

## OLFACTORY BEHAVIOR AND ELECTROANTENNOGRAPHIC RESPONSES OF THE COCOA BEETLE, *STEIRASTOMA BREVE* (COLEOPTERA: CERAMBYCIDAE)

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### ABSTRACT

With the aim of studying the olfactory behavior of one of the main pests in neotropical cocoa plantations, the cocoa beetle *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae), we studied behavioral and antennal responses towards different odor sources in a two-choice olfactometer and an electroantennographic system, respectively. Odor sources tested as stimuli in olfactometric experiments were chopped pieces of cocoa branches, adult males, adult females of *S. breve*, and combinations of these. Extracts of female and male body parts in *n*-hexane were tested in electroantennographic experiments. Statistically significant attraction responses in the olfactometer were observed only when *S. breve* individuals were stimulated with odors from pieces of cocoa branches. Both sexes showed active EAG responses to odors of cocoa branches, and females showed active EAG responses to adult male odors. These results suggest that olfactory behavior of *S. breve* is mediated by volatiles derived from cocoa trees and from adult male insects.

**Key Words:** behavior, cocoa, *Theobroma cacao*, pheromone, kairomone, ethological control, electroantennography

### RESUMEN

Una de las principales plagas del cultivo de cacao en el neotrópico, es la comúnmente conocida “Gota del Cacao”, *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae). A fin de conocer el comportamiento olfativo de *S. breve*, se evaluó la respuesta del insecto hacia diferentes fuentes de aroma. Para ello se usó un dispositivo olfatométrico de dos vías de selección y se evaluaron las respuestas de antenas de machos y hembras en un electroantenógrafo. En el olfatómetro se evaluaron tres fuentes de aroma: trozos de ramas cacao, machos adultos, hembras adultas y sus combinaciones; y en el electroantenógrafo adicionalmente se midieron las respuestas de las antenas hacia extractos de partes del cuerpo de machos y hembras. Se observó que las únicas fuentes de aroma que demostraron un efecto significativo de atracción en el comportamiento olfativo y respuestas electroantenográficas de *S. breve* fueron: los trozos de ramas de cacao que resultaron atractivos para hