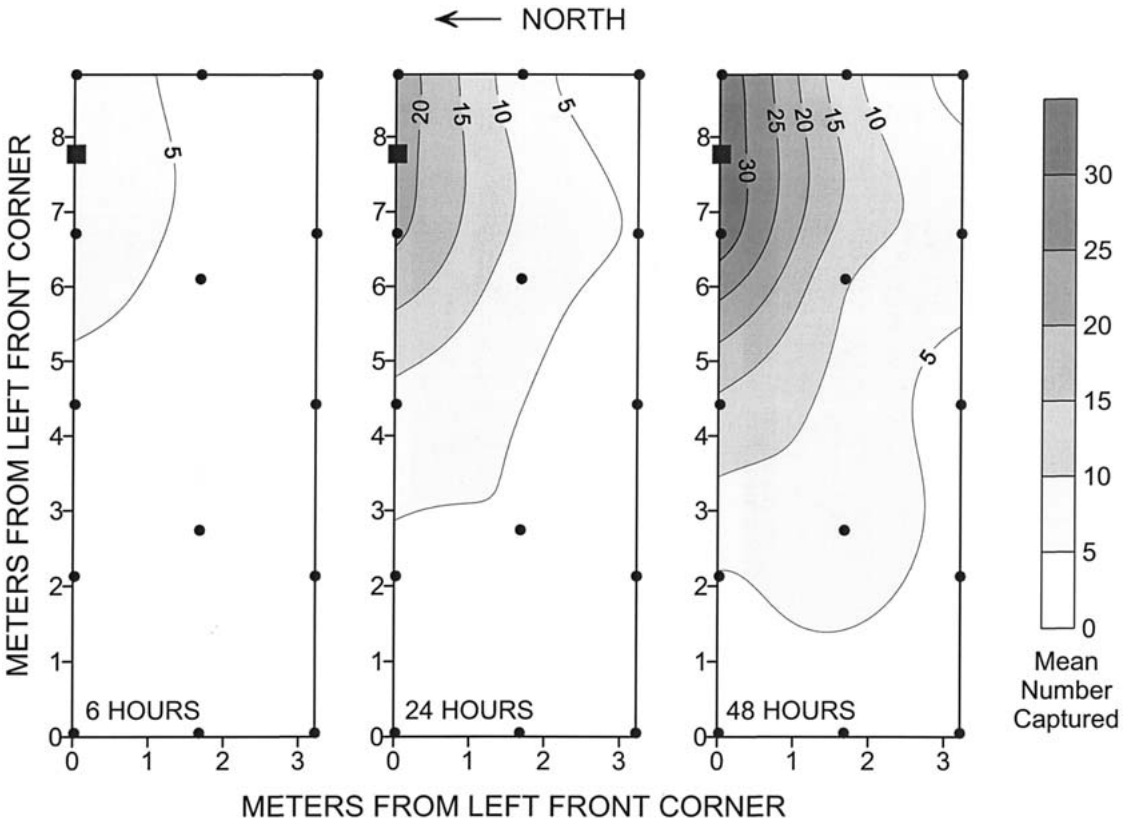

Fig. 3. Contour maps illustrating the changing spatial distribution of adult *Lasioderma serricorne*, as indicated by trap catch 6, 24, and 48 h after the beetles were released near the northeast corner of the shed. The release point is indicated by a solid square and trap positions by solid circles. The contours indicate mean numbers captured (5 replicates).

locating infestation is more difficult, studies in commercial warehouses (Arbogast et al. 2002) and retail stores (Arbogast et al. 2000) have, nevertheless, shown the method to be useful.

The rate of capture in the shed was highest immediately after release of the beetles and declined with time, rapidly at first and then more slowly until it became nearly constant (Fig. 4). Without further research, we can only speculate about the cause of this initially rapid decline, but we would expect such a temporal pattern if there were a burst of dispersal immediately after release, followed by a decline and eventual stabilization of dispersal at a much lower rate. The same pattern should also occur when a trap is first set in an infested building, such as a pet store, if the rate of capture is positively correlated with the number of insects available to be captured. In this case, the rate of capture would decline as the adult population becomes depleted and would eventually stabilize when recruitment of adults from infested commodities just balances their removal. In a trapping study of *Plodia interpunctella* (Hübner) and various beetles in-

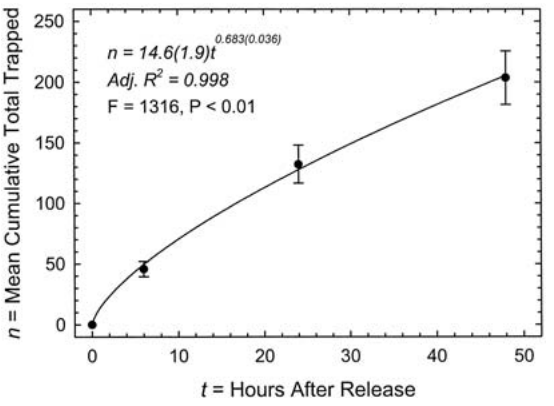

Fig. 4. Variation in rate of capture of adult *Lasioderma serricorne* with elapsed time (t) after release. Mean cumulative totals (n) are based on all trap sites, replicates, and experiments combined (210 observations). Error bars indicate standard errors of the means. The numbers in parentheses are standard errors associated with estimates of the parameters a and b in the fitted equation: $n = at^b$.

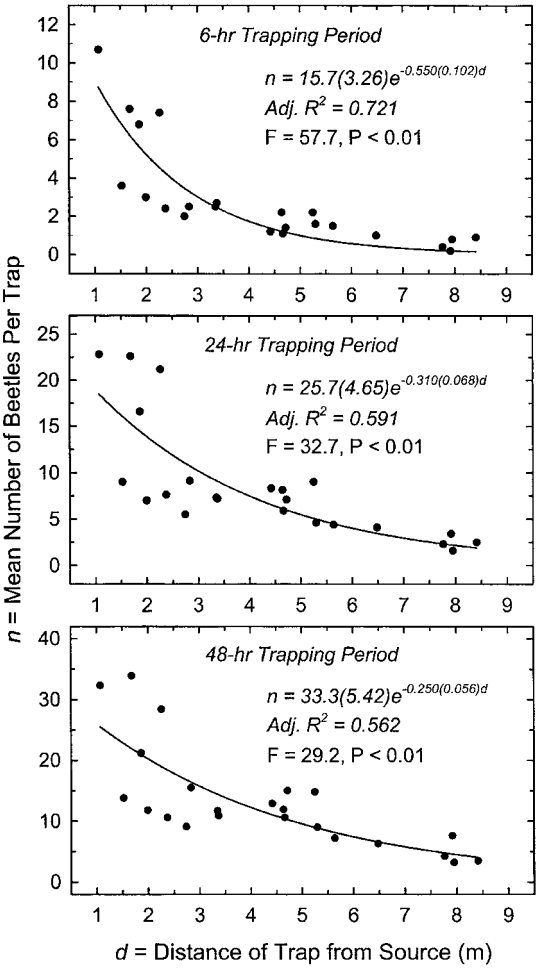

Fig. 5. Relationship between mean number of adult *Lasioderma serricorne* per trap (n) and distance (d) of the traps from a source of infestation (point of release) after 6, 24, and 48 h. The plots are based on all 3 experiments (points of release) combined. The number of counts in each mean ranged from 5 to 20. The numbers in parentheses are standard errors associated with estimates of the parameters a and b in the fitted equation: $n = ae^{-bd}$.

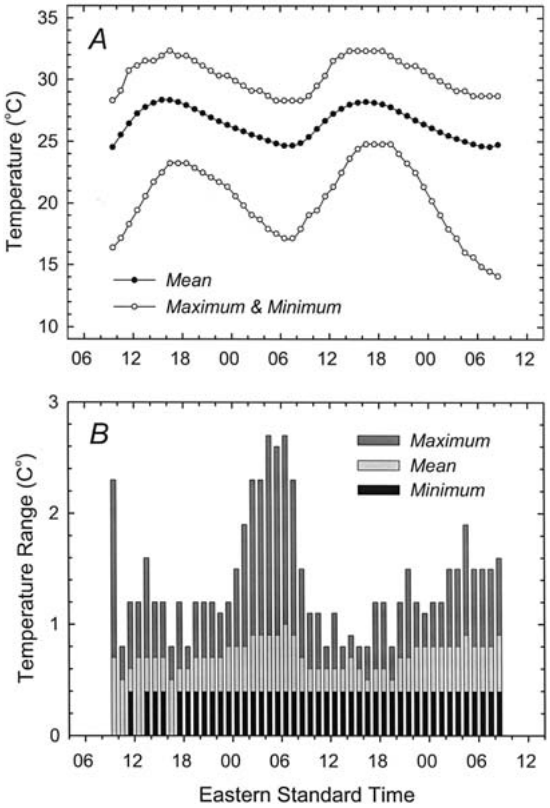

Fig. 6. Variation in temperature on the floor of the shed with time of day. (A) Mean, minimum, and maximum temperatures for each hour based on temperature records for all trap sites, replicates, and experiments combined (210 records). The maxima and minima for each time of day are the highest and lowest temperatures recorded for that time over the course of the entire study. (B) Mean, minimum, and maximum temperature ranges (differences between trap sites with the highest and lowest readings) for each time of day. Ranges were determined for each hour of each replicate, and all replicates were then combined to determine mean, minimum, and maximum ranges for the study. The range of each temperature statistic can be read from the top of the shading representing that statistic.

festing pet and department stores, Arbogast et al. (2000) found that the relationship between days of trapping and cumulative numbers captured over periods of 4-5 days was well described by straight lines. However, these authors noted some evidence that the rate of capture may actually have decreased with time during the first day or two.

The number of beetles (n) that had been captured by any trap in the shed 6, 24, or 48 h after release declined as an exponential decay function of distance (d) from the source of infestation (Fig. 5). The effect of distance on numbers captured became less pronounced with time as the dispersing beetles spread out and occupied more of the shed. Pierce (1994) successfully located infestations of *L. serricorne* and pyralid (phycitine) moths using a triangulation method based on the assumption (implied although not explicitly stated) that there is an inverse relationship between trap catch and the distance of the trap from a source of infestation. The results of the present study, as well as Pierce's success in locating infestations, support the validity of his assumption.

Temperature inside the shed varied over the course of the study (Fig. 6), but the seasonal range of variation was apparently insufficient to affect dispersal of beetles from the point of release, or the

TABLE 1. NUMBERS OF CIGARETTE BEETLES THAT DISPERSED FROM RELEASE SITES AND NUMBERS THAT WERE CAPTURED IN PITFALL TRAPS DURING 48-H TRAPPING PERIODS.

	Beetles dispersed[1]		Beetles trapped[2]	
Trapping run	Total	Percentage	Total	Percentage[3]
Experiment 1: Beetles released at center of shed				
27-29 Jun	1570	78.5	251	16.0
10-12 Jul	1629	81.4	326	20.0
17-19 Jul	1103	55.2	150	13.6
23-25 Jul	1334	66.7	162	12.1
06-08 Aug	1923	96.2	319	16.6
Experiment 2: Beetles released near southwest corner of shed				
14-16 Aug	1507	75.4	220	14.6
23-25 Aug	938	46.9	80	8.5
28-30 Aug	1558	77.9	326	20.9
05-07 Sep	1498	74.9	217	14.5
10-12 Sep	976	48.8	297	30.4
Experiment 3: Beetles released near northeast corner of shed				
19-21 Sep	838	41.9	142	17.0
24-26 Sep	683	34.2	119	17.4
02-04 Oct	1060	53.0	83	7.8
09-11 Oct	1198	59.9	172	14.4
15-17 Oct	1745	87.2	188	10.8

[1]Number of beetles out of 2,000 that dispersed from the plastic boxes at the release point during the 48-h trapping run.
[2]Combined number of beetles captured by 14 traps during the 48-h trapping run.
[3]Of the beetles that dispersed, the percentage that were trapped.

numbers captured. The number of beetles (out of a possible 2000) that dispersed from the diet during any 48-h trapping run ranged from 683 to 1923, and the total number of dispersing beetles captured by the 14 traps ranged from 80 to 326 (Table 1). Correlation analysis of data pairs for all three experiments combined showed no significant association between number of beetles dispersed and mean ($R_s = 0.12$, $P = 0.67$), minimum ($R_s = 0.11$, $P = 0.70$), or maximum ($R_s = 0.15$, $P = 0.58$) temperature. Correlation analysis also indicated no significant association between trap catch and mean ($R_s = 0.36$, $P = 0.18$), minimum ($R_s = 0.38$, $P = 0.15$), or maximum ($R_s = 0.35$, $P = 0.19$) temperature. The temperature range among trap sites varied with time of day, but the ranges of the means, maxima, and minima never exceeded 0.4, 1.0, and 2.7°C, respectively (Fig. 6B). A frequency distribution of the temperature ranges for all experiments, replicates and hours combined (720 ranges) showed that 86.5% were ≤1.0°C and that 99.0% were ≤1.9°C. Consequently, isothermal maps showed very weak temperature gradients on the floor of the shed, and comparison of these maps with contour maps of trap catch revealed no clear effect of temperature gradients on movement of the beetles.

Several studies have shown that a combination of trapping and contour analysis of trap catch provides a useful, albeit less than perfect, method for monitoring stored product insects and locating foci of infestation in buildings such as warehouses, mills, and retail stores (Rees 1999; Arbogast et al. 2000, 2001, 2002; Campbell et al. 2002). The spatial pattern of trap catch relative to sources of infestation indicated by the contour maps in the present study, and the inverse relationship of trap catch to distance from the source, further support the validity of contour mapping as a method of monitoring stored-product insects and locating foci of infestation. Although the action of one or more factors other than distance from a source of infestation was evident in two of the experiments, the highest trap catch, nevertheless, occurred at one of the sites closest to the source. Campbell et al. (2002), however, noted that high trap captures in the warehouse portion of a food processing plant may have resulted from three distinct factors: proximity to a large infestation, proximity to a major route of insect movement, and proximity to a major source of attractive odor. The influence of various factors (in addition to proximity of infestation) on the spatial distribution of trap catch clearly needs further investigation.

Contouring has some advantages over the triangulation method used by Pierce (1994). One advantage is the utility of contour maps in documenting and communicating insect problems, as

already noted. Another is the fact that traps need not be arranged in a regular rectangular array as required by Pierce's method, a requirement that cannot always be satisfied in commercial settings. Contouring software employs various algorithms to create regular arrays of data points by interpolation between irregularly spaced observations, and contours are then fitted to the interpolated values at these points. This advantage, however, is not as great as it may appear at first glance, because the more widely an arrangement of traps deviates from a regular rectangular array, the weaker the agreement between observed and predicted values at the trap sites. Arbogast et al. (2003) examined this and other sources of error in predicting insect distribution by trapping and contour analysis, and pointed out measures that can be taken to minimize them.

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LIFE HISTORY AND BIOLOGY OF *PHYCIODES PHAON* (LEPIDOPTERA: NYMPHALIDAE)

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ABSTRACT

The butterfly *Phyciodes phaon* (Edwards), the Phaon crescent, was reared in the laboratory on its host plant, *Phyla nodiflora* (L.) Greene, at 27°C with 16:8 (L:D) photoperiod and fluorescent lighting. Eggs are laid in clusters on the underside of host leaves and hatch in about 5 days. Newly hatched larvae aggregate and feed on the underside of the leaf. Later instars disperse on the host plant and continue to feed on the leaves. Larvae develop through five instars based on head capsule, weight, and size measurements. The duration of each instar and the pupal stage were determined. Adults mate 2-3 days after emergence, and females begin laying eggs after 2 more days. The life cycle from egg to adult requires 23-31 days. The butterfly is easy to rear and mating occurs in laboratory cages under artificial lighting. The butterfly has been reared continuously in the laboratory for about 3 years with no evidence of disease in the colony.

Key Words: *Phyciodes phaon* (Edwards), butterfly, Phaon crescent, Nymphalidae, Lepidoptera, insect-host plant interaction, *Phyla nodiflora* (L.) Greene, Verbenaceae

RESUMEN

La mariposa, *Phyciodes phaon* (Edwards), fue criada en el laboratorio en su planta hospedera, *Phyla nodiflora* (L.) Greene, a los 27°C con un fotoperíodo de 16:8 (L:D) e iluminación florescente. Los huevos son puestos en grupos en el envéz de la hoja del hospedero y se eclosionan en aproximadamente 5 dias. Las larvas recien nacidas se agregan y se alimentan en el envez de las hojas. Los estadios tardios se dispersan en la planta hospedera y continuan alimentandose sobre las hojas. Las larvas pasan por cinco estadios basado sobre la cápsula de la cabeza, el peso y las medidas del tamaño. La duración de cada estadio y el estado pupal fue determinada. Los adultos se aparearan 2-3 dias después de la salida, y las hembras empiezan poner huevos 2 dias después. El ciclo de vida desde el huevo hasta el adulto requiere 23-31 dias. La mariposa es fácil criar y el apareamiento ocurre en el laboratorio bajo iluminación artificial. La mariposa ha sida criada continuamente en el laboratorio por alrededor de 3 años sin evidencia de una enfermedad en la colonia.

Species of the butterfly genus *Phyciodes* Huebner (Nymphalidae) are restricted to the Americas, and many of the species are tropical. There are 12 species in the United States that have been divided into three species-groups (Scott 1994). The Phaon crescent, *Phyciodes phaon* (Edwards), occurs in Florida (Opler & Krizek 1984; Minno & Minno 1999) and is distributed from coastal North Carolina throughout the southern parts of the Gulf States to southern Texas and westward to southern California, and sometimes migrates north to Iowa and Nebraska. The Phaon crescent adult is characterized by a strong contrasting orange and black coloring of the forewings and upper side of the hindwings. The undersides of the hindwings are pale with brown markings. The Phaon crescent is distinguished from other *Phyciodes* species by having a creamy yellow band evident across both upperside and underside of the forewing.

The host plant utilized by the Phaon crescent in Florida is *Phyla nodiflora* (L.) Greene (previously described as *Lippia nodiflora* L.) in the Verbenaceae (Riley 1975), and it is known by a number of common names including fog fruit, frog fruit, matchweed, capeweed, creeping Charlie and match heads (Verdcourt 1992). It is a perennial herb with long creeping stems and small white to light yellow flowers with a purple center (Fig. 1). It is widely distributed in the southern United States. It roots readily at the nodes and spreads as a ground cover. Leaves are opposite, wedge shaped, thick, leathery, and finely serrated along the edges but rounded at the tip. The plant prefers moist areas and disturbed habitats such as along roadsides and sidewalks, and the margins of wetlands and rivers. Two other butterflies reported to use *Phyla nodiflora* as a larval host are the common buckeye, *Junonia coenia* Hübner, and the white peacock, *Anartia jatrophae* Munroe (Minno & Minno 1999). Little is known about the biology of *P. phaon*. The aim in this paper is to describe the life history, biology and immature stages of *P. phaon* feeding on its host plant in the laboratory.

Fig. 1. *Phyla nodiflora* used as a larval food plant by the Phaon crescent.

Materials and Methods

During the summer of 1999, *P. phaon* adults (n = 30) were captured in the vicinity of Gainesville, Florida. Eggs were obtained from these adults by placing them in a screen cage with potted host plants, *P. nodiflora*. Adults were given access to 10% honey solution or Fruit Punch Gatorade® on small cotton balls. Eggs were removed daily, counted, and kept in a Petri dish on moist filter paper. Larvae were fed freshly cut host-plant material. Larval food was changed every other day by transferring all larvae to new plants. Pupae were harvested daily, and transferred to a new cage with a potted host plant. The colony was maintained under controlled laboratory conditions at 27°C, 16:8 (L:D) h photoperiod. The number of instars was determined from data collected from 10 larvae examined each day. Shed larval head capsules were collected, measured, and preserved in 70% ethyl alcohol. Larvae also were weighed and their length measured daily for the 10 individuals to determine the number of instars. Larvae were weighed individually. Data were analyzed by one way ANOVA with Statpak (Northwest Analytical, Inc., Portland, OR), and when the F value was significant, means were separated by Fisher's Least Significant Difference. Significance was accepted with $P \leq 0.05$.

Results

Description of *Phyciodes phaon* Edwards Immature and Adult Stages

Eggs

Females laid eggs in clusters on the undersurface of host leaves (Fig. 2A). In the laboratory, as few as 5 and as many as 187 eggs occurred in clusters. Sometimes eggs were stacked on top of each other. The light green eggs were elliptical, about 0.63 ± 0.03 mm in length and 0.36 ± 0.01 mm in diameter (N = 25 eggs) with a flattened base and slight depression at the micropyle. They were sculptured with 18-20 vertical raised ridges (Fig. 2A, B). Development to hatching required 5.1 ± 0.3 days at 27°C, and the color of the egg changed from light green to brownish black at about 4 days as the mandibles and head of the larva became visible through the chorion.

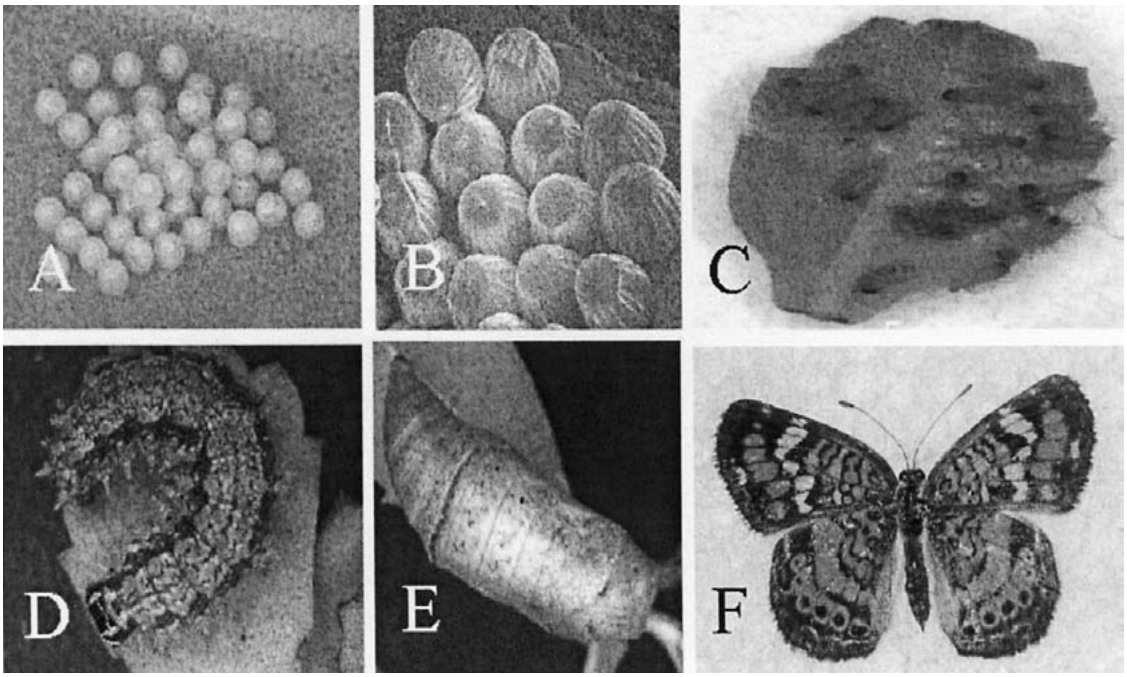

Fig. 2. A composite photo of some developmental stages of the Phaon crescent *Phyciodes phaon*. A, A cluster of eggs on the underside of the host plant; B, Scanning electron micrograph (SEM) of an egg cluster; C, Newly hatched first instars on the underside of a leaf; D, The fourth instar; E, A *P. phaon* pupa attached by the cremaster to a stem of the host plant; F, An adult butterfly.

Larvae

Larvae developed through five instars. Larval weight, length, and head capsule measurements (n = 10) in each instar are shown in Table 1. The first instar was olive green to olive brown, with long setae over the body (Fig. 2C). The head was cream colored with two large brown to black patches. The legs and prolegs were light brown and tarsal segments were black to brown. The anal prolegs were dark brown. Antennae were cream in color, with brown basal area. The labrum was brown, the labial and maxillary palpi were light cream in color, and ocelli were black. The facial suture margins were darkened. Head capsule setae were numerous and oriented anteriorly. Brown and cream spots were randomly distributed on the integument. First instars ate their eggshells and stayed aggregated on the underside of the leaf, typically spinning some silk web on the leaf. Generally, larvae rested on top of the silk web, but sometimes larvae rested and fed beneath part of it. They ate small amounts of the underside of the leaf, creating a small pit, which they continued to enlarge as they fed on internal leaf tissue. The duration of the first instar in the laboratory was 3.6 ± 0.8 days (n = 25).

The second instar was light brown in color with dark subdorsal bands. Each segment contained a row of short, branching small spines. The head was black with two long cream dorsal stripes extending posterior to the neck. The mouthparts were dark brown. The head capsule setae were more numerous than in the first instar. The integument was textured with brown, dark brown, and cream spots. The longitudinal, dorsal and subdorsal bands were more evident in the second instar than in the first instar. The thoracic legs were light brown or cream in color with the tarsal claws darkened. The spiracles were brown. The duration of the second instar was 3.8 ± 0.8 days (n = 25).

Third instars were similar in appearance to second instars, but cream patches on the head capsule were more evident. Third instars generally rested on the upper side of leaves and fed on the edges. They no longer aggregated, but distributed themselves over the whole plant. They spent 4.1 ± 0.8 days in the third instar (n = 25).

Fourth and fifth instars were similar in appearance to each other and to third instars (Fig. 2D). These last two instars consumed a large quantity of host leaves. The duration of the fourth instar was 4.3 ± 0.8 days (n = 25), and duration of the fifth instar was 3.9 ± 0.8 days (n = 25).

Prepupae

Mature larvae attached with the cremaster to a stem, leaf or other support and remained in a cres-

TABLE 1. MEASUREMENTS OF HEAD CAPSULE, WEIGHT, AND LENGTH OF LARVAL *PHYCIODES PHAON* IN EACH INSTAR (MEAN ± SD, N = 10).

Instar	Head capsule measurements (mm)	Weight (mg)	Length (mm)
First	0.296 ± 0.008 a	5.1 ± 0.7 a	2.06 ± 0.08 a
Second	0.593 ± 0.008 b	15.5 ± 4.9 b	6.07 ± 0.12 b
Third	0.798 ± 0.004 c	37 ± 4.8 c	12.63 ± 0.43 c
Fourth	1.295 ± 0.007 d	81 ± 7 d	18.95 ± 0.83 d
Fifth	1.90 ± 0.007 e	163 ± 9 e	28.3 ± 0.67 e
LSD*	0.0063	0.0054	0.0456

*LSD = Fisher's Least Significant Difference between any two means. The means within a column followed by a different letter are different from each other ($P < 0.05$) (ANOVA and Fisher's Least Significant Difference tests).

cent shape about 8-10 hours. Then, hanging straight down, they changed within 2-3 minutes into the characteristic pupal shape and appearance.

Pupae

Pupae were initially very soft and light tan, speckled with black and white (Fig. 2E). They had darker and paler areas over the wings, and a brown "U-shaped" mark around the front of the head. Some pupae were very dark, almost black, in color, but the cause of this color variation was not explored. The pupal abdomen consisted of 10 segments, with the 10th segment bearing the cremaster by which pupae attached to a support. Pupae measured 12.2 ± 0.1 mm in length, 5.8 ± 0.1 mm in width (measured dorsoventrally in the thoracic region), and weighed an average 82 ± 40 mg (n = 25). The duration of the pupal stage was 4.6 ± 0.8 days.

Adults

Males and females were similar in appearance (Fig. 2F). The wingspan was 30.7 ± 0.02 mm in females and 23.4 ± 0.01 mm in males (n = 25). Mating pairs often rested quietly together 4-5 hours. Mated females started laying eggs about 2 days after mating. A single female laid from 200-250 eggs (n = 25). Adults survived in the laboratory about 2 weeks. The duration from egg to adult was 23-31 days at 27°C, 16:8 (L:D) photoperiod in the laboratory.

DISCUSSION

Species in the genus *Phyciodes* are believed to be a monophyletic group based upon mitochondrial DNA sequences (Wahlberg & Zimmermann 2000). Most of the species feed as larvae on host plants in the family Asteraceae and Acanthaceae (Scott 1994; Brock & Kaufman 2003). In addition to feeding upon the Asteraceae, *P. picta* also colonizes Convolvulaceae, and larvae of the phaon crescent feed on several species in the genus *Phyla* in Verbenaceae and one species in Acanthaceae (Scott 1994; Wahlberg 2001). Larval food plants for several species are still unknown (Brock & Kaufman 2003).

The ranges of the phaon crescent and pearl crescent overlap in northern Florida and parts of the southern United States, but the larval host plants belong to two different plant families, the Verbenaceae and Asteraceae, respectively (Oliver 1982; Emmel & Kenney 1997; Brock & Kaufman 2003). Oliver (1982) succeeded in achieving hand-paired matings between adults of the phaon crescent and pearl crescent, and obtained F_1 hybrids from some crosses that would feed upon both *P. nodiflora* and various asters.

In our study, adult phaon crescents mated readily in small to large laboratory cages, and cage size and lighting seemed not to be critical. Although the host plant is widely available in much of the southern United States, it also can be cultured easily in small pots. Remarkably, during three years of rearing the butterfly we have seen no evidence of disease. These ease-of-rearing characteristics and the availability of the host plant all year in the Gainesville area (and possibly further north in protected places) make the phaon crescent a potentially useful teaching tool in schools and a convenient display butterfly for butterfly houses. Moreover, the Phaon crescent seems to be a valuable model butterfly for further research in genetics, mating behavior, pheromone biology, and physiology.

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IDENTIFYING HOST STRAINS OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) IN FLORIDA USING MITOCHONDRIAL MARKERS

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ABSTRACT

Two molecular techniques were used to identify host strains of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) from male moths captured in pheromone-baited traps in north-central and central Florida. Moths collected in 1998 were analyzed using direct detection of mitochondrial DNA (mtDNA) RFLPs generated from restriction endonuclease digestion of total DNA, while moths collected in 2000 and 2001 were analyzed using a mitochondrial cytochrome oxidase subunit I (COI) gene PCR-RFLP marker. Both techniques could distinguish between rice and corn strain moths, however, the COI PCR-RFLP marker was more robust as indicated by a time interval experiment that showed that moths held for up to 15 days in a "bucket trap" could still be used for strain diagnosis. In a field study, our strategy gave results consistent with expectations. Rice strain moths were common in habitats with large areas of small grasses, corn strain moths were common in large areas planted to corn, and habitats with mixed large- and small-grass plantings contained both strains. Our methodology of combining pheromone traps with PCR-RFLP analysis will provide a valuable sampling system to determine the population ecology habits and strain isolating mechanisms of fall armyworm populations in numerous habitats, including overwintering areas of southern Florida.

Key Words: *Spodoptera frugiperda*, host strain identification, PCR-RFLP

RESUMEN

Dos ténicas moleculares fueron utilizadas para identificar razas hospederas del cogollero, *Spodoptera frugiperda* (J. E. Smith) a partir de polillas machos capturadas en trampas cebadas con feromonas en la región centro-norte y central de Florida. Las polillas colectadas en 1998 fueron analizadaas utilizando la detección directa de los PLFR (Polimorfismo en la Longitud de los Fragmentos de Restricción [RFLP en ingles]) del ADN mitocondrial (mtADN) generados a partir la digertión por la endonucleasa de restricción del ADN total, mientras que las polillas colectadas en el 2000 y 2001 fueron analizadas utilizando un marcador PCR-RFLP de la subunidad I del gen citocromo oxisasa (COI) miticondrial. Ambas técnicas pudieron distinguir entre las razas de polillas del arroz y las del maíz, sin embargo, el marcador COI PCR-RFLP fue masrobusto tal como lo indico un experimento de intervalo de tiempo en el cual las polillas que se mantuvieron en una "trampa de balde" hasta por 15 días, todavía podían ser utilizadas para diagnosticar su raza. En un estudio de campo realizado, nuestra estrategia produjo resultados consistentes con las expectativas. Las razas de polillas de del arroz fueron communes en habitats con amplias áreas de pastos bajos, las razas de polillas del maíz fueron mas comunes en amplias áreas sembradas con maíz, y en áreas sembradas con una mezcla de pastos altos y bajos se consiguieron ambas razas. Nuestra metodología de combinar las trampas de feromonas con el análisis de PLFR-PCR proveerá un importante sistema de muestreo para determinar hábitos ecológicos de las poblaciones y los mecanismos de aislamiento de ls poblaciones de cogollero en numerosos habitats, incluyendo las áreas de hibernación en el sur de Florida

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a migratory polyphagous pest that attacks several important crops such as maize, sorghum, forage grasses, rice, cotton and peanuts (Luginbill 1928; Sparks 1979; Knipling 1980). Two morphologically indistinguishable host strains have been identified that are possibly in the initial stages of speciation (Pashley 1986; Prowell 1998). One strain was identified from populations feeding on corn and sorghum (corn strain), and the other strain was identified from populations feeding on rice and bermudagrass (rice strain). Strains exhibit polymorphisms at five allozyme loci (Pashley 1986), in their mitochondrial DNA (mtDNA) (Pashley 1989; Lu & Adang 1996) and in their nuclear DNA (Lu et al.

1992). Additionally, a tandemly repeated (189 bp) DNA sequence has been shown to be unique to the rice strain (Lu et al. 1994). Two recent techniques have been used to improve strain discrimination, including one that uses amplified fragment-length polymorphisms (AFLPs) (McMichael & Prowell 1999), and another that employs amplification of a region of the mitochondrial cytochrome oxidase C subunit I gene (COI) followed by restriction enzyme digestion (PCR-RFLP) (Levy et al. 2002).

Strain identification is important because research has shown biological, behavioral, toxicological, and host genotypic differences between strains. Both strains attained similar larval and pupal weights when fed bermudagrass or rice, but when reared on maize, corn strain larvae attained larger weights (Pashley et al. 1995; Veenstra et al. 1995). Behavioral reproductive incompatibilities, such as the lack of successful mating between corn strain females and rice strain males, have been tentatively identified (Pashley & Martin 1987), although successful matings were achieved with moths held in culture for over three years (Whitford et al. 1988). Temporal partitioning of calling/mating times has been presented as a strain isolation mechanism (Pashley et al. 1992). Rice strain larvae were shown to be more susceptible to various insecticides such as carbaryl, diazinon, cypermethrin, methyl parathion, and methomyl, while corn strain larvae were more susceptible to carbofuran (Pashley et al. 1987b; Adamczyk et al. 1997). Rice strain larvae were also more susceptible to transgenic *Bacillus thuringiensis* Berliner (Bt) cotton than corn strain larvae (Adamczyk et al. 1997). Laboratory and field studies have shown distinct differences in feeding of bermudagrass genotypes, with rice strain larvae generally able to gain more weight and consume more plant material than corn strain larvae (Pashley et al. 1987a; Quisenberry & Whitford 1988).

Although fall armyworm overwinters in southern Florida counties and can build up large populations in central and north-central Florida (Pashley et al. 1985; Mitchell et al. 1991), strain identification of Florida populations is limited. Late instar larvae collected from corn in southern Florida (Hendry Co.) were identified as corn strain in 1983 and 1984 (Pashley et al. 1985). Both corn and rice strain populations were identified from southern Florida, although no information regarding location or collection habitat were provided (Pashley 1988). Corn strain larvae were collected in early 1989 from southern Florida, but again collection sites and habitats were not disclosed (Pashley et al. 1992). Strain identification of populations from non-overwintering areas in north or central Florida has not been attempted. The objective of this research was to identify the host strain of fall armyworm moths collected from sex pheromone traps for periods up to 15 days in north-central and central Florida using suitable molecular methods.

Materials and Methods

Moth Collection

Standard plastic Unitraps (bucket traps) baited with commercial fall armyworm sex pheromone [(*Z*)-9-tetradecen-1-ol acetate, (*Z*)-11-hexadecen-1-ol acetate and (*Z*)-7-dodecen-1-ol acetate; either Scentry® (Ecogen, Inc., Langhorne, PA) or Scenturion® (Scenturion, Inc., Clinton, WA) lures] were placed in field locations in 1998, 2000, and 2001. All traps contained insecticide strips (Hercon® Vaportape II containing 10% 2,2-dichlorovinyl dimethyl phosphate, Hercon Environmental Co., Emigsville, PA) to kill the moths. In 1998, locations in Alachua Co., FL were used to collect moths. One was near the Dairy and Agronomy Forage Research Unit of the University of Florida, and the second location was a commercial corn field near the town of Alachua. The research unit was located in the northern half of the county and contained plantings of field corn and pasture grasses.

In 2000, three traps were placed along State Route 121 in Levy Co., FL. Several hundred hectares of forage grasses bordered the route. In 2001, three traps were placed at the University of Florida Range Cattle Research and Education Center in Ona, FL. This center has over 1150 h of natural and improved forage grasses. Two traps were also placed beside sugarcane plantings in the Everglades Agricultural Area near the University of Florida Everglades Research and Education Center, Belle Glade, FL, and the USDA, ARS, Sugarcane Field Station, Canal Point, FL. Fall armyworm larvae were also collected from Ona and resulting adults were analyzed to determine their host strain.

Interval Testing

This test was designed to determine if the host strain of moths held for up to 15 d in a pheromone trap could still be accurately identified. Fall armyworms used in this test were reared in the laboratory on a pinto bean-based artificial diet according to the procedures of Guy et al. (1985). Pupae were sexed and placed in 163-ml (5.5 oz.) paper cups (Sweetheart, Chicago, IL) that were placed in 24 × 24-cm screen cages for eclosion. Pupae were maintained under reversed photoperiod (14:10, light:dark) in an environmental chamber held at 26°C and 70% RH. Adult males had access to cotton balls saturated with distilled water and a honey-sugar solution. Live male moths aged 2-5 d were placed in bucket traps with insecticide strips and removed for testing at 1, 2, 4, 7, 10 and 15 d. Moths usually died within an hour after ex-

posure to the insecticide strips. Three to 10 moths were sampled for each time point.

Total DNA Extraction and Strain Identification

Two techniques were used to identify strains. For both techniques, total DNA was extracted from one fall armyworm adult that was ground in liquid nitrogen and then homogenized in 1 ml of extraction buffer (100 mM Tris-HCl, pH 7.5, 20 mM EDTA, pH 8, 500 mM NaCl, 2% (w/v) SDS). The remainder of the extraction procedure was performed according to Lu et al. (1992) except that final resuspension of the DNA was in 100 µl of TE buffer (10 mM Tris, 1 mM EDTA, pH 8.1). The technique of Lu & Adang (1996) was employed to identify strains of moths collected in 1998. Approximately 50 µg of total DNA was double-digested with the endonucleases *Hae*III and *Msp*I, and 8 µg of the digested DNA was electrophoresed on a 1% agarose gel in TBE buffer. Gels were then stained with ethidium bromide and visualized under UV illumination. The corn strain produces four mitochondrial bands of 5.5, 4.3, 3.8 and 1.3 kb (cannot see this smaller band due to masking by nuclear DNA), while the rice strain produces only two bands of 10.4 and 4.4 kb.

Strain identification of moths collected in 2000 and 2001 was accomplished using a mitochondrial PCR-RFLP marker (Levy et al. 2002). Two PCR primers (5'GAGCTGAATTAGGGACTCCAGG3'-forward and 5'ATCACCTCCACCTGCAGGATC3'-reverse) flanking a diagnostic *Msp*I restriction site (CCGG) within the mitochondrial COI gene sequence were used to amplify a 569 bp product. The PCR mixture contained 25 ng of total DNA, 0.25 µM primers, 200 µM dNTPs, 0.5 U *Taq* polymerase, and 1× PCR buffer (Perkin Elmer GeneAmp kit) in a total volume of 25 µl. Amplification conditions were as follows: denaturation, 94°C for 30 sec; annealing, 58°C for 1 min; extension, 72°C for 1 min, with a final cycle extension of 72°C for 10 min. Reactions were run for 40 cycles in a thermal cycler (MJ Research). Following amplification, 5 µl of the PCR mixture was used for *Msp*I digestion and the resulting products were surveyed using 2% agarose gel electrophoresis. The corn strain PCR product contains the *Msp*I restriction site while this site is missing in the rice strain product. Consequently, *Msp*I digestion of the corn strain 569 bp PCR product results in two bands of 497 bp and 72 bp, whereas this PCR product from the rice strain moths is unrestricted and remains intact.

Results and Discussion

Both corn and rice strain moths were collected from north-central and central Florida (Table 1). Digestion of total DNA with *Hae*III fragments genomic DNA made observation of mtDNA fragments easier. Digestion of mtDNA with *Msp*I produced three visible bands of 5.4, 4.3, and 3.8 kb representing corn strain moths, and two bands of 10.4 and 4.3 kb representing rice strain moths as previously established by Lu & Adang (1996) (Fig. 1). The size of the mtDNA genome of fall armyworm was estimated as approximately 14.8 kb (Lu & Adang 1996). The smallest band (1.3 kb) from corn strain moths was not visible because of masking by nuclear DNA. This method therefore resulted in a "3-band pattern" and "2-band pattern" of corn and rice strain moths, respectively (Lu & Adang 1996). The PCR-RFLP marker correctly identified corn strain moths by way of *Msp*I digestion of the 569 bp COI amplified fragment into two bands of 497 bp and 72 bp (Fig. 2). The rice strain PCR product was not digested by *Msp*I (Levy et al. 2002).

Rice strain moths predominated in large areas of small grasses such as Levy Co. Rt. 121 and Ona (Table 1), while corn strain moths were found more frequently in the only large corn site tested in Alachua Co. (Table 1). Mixed areas of corn and small grasses, and the peanut habitat contained moths of both strains. The agroecosystem near

Table 1. Corn and rice strains of fall armyworm adult males collected in pheromone traps at different field sites in Florida, 1998, 2000, 2001. Moths collected in 1998 were analyzed using the method of Lu & Adang (1992); moths collected in 2000 and 2001 were analyzed using the method of Levy et al. (2002).

Location	Date	Habitat	Strains (No.)	
			Corn	Rice
Dairy/Forage, Alachua Co.	4/22, 5/29, 6/12/98	grass, corn	3	1
Corn fields, Alachua Co.	5/25/98	corn	7	1
Levy Co., Rt. 121	8/10, 8/14, 8/16/00	grass	1	13
Levy Co.	8/20, 9/3/01	peanuts	17	10
Everglades Agric. Area	8/17/01, 11/14/01	sugarcane	1	6
Ona, RCREC	6/7, 7/12, 8/30, 11/14/01	grass	0	39
Ona, RCREC	11/14/01	colony	0	5

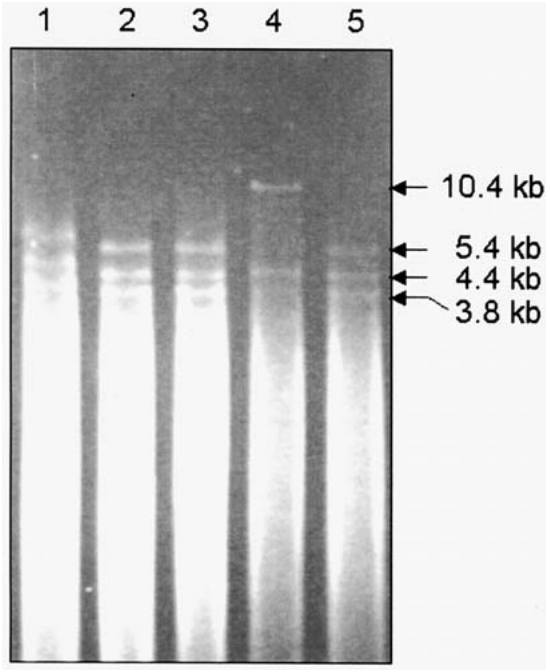

Fig. 1. Fall armyworm total DNA digested with *Hae*III and *Msp*I. Lanes 1-3 and 5 are corn strain, lane 4 is a rice strain.

the peanut site also contained large tracts of pasture, easily accessible by moths. Therefore, the presence of both strains was expected, although it is not known which strain is physiologically better adapted to peanut as a host plant. Previous studies of peanut host plant resistance used laboratory colonies that were probably corn strain (Leuck & Skinner 1971; Garner & Lynch 1981; Lynch et al. 1981), although strain analysis was not performed on these colonies. Further studies are underway to determine whether one strain is better adapted to peanuts than the other. The Everglades Agricultural Area also contained moths of both strains. The habitat in this area is dominated by large grasses such as sugarcane and corn and small grasses such as rice and "wild" grass species. Larger sample sizes from these habitats are needed to determine which strain is more common in this important and fragile agroecosystem.

Previous physiological studies suggested that rice strain larvae were more specialized and affected by their host plant than were corn strain larvae (Pashley et al. 1995; Veenstra et al. 1995). However, larval collections in the field disclosed that rice strain larvae occur in both large and small grass habitats, whereas corn strain larvae rarely occupied small grass habitats (Pashley 1988; Pashley et al. 1995; McMichael & Prowell 1999). Our study detected few corn strain moths in small grass habitats (Levy Co. Rt. 121, Ona). Although host-plant specialization is likely mediated by adult behavioral attributes rather than larval physiological characteristics (Pashley et al. 1995), studies determining adult attributes such as mating behavior and ovipositional preference have not provided clear results.

Pheromone traps provide a convenient means of collecting wild males in the field and represent one of the few methods of directly trapping adult fall armyworm. However, such field-collected specimens may be in traps for up to two weeks before they can be analyzed, with significant degra-

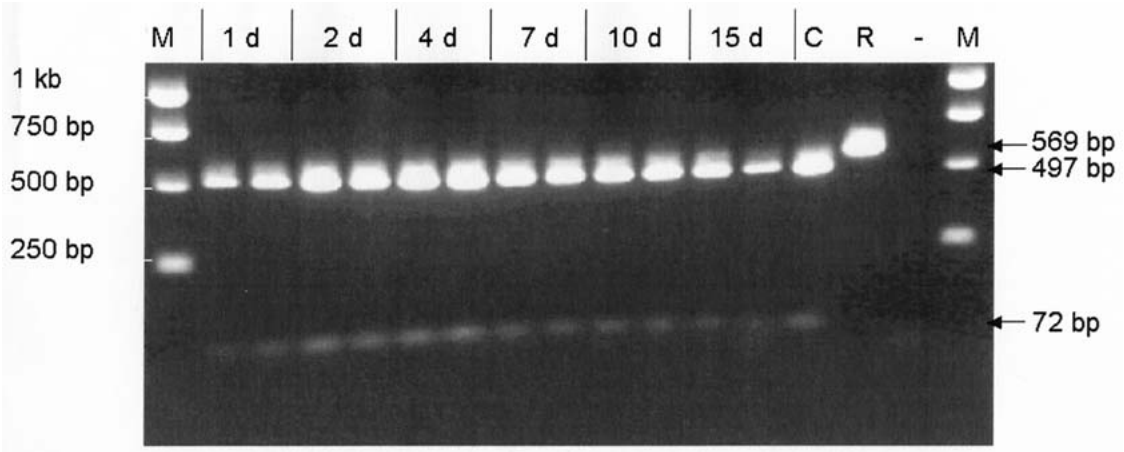

Fig. 2. Time interval experiment showing that adult fall armyworm held for up to 15 days could still be used for strain diagnosis by using the COI PCR-RFLP marker. All laboratory-reared moths from a corn strain colony were killed in the traps and exposed to outdoor climate for 1, 2, 4, 7, 10 or 15 d before collection for strain diagnosis. Positive controls included freshly collected corn [C] and rice [R] strains and the negative control was a PCR reaction containing no DNA template (-). M = 1 kb ladder.

dation of DNA likely. Therefore, it is necessary that the diagnostic molecular techniques employed for strain identification be robust enough to distinguish between the strains under these field conditions. The time interval experiment showed that moths held for at least 15 d could still be used for strain diagnosis when using the COI PCR-RFLP marker (Fig. 2). In comparison, results from the non-PCR based mt DNA RFLP method of Lu & Adang (1996) were highly variable and this method could not be used to identify strains held in traps longer than four days (data not shown). Therefore, the PCR-based method now makes it possible to obtain consistent and accurate strain identification of moths collected by standard pheromone trapping methods.

The combination of the pheromone trapping method with PCR-RFLP provide a valuable sampling system. Biological attributes such as strain isolating mechanisms, intra- and inter-strain mating behavior, and within-field populations in monocot and dicot crops are potential future studies. Additionally, strain analysis of overwintering fall armyworm populations in southern Florida is a important component to understanding population flow of this neotropical migrant.

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ATTRACTIVENESS AND EFFECTIVENESS OF AN ARTIFICIAL DIET FED TO HYBRID IMPORTED FIRE ANTS, *SOLENOPSIS INVICTA* × *RICHTERI* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Attractiveness of freeze-dried and reconstituted entomophage diet to hybrid fire ants (*Solenopsis invicta* × *richteri*) was investigated in choice tests using freeze-killed, crushed cricket (*Acheta domestica* L.) as a standard. Worker ants were strongly attracted to both crickets and reconstituted diet. Foragers collected approx. 27 times more reconstituted diet than freeze-dried diet, and collected statistically equivalent amounts of artificial diet and crickets (36.0 ± 7.0 and 26.0 ± 0.3 mg/h, respectively). Even though workers were strongly attracted to the artificial diet, all measures of colony growth (mean mass of brood, workers, and queen) were at least 30% lower in colonies fed sugar water + artificial diet than in colonies fed sugar water + crickets or sugar water + artificial diet + crickets. While this diet may have some utility as a bait for monitoring fire ants in the field, it offers no advantage over a standard diet of crickets and sugar water for rearing fire ants in the laboratory.

Key Words: Colony growth, laboratory rearing, foraging, bait

RESUMEN

La atracción de alimentos entomófagos reconstituidos y liofilizados hacia *Solenopsis invicta* × *richteri* fue investigada en un experimento de preferencia usando como un grillos (*Acheta domestica* L.) molidos y matados por congelación. Las hormigas trabajadoras fueron fuertemente atraidas de igual manera hacia los grillos y el alimento reconstituido. Las hormigas colectoras de alimentos colectaron la dieta reconstituida aproximadamente 27 veces más que la dieta liofilizada, y colectaron estatisticamente una misma suma de dieta artificial y grillos (36.0 ± 7.0 y 26.0 ± 0.3 mg/h, respectivamente). Aunque las hormigas trabajadoras són fuertemente atraidas a la dieta artificial, todas las medidas del crecimiento de la colonia (promedio de cantidad de cría, trabajadoras, y reina) fueron por lo menos 50% más bajas en las colonias alimentadas con agua zucarada+dieta artificial en comparación con colonias alimentadas con agua azucarada+grillos o agua zucarada+dieta artificial+grillos. Mientras que esta dieta puede tener algun utilisaje como cebo para el chequeo de hormigas de fuego en el campo, no ofrece ninguna ventaja sobre la dieta de grillos y agua azucarada para la crianza de hormigas en el laboratorio.

Translation provided by Demian Kondo.

Various diets have been proposed for rearing imported fire ants (*Solenopsis invicta* Buren, *Solenopsis richteri* Forel, and *Solenopsis invicta* × *richteri*, the red, black, and hybrid imported fire ants, respectively) (Khan et al. 1967; Bhatkar & Whitcomb 1970; Banks et al. 1981; Porter 1989); however, none have proven satisfactory without whole insects, offered separately or as a diet component.

I tested the attractiveness of a liver and ground beef-based artificial entomophage diet (Cohen, U.S. Patent #5,834,177. November 10, 1998) to foraging hybrid imported fire ants, *S. invicta* × *richteri*. For a description of the diet, see Cohen & Smith (1998). This diet was tested because it has been used to successfully rear several generations of *Chrysoperla rufilabris* (Burmeister). A reasonable start to assessing potential benefit of the diet to fire ant colonies would be to test its palatability; thus, we addressed the following questions: Does attractiveness of the diet warrant further study on its use as a supplement for laboratory colonies? Is the diet more attractive in its freeze-dried form or its reconstituted form? Is fresh diet more attractive than freeze-dried and reconstituted diet? Finally, an experiment was conducted to determine growth of laboratory colonies fed the artificial diet, crickets (*Acheta domestica* L.), or a combination of both.

MATERIALS AND METHODS

Diet Attractiveness

Colonies of *S. invicta* × *richteri* were collected from the field (Oktibbeha Co., MS) and maintained in trays (56 cm L × 44 cm W × 12 cm H) with castone nests (150 mm × 25 mm), a water source (150 mm × 25 mm test tube filled with

water, and plugged with cotton), and 1 M sucrose solution in a 150 mm × 15 mm test tube plugged with cotton. All colonies had a functional queen, 50,000-100,000 workers, and 15-20 g of brood. Colonies were fed crickets 2×/week, occasionally supplemented with boiled hen's egg yolk. Crickets and egg yolk were removed from the colonies 2 d prior to all tests to insure uniform levels of hunger. All trials took place in a climate-controlled room (28°C, approx. 60% RH). Hybrid status of colonies was confirmed by chemotaxonomy (Vander Meer & Lofgren 1990).

In experiment 1, freeze-killed crickets, freeze-dried entomophage diet, and reconstituted entomophage diet (2:1 diet:water by weight) were tested for recruitment time and attractiveness to foraging ants. Crickets were macerated using a mortar and pestle prior to testing. Macerated crickets and reconstituted diet were similar in consistency, with the exception of some small (<3 mm^2) pieces of exoskeleton in the macerated crickets; freeze-dried diet was composed of fine (5 to 40 μm) particles with relatively few larger, stringy solids. Four test colonies were used; each was connected to a foraging arena (41.75 cm L × 27.5 cm W × 12 cm H tray) with Tygon® tubing. Diets were placed in the barrels of 10 ml syringes cut at the 7 cc mark to present a 154 mm^2 surface area of diet. Each syringe contained 3 cc of the appropriate diet. Syringes were placed in the foraging arena, equidistant to the arena entrance (approx. 15 cm). A pair of observers, each observing 2 colonies, recorded discovery time and recruitment time for each syringe. Recruitment was assumed to have taken place once 10 foragers were present at the food surface. Once recruitment had taken place, the number of ants on the surface of the food sources was estimated at timed intervals. As foragers removed material from the syringes, the plunger was pushed forward so that the surface area presented to foragers remained constant.

In experiment 2, freeze-dried and reconstituted entomophage diets were presented in 1 oz plastic soufflé cups. Each cup had 2 small (approx. 3 mm dia.) holes cut in the side for ant access, and a plastic lid to minimize desiccation of the material. Cups containing diet were preweighed to the nearest 0.01 g, dried for 24 h at 60°C and re-weighed. Water (Millipore) was then added to the reconstituted diet treatment (2:1 water:diet ratio), exposed to the ants, weighed again, then dried for 24 h at 60°C to obtain dry weight of material removed. Eight controls (4 freeze-dried and 4 reconstituted) were placed in the room outside of the ant colonies. Paired cups were placed directly in colony trays; care was taken to place the cups equidistant from nest cells. Ants were allowed to forage for approx. 5 h (exact time noted for each cup), then all cups with were removed along with their contents, dried, and weighed.

In experiment 3, foraging ants were allowed access to reconstituted entomophage diet and a cricket standard, to compare attractiveness/retrieval rate. Prior to conducting this experiment, samples of macerated cricket (N = 6) were weighed, dried for >24 h at 60°C, and reweighed to obtain water content. Data were used to express retrieval rates in terms of dry weight. Presentation of food sources was done in the same manner as in experiment 2.

In experiment 4, freeze-dried, reconstituted diet (commercially prepared and canned approx. 3 yr. prior to testing) and fresh diet (made the day prior to testing) were presented to foraging ants using the same methods as in experiment 2. Water content of fresh and reconstituted material was obtained by weighing samples of each (N = 3), drying them in a 60°C oven for >24 h, and reweighing them.

Discovery and recruitment data were subjected to Proc Mixed (Little et al. 1996) to test for differences between treatments, with colony as a blocking factor. Timed observations from experiment 1 were analyzed as a randomized complete block with repeated measures and subjected to Proc Mixed to test for differences in attraction between treatments, and changes in attraction over time. Data from the other experiments were analyzed using Proc Mixed with source colony as a random blocking factor to test for differences in retrieval or attractiveness between treatments. Analysis of variance was used to examine controls for differences between treatments. Data are presented as mean ± SE, and were tested for significance at the $\alpha = 0.05$ confidence level.

Growth of Laboratory Colonies

An experiment was designed to compare colony growth of hybrid fire ant colonies fed sugar water (SW) + crickets (C), SW + artificial diet (AD), and SW + C + AD. Colonies (n = 15) were collected from the field (Oktibbeha Co., MS) and standardized just prior to beginning the experiment. Each standard colony contained 1 physogastric queen, 5 g workers, and 2 g brood. Colonies were housed in trays (41.75 cm L × 27.5 cm W × 12 cm H) and provided a castone® nest (150 mm × 25 mm), water, and 1 M sugar solution. Crickets and artificial diet were offered separately in 1 oz. plastic soufflé cups, with small (0.3 mm) holes drilled in the side for forager access, and lids to slow desiccation of the contents. Cups were checked daily for mold or desiccation, and replaced as necessary. Colonies were provided the appropriate foods *ad libitum* for a total of 8 wk, replacing diet cups at least every 2 d. The experimental design was a completely randomized design replicated 5 times. Data were analyzed using Proc Mixed followed by Least Squares Means to test for treatment effects on queen mass, total

brood mass, total worker mass, and total colony mass (live weights). Data are reported as mean ± SE.

RESULTS

Diet Attractiveness

Experiment 1. Foraging ants discovered all food sources in <4 min, and recruited within <13 min. No significant differences existed in discovery or recruitment times ($P > 0.05$), which were quite variable (e.g., recruitment to freeze-dried diet ranged from 5.9 to 12.8 min). In a mixed model with colony, treatment (food type), and time as fixed effects, and colony by treatment as the subject of the repeated statement, treatment ($F = 36.2$; df = 2,6; $P = 0.0004$) and time (h) ($F = 4.9$; df = 22,198; $P < 0.0001$) significantly influenced number of foragers per bait (Fig. 1).

Experiment 2. Pre- and post-drying weights of freeze-dried diet indicated 2 to 6% water content, so dry weight after exposure to the ants was subtracted from dry weight prior to exposure to obtain amount of diet retrieved. Data were corrected for time exposed to foragers, yielding material retrieved in g/h. Foragers removed approx. 27 times more reconstituted diet from the cups than freeze-dried diet in terms of dry weight ($F = 28.5$; df = 1,7; $P = 0.001$) (Fig. 2). Controls (freeze-dried and reconstituted) remained unchanged during the course of the experiment.

Experiment 3. Freeze-killed crickets used in this trial averaged 67.1 ± 0.4% water. Based on time-corrected data, entomophage diet controls gained an average of 2.4 ± 1.0 g/h, and cricket controls lost an average of 2.4 ± 0.4 g/h; these amounts were applied to post-feeding dry weights as a correction factor. Dry weight of material retrieved by the ants was statistically indistinguishable for the two treatments ($F = 4.45$; df = 1,7; $P = 0.073$) (Fig. 3).

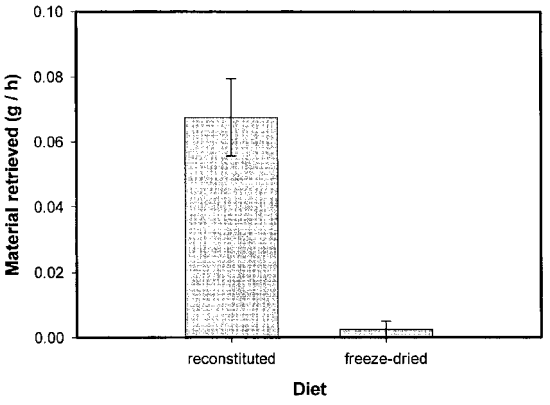

Fig. 2. Retrieval rate (g dry weight/h) for *S. invicta* × *richteri* foraging on reconstituted v. freeze-dried entomophage diet.

Experiment 4. Fresh entomophage diet contained an average of 68.7 ± 0.9% water, while reconstituted diet contained a statistically indistinguishable average of 71.7 ± 0.3% water ($P = 0.12$). Controls lost an average of 0.03 g during the course of the trial; this amount was the same between treatments ($P = 0.10$), and was subtracted from post-feeding data. Since water content of the treatments was similar, I analyzed wet weight of diet retrieved. In approx. 4 h, foragers collected similar amounts of fresh and reconstituted diet (0.49 ± 0.11 g and 0.40 ± 0.08 g, respectively) ($P = 0.09$).

Growth of Laboratory Colonies

Eight weeks after beginning the experiment, all measures of colony fitness and/or growth were significantly lower in colonies fed SW + AD than colonies fed SW + C or SW + AD + C (Table 1). Growth of colonies fed SW + AD appeared to keep

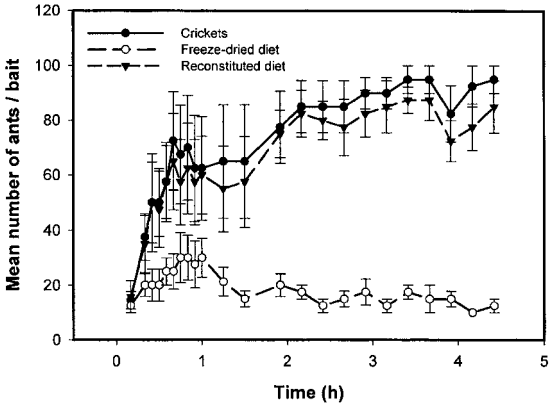

Fig. 1. Foraging activity of *S. invicta* × *richteri* during timed observations at 3 food sources.

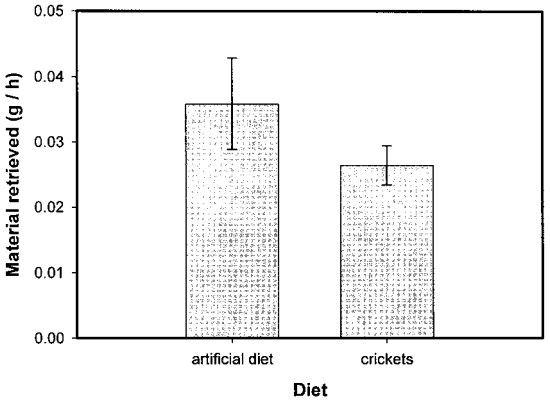

Fig. 3. Retrieval rates (g dry weight/h) for *S. invicta* × *richteri* foraging on entomophage diet v. crickets.

TABLE 1. MEAN (±SE) MASS OF HYBRID IMPORTED FIRE ANT COLONIES FED 3 DIFFERENT DIETS FOR 8 WK.

Treatment	Queen mass (mg)	Brood mass (mg)	Worker mass (mg)	Total mass (mg)
Sugar water + crickets	20.0 ± 2.0 a[1]	24.0 ± 5.1 a	15.1 ± 3.0 a	39.1 ± 7.8 a
Sugar water + artificial diet	14.1 ± 1.3 b	4.3 ± 4.3 b	5.6 ± 3.7 b	9.9 ± 7.9 b
Sugar water + crickets + artificial diet	24.1 ± 1.3 a	23.2 ± 3.9 a	10.6 ± 2.2 ab	33.7 ± 5.8 a

[1]Means in a column followed by the same letter are not significantly different (Least Squares Means, $P > 0.05$).

pace with growth in other treatments until approx. 4 wk into the experiment, but data were only collected at 8 wk. No apparent differences in worker size, color, or behavior were noted at the end of the experiment.

DISCUSSION

Discovery, recruitment, and retrieval rates indicate that the entomophage artificial diet is readily taken by laboratory fire ant colonies. Actual consumption was not measured, but foragers appeared to store large amounts of the diet in and around nest cells. Reconstituting the diet prior to presenting it to colonies increased the rate of retrieval. Low P-value for the analysis in experiment 4 suggests that fresh diet may be slightly more attractive than reconstituted diet; however, foragers were highly attracted to reconstituted diet in all experiments.

Fire ant colonies denied insect prey may cannibalize larvae (Sorensen et al. 1983) or produce abnormal, unmelanized workers (Williams et al. 1987). Workers appeared normal at the end of the colony growth study (e.g., no apparent change in color or size). While workers were not observed cannibalizing larvae during the experiment, that behavior could have contributed to the sharp decline in brood for colonies fed SW + AD. The decline in brood could explain lower queen weight in those colonies, as queen fecundity and ovarian development is tightly linked to presence of 4th instar larvae (Tschinkel 1995). The total mass of colonies fed SW + AD was 75% lower than mass of colonies fed SW + C. Porter (1989) reported that colonies fed crickets, sugar water, and an artificial diet based on Bhatkar and Whitcomb (1970) also exhibited growth indistinguishable from colonies fed crickets and sugar water only.

The artificial entomophage diet tested in these studies may have some utility as an attractive bait for monitoring fire ant presence or activity in the field, or as a hydratable carrier for toxins in home bait stations; however, it offers no advantage alone or in combination with a standard cricket diet for rearing fire ant colonies in the laboratory.

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ANTS (HYMENOPTERA: FORMICIDAE) ON NON-NATIVE NEOTROPICAL ANT-ACACIAS (FABALES: FABACEAE) IN FLORIDA

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ABSTRACT

One of the best-known symbioses in the Neotropics is the association between ant-acacias and *Pseudomyrmex* ants that live in the acacia's hollow thorns. We surveyed ants on two species of ant-acacia, *Acacia cornigera* (L.) and *Acacia sphaerocephala* Schlechtendal & Chamisso, growing outside their native range at five sites in Florida. We found eleven ant species: five native Florida ants (*Brachymyrmex* sp. nr. *obscurior*, *Camponotus floridanus* (Buckley), *Pseudomyrmex cubaensis* (Forel), *Pseudomyrmex ejectus* (Smith), and *Pseudomyrmex elongatus* (Mayr)), two Neotropical exotics (*Camponotus sexguttatus* (Fabr.) and *Pseudomyrmex gracilis* (Fabr.)), and four Old World exotics (*Monomorium floricola* (Jerdon), *Paratrechina longicornis* (Latreille), *Pheidole megacephala* (Fabr.), and *Technomyrmex albipes* (Smith)). Only the two Neotropical exotics, *Ps. gracilis* and *C. sexguttatus*, inhabited thorns with holes that appeared to have been perforated by ants as entrances. For *Ps. gracilis*, and perhaps also for *C. sexguttatus*, their association with ant-acacias in Florida represents the reconstitution in an exotic locale of a facultative symbiosis evolved in the Neotropics.

Key Words: Acacia-ants, ant-plants, *Camponotus*, *Pseudomyrmex*, symbiosis

RESUMEN

Una de las simbiosis Neotropicales más conocidas es la asociación entre los cornizuelos y las hormigas de *Pseudomyrmex* que viven en las espinas ahuecadas del cornizuelo. Estudiamos las hormigas en dos especies del cornizuelo, *Acacia cornigera* (L.) y *Acacia sphaerocephala* Schlechtendal & Chamisso, creciendo fuera de su rango nativo en cinco sitios de la Florida. Encontramos once especies de la hormiga: cinco hormigas nativas de la Florida (*Brachymyrmex* sp. nr. *obscurior*, *Camponotus floridanus* (Buckley), *Pseudomyrmex cubaensis* (Forel), *Pseudomyrmex ejectus* (Smith), y *Pseudomyrmex elongatus* (Mayr)), dos exóticas Neotropicales (*Camponotus sexguttatus* (Fabr.) y *Pseudomyrmex gracilis* (Fabr.)), y cuatro exóticas del Viejo Mundo (*Monomorium floricola* (Jerdon), *Paratrechina longicornis* (Latreille), *Pheidole megacephala* (Fabr.), y *Technomyrmex albipes* (Smith)). Solemente las exóticas Neotropicales, *Ps. gracilis* y *C. sexguttatus*, ambas habitaban las espinas con los agujeros que aparecían haber sido perforados como entradas por las hormigas. Para *Ps. gracilis*, y quizás también *C. sexguttatus*, esta asociación con los cornizuelos en la Florida representa la reconstitución en un locale exótico de una simbiosis facultativa desarrollada en el Neotrópico.

Translation provided by author

One of the best-known Neotropical symbioses is the association between *Acacia* trees and *Pseudomyrmex* ants (Belt 1874; Janzen 1966; 1967; Hölldobler & Wilson 1990). Thirteen Neotropical *Acacia* species are "ant-acacias," specialized myrmecophytes that house ants in their thorns and provide ants with extrafloral nectaries and nutritious Beltian bodies (Seigler & Ebinger 1995). Thirteen Neotropical *Pseudomyrmex* ant species obligately live in ant-acacias. Nine of these species vigorously defend the *Acacia* from herbivory and overgrowth by vines, whereas the other four provide little or no defense (Ward 1993). In addition, numerous other ant species live opportunistically in ant-acacias, but also nest elsewhere, typically in hollow twigs (Wheeler 1913). Only two of these facultative symbionts, *Pseudomyrmex gracilis* (Fabr.) and *Camponotus planatus* Roger, are known to show specialized behaviors in exploiting ant-acacia thorns.

The present study was motivated by our observation in September 1999 of *Pseudomyrmex* ants living in an ant-acacia tree growing at Mounts Botanical Garden in West Palm Beach, Florida. We noticed a thorn on an *Acacia cornigera* (L.) tree that had a round hole indicative of an ant entrance. Breaking open the thorn, we found it full of adult *Pseudomyrmex gracilis* ants and brood. This unexpected discovery of ants inhabiting domatia of a non-native myrmecophyte contrasted with an earlier finding concerning another well-known Neotropical myrmecophyte,

Cecropia obtusifolia Bertol. In Hawaii, where neither *Cecropia* nor ants are native, Wetterer (1997) found no ants inhabiting the hollow trunks and branches of the exotic *Cecropia obtusifolia* trees that grow abundantly in the disturbed lowlands.

In the present study, we wished to determine what ant species live on and in exotic ant-acacias growing in Florida, a region devoid of both native ant-acacias and obligate acacia-ants.

Methods and Results

We contacted and visited numerous botanical gardens around Florida looking for live ant-acacia specimens, and found ant-acacias growing at four locations in addition to Mounts Botanical Garden: Fairchild Tropical Garden in Miami, University of South Florida Botanical Garden in Tampa, Walt Disney World in Orlando, and on the property of G. Joyner in West Palm Beach.

On 10 May 2000, at Fairchild Tropical Garden, we collected ants on three ant-acacias: one bullhorn acacia tree, *Acacia cornigera*; and two bee wattles, *Acacia sphaerocephala* Schlechtendal & Chamisso. We found five ant species: *Brachymyrmex* cf. *obscurior*, *Camponotus floridanus* (Buckley), *Monomorium floricola* (Jerdon), *Pseudomyrmex cubaensis* (Forel), and *Technomyrmex albipes* (Smith) (Table 1). We found no ant entrance holes on any thorns.

On 13 May 2000, at Mounts Botanical Garden, we collected ants on one *A. cornigera*, grown from seed. We found four ant species: *Paratrechina longicornis* (Latreille), *Pseudomyrmex cubaensis*, *Pseudomyrmex ejectus* (Smith), and *Pseudomyrmex gracilis* (Table 1). As in December 1999, we again noted smooth, round ant entrance holes on thorns of this tree. The ants inhabiting the hollowed thorns were *Ps. gracilis*.

On 19 May 2000, at University of South Florida Botanical Garden, we collected ants on one small *A. cornigera*. We found a single *Ps. gracilis* worker (Table 1) but did not observe any ant entrance holes.

On 21 May 2000, at Animal Kingdom, a part of Walt Disney World, we collected ants on one *A. cornigera* growing in a planter in the Africa section. The tree was purchased in Miami; the store had received it from a customer (J. Thompson, pers. comm.). On this tree we found *Brachymyrmex* cf. *obscurior* workers on the trunk (Table 1). We found one thorn with a smooth, round ant entrance hole. We broke open this one thorn and found *Camponotus sexguttatus* (Fabr.) workers and brood inside (Table 1).

On 12 June 2000, on Alexander Street in West Palm Beach, we collected ants on an *A. cornigera* grown from seed by G. Joyner. This tree differed from the well-pruned trees we had examined in botanical gardens because it had many dead branches and twigs, which we were free to break open. We also broke open many dead and live thorns. We found four ant species: *Brachymyrmex* cf. *obscurior*, *Pheidole megacephala* (Fabr.), *Pseudomyrmex elongatus* (Mayr), and *Pseudomyrmex ejectus* (Table 1). Inside many dead twigs, we found *Ps. ejectus* colonies with brood, including alates. In a few dead thorns we found *Pheidole megacephala*, *Pseudomyrmex elongatus*, and *Pseudomyrmex ejectus*, in some cases with brood. None of the entrance holes to these thorns were smoothly rounded, suggesting that none were created by ants.

Discussion

We found eleven ant species living on or in exotic ant-acacia trees in Florida. Five of these ant species are native to Florida (*Brachymyrmex* cf. *obscurior*, *Camponotus floridanus*, *Pseudomyrmex cubaensis*, *Pseudomyrmex ejectus*, *Pseudomyrmex elongatus*), though the last three are also found in the Neotropics (native/exotic designations from Deyrup et al. 1988, 1989). Two ant species we found are New World exotics (*Camponotus sexguttatus* and *Pseudomyrmex gracilis*) and four are Old World exotics (*Monomorium floricola*, *Paratrechina longicornis*, *Pheidole megacephala*, and *Technomyrmex albipes*). Of greatest interest were *Ps. gracilis* and *C. sexguttatus*, the only ants we found inhabiting *Acacia cornigera* thorns that appeared to have been perforated by ants.

Pseudomyrmex gracilis ranges from Argentina to Texas and the Caribbean (Kempf 1972; Jaffe & Lattke 1994) and has invaded Hawaii and Florida (Beardsley 1979; McGlynn 1999). The earliest *Ps. gracilis* records in Florida were from Miami ca. 1960 (Whitcomb et al. 1972). By 1970, *Ps. gracilis* was common throughout southeastern Florida, as far north as West Palm Beach (Whitcomb et al. 1972), and by 1988, *Ps. gracilis* was found from the Florida Keys north to Duval County, near the Georgia border (Johnson 1986; Deyrup et al. 1988, 1989). *Pseudomyrmex gracilis* opportunistically nest in acacias, providing little or no defense for the tree, but also commonly nests in hollow branches, twigs, and stems, as well as building crevices (Buren & Whitcomb 1977; Cassani 1986; Ward 1993; Klotz et al. 1995). Wheeler (1942) found that *Ps. gracilis*, "though a very frequent tenant of dead twigs and *Cordia* domatia in regions where there are no Acacias, nevertheless exhibits a strong proclivity not only to inhabit the spines of these plants [ant-acacias], wherever they are available, but also to perforate them at the same point, to visit the foliar nectaries and to collect food-bodies."

Camponotus sexguttatus ranges from Argentina to Nicaragua and the Caribbean (Kempf 1972) and has invaded Florida and Hawaii (McGlynn 1999). The earliest known Florida specimens date to 1993 (Deyrup et al. 2000). Our

TABLE 1. ANTS ON TWO SPECIES OF EXOTIC ANT-ACACIAS (*ACACIA CORNIGERA* AND *ACACIA SPHAEROCEPHALA*) IN FLORIDA.

	Acacia cornigera					*Acacia sphaerocephala*	
	M	F	D	U	W	F	F
Brachymyrmex "obscurior"		X	X		X	X	X
Camponotus floridanus		X					
Camponotus sexguttatus			X				
Monomorium floricola		X				X	
Paratrechina longicornis	X						
Pheidole megacephala					X		
Pseudomyrmex cubaensis	X					X	X
Pseudomyrmex ejectus	X				X		
Pseudomyrmex elongatus					X		
Pseudomyrmex gracilis	X			X			
Technomyrmex albipes		X				X	X

M = Mounts Botanical Garden, F = Fairchild Tropical Garden, D = Walt Disney World, U = University of South Florida Arboretum, W = West Palm Beach.

observation is the first record of *C. sexguttatus* inside the thorns of an ant-acacia. It is unclear whether the *C. sexguttatus* simply occupy previously prepared thorns or if they perforate and hollow out the thorns themselves, as do another species of carpenter ant, *C. planatus*. *Camponotus planatus* ranges from Columbia to Texas and the Caribbean (Kempf 1972) and has invaded Florida, Hawaii, and the Galapagos Islands (McGlynn 1999). Like *Ps. gracilis*, Wheeler (1942) considered *C. planatus* "of special interest" because of its specialized behaviors in exploiting ant-acacias. Wheeler (1913) observed *C. planatus* workers perforating a new thorn, indicating that this species does not merely take possession of thorns excavated and abandoned by other ants, but actually opens and excavates its own acacia thorns. Wheeler (1913) considered it "extraordinary that *C. planatus*, which throughout tropical America so constantly lives in hollow twigs, should be able in widely separated localities to utilize the acacia thorns as perfectly and in precisely the same manner as the regular Pseudomyrmas." *Camponotus planatus* occurs in southernmost Florida (Deyrup et al. 1988), though we did not find any on the ant-acacias we examined.

The native range of *A. cornigera* trees in Central America and Mexico (Seigler & Ebinger 1995) overlaps with the native ranges of both *Ps. gracilis* and *C. sexguttatus* ants. Thus, for *Ps. gracilis*, and perhaps also *C. sexguttatus*, their association with *A. cornigera* in Florida represents the reconstitution in an exotic locale of a facultative symbiosis evolved in the Neotropics. Seigler and Ebinger (1995) report that naturalized populations of *A. cornigera* occur in the Caribbean and in southern Florida. In the future, we hope to study the ant fauna of these and other non-native ant-acacias populations.

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TAXONOMY AND BEHAVIOR OF *PHOTURIS TRIVITTATA* SP. N. (COLEOPTERA: LAMPYRIDAE: PHOTURINAE); REDESCRIPTION OF *ASPISOMA TRILINEATA* (SAY) COMB. N. (COLEOPTERA: LAMPYRIDAE: LAMPYRINAE: CRATOMORPHINI)

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ABSTRACT

Photuris trilineata (Say) is assigned to *Aspisoma* Laporte, and the type female is redescribed. *Photuris trivittata* sp. n. is described from behavior voucher specimens, and behavioral data are presented and discussed. Aspects of abdominal segmentation and aedeagal structure of *Aspisoma* and *Photuris* are described.

Key Words: flash patterns, ecology, predation, neotropical fireflies

RESUMEN

Photuris trilineata (Say) es asignado al *Aspisoma* Laporte, y el tipo de hembra es redescrito. *Photuris trivittata* sp. n. se describe de acuerdo a los comportamientos de los especímenes comprobantes y los datos de los comportamientos son presentados y discutidos. Aspectos sobre la segmentación abdominal y el edeago del *Aspisoma* y *Photuris* son descritas.

Translation provided by author.

Say (1835) described *Lampyris trilineata* from a female now housed in the Museum of Comparative Zoology at Harvard University. Olivier (1886) assigned *L. trilineata* to *Photuris* but did not examine the type and appears to have based his action on the similarity of the described color patterns. Lloyd tentatively assigned the behavior voucher specimens included here to *Photuris trilineata* (Say), but after locating and examining the type female of *L. trilineata* he determined that *trilineata* should be assigned to *Aspisoma* and that his specimens were of a new species. Olivier's collection in the Paris Museum was examined by Ballantyne in November 1993. Olivier's methodical collection often reflects the chronology of his published work, and standing in the Olivier collection under *Photuris trilineata* (Say) were specimens of *Photuris* which are conspecific with the specimens described below. *Lampyris trilineata* Say is assigned to *Aspisoma*; Olivier's firefly apparently has remained unnamed, and is herein described as *Photuris trivittata*. JEL provided the biological data; LAB provided the taxonomic framework.

Taxonomy

Taxonomic characters follow Ballantyne (1987a, 2000) with exception of abdominal segmentation and aedeagal structures, which are discussed separately below. Descriptions are ordered so that features on the dorsal surface are described in sequence from the anterior to posterior end, and then the ventral surface is described in the same manner. This facilitates handling under the microscope. Length, measured as median length of pronotum plus maximum length of an elytron, is sometimes a misleading representation since the pronotum droops in pinned specimens, and the specimen will always appear shorter than the figure given. The length of the head, which may protrude to a variable extent in males, is not included. Measurements (i.e., lengths) taken at the longest and widest areas respectively, such as pronotal width, greatest head width in anterior aspect, are used on a comparative rather than absolute basis (Lampyrids being soft bodied are subject to much distortion)—for example the distance between the antennal sockets is given as a function of the nearest convenient point of reference, the width of an antennal socket.

McDermott (1964) distinguished the Photurinae with a "membranous labrum arising from the ventral surface of a strongly sclerotized clypeus." The nature of the labrum was reinterpreted by John Lawrence (1987): in the cantharoids there is probably never a well-developed clypeus separated from the frons by a complete frontoclypeal suture. In most Lampyridae the labrum is at least slightly sclerotized and separated from the clypeus

by a strip of membrane. The anterior strongly sclerotized plate on the Photurinae head is here interpreted as the labrum.

While specimens were still soft and flexible many aedeagi were extruded by the collector (JEL), and remain attached to the specimen, usually with the basal piece still encased between ventrite 9 and tergite 9 (the "aedeagal sheath" of Ballantyne 1987a, b), and often hidden. Some aedeagi were removed and mounted on transparent points, using transparent glue, and remounted beneath the specimen (specimens were softened for 2-3 days in a humid atmosphere in an airtight container with moist sand with a few drops of Lysol® to retard mold).

The two specimens selected for scanning electron microscopy were dried pinned specimens which had the aedeagus extended. They were mounted on aluminum stubs using double sided semitransparent tape and coated with gold in a Denton Vacuum Desk II Cold Sputter Etch Unit, and examined in a Hitachi 570 scanning electron microscope at an accelerating voltage of 15 KV. The operation was carried out as part of a class exercise in the Department of Entomology and Nematology at the University of Florida in Gainesville, under the direction and assistance of Prof. Harvey Cromroy. The type female of *Lampyris trilineata* Say was not dissected, and drawings represent the specimen in its actual state at the time of examination. Specimens are currently housed in the JEL collection in Gainesville (JELC), or the Florida State collection of Arthropods (FSCA).

Abdominal segmentation. Abdominal segmentation is interpreted from Ballantyne (1987a, 1992). Terminology of the ventral abdominal plates has varied. Green (1956) used "sternite" for the median half of each ventral segment in *Photinus* and considered the lateral area on each side the pleurite, which is narrowly inflexed dorsally and bears the spiracles. Crowson (1972) called the ventral abdominal plates "ventrites" but (page 39) referred the spiracles to the "inflexed edges of the sternal plates." A refinement of the definition of the term "ventrite" in the Lampyridae was proposed (Branham & Archangelsky 2000).

The abdomen of *Aspisoma* and *Photuris* males (Figs. 4 and 15) consists of 8 visible tergites, although the first may be difficult to distinguish. Segments 2-8 are distinguishable ventrally. The light organs occupy the ventral plates of segments 6 and 7. Ventrite 8 is well developed although it is usually shorter than 7. Ventrite 9 (which surrounds the aedeagus) is usually visible externally, protruding beyond the posterior margin of ventrite 8, and completely covered dorsally by the relatively large tergite 8.

The *Aspisoma* abdomen is broad, flattened, tapering at front and behind (Figs. 3 and 4); the dorsally reflexed lateral margins of the ventrites bear the spiracles which are covered by the lateral tergal margins and difficult to see in pinned specimens; the light organ in males and females occurs in ventrites 6 and 7 (Figs. 3 and 4) but may be considerably retracted from the lateral areas in females; depressions in lateral areas of ventrites 6 and 7 probably house sense organs (Lloyd & Ballantyne, pers. obs.); ventrite 8 is transverse, about half as long and wide as 7, with the median posterior margin emarginate; aedeagal sheath (= ventrite and tergite 9) when visible externally is turned on its side; tergite 8 about as long as, but narrower than, tergite 7.

The male *Photuris* abdomen (Fig. 15) has ventrites 6 and 7 often medially emarginate posteriorly; ventrite 8 always tapers posteriorly, is usually about half as long as 7, although sometimes retracted beneath 7; the median posterior margin of ventrite 7 always has a pointed projection of varying length; tergite 8 often has lateral margins reflexed; aedeagal sheath ventrite and lateral projections of aedeagus sometimes visible behind ventrite 8. The female abdomen has light organs apparently contained in ventrites 6 and 7; ventrite 8 tapers posteriorly and may be medially incised (Fig. 16).

Aedeagal structure. The aedeagus of *Aspisoma* most closely approaches that of the Luciolinae (Ballantyne 1987a, b, 2000), in having a clearly defined median lobe, lateral lobes (which may be slightly longer or shorter than the median lobe), and an elongate well defined basal piece. The median lobe is elongate, slender, and often medially carinate along the dorsal surface; lateral margins of the median lobe can expand and are variably developed. Small hooks may arise from the inner face of the lateral lobes and in *A. physonotum* they engage against the median dorsal carina of the median lobe (Ballantyne 1992).

The aedeagus of *Photuris* spp. consists of median and lateral lobes and a basal piece, and paired long slender processes extending from the sides of the basal piece (Figs. 17 and 18). These pieces "splay" to varying degrees in pinned specimens, and the full extent of the basal piece is not always visible in specimens where the aedeagus is still attached to the abdomen.

Barber (1951) described the *Photuris* aedeagus: "sides of the 'basal piece' are produced into long slender, clubbed, lateral processes extending beyond the apex of a slender median lobe". McDermott (who completed Barber's manuscript after his death), included figures (Figs. 2 and 3) of *Photuris lucicrescens* aedeagus which was unlabeled but described in the text as having "the lateral lobes fuse with the dorsal surface of the median lobe at about basal third, and are armed internally opposite this point with a strong transverse ridge, which is sharply angulate at inner third". McDermott (1962) figured 3 unlabeled *Photuris* spp. aedeagi and (1964) referred to the *Photuris*

aedeagus with 2 long slender lateral processes, but did not determine their origin. Lloyd (1979, 1981) pictured a copulating *Photuris* spp. pair, and attributed the lateral processes of the aedeagus to the basal piece (as Ballantyne does here) (Lloyd 2002); the picture shows that these pieces remain outside the female during intromission.

Photuris trilineata was used (as *Photuris* sp.) as the outgroup in a cladistic analysis of Australian Luciolini (Ballantyne & Lambkin 2000).

Aspisoma trilineata (Say) comb. n.
Figs. 1-3

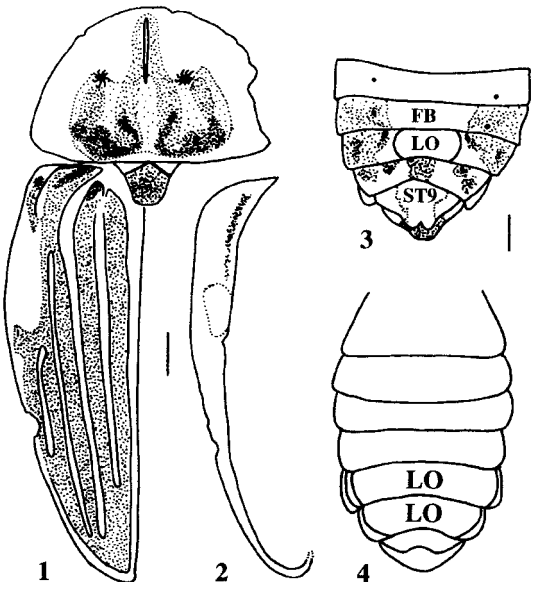
Lampyris trilineata Say, 1835, p. 157.

Photuris trilineata (Say).Olivier, 1886, p. 232; 1910, p. 52. McDermott, 1966, p. 92 (misidentification).

Type. Holotype female, Mexico (Museum of Comparative Zoology, Harvard University).

Redescription of type female. Length:13.7 mm. Color: Pronotum dingy yellow with dark brown markings on median area of dorsal surface (Fig. 1); pronotum largely semitransparent, and pale pink and yellow fat body visible through the cuticle; mesonotum light brown; mesoscutellum dark brown, darker in posterior area; elytra medium brown, with lateral, apical and sutural margins, and longitudinal interstitial lines dingy yellow (Fig. 1); head yellow; antennae, palpi and labrum light brown; ventral surface of pro- and mesothorax medium brown, of metathorax moderately dark brown; legs medium brown with dark brown tarsi; abdominal ventrites yellow with brown mottling; compact light organ material defined in median area of ventrite 7 only, although ventrite 6 bears a diffuse median area of fat body (Fig. 3).

Body covered with fine short pale setae, which have been abraded in certain areas. Pronotum (Fig. 1) 4.2 mm long, 6 mm wide; with dense covering of short fine pale setae; setal swirls originate in positions marked (Fig. 1); dorsal surface with median ridge extending posteriorly from anterior margin for about ⅓ length of the pronotum; anterolateral corners of pronotum rounded obtuse; posterolateral corners rounded; lateral margins diverging posteriorly along most of their length, and widely flattened especially in the posterior half. Elytra (Fig. 1) 9.5 mm long; convex-sided when closed; laterally explanate margins well developed, especially in anterior ½ (Fig. 2); 4 interstitial lines present, of which the most lateral line is evanescent anteriorly and posteriorly; epipleuron and sutural ridge extending to and around apex of elytron. Head small, completely retracted into and beneath pronotum in resting condition; greatest head width 2.2 mm; smallest interocular width 0.8 mm; antennal sockets separated by more than width of an antennal socket; head not depressed between eyes; mouthparts well developed. Terminal ventrites as figured (Fig. 3).



Figs. 1-4. (1-3) *Aspisoma trilineata* holotype female: (1) dorsal aspect of pronotum and left elytron (position of hair swirl pattern indicated on pronotum); (2) ventral surface of epipleural margin of left elytron; (3) ventral surface of terminal abdomen. (4) *Aspisoma physonotum* male: ventral surface of terminal abdomen. Scale lines are 1 mm. FB, fat body; LO, light organ; ST9, ventrite 9.

Photuris trivittata sp. n.
(Fig. 23 habitus)

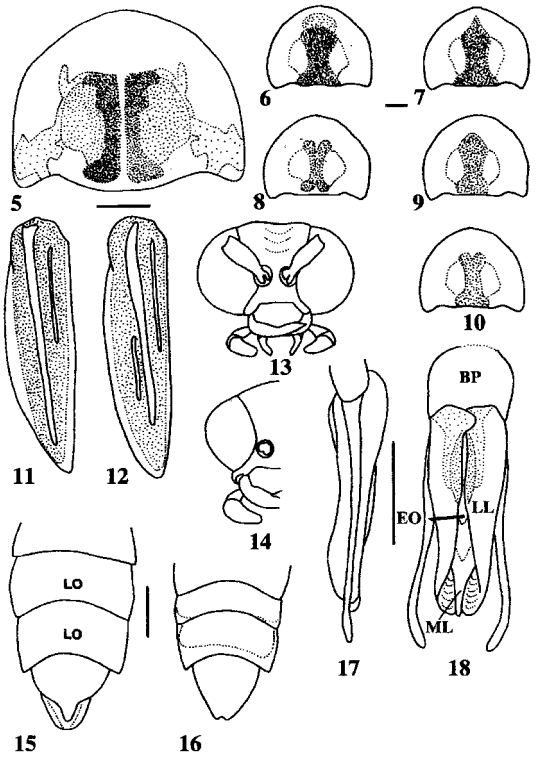
Type Specimens (Currently housed in JELC, Gainesville). Holotype male: MEXICO. Tabasco: 27 km w Cardenas at CSAT, 1980, J E Lloyd (M805*). Paratypes: same locality as holotype, 16.X.1980, 3 males (M809*, M8010*, M8015*); 18.X.1980, 1 male (M8027* used for SEM); 23.X.1980, 1 male (M8048*); 28.X.1980, 3 males, 1 female (M80104*, M80108*, M80103*, M80116); same locality and collector as for holotype, 1980: 16.X.1980, 1 female (M8050), 1 male (M803, CSAT); 17.X.1980, 1 female (M8025); 20.X.1980, 1 male (M8037); 21.X.1980, 2 males (M8044 macerated, M8047), 1 female (M8046); 23 X.1980, 3 males (M8052, 8053, 8055), 1 female (M8049); 28.X.1980, 2 males (M80109, 80114), 5 females (M80106, M80111-113, 80115). Cardenas, nr hotel Siglo XX, 27.X.1980, J E Lloyd, 1 male (M8093*), 1 female (M8059) (JELC). Cancun, Quintana Roo State, D Thomas & J Burne, 10.VIII.1990, 1 male (FSCA). Chiapas: Palenque, D. Thomas, 16-20.V.1985, 1 male, 2 females (FSCA); Parque Lag. Belgica, D Thomas, J Burnie, 5-6. VII.1989, 1 female (FSCA); 5 mi N Ixhuatan, B Ratcliffe, C Messenger, 9-16.IX.1985, 1

female (FSCA); Lago Montebello, D Thomas, J Mackley, 15.VI.1985, 1 female (FSCA); Simojovel, D Thomas, 23.VIII.1987, 1 female (FSCA); Chicoasen Dam area, D & A Thomas, 10.IX.1988, 1 female (FSCA). Comitan, 31 mi SE of Chis, Burke et al., at light, 17.VI.1965, 2 females. Veracruz, Dos Amates Mun. Catemaco, P Hubbell, 4-14.XI.1972, 1 female. BELIZE. Orange walk, Sept 1986, D Thomas, 1 male, 1 female (FSCA). COSTA RICA. Guanacaste, 2.7 mi NE La Cruz on Pan Amer. Hwy, 27.IX.1961, Hubbell et al., 1 female (pink), GUATEMALA. Dept. Isabel, Quirigua, 11.I.1937, 240 ft C. Roys, 1 male (pink); Peten, Pasion River at Cambio, 20.IV.1935, Hubbs-Vander Schalie, 1 female (pink); Suchitepe-quez, Dept. E of Cocales, 400-500 m, 2.XII. 1983, flsh on grnd, J Schuster, 2 females (JELC); Peten Tikal, 100 ft, I. Cantrall, 1 female 7.II.1956, at light at camp (pink), 1 female 17.II.1956 (pink), 1 female 13.III.1956 (pink), 1 female 31.III.1956 (pink). HONDURAS. Dept. Morazán, Esc. Agr. Pan. Zamborano, T. Hubbell, 2550 ft 18.VIII.1948 (vega Yeguare R., 1 female (pink); 2600 ft (hortaliza), 13.VII.1948, 2 females (pink); 2600 ft (creek bank) 19.VII.1948, 2 males (pink); 2600 ft 30.VII.1948, 1 female (pink). Tela, 6.IV.1923, T Hubbell, 1 male, 1 female (pink).

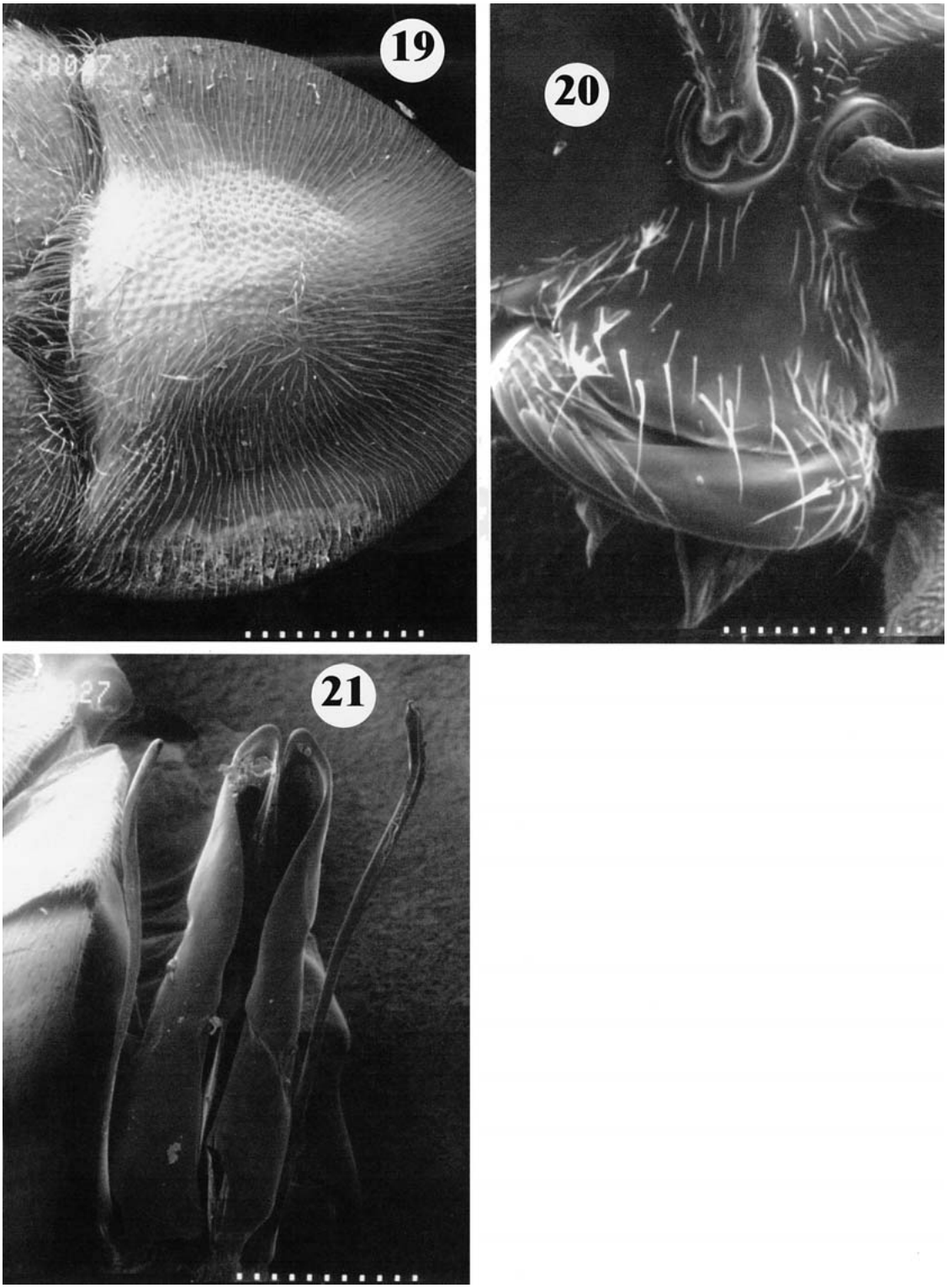
(Specimens "*" in the collection of JEL may bear a green label "semiosystematic voucher specimen, James E Lloyd". A further lettered and numbered label on each specimen relates to field records kept by the collector.)

Male. Length: 13-15 mm long (holotype 14 mm). Color: Pronotum yellow with median brown markings (Figs. 5-10), semitransparent; fat body visible through cuticle in posterolateral corners is yellow, in median area is pink; mesoscutellum and metanotal plates yellow; elytra brown, with broad lateral, narrow apical and narrow sutural margins yellow, and 2-3 longitudinal yellow interstitial lines; (Figs. 11 and 12 show variation; the coloration gives the appearance of 3-4 brown stripes); head yellow, anterior margin of labrum dark brown; antennae brown, basal portion of all segments narrowly yellow; maxillary palpi mostly brown, penultimate segment yellow at base, enlarged terminal segment yellow on inner face; labial palpi yellow; ventral prothorax yellow; legs 1 yellow with brown tarsi, brown apices of tibiae, and brown markings on femora at inner and outer basal and apical surfaces; mesopleura brown, mesosternum yellow; legs 2 marked as for legs 1 except for basal tarsomere which is brown at apex only; ventral metathorax brown; basal abdominal ventrites dingy to brownish yellow, semitransparent and fat body is visible through cuticle; light organ in ventrites 6 and 7 creamy yellow; ventrite 8 yellow; basal abdominal tergites medium brown, terminal 3 tergites pale yellow.

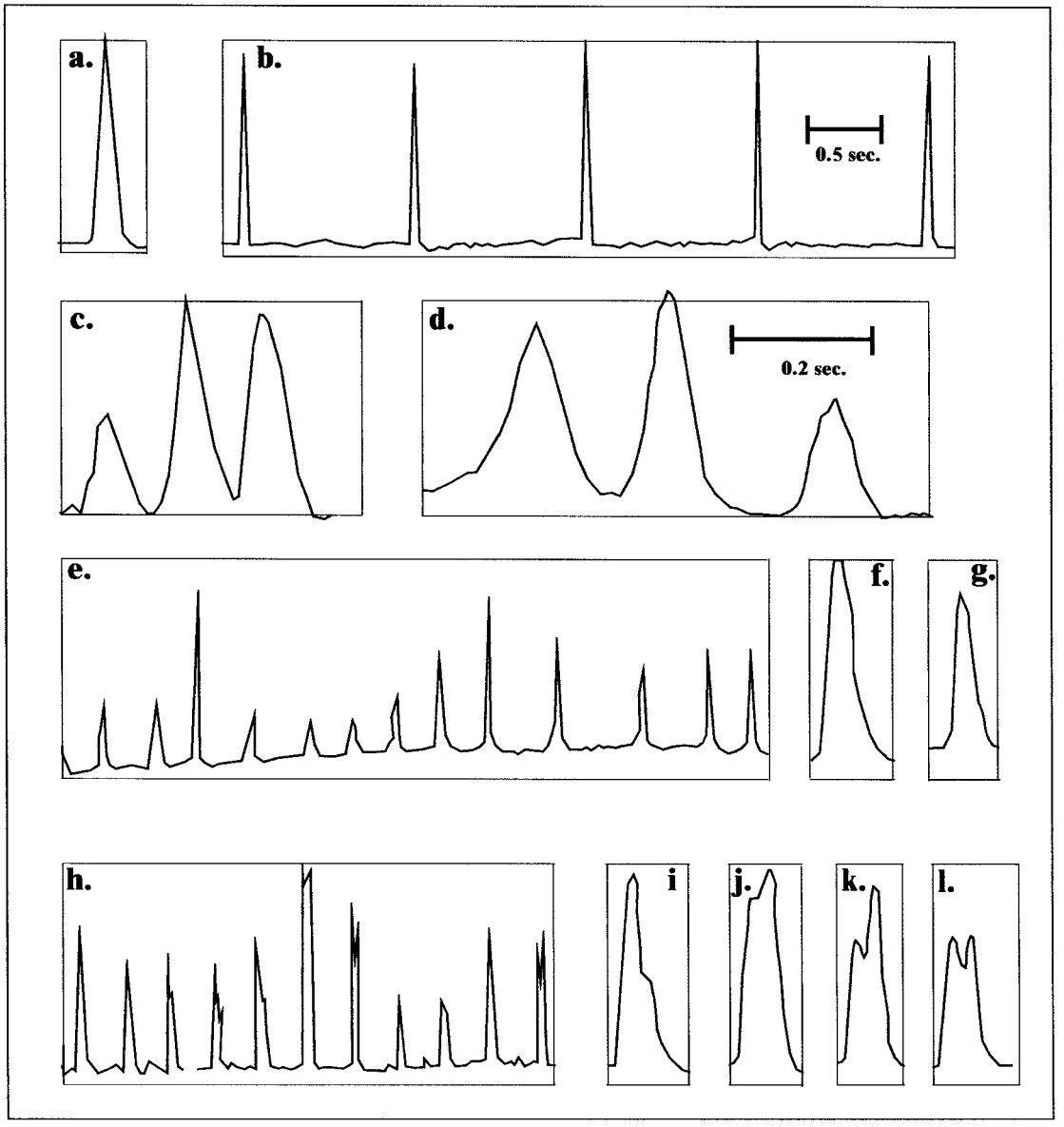
Pronotum 3.3-3.9 mm long; 5.2-5.9 mm wide; setal swirl pattern distinctive (Fig. 19); pronotal punctures small, shallow, separated by up to their width and evenly distributed over dorsal surface; hypomera open in front; lateral pronotal margins diverging along anterior half or more with some convergence in posterior area; anterolateral corners obliterated; posterolateral corners rounded obtuse; anterior margin narrowly explanate; lateral margins widely flattened along their length and anterior area as wide as posterior area; outline as figured (Figs. 5 and 19). Elytra with 3 interstitial lines well defined by their pale color but not well elevated relative to the sutural ridge (Fig. 23); epipleuron not extending posteriorly beyond mid point of elytron; sutural ridge evan-



Figs. 5-18. *Photuris trivittata* sp. n. (5-10) Dorsal surface of pronotum (5) M805 detail—dense stippling represents dark brown markings, less dense stippling represents pink fat body, least dense stippling represents yellow fat body; (6) M80116 female; (7) M809 male; (8) M8010 male; (9) M80104 female; (10) M8015 male (single dotted line represents extent of fat body visible through cuticle). (11, 12) dorsal surface of left elytron (11) M8027 male; (12) M809 male. (13, 14) anterior aspect of head (13) M8048 male; (14) right side only M80116 female. (15, 16) ventral view of terminal abdominal segments (15) M8027 male; (16) M80116 female. (17, 18) aedeagus (17) left lateral M805 holotype male; (18) dorsal M805 holotype male. Scale lines are 1 mm. BP, basal piece of aedeagus; EO, ejaculatory orifice; LL, lateral lobe aedeagus; LO, light organ; ML, median lobe aedeagus.



Figs. 19-21. *Photuris trivittata* sp. n. Electron micrographs. (19) M8027 male, dorsal surface of pronotum, dotted scale line 1.36 mm; (20) M80108 male, anterior aspect of head, antennal sockets, dotted scale line 0.5 mm; (21) M8027 male, dorsal aspect of aedeagus, dotted scale line 0.75 mm.



Figs. 22a-l. Chart traces of *Photuris trivittata* flashes, except for "d" an *Aspisoma* sp., detected in the field with a photomultiplier system, recorded on magnetic tape, then chart traced at two different speeds. Horizontal axis is time; vertical axis, relative intensity. Time scale is indicated by bars: bar in "b" applies to b, e, h; bar in "d" applies to all others. (a) Single flash of male; (b) five single flashes in sequence emitted by a perched male; (c) modulated flash of about 8.3 Hertz (Hz, cps); (d) modulated flash of co-active *Aspisoma* sp. with form similar to that of certain flashes emitted by *P. trivittata*, but at a much slower modulation rate, averaging 4.8 Hz (5.3 and 4.2 Hz); (e) flashes of female with short train of rapid flashes; (f, g) individual female flashes; (h) train of bimodal rapid flashes of a perched male; (i-l) male flashes from train in "h".

escent before elytral apex. Head slightly to moderately exposed in front of pronotum in withdrawn condition; gently excavated between eyes; eyes widely separated on ventral surface; greatest head width 2.8-3.3 mm; antennal sockets close but not contiguous (Figs. 13 and 20); mouthparts well developed, apical segment of labial palpi lunate (Figs. 13 and 20); labrum wider than long, well sclerotized, separated from head by an inflexible suture and bearing short rounded projections along its anterior margin; antennal length 2-3 times greatest head width; flagellar segment 1 short, half as long as flagellar segment 2, remaining flagellar segments long, slender,

Fig. 23. Habitus of *Photuris trivittata* male from near Cardenas, Tabasco, Mexico. Note the distinctive and diagnostic (for the present) elytral vittae. The split, median pronotal vitta in Cardenas specimens differs considerably from vittae occurring in North American *Photuris* species. This is a carbon dust drawing by Laura Line.

simple, much longer than wide, and narrowing towards apex. Abdomen with median posterior margin of ventrite 8 narrowly prolonged and apically rounded (Fig. 15). Aedeagus (Figs. 17, 18, and 21) with median lobe narrowing at apex, not projecting posteriorly beyond apices of lateral lobes; lateral lobes closely approximate dorsally, narrowly overlapping at base in ventral aspect, and shallowly excavated in apical 1/5; aedeagus bearing elongate slender projections bearing sense organs on their apical inner surface (Fig. 21).

Female. Length: 13 mm long. Macropterous; colored as for male; head slightly smaller than that of male (Fig. 14); light organ in ventrites 6, 7; ventrite 8 narrowing posteriorly, median posterior margin emarginate (Fig. 16).

Flashing and Ecology

Photuris trivittata was observed on the campus of the Agriculture School at Cardenas, Tabasco, Mexico, at the edge of small woods along an irrigation ditch and mowed roadside. Occurring with it at this site were about a dozen flashing species of *Photinus*, *Photuris*, and *Aspisoma*. Female *trivittata* hunted males of at least one other *Photuris* species in an adjacent field, and an *Aspisoma* species near the woods, via aggressive mimicry (sensu Wickler 1968; Pasteur 1982; Lloyd 1964, 1984). At a nearby site near an irrigation canal, this same firefly displayed a sedentary flashing-feeding behavior previously unreported for *Photuris* fireflies.

Evening flashing activity at the ditch site began about one-half hour after sunset (x-bar = 33 min, 1.5 crep (i.e., civil twilight duration, see Neilsen 1963); range = 24-40 min, 1.1-1.8 crep; n = 6, 17-28 Oct 1980), in the shrubs and understory, and quickly moved up and around the canopy foliage of the trees. The most common male flashing pattern observed at this site was a short flash (base duration ca 52 mSec, Fig. 22a), that was emitted in continuous sequence at 1.2-1.4 sec, quite-regular intervals (Fig. 22b; 27.2°C; Table 1). Perched males also emitted this pattern, and they as well as flying males could be attracted (via penlight) close from 20-30 meters above ground, by flashing a short flash immediately after each of their flashes. On one occasion about 15 males were seen perched in a low tree, each facing outward with head and neck extended, flashing this pattern. This pattern was also emitted amongst and around the tips of the fronds of oil palms at the second site.

Males high in the trees at the first site occasionally appeared to emit a bimodal flash pattern, with the two peaks appearing 20-30 mSec apart. However, photo-multiplier recordings of what were verbally noted as this “fast double”, showed that it was a short flicker of 3-4 modulations (Fig. 22c) with a mean modulation rate of ca 8.0 Hertz. The mean pattern period of this signal was 2.4 sec (27.2°C; Table 1). This pattern is similar in form to that of a co-active *Aspisoma* species at this ditch site, but the modulation rates of the two are different (cf. Figs. 22c, d). Male *P. trivittata* sometimes emitted a longer flash, which had an estimated duration of about 300 mSec.

Across campus at the second site, near a large irrigation canal, male and female *Photuris* of two species perched in aggregations on the seedheads of a tall Bahia grass, *Paspalum virgatum*. They “mouthed”, chewed or licked, the seeds, which were coated with a sticky material. Each of the two mixed groups observed numbered 20-30 individuals and extended along the canal about 30 ft. The groups were about 200 ft apart and a few isolated individuals perched and flashed in the grass extending along the canal bank between them. Males and females on the grass heads emitted sequences (trains) of short flashes, and fireflies in these aggregations had a tendency to flash together in bouts of up to about 1 minute duration, separated by relatively dark periods.

TABLE 1. FLASH DATA FROM ELECTRONIC RECORDING AND STOPWATCH MEASUREMENTS. FLICKER MODULATION RATES ARE EACH FOLLOWED BY THE NUMBER OF MODULATIONS USED TO CALCULATE THE RATE INDICATED. MEANS ARE INDICATED BY "$\bar{x}$"; STANDARD DEVIATION BY "s". NUMBERS IN BRACKETS ARE ID NUMBERS OF INDIVIDUAL MALES ON CHART RECORDS THAT ARE ARCHIVED WITH THE VOUCHER SPECIMENS AND FIELD NOTE BOOKS.

A. Data from photo-multiplier recordings

Male no.	Observations	$\bar{x}$	s.d.	Temp.
Short flash period				
1.	1.35, 1.35, 1.36	1.35	0.01	26.1°C
3.	1.19, 1.18, 1.18, 1.18, 1.18, 1.17, 1.14, 1.16	1.17	0.02	25.8°C
4.	1.27, 1.23, 1.24, 1.22, 1.20, 1.20, 1.20, 1.21, 1.22, 1.22, 1.22, 1.23, 1.23, 1.24, 1.24, 1.22, 1.22 (perched)	1.22	0.02	27.2°C
5.	1.25, 1.24, 1.23, 1.25, 1.25, 1.23	1.24	0.01	27.2°C
6.	1.25, 1.25, 1.24, 1.24	1.25	0.01	27.2°C
7.	1.24, 1.26, 1.25, 1.18, 1.19, 1.11, 1.19, 1.23, 1.23, 1.23	1.21	0.05	27.2°C
8.	1.28, 1.28, 1.27, 1.27, 1.25, 1.27, 1.23, 1.20, 1.22, 1.24, 1.23, 1.22, 1.21, 1.21, 1.21	1.24	0.03	27.2°C
	Combine 5 males @ 27.2°C: $\bar{x}$ = 1.23 Sec., s.d. = 0.02			
Flicker pattern period				
9.	1.80, 2.26, 1.94	2.00	0.24	25.8°C
10.	2.30			27.2°C
11.	2.48			27.2°C
	Combine 2 males @ 27.2°C: $\bar{x}$ = 2.39 Sec., s.d. = 0.13			
Flicker modulation rate				
9.	9.6/3, 9.6/2, 8.9/3, 8.8/3, 9.0/3	9.2	0.4	25.8°C
10.	8.5/3, 8.2/3	8.4	0.2	27.2°C
11.	7.5/4, 7.4/4	7.5	0.1	27.2°C
	Combine 2 males @ 27.2°C: $\bar{x}$ = 8.0 Sec., s.d. = 0.6			

Short flash duration

Σ = 70 flashes (8 males) pm-recorded; 64 flashes from 8 males usable:
8 flashes, 2 males: $\bar{x}$ = 53 mSec, r = 51-56 mSec, 26.1°C, 23-X-80.
6 flashes, 1 male: $\bar{x}$ = 48 mSec, r = 46-51 mSec, 25.8°C, 26-X-80.
50 flashes, 5 males: $\bar{x}$ = 52 mSec, r = 48-62 mSec, 27.2°C, 28-X-80.

B. Flash pattern period data from stop watch records

3 males: 1.4 1.4 1.4; $\bar{x}$ = 1.4, 26.1°C
3 males: 1.4 1.4 1.4; $\bar{x}$ = 1.4, 26.1°C
6 males: 1.4, 1.3-1.4; $\bar{x}$ = 1.4, 26.1°C
2 males: 1.6 1.6; $\bar{x}$ = 1.6, 24.4°C
1 male: 1.3, 26.7°C

Flash rate within an individual's train was not constant. A few (5-15) rhythmic flashes were emitted in rapid succession, then rate slowed and became much less regular (Fig. 22e). Brief (ca 10-sec, 25.8°C) recordings of the flashes of several individuals on seeds suggest that there may be sexual differences. In a sample of four males and four females, the flashes of two males are nearly all bimodal, while those of females are all unimodal (Figs. 22e-l). Male flashes are longer on average (duration 91 mSec versus 75 mSec for females). In the short bouts of regular, rhythmic flashing, the flash rate of males is lower (x = 2.8 Hz versus x = 3.4 for females; Table 1). However, no overt sexual behavior such as mounting or rapproachment was observed.

Several kinds of insects occurred on the grass, including mosquitoes, crane flies, leaf beetles, roaches, grasshoppers, and moths, all apparently feeding on the seeds, except for a cone-headed katydid that was eating another insect. *Photuris trivittata* captured and fed upon mosquitoes, crane flies and beetles. To our knowledge, this is the only time that adult *Photuris* have been found eating prey other than Lampyridae in the field,

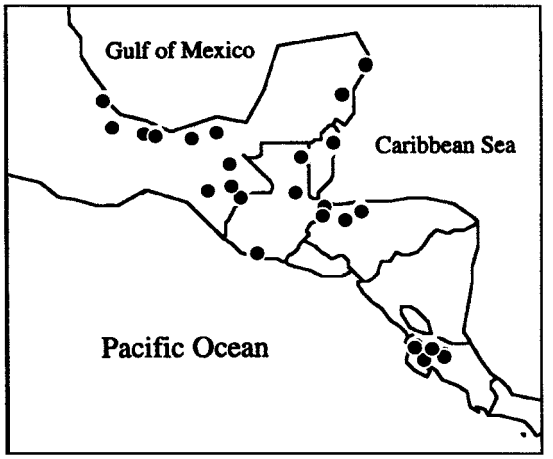


Fig. 24. Known distribution of *P. trivittata* as presently recognized, from specimens located in several collections.

though captive specimens have fed upon other insects. Firefly prey (*Photinus*, *Pyractomena*) provides defensive chemical substances that *Photuris* fireflies use in their own defense (Eisner et al. l997 and refs). However, non-firefly prey may also be captured by Nearctic *Photuris*, but because they don't glow while being eaten, go unnoticed. In the field adjacent to the first site, female *P. trivittata* perched down in dense grass within a foot of the ground, flashed responses to flash patterns of *Photuris* males of another species and attracted them to within 1 meter distance. *Photuris trivittata* occurs broadly through Central America, from southern Mexico to Costa Rica (Fig. 24), and its predation certainly has had an important influence on the signaling behavior of other lampyrids, and perhaps the behavior of other insects as well.

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EFFECT OF IMIDACLOPRID ON WING FORMATION IN THE COTTON APHID (HOMOPTERA: APHIDIDAE)

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The cotton aphid, *Aphis gossypii* Glover, is considered one of the most important pests of many vegetable and field crops (Leclant & Deguine 1994). In 2002, aphids were present in 70% of US cotton fields, infesting 9.4 million acres of cotton (Williams 2003).

Imidacloprid is an effective systemic insecticide (Nauen et al. 1998) with a high degree of residual activity against cotton aphids although the compound is slow acting (Boiteau & Osborn 1997). Imidacloprid acts on the nicotinic acetycholine receptor, causing the insect to reduce or stop feeding, and reduces mobility (Gourment et al. 1994; Boiteau & Osborn 1997).

As a result of casual observations of an increased proportion of alate aphids in imidacloprid-treated fields compared to untreated fields, laboratory spray tests were conducted on apterous adult aphids to evaluate the extent of wing formation and fecundity in offspring due to exposure to imidacloprid. Increased wing formation due to insecticide treatment has not previously been reported.

Probit analysis using five application rates from 0 to 0.05 Lb ai/A imidacloprid (Provado® 1.6F, Bayer Corporation, Kansas City, MO 64120) were used to establish an LC_{50} for imidacloprid of 0.122 ppm (0.0125 Lb ai/A). In imidacloprid-treated fields, aphids may not obtain a lethal amount of insecticide because of insufficient dosage, inadequate coverage, or active avoidance of insecticide residues (Kerns & Gaylor 1992).

Each treatment was replicated eight times on greenhouse-produced four-leaf stage cotton (Deltapine 51 and Deltapine 428B; Delta and Pine Land Company, Scott, MS 38772). Ten pots (1 plant/pot) were used in each replication. Ten adult apterous cotton aphids were transferred onto each of the four true leaves of each plant with a fine camel hair brush. Treatments were applied to cotton plants in a spray booth (Research Track Sprayer SB6-079, DeViries Manufacturing, Hollandale, MN 56045). The aphids were allowed to settle and recounted to insure all leaves contained 10 aphids prior to treatment.

Five plants were randomly selected and sprayed with the LC_{50} solution of 0.0125 Lb ai/A imidacloprid and the other five plants were sprayed with water. Following spraying, plants were allowed to dry and placed in a chamber at 20 ± 3°C, (13:11, L:D). Aphids were counted on each of the four leaves/plant 48 h post-treatment to check mortality and to establish the number of surviving adults for fecundity assessment. The 48 h reading was selected based on mortality assessment from probit analysis. Ten days post-treatment, aphids on each leaf were examined and classified as either apterous or alate based on the presence or absence of wings or wing pads. All replicates were sampled using the same protocol with a total of 80 plants sampled (4 leaves/plant; 10 plants/replicate; 8 replicates). Data on wing formation with $\log_{10}$ transformed means and on fecundity were analyzed by analysis of variance with means separated using PROC GLM, ANOVA, and t-tests (SAS institute 1997, 1999).

Two days post-treatment 89.2% (±0.11) of the aphids survived in the control and 51.4% (±0.13) in imidacloprid treatments (±SE). No significant difference occurred among replications. A significant difference ($P \leq 0.0001$) in wing formation was observed between treatments (Table 1). Imidacloprid-treated plants had 12.0% (±1.30) alate offspring compared with 2.0% (±0.24) in the control plants. Further, a significant decrease ($P \leq 0.0003$) in fecundity of treated aphids occurred with aphids on control plants having 9.2 ± 0.97 offspring per adult and aphids on imidacloprid plants having 4.9 ± 0.50 offspring.

Alate aphids can migrate, have a longer developmental time, produce fewer offspring, and have

TABLE 1. EFFECT OF IMIDACLOPRID ON WING FORMATION OF OFFSPRING AND THE FECUNDITY OF SURVIVING ADULT COTTON APHIDS.

Treatment	Wing Formation ± SE (%)	Fecundity ± SE (offspring/adult)
Water control	2 ± 0.24 b	9.2 ± 0.97 a
Imidacloprid (0.0125 Lb ai/A)	12 ± 1.30 a	4.9 ± 0.50 b

Means in the same column followed by the same letter are not significantly different, df = 79, $P \leq 0.05$ (LSD, SAS Institute 1997). Wing formation data were $\log_{10}$ transformed. Percentage non-transformed data are shown in the table.

an increased risk of mortality when moving than apterous aphids (Noda 1959; Dixon 1977). Additionally, alatiform nymphs and adults are more tolerant than the apterous form to pesticides, possibly due to size difference, amount of sclerotization, and/or difference in behavior (Crafton-Cardwell 1991).

The formation of wings is influenced by prenatal (inside the mother), postnatal (early nymph), and a combination of both prenatal and postnatal conditions (Dixon 1998). *Aphis gossypii* has developmental flexibility as late as the second instar (Shaw 1970). The longer the delay, before wing development, the quicker the aphids respond to rapid environmental changes (Dixon 1998). Our results could be due to either prenatal or postnatal effects.

Crowding and nutritional factors are the two main forces involved with the production of alates in most aphids (Dixon 1998). Colonies with fewer than three aphids seldom produce alates, while colonies with three or more aphids often produce alate offspring (Reinhard 1927). Unfortunately, research has not identified the relative importance of nutrition versus crowding, and just two aphids can promote wing induction from tactile stimulation (Muller et al. 2001). Aphids on a more nutritious host produce more offspring and are less likely to move frequently. However, on poor quality hosts, aphids are more restless and more likely to contact other aphids, producing a crowding response (Tamaki & Allen 1969). The physical influence of spray likely induced movement and may have simulated crowding in some of the aphids in our tests, but this influence was controlled because the control plants received a water spray.

Imidacloprid treatment induced increased wing formation in the cotton aphid independent of aphid crowding and associated decline in plant quality due to aphids or plant senescence. Imidacloprid reduces aphid feeding and may lower plant nutrition; these effects may cause wing production. Further, the production of wings could be caused by the insecticide acting on the endocrine system in a way similar to that of precocenes (Hardie 1986; Hardie et al. 1996) or by the impact of the insecticide on the plant, or a combination of these or another unknown mechanism.

Summary

When treated with imidacloprid, cotton aphids produce a significantly higher percentage of alate offspring with significantly fewer offspring per adult. In addition to potential increased emigration by alate aphids, an increase in the proportion of alate aphids among survivors of an imidacloprid treatment may have further caused a decrease in the number of aphids in the treated field because alate aphids required a longer developmental time, produced fewer offspring, and had an increased risk of mortality. An increase in the proportion of alate offspring could ultimately decrease the overall number of aphids in the field and thus increase the apparent efficiency of the insecticide. Conversely, applications of imidacloprid could serve to worsen area-wide problems via increasing the dispersal of winged aphids to other fields, assuming the surviving alates are fit and disperse normally.

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HOMALODISCA COAGULATA (HEMIPTERA: CICADELLIDAE) EMBRYONIC DEVELOPMENT AT CONSTANT TEMPERATURES

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Homalodisca coagulata (Say) (glassy-winged sharpshooter), a species exotic to California, is an important vector of the xylem-limited bacterium, *Xylella fastidiosa*, which causes diseases on several crops and ornamentals including Pierce's disease of grapes, citrus variegated chlorosis, phony peach disease, almond leaf scorch, alfalfa dwarf, and oleander leaf scorch (Blua et al. 1999; UCOP 2000; Varela et al. 2001). Little is known about the developmental biology of *H. coagulata*. Eggs of this insect are laid below the epidermis of leaves as a cluster of eggs oriented nearly parallel to one another. The number of eggs per egg mass on chrysanthemums averages 8.8 eggs (range: 1-30) (A.K.A., unpublished data). Data regarding the effect of temperature on the development of the egg stage of this insect are lacking. Such data are useful for two basic purposes: cold storage of eggs for later use in rearing parasitoids (Leopold & Yocum 2001) (needed because of reproductive dormancy of female sharpshooters during fall and winter) and the ability to predict egg hatch in the field.

Egg masses were produced by caging field-collected leafhoppers on small chrysanthemum plants (rooted cuttings) in sleeve cages at 23°C. Plants were checked every 12 h for fresh egg masses. Egg masses were then immediately incubated in situ (inside leaves intact on plant) in growth chambers set at different temperatures: 10, 15, 20, 25, 30, 32, 33, 35, and 40°C. For all temperature treatments, RH was set at ca. 50%, and the light regime at 14:10 L:D. Temperature inside the chambers was recorded using HOBO data loggers (Onset Computer Co., Bourne, MA) in order to arrive at actual incubation temperatures (11.5, 16.7, 19.7, 25.6, 31.2, 32.9, 33.4, 35, and 40.4°C) which corresponded respectively to the set temperatures stated earlier. During embryonic development, egg masses were checked daily (at or above 20°C); or weekly (10, 15°C) until eye spots became faintly visible within eggs, and then daily afterwards. When hatching was imminent (large dark eye spots), leaves containing egg masses were excised and placed inside petri dishes with moist tissue paper. This allowed easier and more accurate observation of emergence of nymphs from individual eggs within an egg mass. At this stage, egg masses were monitored in the morning and evening. Records of time of hatching of each egg and stage of development for each egg mass were kept. Developmental periods and rates were calculated and plotted against actual mean temperatures. Linear regression was used to arrive at estimates of the minimum developmental threshold and degree-days required for development. ANOVA on arcsine transformed data was used to compare egg hatch rates (proportion of hatched nymphs). Statistical analyses were done using JMP IN (SAS Institute 1996).

Complete development (for at least 1 egg) occurred at all temperatures except 11.5 and 40.4°C. At 11.5°C, development was retarded and aborted during early stages of eye spot formation; egg masses desiccated at 40.4°C. The relationship between development rate and temperature was linear for temperatures in the range 16.7 to 25.6°C, peaked at ca. 32.9°C, and then declined at higher temperatures (Fig. 1). Regression of the linear part of the curve yielded the linear equation shown in Fig. 1. From the linear equation, it is estimated that *H. coagulata* requires 113.8 degree days to complete embryonic development with a minimum developmental threshold temperature of 11.9°C. Hatch rate (proportion of hatched

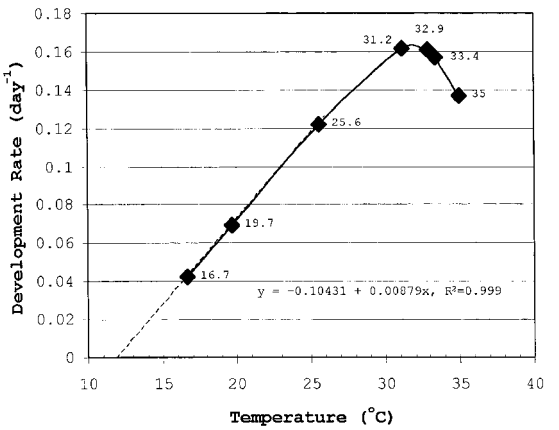

Fig. 1. The relationship between mean embryonic development rate and temperature for *H. coagulata*. 16.7°C, n = 255, SEM = 0.00007; 19.7°C, n = 465, SEM = 0.00013; 25.6°C, n = 212, SEM = 0.00089; 31.2°C, n = 202, SEM = 0.00105; 32.9°C, n = 133, SEM = 0.00099; 33.4°C, n = 137, SEM = 0.00074; 35°C, n = 25, SEM = 0.00189 (n is the number of eggs held at indicated temperatures, and SEM is the standard error of the mean). Linear regression equation for the three lower temperatures is shown. The dotted line represents extrapolation of the linear portion of the curve to the temperature at which development rate equals zero.

nymphs) did not differ significantly among temperatures in the low range (16.7-32.9°C). However, hatch rate was significantly lower at 33.4 than at 19.7°C (Fig. 2). Hatch rate was also significantly lower at 35°C than at all other temperature treatments except 33.4°C (Fig. 2).

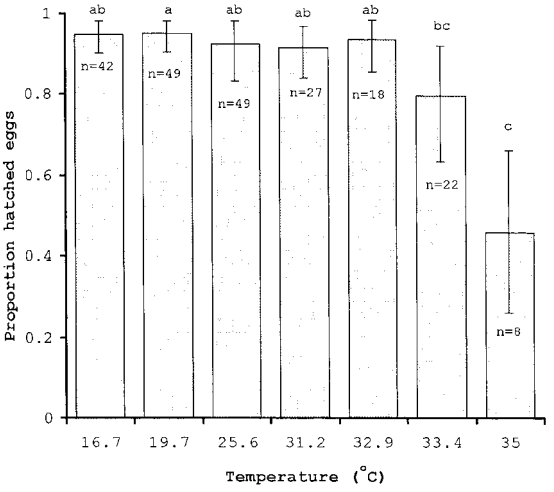

Fig. 2. The effect of temperature on the hatch rate of *H. coagulata* eggs. Hatch rate is based on the proportion of hatched eggs per egg mass for each of the temperature treatments. Original data were arcsine transformed and ANOVA was conducted on transformed data. Means represented by columns were calculated by back transformation of means produced by ANOVA. Bars through the top of columns are 95% confidence limits of the means. Means topped by the same letter are not significantly different (P = 0.0001, Tukey-Kramer HSD). The number of replicates shown within the top of each column refers to the number of egg masses held at each temperature.

This work provides an important tool to predict the time of hatch of *H. coagulata* eggs in the field. It also indicates that constant temperatures equal to or above 35°C will result in high mortality and that development is incomplete at ca. 11.5°C. It appears that the optimal temperature range for successful development of eggs of *H. coagulata* is in the 16.7-32.9°C range. Work is underway to investigate the circadian rhythm of nymphal hatch at different temperatures.

Summary

Development rate of *Homalodisca coagulata* (Say) was linearly related to temperature from 16.7 to ca. 30°C. It is estimated that *H. coagulata* requires 113.8 degree-days from oviposition to egg hatch, with a minimum developmental threshold of 11.9°C. At higher temperatures, hatch rate was significantly reduced, especially at 35°C.

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DIFFERENTIAL ATTRACTION OF A PARASITOID TO DEAD HOST ANTS

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a major economic pest that has spread throughout the southern United States and beyond in the mere 60 years since its introduction to Mobile, Alabama (Vinson 1997). Efforts to control its spread without the use of non-specific and environmentally harmful pesticides (Banks 1990) has led researchers to explore every available option, including the use of a parasitoid fly as a biocontrol. A species-specific phorid fly parasitoid of the RIFA in its native South America, *Pseudacteon tricuspis*, has been introduced into areas infested with the RIFA in the southern United States, including sites around Austin, Texas, where this study was conducted (Gilbert & Patrock 2002).

In South America, many different species of *Pseudacteon* flies parasitize the RIFA (Disney 1994; Porter & Pesquero 2001), and these species differ in the context of their attraction to the ants. Some flies are more likely to be attracted to ants at their mound, as is the case with *P. tricuspis*, while others to ants foraging away from the mound (Orr et al. 1997). In the introduced populations in Austin, Texas, the flies have most commonly been seen attacking worker ants naturally at nuptial flight swarms, but have also been found at mound disturbances and at food baits with workers. The flies can also be attracted by presenting them with dead *S. invicta* workers; using freshly killed and crushed ants is a very effective method of attracting the flies.

Pseudacteon tricuspis flies are known to mate at the same place as they attack their hosts (Porter 1998). Although only females lay eggs on the ants, males will typically hover over the ants and often "harass" them; the male fly behavior is "harassment" in that they elicit an alarm response from the worker ants. While both sexes are attracted to the host ant, whether males arrive first, disturb the ants and attract females, females arrive first and attract males, or both males and females are equally likely to find their host ants, has not been reported.

In this study we investigated the relative attraction of the flies to two ant castes (workers and female reproductives), and documented the sex ratio of flies attracted. The sex ratio of the first fly to arrive was also noted in order to gain insight into whether fly sex is important in host location.

Given the flies' attraction to workers participating in nuptial flight swarms, and the role of ant sexuals in inducing nuptial flight worker behavior (Obin & Vander Meer 1994), we investigated the possible role of winged female sexuals (alates) in attracting the flies. Boxes that contained equal weights of either dead workers or alates (0.5 grams ants crushed a la mortar and pestle) were paired. The date of death (by freezing) and the colony of origin of both ant castes (workers and female sexuals) in each pairing were identical. The boxes in each pair were placed 1 meter apart in an area where *P. tricuspis* had been consistently recovered. If multiple pairs were used per trial, 15 minute observation period, the pairs were separated by 20 meters and the results for all pairs in the trial were pooled, thus making any conclusions more conservative; 1, 2, or 3 pairs were used per trial. Boxes were separated by 1 meter so that the flies would be able to detect both boxes and choose between the two, yet far enough away so that the choice they made would be distinct. Pairs of boxes were separated by 20 meters in order to increase the area over which flies were attracted; independence of pairs was not considered since the data over all pairs was pooled. Flies attracted to the ants in the boxes were aspirated, sexed, and released at the conclusion of each 15 minute trial. After their collection, females were confirmed to be *P. tricuspis* via their ovipositor, but males were only positively identified to family and behaviorally identified to species; low hovering over corpses or live ants by the males is sufficient to distinguish this species since no other phorids in the area have been previously attracted to *S. invicta*, whether live or dead (personal observation). Since our methods included sampling the flies with replacement, efforts were made to spatially and temporally segregate samples.

A follow-up experiment was performed pairing crushed alates with an equal weight of crushed crickets (control), using the same protocol described for the above experiment. This was done in order to assess the flies' ability to detect alates in the absence of workers.

A significantly greater number of *P. tricuspis* were collected at the worker boxes in comparison with the alate boxes (Mann-Whitney U-test, $n = 12$ trials, $U = 26.5$, $p < 0.01$), 51 flies were col-

lected at the workers versus 23 at the alates. However, when alates were paired with crickets (n = 7), only in one of the trials was a fly collected at the alates, none were collected at the crickets; at the conclusion of each trial the presence of flies was verified using dead crushed workers. No flies were ever observed trying to attack the ant cadavers. All trials were performed in areas where flies had been collected within the past 48 hours and during meteorological conditions within the active range of *P. tricuspis*.

The sex ratio (female: male) of the first flies arriving at the boxes, the "discovering flies" (1:2.4, n = 17), was similar to that of the total collected (1:2.2, n = 74) ($\chi^2 = 2.88$, $p > 0.05$, d.f. = 1).

This study shows that dead alates are attractive primarily in the context of nearby dead workers since alates alone proved to be only weakly attractive to *P. tricuspis*. Dead workers on the other hand are very effective in attracting the flies. Fresh dead workers appear to be just as attractive, if not more so, as live workers (personal observation). It appears the workers are the primary source of long range cues that attract this fly to its host. Thus, using dead workers to assess fly presence is an effective tool for monitoring. This method is essential in drought-prone Texas because it can be employed independent of ant activity and density.

The similarity between the sex ratio of the discovering fly and that of all flies collected during the observation periods indicates that both sexes are equally likely to discover their hosts. Therefore, it is unlikely that either males or females attract the other to mate at the site of their host, but instead, it is the host that attracts both of them.

The use of odors of both dead and living workers as host orientation cues may account for the tendency of species like *P. tricuspis* to be associated with mounds versus foraging trails (e.g., Orr et al 1997).

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Summary

In a field study conducted in Austin, Texas, a greater number of *Pseudacteon tricuspis*, a species-specific phorid fly parasitoid of the red imported fire ant (RIFA), were collected at worker corpses than alate corpses. Neither sex of fly discovered corpses more frequently than the other.

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COMMERCIAL ADOPTION OF BIOLOGICAL CONTROL-BASED IPM FOR WHITEFLIES IN POINSETTIA

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The whiteflies *Bemisia argentifolii* Bellows and Perring and *Trialeurodes vaporariorum* Westwood continue to be the most important insect pests in commercial poinsettia (*Euphorbia pulcherrrima* Willd. ex Koltz) production in the northeastern United States. Most crops are chemically treated to suppress whiteflies, either preventatively with pot applications at planting of the systemic insecticide imidacloprid (Marathon®) or, later in the crop cycle, with foliage applications of various other insecticides. In the fall of 2000, a survey of 22 Massachusetts poinsettia growers found they used an average of 8.3 pesticide applications for this pest, at a cost of $0.14 ± $0.02 (SE) per plant (Van Driesche et al. 2002). Significantly, only 7 of 22 growers were able to achieve full season whitefly suppression with only the use of Marathon® at planting; the other 15 growers all needed to apply additional foliar pesticides later.

As an alternative approach, the use of parasitoids for suppression of whiteflies in poinsettia crops has been developed over the past decade (Hoddle and Van Driesche 1996, 1999a,b; Hoddle et al. 1996a,b, 1997a,b,c,d, 1998, 1999, 2001; Van Driesche et al. 1999a,b, 2001a,b, 2002). Unlike most implementation of augmentative biological control, the release pattern and rate was not based on testimonials but rather replicated controlled research trials in experimental and commercial greenhouses. This research considered three initial parasiotids (*Encarsia formosa* Gahan, *E. formosa* Beltsville strain, and *Eretmocercus eremicus* Rose and Zolnerowich), three release patterns (constant, front end loaded and back end loaded) and three release rates (3, 1 and 0.5 females per plant per week), as well as in combination with insect growth regulators. Cost of use, while at first uneconomical ($2.70 per plant per season) was reduced steadily through research and changes in product price, reaching $0.25 per plant (including the cost of shipping) (Van Driesche et al. 2002), a 93% reduction in cost.

Here, we report results of the first large scale commercial adoption of this biological control program, which was implemented by one of the largest Massachusetts poinsettia growers in 2002 on the grower's initiative. A single large greenhouse with 15,408 potted plants (wholesale value, $77,737) was managed through releases of *E. eremicus* (purchased from Syngenta) released at 0.5 females per stem. Whitefly populations were monitored in alternate weeks by staff of our laboratory and an employee of the producer, using the same protocol as employed in Van Driesche et al. (2002). The grower purchased, received, and released his own parasitoids. Here we report on the degree of suppression obtained and the degree of grower satisfaction with the outcome in terms of crop quality and production cost. We also discuss management errors that occurred and how they affected the ease of maintaining biological control.

The greenhouse range under biological control management was divided into east and west blocks that were separated by an internal space for movement of machinery. Both sections were physically inside one very large greenhouse (23,520 ft^2 = 2219 m^2). The trial began 9 September, 2002 when the range was filled with untreated plants (potted in mid-August in another greenhouse), which were immediately sampled to measure whitefly density. The trial ended 4 December, once the majority of plants had been removed for sale. A total of 14,625 plants were initially placed under biological control, 7894 in the east and 6731 in the west blocks. Approximately 16 October, the grower introduced an additional 783 "Winter Rose" poinsettia plants from a different greenhouse, for a final total of 15,408 plants in the test area. This variety has crumpled bracts, creating a false rose appearance. These plants had not been treated with Marathon® prior to their introduction into the biological control area and were highly infested with whiteflies (4.2 ± 1.1 SE live nymphs and pupae per leaf when introduced). These plants were placed as a group on the far west side and acted as an undesired source of adult whiteflies for the remainder of the plants in the test greenhouse, especially those in the west block.

In the east block, 6 parasitoid releases were made, in weeks 3, 6, 7, 8, 9, 10 (on 25 September; 16, 23 and 30 October; and 6 and 13 November, respectively) and three insect growth regulator applications (using Enstar II® because Precision®, the material used in our previous tests, was no longer available) were made in weeks 4, 5, and 9 (2 and 10 October and 5 November). These applications were timed to suppress whiteflies at mid-crop but before bract coloration. (We did not recommend the third treatment, which was only applied by the grower because the other half of the greenhouse was being treated). Whiteflies were suppressed below the at-harvest target threshold of 2.0 live nymphs and pupae per leaf for the entire cropping period and at harvest had 1.1 ± 0.1 SE live nymphs and pupae per leaf (Fig. 1).

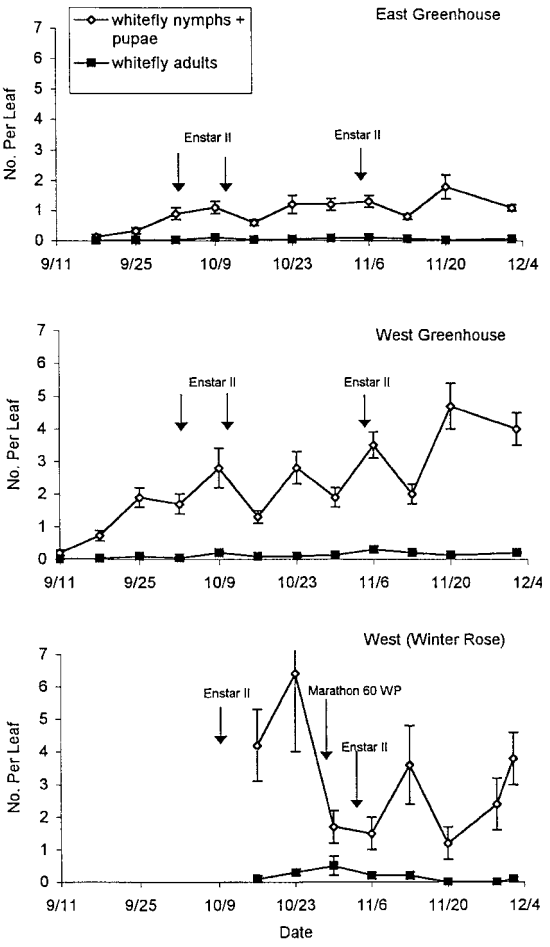

Fig. 1. Densities of live whiteflies per leaf in poinsettia in three parts of a greenhouse managed with releases of the parasitoid *Eretmocerus eremicus* near Boston, Massachusetts in 2002.

West block was filled with plants one week before east block. The grower made 8 parasitoid releases, in weeks 2, 3, 6, 7, 8, 9, 10,11 (on 18 and 25 September; 16, 23, and 30 October; and 6, 13 and 20 November, respectively). Enstar II was applied three times, in weeks 4, 5, and 9 on the same dates as East block. West block whitefly counts exceeded the target threshold (2.0) on two dates each in October and November and had 4.0 ± 0.5 SE live nymphs and pupae per leaf at sale on 4 December (Fig. 1). Higher whitefly densities in West block were caused in large measure by the introduction on 16 October of the "Winter Rose" plants. The edge of the block in contact with the "Winter Rose" plants was the most strongly affected. At harvest, west block plants exceeded our target threshold, but grower assessment of plant quality was favorable and plants were readily sold.

"Winter Rose" plants, which were placed next to the west block plants on 16 October, were also sampled weekly. These plants had 4.2 ± 1.1 SE live nymphs and pupae per leaf when introduced, but this increased to 6.4 ± 2.4 SE within 1 week. We immediately recommended treatment with Marathon®, as removal to another greenhouse was not possible. Marathon® was not applied until 30 October. In addition, this block of plants was treated twice with foliar applications of Enstar II® (10 October and 6 November), even though it was difficult to obtain effective coverage. At harvest, this group of plants had 3.8 ± 0.5 SE live nymphs and pupae per leaf.

Costs of the parasitoid releases (inclusive of shipping) and the IGR applications for the east and west blocks were $0.10 per plant and $0.14, respectively. This was based on the application of two packages of 10,000 *E. eremicus* pupae on each release date. This number of pupae and the numbers of plants in the test greenhouse, together with an assumed 50/50 sex ratio and 70% emergence rate, suggests a parasitoid release rate of ca. 0.45 females per plant was achieved. The price for biological control in this trial is lower than in previous trials because fewer total applications were made, in part because the grower did not start the biological control program until ca 3 weeks after planting, and applied an IGR in 3 weeks (rather than 2 as recommended), thus reducing the number of parasitoid applications in his 15 week crop from an expected 13 to actual 6-8. However, it is noteworthy that even this reduced frequency maintained control, in the absence of a source of whitefly-contaminated plants (i.e., the "Winter Rose" plants).

The per plant cost of whitefly control in this crop ($0.10 to $0.14 for the parasitoids, including shipping, and the IGR applications) compares to $0.14 for chemical control (exclusive of labor) for the same grower in 2001, when he applied Marathon® and nine other pesticides (one or more applications of each) to suppress whiteflies in the same greenhouse.

An exit interview with the grower found a high level of satisfaction with the biological control program. Production of this crop (as part of a Massachusetts extension effort to assist growers interested in implementing biological control measures) has demonstrated that sufficient information exists for northeast poinsettia growers to be successful in use of biological control for whitefly management and produce crops that meet the target threshold for whitefly suppression, with consequent good market acceptance. Costs were also acceptable to the grower relative to his past need for application of ten different pesticide products in a comparable crop in the previous year. This is the first published demonstration of successful implementation of biological in poinsettia in the United States at a price competitive with pesticides, meeting fully all grower concerns.

SUMMARY

Releases of *Eretmocerus eremicus* at the reduced rate of 0.5 females per plant per week, combined with three mid-season applications of the insect growth regulator kinoprene (Enstar II), successfully maintained densities of live nymphs+pupae of pest whiteflies (*Bemisia argentifolii*) at or below threshold (2 per leaf), barring management errors (introduction of highly infested plants). This program had a cost of $0.10-0.14 per plant, including the cost of the pesticide, the parasitoids and their shipping. This price was equal to or lower than the average cost of chemical control ($0.14 per plant) for 22 Massachusetts poinsettia growers whose pesticide application records were examined in a separate survey. This trial demonstrates that effective whitefly biological control on poinsettia can be achieved in the northeastern United States at prices competitive with current pesticide use.

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MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE) IN JAMAICA

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The only species of mole cricket reported for Jamaica in Gowdey's (1926a,b, 1928) catalogue of the insect fauna is *Scapteriscus didactylus* (Latreille). That mole cricket species was the subject of many reports in Puerto Rico in the latter part of the 19th century and first half of the 20th because of its destructiveness to cultivated crops and grasses (e.g., Barrett 1902). Seemingly, in every West Indian island where it occurs, it has been blamed as a pest; yet, nothing seemed to have been published about it in the Jamaican literature. The contrast—many reports from Puerto Rico and other West Indian islands where *S. didactylus* occurs, but none from Jamaica—did not make sense, because entomologists of the Jamaica Department of Agriculture (later Ministry of Agriculture) published numerous reports about other pest insects. For that reason, we decided to verify existence of *S. didactylus* in Jamaica by examining specimens of mole crickets in Jamaican collections. Our effort had a biogeographic focus (the history of colonization of the West Indies by mole crickets) and a practical implication. The practical implication was that we have worked with biological control agents of *Scapteriscus* mole crickets in Florida, and could have offered help in Jamaica if help had been needed; on the other hand, if some natural enemy had been suppressing *S. didactylus* populations in Jamaica, information that we might glean from Jamaica could be useful in other islands.

The senior author, newly employed by the Sugar Research Department (SRD) of the Sugar Manufacturers' Association (of Jamaica), encountered mole crickets in Jamaica as pest insects. The one incident was in January 1969. The locality was Gray's Inn, an agricultural estate near Buff Bay in the parish of Portland, where he was called by Brian Michelin (Farm Manager) to examine and recommend treatment for this occurrence. A field of banana had been replanted with sugarcane (this was done, as usual in planting sugarcane, by planting cut sections of sugarcane stem), and mole crickets were damaging the roots and shoots produced by the cut sections. Recommendations for insecticidal treatment were given, and specimens were collected and placed in vials of alcohol in the SRD collection. The specimens were not submitted for expert identification by specialists and are no longer available. There was no evidence of mole cricket damage to sugarcane in Jamaica from 1972 to the present (Trevor Falloon, pers. comm.). This assertion corroborates an earlier report by Frank & Bennett (1970) based on lack of mention of these pests in the pre-1970 literature on Jamaican agricultural pests and the direct observations by the senior author in 1969-1970. Other entomologists in Jamaica (Dionne Newell and Eric Garraway, pers. comm.) confirmed these findings not just for sugarcane but for all other crops. It is said that hindsight is a good teacher. The senior author should, in 1969, have sent specimens for expert identification because no key to the West Indian mole cricket species was then available; the key by Nickle & Castner (1984) was 15 years in the future.

Gowdey (1926a) does not specify how he identified most insects whose names appear in his catalogue. In his introduction, he acknowledges obligations to various specialists in England, Canada, and the USA. Among these, he mentions J. A. G. Rehn, of the Academy of Natural Sciences of Philadelphia, a specialist in Orthoptera. It is thus possible that Rehn examined a mole cricket specimen that Gowdey sent to him, or made a presumption, or that Gowdey himself made a presumption without sending a specimen to Rehn. Unfortunately, Rehn (1909) had earlier catalogued *S. didactylus* as being present in Cuba—which later was denied by others, summarized by Frank et al. (2002): *S. didactylus* does not occur in Cuba. Thus, Rehn may have allowed his presumptions to get in the way of hard evidence (examination of specimens) at least once and perhaps twice. In St. Croix (U.S. Virgin Islands) *S. abbreviatus* Scudder had been misidentified as *S. didactylus* (Frank & Keularts 1996).

The specimens housed in the Institute of Jamaica are the best evidence of identity of the mole cricket species in Jamaica. There are only five, and all are *Scapteriscus abbreviatus*. The earliest specimen (1) has no label and is from Gowdey's collection (*teste* Dionne Newell), presumably collected before 1926. All of the other specimens are from the parish of Kingston and St. Andrew. Collection data are: (2) St. Andrew, Sandy Gully nr. Barbican, 7-VII-1957, Peter Drummond, (3) Kingston, beneath seashells at foot of Paradise Street, 9-IX-1961, K. Eldemire, (4) St. Andrew, Port Royal, 14-I-1975, Donna Clark, (5) Kingston, Sutton Street, 9-V-1992, E. Sterling. These data suggest restriction of mole crickets to one parish (Kingston and St. Andrew), but evidence of mole crickets in Portland Parish (above) suggests a wider distribution. The first specimen of *S. abbreviatus* is from the collection that Gowdey assembled. It was the first general collection of insects formed by a Department of Agriculture entomologist (Gowdey 1926a). This is crucial evidence because Gowdey (1926a,b, 1928) acknowledges the presence only of *S. didactylus* in Jamaica. A misidentification was made. The mole cricket present in Jamaica is *S. abbrevia-*

tus (not *S. didactylus*). We must presume that *S. abbreviatus* arrived in Jamaica before 1926, possibly in ship ballast to Port Royal or to the port of Kingston, or both, as it is believed to have done in Cuba, Haiti, Puerto Rico, and St. Croix in the West Indies, and Florida and Georgia in the USA. Restriction of *S. abbreviatus* to the vicinity of its port of arrival (the parish of Kingston and St. Andrew), because adults are flightless, may account for the lack of widespread damage by it. Its presumed presence in Portland Parish on Jamaica's north coast is then more interesting, and suggests a separate arrival, perhaps at Buff Bay, or Port Antonio.

Neither of us has visited Jamaica in many years. We have not interviewed golf course superintendents about insect damage to turfgrass. It is golf course superintendents who may bear the brunt of damage by pest mole crickets, if there is any, because of the highly attractive habitat that they provide to these insects.

This note would not have been possible without the collaboration of Mrs. Dionne Newell (Natural History Department, Institute of Jamaica) who lent the five mole cricket specimens housed in the Institute's collections, and Dr. Eric Garraway (University of the West Indies, Mona, Kingston) and Mr. Trevor Falloon (Sugar Industry Research Institute, Mandeville) who made helpful comments. We acknowledge critical reviews of the manuscript of this note by Drs. Pauline Lawrence and Norman Leppla (University of Florida). This is Florida Agricultural Experiment Station Journal Series No. R-09469.

Summary

One species of mole cricket is proven to occur in Jamaica, and it is *Scapteriscus abbreviatus* Scudder. It is not native to Jamaica, and it arrived there before 1926. This species occasionally damages crops, but has not heretofore in print, to the best of our knowledge, been reported to do so in Jamaica. In the West Indian islands of St. Croix and Cuba, *S. abbreviatus* was apparently misidentified as *S. didactylus* (Latreille), and here we report that the same misidentification was made in Jamaica in the 1920s, uncorrected until now.

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NEW HOST RECORDS FOR TWO SPECIES OF *GONATOCERUS* (HYMENOPTERA: MYMARIDAE), EGG PARASITOIDS OF PROCONIINE SHARPSHOOTERS (HEMIPTERA: CLYPEORRHYNCHA: CICADELLIDAE), IN PERU

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Exploration for egg parasitoids of proconiine sharpshooters (Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Proconiini) was conducted by the senior author in Junín State of Peru during May 2002 as part of the ongoing classical biological control program against glassy-winged sharpshooter, *Homalodisca coagulata* (Say), in California (Jones 2001). Adults of *Pseudometopia amblardii* (Signoret), *P. phalaesia* (Distant) and *Oncometopia* n. sp. were collected by hand and caged on Satsuma mandarin, *Citrus reticulata* var. *satsuma* Blanco, trees in the Fundo Genova farm orchard near La Merced, Chanchamayo County, which is surrounded by a tropical jungle. These sentinel egg masses were obtained and marked on the leaves (individual eggs and egg masses of *Oncometopia* n. sp. are much larger than those of *P. amblardii* and *P. phalaesia*) and then were exposed to parasitization for 1-3 days prior to their removal and shipment to University of California, Riverside (UCR) and USDA-APHIS-PPQ Mission (Edinburg, Texas) quarantine laboratories under appropriate importation permits.

Two species in the family Mymaridae (Hymenoptera), both belonging to the *ater* species-group of the genus *Gonatocerus* Nees, which is known to contain egg parasitoids of proconiine sharpshooters in the New World (Triapitsyn et al. 2002), and one species in the family Trichogrammatidae (Hymenoptera) emerged in quarantine from these samples. Four female specimens of this trichogrammatid, an undescribed species belonging to an undetermined genus near *Zagella* Girault, was reared at the UCR facility from an egg mass of *P. amblardii*, or *P. phalaesia*. Its female antennal clava is two-segmented whereas that of *Zagella* species, some of which parasitize eggs of proconiine sharpshooters in Argentina and southeastern USA, are three-segmented (Triapitsyn 2003). According to J. D. Pinto (UCR, pers. comm.), this unnamed genus is quite common and diverse in the Neotropical region. This is the first reported host association for any of its members.

The two mymarids were *G. triguttatus* Girault and an undetermined species of *Gonatocerus* near *ashmeadi* Girault. Two females and one male of *G. triguttatus* emerged at UCR quarantine from a single egg mass of *P. amblardii*, or *P. phalaesia*. Previous known host records of *G. triguttatus* include *O. clarior* (Walker), *O.* sp., and *H. coagulata* in Texas and northeastern Mexico (Triapitsyn & Phillips 2000; Jones 2001; Triapitsyn & Hoddle 2001; Triapitsyn et al. 2002) and also *O. nigricans* (Walker) in central Florida (Triapitsyn et al. 2002). A species very closely related to *G. triguttatus*, *G. metanotalis* (Ogloblin), was reared by the senior author during December 2000 and January 2001 in Misiones, Salta, and Tucumán Provinces of Argentina from sentinel eggs of the proconiine sharpshooter *Tapajosa rubromarginata* (Signoret) on citrus (*Citrus* spp.) leaves. A culture of *G. metanotalis* has been successfully maintained since March 2002 at the USDA-APHIS Mission quarantine laboratory using eggs of a factitious host, *H. coagulata*.

Numerous female and male adults of *G.* sp. near *ashmeadi* emerged from egg masses of all three proconiine sharpshooter species from Peru, varying in body size in direct correlation with the size of the host's egg. This is the first known record of an egg parasitoid attacking a host in the genus *Pseudometopia* Schmidt. Parasitoids were given time to mate and then females were exposed to egg masses of *H. coagulata* on *Euonymus japonica* Thunberg leaves at the UCR and on leaves of three plant species (hibiscus, *Hibiscus rosa-sinensis* L. var. "Brilliant Red", sweet potato, *Ipomoea batatas* (L.) Lamarck, and cowpea, *Vigna unguiculata* (L.) Walpers) at the USDA-APHIS Mission quarantine laboratories, respectively. Colonies of this species were successfully established at both facilities. At UCR quarantine, three full generations were maintained at 20.5-25.5°C and 30-50% RH. Under these conditions, the developmental period of *G.* sp. near *ashmeadi* from egg to adult was 16-18 days. The UCR colony was lost after females of the fourth generation wasps. The two colonies of this species at the USDA-APHIS Mission quarantine were lost in the first and second generations.

Taxonomically (based solely on morphology), *G.* sp. near *ashmeadi* from Peru seems to be con-

specific to an undetermined, and possibly undescribed, species of *Gonatocerus* reared in January 2001 by the senior author in Santa Clara, Salta Province of Argentina from sentinel eggs of *T. rubromarginata* on citrus leaves. Both these forms are definitely different from, but nevertheless related to, *G. ashmeadi* Girault, a common egg parasitoid of *H. coagulata* and other proconiine sharpshooters in the USA and northeastern Mexico (Triapitsyn et al. 2002), and also to an undescribed species of *Gonatocerus* from Tamaulipas, Mexico, which was reported as an unusual form of *G. ashmeadi* by the same authors (S. V. Triapitsyn, unpublished data).

All proconiine sharpshooter and parasitoid specimens resulting from this study were determined by Pedro Lozada (Senasa, Lima, Peru) and S. V. Triapitsyn, respectively; vouchers specimens of the parasitoids are deposited in the Entomology Research Museum, University of California at Riverside, California, and those of proconiine sharpshooters (along with some specimens of *Gonatocerus*) were deposited in Senasa, Lima, Peru.

We thank Laura Varone (USDA-ARS South American Biological Control Laboratory, Hurlingham, Buenos Aires, Argentina) for assistance in the field, Vladimir V. Berezovskiy (Department of Entomology, University of California, Riverside, CA) for help with quarantine work and specimen preparation, as well as David J. W. Morgan (Pierce's Disease Control Program, California Department of Food and Agriculture, Mount Rubidoux Field Station, Riverside, CA) and Isabelle Lauzière (Quarantine Laboratory, USDA-APHIS-PPQ Mission Plant Protection Center, Moore Air Base, Edinburg, TX) for supplying fresh egg masses of glassy-winged sharpshooter and assistance in rearing the parasitoids in quarantine. This project was funded by USDA-APHIS and USDA-ARS.

Summary

Exploration for egg parasitoids of proconiine sharpshooters was conducted in Junín State of Peru in May 2002. Adults of three leafhopper species, *Pseudometopia amblardii*, *P. phalaesia*, and *Oncometopia* n. sp., were collected and caged on Satsuma mandarin trees in an orchard near La Merced. Two species of the mymarid wasp genus *Gonatocerus*, *G. triguttatus* and *G.* sp. near *ashmeadi*, emerged from these egg masses, the latter from all three hosts but the former from eggs of *P. amblardii*, or *P. phalaesia*. These are the first known records of egg parasitoids of *Pseudometopia* species and also new host records for both species of *Gonatocerus*. An undetermined trichogrammatid species of a genus near *Zagella* was also reared from an egg mass of *P. amblardii*, or *P. phalaesia*.

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ANASA TRISTIS (HETEROPTERA: COREIDAE) DEVELOPMENT, SURVIVAL AND EGG DISTRIBUTION ON BEIT ALPHA CUCUMBER AND AS PREY FOR *COLEOMEGILLA MACULATA* (COLEOPTERA: COCCINELLIDAE) AND *GEOCORIS PUNCTIPES* (HETEROPTERA: LYGAEIDAE)

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The Beit alpha cucumber, *Cucumis sativus* L., a crop grown under protective structures in the Middle East, is a new greenhouse commodity in Florida that will compete in the marketplace with the traditional Dutch-type cucumber (Shaw et al. 2000). While it produces a seedless fruit with a thin smooth skin like the Dutch cultivars, productivity can be much higher than other cucumbers. The Beit alpha cucumber can be grown successfully year-round in greenhouses but pests must be controlled for optimal production. The Protected Agriculture Project of the Horticultural Sciences Department at the University of Florida (www.hos.ufl.edu/ProtectedAg/) is seeking to implement biological control and reduce insecticide use as part of an integrated pest management program for this crop. Some of the common cucumber pests encountered by the project are aphids, spider mites, thrips, and whiteflies.

During the spring of 2002, a sporadic pest, the squash bug, *Anasa tristis* DeGeer (Heteroptera: Coreidae), infested the Beit alpha cucumber crop in the project's greenhouse three weeks before harvest and caused considerable damage. The squash bug is considered an important pest of cucurbits in open fields in the U.S. (Beard 1940; Nechols 1987; Cook & Neal 1999). Host preference includes squash, pumpkin, cucumber, and melon (Nechols 1987; Bonjour & Fargo 1989). Important natural enemies of the squash bug are the tachinids, *Trichopoda pennipes* (Fab.), and sceleonids, *Eumicrosoma* spp. (Metcalf & Metcalf 1993; Van Driesch & Bellows 1996). We evaluated 3rd instar larvae and adults of the two predators, *Coleomegilla maculata* DeGeer, and *Geocoris punctipes* (Say), as candidates to control the squash bug. These predators were selected because they are being used extensively to control other pests in the project's greenhouses. We also observed the location and number of eggs deposited by adults in the crop, and the development and survival of squash bug nymphs on Beit alpha cucumber.

The spatial distribution of egg masses in the greenhouse indicated the presence of female squash bugs and subsequent nymphs. Squash bug egg mass distribution on Beit alpha cucumber was determined by randomly selecting 20 cucumber leaves in each of the lower, middle, and upper levels of cucumber plants. Counts were made every other day for three weeks. Beit alpha plants were 3.7 m tall and each level was approximately 1.2 m wide. For each plant level and day, the number of egg masses and eggs per mass were counted (n = 20). Plants in the outside row proximal to the east wall screen were used because the pest appeared there first.

Squash bug adults (n = 20) were collected from the Beit alpha cucumber crop and taken to the laboratory (28 April). The colony was maintained at 21°C and 65% RH with a 16:8 (L:D) photoperiod. The squash bugs and cucumber plant material were kept in 3.8-liter Mason jars and egg masses were collected daily. Five egg masses from a single day were transferred to individual 7-cm plastic cups and kept moist with wet cotton balls. After the eggs hatched, about 20 nymphs were removed from each mass (n = 100) and isolated in individual 10 cm diameter plastic cups. Daily observations were made until individuals died. Each nymph was fed one-fourth of a Beit alpha cucumber leaf and a section of cucumber fruit. Food was replaced every two days. The developmental period was recorded for eggs and first and second instar nymphs.

To test squash bugs as prey, an experimental unit was used consisting of a section of Beit alpha cucumber leaf, a predator, and five first instars of the squash bug in an 8.2 cm diameter petri dish (Fisherbrand, Suwanee, GA) sealed with parafilm. Third instar and adult predators were used based on the results of a pilot experiment. Predators were not fed 8 h prior to the experiments. The control consisted of five prey without a predator. The mean number of prey consumed per day by each kind of predator was recorded and LSD was used to determine significant differences among treatment means (SAS Institute 2002).

Most egg masses were laid in the upper level of the crop, since it was frequented by the adults, averaging 11 ± 1.6. The mean number of egg masses in the middle third was 6 ± 1.2 and in the lower third 3 ± 0.8 (LSD, 0.05 = 4.75). Combining data from all locations, the average number of eggs laid per mass was of 20 ± 3.3. Egg masses were roughly circular (Fig. 1). The squash bug nymphs reared on Beit alpha cucumber advanced through the 2nd instar only. Bonjour and Fargo (1989)

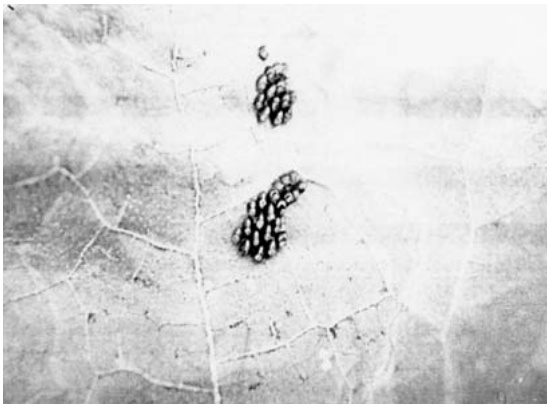

Fig. 1. Egg masses of *A. tristis* on the Beit alpha cucumber, *C. sativus* (12 mm length × 9.5 mm width). Photographed by Elio Jovicich.

Fig. 2. Damage caused by the squash bug on Beit alpha cucumber, *C. sativus* (insect length 90 mm). Photographed by Daniel J. Cantliffe.

obtained similar results when squash bugs were fed cucumbers; however, they did not specify if squash bugs were fed leaves or a combination of leaves and fruits. The nymphs were not able to molt and reach the 3rd instar. The mean developmental time for egg, 1st instar, and 2nd instar was 6.7 ± 1.8, 5.0 ± 1.2, and 3.2 ± 1.0 days, respectively.

The inability of the squash bugs to reach the adult stage under laboratory conditions does not eliminate the possibility of them causing severe damage in a greenhouse, especially if two related crops are being grown simultaneously and one is a true host plant. Our greenhouse contained the Beit alpha cucumber and the Galia muskmelon, *Cucumis melo* L. *reticulatus* group (Shaw et al. 2001). Egg masses, nymphs, and adults were observed only in the Beit alpha cucumber crop, causing wilting and eventually death of the plant. However, relocation of bugs from Beit alpha cucumber border rows toward the center of the cucumber crop and the melons was in progress. Adults, but not egg masses, were found in the melons. The melon crop was terminated 3 weeks after the cucumber crop.

Both nymphs and adults of *C. maculata* consumed more squash bug first instars than did either stage of *G. punctipes* (Table 1). Considering both species and stages, *C. maculata* adults consumed the most first instar squash bugs by the end of the 5-day trial (LSD = 1.61). Field observations indicated that neither predator could consume later instars of the squash bug.

Since no single pest management tactic (chemical, cultural, or biological control or host plant resistance) has been entirely effective in controlling squash bugs (Zavala 1991; Olson et al. 1996), their integration is necessary (Margolies et al. 1998). Early detection of squash bugs in the greenhouse is imperative because moderate infestations can cause plant wilt in cucumber (Fig. 2). *C. maculata* and *G. punctipes* can control early instars of the squash bug, thereby providing growers with a control tactic in addition to the use of chemicals and resistant varieties.

TABLE 1. CUMULATIVE MEAN NUMBER (±SEM) 1ST INSTAR SQUASH BUGS, *A. TRISTIS* DEGEER, CONSUMED BY TWO SPECIES OF PREDATORS (TREATMENTS REPEATED THREE TIMES, FIVE REPLICATES, N = 15).

	Mean number of prey consumed (n = 5)					
	Days of observations					Not consumed in 5 days
Predator	1	2	3	4	5	
Coleomegilla maculata (3rd instar)	0.75 ± 0.48	2.25 ± 0.85	3.00 ± 1.08	3.25 ± 1.18	3.25 ± 1.18	1.75
Coleomegilla maculata (adult)	3.00 ± 1.08	3.00 ± 1.08	3.75 ± 0.63	4.00 ± 0.71	4.25 ± 0.48	0.25
Geocoris punctipes (3rd instar)	0.25 ± 0.25	1.00 ± 0.41	2.00 ± 0.82	3.00 ± 0.41	3.00 ± 0.41	2.75
Geocoris punctipes (adult)	0.75 ± 0.48	0.75 ± 0.48	1.75 ± 0.85	1.75 ± 0.85	1.75 ± 0.85	3.25
Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	5.00
LSD, 0.05	1.64	1.61	2.17	2.01	2.00	1.61

Thanks to Nicole Shaw for detecting the squash bugs on her Beit alpha cucumber crop. We also thank Alex P. Diaz for his collaboration. Entomos, a commercial supplier located in Gainesville, Florida, provided the predators. Norm Leppla, Phil Stansly, and anonymous reviewers helped to improve the manuscript. This research was supported by the Florida Agricultural Station and approved for publication as Journal Series No. 08283.

Summary

During the spring 2002 growing season, the squash bug, *Anasa tristis* (De Geer) (Heteroptera: Coreidae), appeared for the first time in damaging numbers on the Beit alpha cucumber, *Cucumis sativus* L., a new greenhouse commodity in Florida. Adult squash bugs distributed egg masses mostly in the upper areas of the cucumber plants. However, the nymphs did not develop beyond 2nd instar when fed solely on cucumber leaves and fruits. Although squash bugs may not be the preferred prey for *Coleomegilla maculata* DeGeer or *Geocoris punctipes* (Say), 1st instar squash bugs were consumed by 3rd instars and adults of these predators. *C. maculata* adults consumed more prey than did the nymphs or either stage of *G. punctipes*. Early detection of the squash bug and immediate releases of these predators would be required to affect a significant level of control.

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THE ESTABLISHMENT OF *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE) IN MISIONES, NORTHEASTERN ARGENTINA

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Diachasmimorpha longicaudata originated from the Indo-Pacific region, has been widely disseminated into America via Hawaii, and can be considered successfully established in most of the importing countries, such as Colombia, Costa Rica, Guatemala, El Salvador, Mexico, Nicaragua, Trinidad, United States of America (Florida), Venezuela (Ovruski et al. 2000), and Brazil (Carvalho & Nascimento 2002). This exotic parasitoid species is the most widely employed parasitoid now in use for augmentative biocontrol programs against fruit flies in Latin America and the southern United States, primarily because it is easily mass-rearing and it adapts readily to different fruit fly species of economic importance such as *Anastrepha fraterculus*, *A. suspensa* (Loew), *A. ludens* (Loew), *A. obliqua* (Macquart), *A. striata* Schiner, *A. serpentina* (Wiedemann) and *Ceratitis capitata* (Sivinski 1996), (Ovruski et al. 2000). Interestingly, *D. longicaudata* is a common parasitoid species of *Anastrepha* larvae particularly in exotic commercial fruit in the state of Veracruz, México (López et al. 1999), and it may enter a dry season diapause (Aluja et al. 1998).

In 1961, Argentina's Ministry of Agriculture and Cattle, together with the National Institute of Agricultural Technology (INTA), introduced the braconid parasitoid of fruit flies *D. longicaudata* to the country from Mexico. This biological control program was a direct response to the establishment of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in Argentina However, the effort also hoped to control *A. fraterculus*, The South American fruit fly, in several citrus-growing areas where both tephritid species coexisted in wild and commercial fruit. *Diachasmimorpha longicaudata* was released in 100,000 in the 1960s in the northeastern provinces of Misiones (Montecarlo county) and Entre Rios (Concordia county), in the northwestern provinces of Tucumán (San Miguel de Tucumán city) and Jujuy (Calilegua county) and the central province of Córdoba (Cruz del Eje and Yacanto counties) Turica (1968). New releases of *D. longicaudata* were made in citrus orchards in the Tucumán province in 1977 and 1986 (Ovruski & Fidalgo 1994). As noted by (Ovruski et al. 1999), *D. longicaudata* was recovered immediately following release in all sites. However, until now, there was no evidence of permanent establishment in any release site.

Recent fruit fly parasitoid surveys made in Montecarlo county (Misiones province) included specimens of *D. longicaudata*. The first record of this species was in March 2000 attacking *A. fraterculus* larvae from *Feijoa sellowiana* L. (Myrtaceae), and it was subsequently found in February and March 2001 and April 2002 (all collection data are listed at the end of the paper), so that *D. longicaudata* was recovered approximately 40 years after its first release in Argentina. Thus this exotic parasitoid has become established in at least one province of Argentina, albeit in small numbers(≈1% parasitism).

Fruit flies and parasitoids were identified to species by the authors using Zucchi's (2000) and Wharton and Gilstrap's (1983) taxonomic keys, respectively. Voucher specimens were deposited in the Fundación Miguel Lillo (FML) entomological collection in San Miguel de Tucumán, Argentina.

Collection Data

Argentina, Misiones, Montecarlo, Chacra Laharrague, 21-03-00, ex. *Anastrepha fraterculus* on *Feijoa sellowiana*, 5 males, col.: O.R. De Coll, Ident.: S.M. Ovruski—P. Schliserman.

Argentina, Misiones, Montecarlo, Chacra Laharrague, 26-02-01, ex. *Anastrepha fraterculus* on *Feijoa sellowiana*, 1 female and 2 males, col.: O.R. De Coll, Ident.: S.M. Ovruski—P. Schliserman.

Argentina, Misiones, Montecarlo, Chacra Laharrague, 05-03-01, ex. *Anastrepha fraterculus* on *Feijoa sellowiana*, 2 males, col.: O.R. De Coll, Ident.: S.M. Ovruski—P. Schliserman.

Argentina, Misiones, Montecarlo, Chacra Laharrague, 08-04-02, ex. *Anastrepha fraterculus* on *Feijoa sellowiana*, 3 females and 1 male, col.: O.R. De Coll, Ident.: S.M. Ovruski—P. Schliserman.

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Summary

The establishment of *Diachasmimorpha longicaudata* (Ashmead) on *Anastrepha fraterculus* (Wiedemann) in the northeastern province of Misiones, Argentina, is described.

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BOOK REVIEWS

BOURTZIS, K. A., AND T. A. MILLER, EDITORS. 2003. Insect Symbiosis. CRC Press; Boca Raton, FL. 347 p. ISBN 0-8493-1286-8. Hardback. $119.95.

This edited volume contains a wealth of information about symbionts of insects which all entomology graduate students (and their advisors) need to be aware of. Information contained within this multi-authored volume may change the way we conduct our research or interpret data. The chapters contained within it make it clear that the biology, behavior, ecology and genetics of insects cannot be considered without considering the role of symbionts in each of these topics. This volume provides an overview of a rapidly advancing field of study, although it is not an exhaustive review of all that is known about symbionts. The entire field of symbiosis is expanding quickly and various books (including Margulis 1970, 1993; O'Neill et al. 1997; Sapp 1994; Douglas 2002; Paracer & Ahmadjian 2000; Majerus 2003) are available to discuss the symbioses found in an incredible array of organisms, giving symbiosis a prominent role in understanding ecology, behavior and evolution.

The entomological world is in the process of being revolutionized by the discovery, and increased understanding, of the various roles played by the multitude types of symbionts found in arthropods. There are several definitions of symbiosis, but in this book it involves an association where one organism (the symbiont) lives within the body of another organism (the host), regardless of the actual effect on the host; some symbioses are mutualistic, some parasitic, and some involve commensalism, in which one partner derives some benefit without either harming or benefiting the other organism.

The advent of molecular biology and the polymerase chain reaction (PCR) has made it possible to identify a wealth of hitherto-unknown microorganisms associated with their arthropod hosts that are not culturable by traditional methods. Novel fungi, bacteria, viruses, microsporidia, and protozoa are being discovered by the PCR and the complete genomic analyses of several (including two symbionts from aphids and *Wolbachia*, which are found in many arthropods and Crustacea) indicate that the field is progressing rapidly. Microscopic and molecular techniques allow scientists to resolve where these symbionts reside and how they are transmitted, while biochemical approaches allow a clearer understanding of their relationships.

Some symbionts, such as *Wolbachia*, are nearly ubiquitous in arthropods and have diverse effects on their hosts including male killing, cytoplasmic incompatibility, sterility, lethality, or 'none apparent'. For example, *Wolbachia*, an intracellular bacterium, has been identified in 76% of arbitrarily chosen insect and mite species within 16 orders and some were found to have multiple types of *Wolbachia* within them (Jeyaprakash & Hoy 2000). Chapters that include discussions of *Wolbachia* include Chapter 15 by M. Huigens and R. Stouthamer on Parthenogenesis Associated with *Wolbachia*, chapter 14 by K. Bourtzis, H. Braig and T. Karr on Cytoplasmic Incompatibility, and Chapter 13 by S. Dobson on *Wolbachia pipientis*: Impotent by Association. In addition, chapter 12 by G. Hurst, F. Jiggins and M. Majerus on Inherited Microorganisms that Selectively Kill Male Hosts: The Hidden Players of Insect Evolution?, include *Wolbachia* as well as other microorganisms that kill male embryos. In Chapter 16, F. Dedeine, C. Bandi, M. Bouletreau and L. Kramer provide Insights into *Wolbachia* Obligatory Symbiosis, describing the relationship between *Wolbachia* and filarial nematodes and contrasting it with the relationships found between *Wolbachia* and insects. In Chapter 17, S. Bordenstein describes what is known about Symbiosis and the Origin of Species, with a special emphasis on the roles of *Wolbachia* in speciation. Finally, in Chapter 18, T. Fukatsu, N. Kondo, N. Ijichi and N. Nikoh describe work that indicates that part of the *Wolbachia* chromosome has been transferred into the nuclear genome of its insect host, the Adzuki bean beetle. Integration of part of the genome of *Wolbachia* into the insect host genome is reminiscent of the transfer of genes from the mitochondrion (originally a microbial endosymbiont of the eukaryotic cell) to the nuclear genome of a eukaryotic host. Such a transfer of genes can ultimately lead to the symbiont becoming an organelle.

H. Ishikawa introduces the book (Chapter 1) with an Introduction and provides an over view of roles of gut microbes, endoparasitism, extracellular and intracellular symbiosis, including mutualistic symbionts (such as those found in aphids, cockroaches, termites, beetles and blood-sucking insects), as well as others such as sex-ratio distorters, *Spiroplasma*, microsporidia and *Wolbachia*. Ishikawa noted that "insects may provide the best material for examining the evolutionary significance of interspecific symbioses."

A. Douglas provides an overview of *Buchnera* Bacteria and Other Symbionts of Aphids in Chapter 2. The *Buchnera*-aphid relationship is ancient and intimate, involving a mutual dependence between the *Buchnera* housed in specialized cells called mycetocytes and their aphid hosts that has extended back 200 million years to the origin of

the Aphidoidea. *Buchnera* provide essential amino acids to their hosts and the relationship has affected the genomes of both aphids and microbes. In chapter 3, I. Tamas and S. Andersson discuss the Comparative Genomics of Insect Symbionts, with a focus on *Buchnera*. The genomes of obligate host-associated bacteria tend to be smaller than the genomes of their closest free-living relatives. The small genome size in *Buchnera* is achieved by loss of phage, transposable elements and repeated sequences, as well as the loss of essential genes. Over the years, the *Buchnera* genome deteriorated, and *Buchnera* genomes are the most highly reduced that have been described. It also appears that the process of eliminating genes is still occurring, although *Buchnera* and their hosts are at a late stage in the co-evolutionary process. Tamas and Andersson suggest that, at this point in the aphid-*Buchnera* relationship, "it may no longer be meaningful to speak about an insect host and a bacterial guest; the two have merged to become a new, single organism."

Another fascinating story is provided in Chapter 4 by S. Aksoy about Symbiosis in Tsetse. Tsetse flies are important agricultural and medical pests in Africa that vector protozoan trypanosomes, causing sleeping sickness in humans and various diseases in animals. Tsetse flies provide a home for several bacterial symbionts, and the relationships between the fly and the bacteria range from obligate mutualists to facultative parasites. The importance of the symbionts in human affairs is demonstrated by the fact that the presence of one, *Sodalis*, has been implicated as enhancing the likelihood that tsetse flies will transmit trypanosomes, thus making tsetse a more effective disease vector. Aksoy also discusses the possibility of harnessing symbionts to control of disease transmission. Several different research approaches, including the genetic modification of one or more of the symbionts, are being evaluated with the goal of conquering human sleeping sickness, which claims over 50,000 lives per year in Africa, as well as devastating livestock production, resulting in serious social and economic problems over much of the continent.

A. Heddi reviews what is known about endosymbiosis in *Sitophilus* weevils, pests of stored cereals, in Chapter 5. These weevils contain bacteria that help their hosts to balance the nutritional deficiencies of their diet. In addition, the weevils contain *Wolbachia,* which induces cytoplasmic incompatibility, which may be a component of the reproductive isolation that can lead to speciation.

In Chapter 6, R. Durvasula, R. K. Sundaram, C. Cordon-Rosales, P. Pennington and C. B. Beard describe the relationship between the kissing bug *Rhodnius prolixus* and its symbiont, *Rhodococcus rhodnii*, an actinomycete that aids in processing B-complex vitamins and in the sexual maturation of the insect host. These authors discuss the possibility of using a genetically modified symbiont to control transmission of Chagas' disease, which kills over 50,000 people annually in Central America and northern regions of South America. The genetic modification of insect gut symbionts results in an insect that is "paratransgenic", rather than transgenic, because it is the genome of the microorganism that is modified rather than the genome of the host insect. The use of a paratransgenic approach to control Chagas' disease would be a novel pest management tactic, but the authors acknowledge that the environmental effects of releasing genetically modified gut symbionts must be understood and issues of regulation and policy pertaining to the release of genetically modified organisms must be resolved before this can be achieved.

Some symbioses involve fungi and in Chapter 7, D. Six discusses bark beetle-fungus symbioses found in the family Scolytidae. Bark beetles are associated with filamentous fungi (Ascomycotina and Basidiomycotina). In addition, bark beetles contain ascomycete yeasts, although the yeasts have been less well studied. Many bark beetles possess specialized structures called "mycangia", and these may involve an invagination of the integument that is lined with glands or secretory cells that are specialized for the acquisition and transport of fungi. Other, less elaborate, mycangia include "any structure that consistently transports fungi". The fungi are considered mutualists, although other relationships may exist, because bark beetle larvae and teneral adults feed on the mycelia and also probably feed on yeasts during development. The fungi are transported to new trees by the beetles, providing the fungi with dispersal mechanisms and protection from the environment in the beetle feeding galleries and during dispersal within the mycangia.

Not all symbioses are obligatory. In Chapter 8, C. Lauzon discusses the Symbiotic Relationships of Tephritids, which appear to be less than obligate, but still interesting for several reasons, including the possible improvement of genetic control programs. The Mediterranean fruit fly and other major agricultural pests such as apple maggot fly contain *Enterobacter agglomerans* and *Klebsiella pneumoniae*, which are present in biofilms within their guts. Lauzon speculates that artificial diets containing antibiotics, which are used in mass rearing programs for sterile insect genetic control programs, affect the health of the fruit flies. An applied aspect of this work suggests that these gut symbionts, which can be cultured, could be fed to sterilized fruit fly adults as probiotics to aid in their nutrition and fitness. Improving the fitness of sterilized males could result in huge cost savings in genetic control programs.

Digestion of cellulose is not easy for most animals but, with the aid of gut symbionts, termites

have been able to exploit an abundant food resource. The relationship between termites and their symbionts is incredibly complex and diverse. The hindgut of a single termite, for example, may harbor hundreds of different types of microorganisms including bacteria, fungi, and protozoa. Although there is a clear relationship between hind gut symbionts and termite nutrition, the relationships are not limited to nutrition. K. Matsuura, in Chapter 9, describes Symbionts Affecting Termite Behavior, including novel termite-bacteria and termite-fungus interactions. Matsuura focuses on the role of symbionts in nestmate recognition, nest odor, and the evolutionary process of egg mimicry by sclerotia of a fungus. Matsuura concludes by noting that ". . . our knowledge of termite-microorganism relationships remain limited." The habits of termites are enmeshed with various microorganisms and, no doubt, additional discoveries remain to elucidate these fascinating interactions.

Symbiosis may involve a parasitic relationship and The Symbiosis of Microsporidia and Insects is described by P. Agnew, J. Becnel, D. Ebert and Y. Michalakis in Chapter 10. Microsporidia are unicellular eukaryotes that are obligate intracellular parasites that cannot live independently outside their host. Recent molecular analyses indicate that microsporidia are related to fungi. During evolution, there was a loss of cytological complexity and reduction in the size of the genomes of microsporidia compared to their free living fungal relatives. Agnew et al. indicate that microsporidia have been highly successful and are among the most common parasites of arthropods (as well as of humans and other animals).

Although *Wolbachia* have been the dominant microorganism recognized to affect sex ratios in arthropods, A. Weeks and J. Breeuwer describe A New Bacterium from the Cytophaga-Flavobacterium-Bacteroides Phylum that Causes Sex-Ratio Distortion in Chapter 11. A new undescribed bacterium from this phylum appears to cause feminization and parthenogenesis in its hosts. In the privet mite (*Brevipalpus phoenicis*), it appears to cause females to be haploid (the first apparent case of haploidy in females in the animal kingdom) and no males are normally found.

Another aspect of sex ratio distortion is described by G. Hurst, F. Jiggins and M. E. Majerus in chapter 12, Inherited Microorganisms That Selectively Kill Male Hosts: The Hidden Players of Insect Evolution?". Scientists have known for a long time that individuals from some insect populations collected from the field would yield progenies with a strongly biased sex ratio in favor of females. The diversity of organisms involved in male killing is reviewed and discussed with regard to fitness costs and effects on population size or extinction. The authors conclude that it is "premature to say we understand the population biology of these elements". However, the authors suspect that the high prevalence achieved by male killers in certain insect species may make them important in evolutionary processes, especially those involving changes in sex determination systems.

This excellent overview of some of the relationships between insects and the various microorganisms that live within them should trigger additional research on the "bugs" of interest to you. The chapters each contain numerous references and excellent illustrations, including color figures in the center of the volume. Run, don't walk, to get a copy of this book; it just may change how you think about your "bugs".

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HARPOOTLIAN, P. J. 2001. Scarab beetles (Coleoptera: Scarabaeidae) of South Carolina. Biota of South Carolina 2L 1-157. ISBN 0-9712527-0-X. Available from Public Service Room, 96 Poole Agricultural Center, Clemson University, Clemson, SC 29634-0129, or see <http://cufan.clemson.edu/> Soft cover. $37.50.

It is a general trend that as scientific studies become more advanced they involve smaller and smaller details of the subjects. Even in some branches of taxonomy, we forget it is whole organisms we are studying and lose track of basic details like how to tell them apart, where are they found, and what do they do; minor details the public wants to know. With a growing interest in biodiversity, we are again realizing how important faunal studies are to our total knowledge of the environment and how little we actually know. *Scarab beetles of South Carolina* is an important step toward correcting that deficiency.

In the introduction, Harpootlian states, "This manual is intended primarily as a guide for identifying adult scarabs of South Carolina." *Scarab beetles of South Carolina* covers what North Americans traditionally call "scarabs," omitting the scarabaeoid families Lucanidae and Passalidae. The book begins with a brief introduction to scarabs, the ecological regions of South Carolina, a scarab illustration with body parts labeled, and a checklist of species. Then it presents keys allowing the user to identify any species known to occur in South Carolina. The entire book is packed with varied illustrations of taxa and distinguishing characteristics which help make the keys user friendly. There is even a glossary of terms to aid the user.

Most of the text is devoted to individual species accounts. These accounts are brief and include a synonymy, diagnosis, biology or comments, and distributions. Considering the fascinating biologies of some scarabs, the species accounts appear too brief. For many, however, that is the state of our knowledge.

Harpootlian expended a great deal of effort to confirm the nomenclature presented. This led to a few new synonymies and changes in authorship of some taxa. These changes are discussed as necessary to present the case. The general public may find that these discussions distract from the book's purpose as an identification guide. Specialists should be grateful he included them.

South Carolina has a tremendous diversity of habitats, from the Appalachians to the coastal dunes, and as such, has a tremendous diversity of scarabs. Most of the species covered have widespread distributions. Although it is not intended to do so, Harpootlian's work can actually be used to identify the vast majority of the scarabs occurring throughout the southeastern coastal plain states (except peninsular Florida). There is no single reference available which can boast such a coverage for any of the other southeastern states.

This book's most obvious shortcomings are in the brevity of coverage and in the varied styles and quality of illustrations. The book contains many wonderful habitus illustrations originally used in the *Scarab beetles of Florida* (Woodruff 1973), plus a myriad of original photographs and line drawings. Line drawings of body parts like legs and genitalia appear to be professionally produced. On the other hand, many of the dorsal habitus drawings appear distorted and only give a diagrammatic representation of the beetle.

This reference work is not up to the quality and coverage of *Scarabs beetles of Florida* (Woodruff 1973; Woodruff & Beck 1989), nor *Scarab beetles of Nebraska* (Ratcliffe 1991). This is because of the brevity with which all of the subjects are covered. For example, larvae are entirely omitted. However, keep in mind that the reference was intended to be an identification guide for adult beetles, to aid others who wish to study scarabs. The *Scarab beetles of South Carolina* more than meets that goal.

Insect identification is difficult only because there are so few references like *Scarab beetles of South Carolina*. This book is a must for anyone interested in scarabs from the southeastern United States. My congratulation to Phil Harpootlian for having produced a much needed resource I will use for years to come!

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HELYER, Y., K. BROWN, AND N. D. CATTLIN. 2003. A color handbook of biological control in plant protection. Timber Press; Portland, Oregon, USA.126 p. ISBN 0-88192-599-3. Hardback, $39.95.

This slim book is the latest entry for a nontechnical handbook on biological control of pests of plants. It has four sections. The first, of 18 pages, is called crop environments. It deals with the environments created by cultivation of arable crops, orchards, and greenhouse-grown crops. It discusses naturally-occurring beneficial organisms and their conservation, the use of chemical pesticides, the use of augmentative biological control, and integrated pest management.

The second section, of 21 pages, is called pest profiles. It first provides small color photographs with accompanying brief text, to allow the novice reader to recognize some of the major groups of arthropod and mollusc pests. But, juxtaposed with the photographs of pests are photographs of beneficial organisms that may be found in the same habitat. Then follow the profiles of "common pest species", order by order, from Coleoptera through Diptera, Homoptera, Lepidoptera, Thysanoptera, Acari, Gastropoda, and Isopoda. These written profiles are in some instances to the level of family, in others to the level of order, depending upon appropriateness. All are accompanied by color photographs of adults, larvae, eggs, or damage caused by the group of pests in question. The pests illustrated are identified to the species level.

The third section, of 63 pages, is called beneficial arthropod profiles. This is where the book exceeds any that I have seen by writing about and illustrating with color photographs, so many beneficial organisms—not just the usual pictures of adults and larvae of *Chrysoperla carnea* and a coccinellid species or two, but scores of predatory insects, mites, and spiders. There are 235 photographs. As in the previous section, almost all the organisms depicted are identified down to the species level.

The fourth section, of 10 pages, is called entomopathogens. It deals with nematodes, bacteria, fungi, and baculoviruses and how these affect their arthropod and mollusc hosts. It, too, is illustrated by color photographs. The book is complete by a page of references, a page of a further reading list, (including websites), a glossary, a taxonomic index, and a subject index.

After lauding the book for its virtues—I think it is very well thought out—I have to admit there is a little snag for readers who live outside western Europe. The fauna illustrated is British. Quite a few of the species photographed and identified down to the species level do not occur in the USA. This is truer of the beneficial organisms than of the pests. How much that matters depends upon whether the reader expects to make a species-level identification simply by using the book.

I think that it would be unrealistic to expect try to identify beneficial organisms down to the species level from this book or any like it. If there are about 25,000 species of terrestrial arthropods in the British fauna, how many can be identified from the 340 photographs in this book? If there are about 100,000 species in the fauna of the USA, how many could be identified to the species level with a book four times this size representing USA species? The book does not pretend to be an identification manual. In that sense, it does not matter that it is British species that are illustrated—they are just representative examples. If the reader wants an identification manual for the terrestrial arthropod fauna of the USA, that will be a whole long shelf of books that have not yet been written.

What we might hope is that authors will use this model as a guide to how to prepare similar volumes for the USA. To keep the bulk and price down, such USA volumes might best be written for regions of the USA instead of trying to cover the entire country with its diverse climates, fauna, and crops, in just one volume. This book's sale price of $39.95 is modest for such a well-illustrated hardbound work with such a mine of useful information. It should be useful to farmers, growers, horticulturists, gardeners, extension agents, and students.

As to errors, I found very few. There is a question of the identity of the beetle larva illustrated in photograph 118 and attributed to Carabidae. It looks remarkably like a staphylinid larva to me, but I could not see its structures in fine enough detail to be sure, because of the screening process used in printing the photographs.

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TONNANCOUR, J. DE 2002. Insects revealed. Monsters or marvels? Comstock Associates (of Cornell University Press), Ithaca, NY, 160 pp. ISBN 0-8014-4023-8. Hardback. $35.00

The text of this book was written in French and was translated to English. This English edition published by Cornell University Press is mirrored by a version published simultaneously in French by Editions Hurtubise of Montreal (not seen by this reviewer). Its large size (9 × 12 inches) and glossy paper display its superb color photographs to great advantage.

Of course the photographs are the pride of the book. Their rendition is clear and the colors are true. The insects photographed are from most continents, and many of them appear to have been photographed in the field. If they were taken in staged settings, then the stage arrangement was done skillfully. As the text says (p. 13) "the result is an anthology of some of the world's most beautiful, most peculiar, and most fascinating insects." With a subject as diverse as insects, very many others (if as well photographed) could have served as examples.

Now you want to hear about the contents of the text? You expect to hear that it is a mishmash of disconnected facts, some of them perhaps erroneous, perhaps poorly translated from French, without any theme that might appeal to you? What I found is that the text is well thought out and is well translated. There are very few spelling errors (those I noticed were just in names of three insect families), but there is an unfortunate tendency to write about insect "varieties" when meaning species, and *Nepenthes* pitcher plants are wrong attributed to the Sarraceniaceae. The chapter titles are: 1, Insects and the human imagination; 2, Fascination: an expanded concept of beauty; 3, The classification of living things: an intellectual need; 4, The origins of insects; 5, Up close: insect morphology; 6. Insects and temperature; 7, Continental drift and the spread of insects throughout the world; 8, The evolution of insects in tropical regions; 9, Ecological niches; 10, Insects and plants; 11, Insects and their coloration; 12, Insects' defense strategies; and 13, The strange need to collect. This is not the arrangement of themes that you would find in a standard entomology textbook, but they make interesting introductions to those subjects. The ultimate challenge to a photographer is to illustrate a textbook of entomology or at least a general guide. Two authors, J. L. Castner (reviewed in Florida Entomologist 85: 298-299) and G. McGavin (reviewed in Florida Entomologist 83: 386-387) have done this, and their reason for a less spectacular presentation almost certainly is their perceived need to keep the sale price of their books low, which they accomplished; is there a need for upscale editions of works such as theirs to try to match the book reviewed?

Of course it is likely that the author/photographer wrote the text to accompany the photographs that he had already taken (rather than setting out to search for and photograph insects that would illustrate themes he had already thought out—a harder task). He has written in a way that shows he understands what he photographed, and which may very well appeal to a general audience: forget the obtuse bureaucratese writing of too many entomologists; forget the fascination of entomologists with the insect digestive system or insect toxicology or insect chromosomes. I define a general audience as one that does not much care about insect classification, but which is willing to look at a beautifully illustrated book and absorb the accounts that accompany it. Such an approach may help to convert the general public from an apprehension of insects to the glimmerings of an understanding, and maybe even enthusiasm. The author, who in earlier years was an artist, has studied those parts of entomology that appeal to him.

Where do we go from here? It is hard to see how a good photographer could surpass this book as an introduction to insects for a general audience at a reasonable price, so it sets a benchmark. I suppose that if the general public has become enthused by this book, there may be a need for books (equally well photographed, please!) to focus on aspects of the life of insects that might appeal to the same audience. But where is the inspiration for such books? Some of the author's ideas on that subject may be the single page of references (p. 160) that he gives. Among them, note *Amazon Insects* (by J. L. Castner) and *Tiger Beetles* (by D. L. Pearson and A. P. Vogler), reviews of which have appeared in the pages of Florida Entomologist and whose authors have already tried the next step.

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NAVARRETE-HEREDIA, J. L., A. F. NEWTON, M. K. THAYER, J. S. ASHE, AND D. S. CHANDLER. 2002. Guía ilustrada para los géneros de Staphylinidae (Coleoptera) de México. Illustrated guide to the genera of Staphylinidae (Coleoptera) of Mexico. Universidad de Guadalajara and CONABIO; Mexico. xii + 401 pp. ISBN 970-27-0180-5. Hardback. $40 (in the USA). Order from José Luis Navarrete (snavarre@maiz.cucba.udg.mx).

This book is a remarkable accomplishment. Its only antecedent in the history of Mexico and Central America was the volume on Staphylinidae (volume 1 part 2, pages 145-824 and plates 5-19) published by David Sharp in 1883-1887 in the series Biologia Centrali-Americana, and also volume 2 part 1, pages 1-46 and one plate by the same author in 1887 in the same series. Sharp's work, based on specimens in the museum now known as The Natural History Museum (London) and on earlier literature persisted as the only synthesis of the Mexican staphylinid fauna for 115 years!

Sharp's work itself was groundbreaking. But it was written with Latin species descriptions and the rest of the text in English. Although it had 14 plates of habitus drawings in color, it had no illustrations of diagnostic characters and no keys. And, of course, it was highly incomplete. Its inadequacies must have been especially evident to Mexican entomologists trying to use it to identify specimens.

The four-person American team (Newton, Thayer, Ashe & Chandler) that produced the 146-page chapter on Staphylinidae in American Beetles (Arnett and Thomas, editors, 2001) joined forces with José Luis Navarrete in tackling the Mexican staphylinid fauna. Now, at last, there is a book on Mexican staphylinids in Spanish and geared to Mexican entomologists.

The new book is much larger (401 pages) than the chapter in American beetles (146 pages). This is not because it deals with more genera: it deals with 384 in contrast with 523 known from America north of Mexico. It is much larger because it had fewer constraints on size and was therefore able to include more information. For example, it includes a 25-page introduction, which deals with the history of studies of staphylinids in Mexico, life cycles and behavior of staphylinids, and collection and preservation methods for them. In short, it tries to provide all the available information that a budding enthusiast needs to know. It provides 16 plates of excellent color photographs of adult specimens of that most difficult subfamily Aleocharinae. A complaint I have heard is that Aleocharinae "all look to be the same", but these plates show that there are huge differences: you just have to use a microscope to see them well. And, for each genus, it provides a habitus drawing of an adult along with a list of the known species and their distribution by (Mexican) state and other countries. It also has an index.

In short, this new book is far more complete (in terms of available information included) than is the chapter on staphylinids in American Beetles. Perhaps one day we may look forward to a book on the staphylinids of America north of Mexico with illustrated keys down to the level of species, and with information of habitats and distribution and behavior. It will have to be a large book, because the fauna as of 2001 was known to have 4100 named species in 523 genera, and the task of species description and generic and tribal revision is not complete.

As to the Mexican fauna, it was known at the time of writing of this book to have only 384 genera with fewer than 2,000 species (with about 500 of these recognized but not yet described). So, how large is the Mexican fauna? I suspect that it is not smaller than that of America north of Mexico. Mexican entomologists can look forward to many decades of taxonomic work, and far more on behavior and ecology of their fascinating staphylinid fauna. Now, at least, thanks to José Luis Navarrete and his talented American collaborators, they have a solid foundation for future studies and the best book on that subject in the New World.

The scenario for studies in South America is much bleaker. There are 397 pages, in a journal issue, about staphylinids of the Amazon Valley, but the journal was published in 1876, and the article has no illustrations, and is in English with descriptions in Latin. The length of specimens is given in "lines" which, in the British system of the time, were each one-twelfth of an inch. Those things hardly encouraged Brazilian entomologists. There is a 392-page book about staphylinids of the province of Buenos Aires, with text in Spanish and descriptions in Latin, with no illustrations, published in 1886. That hardly seems to have promoted further studies by Argentine entomologists. There is a 658-page work, incomplete for the large subfamily Aleocharinae, on staphylinids of the West Indies. It has keys, but it has sketches of some structural characters of adults belonging to just four species in one genus, was published in 1943, and now is sadly out-of-date. The number of species I have seen from Jamaica and Haiti is at least 50% greater than reported in the book, and from those islands I have seen specimens even of genera that are unreported anywhere in the West Indies. I expect much the same will be true for other islands/countries. There are hundreds of scattered, shorter publications, most unillustrated, most without keys, in Latin, German, French, and English (few in Spanish or Portuguese). Against this background, I have received requests from entomologists in Argentina, Brazil, and Colombia, to

identify hundreds or thousands of specimens in some current project, such as an ecological survey of the fauna of a park, or suspected predators in a study in applied entomology. Perhaps they expected I could miraculously identify their specimens in a weekend or two, but the problems caused by lack of adequate taxonomic studies in their countries would take years to resolve. So, now we need an up-to-date book on staphylinids for each major country in South America, one for the Central American countries, and one for the West Indies, like this one from Mexico—and then more, so that identifications can be made not just to genus but to species. We can always hope.

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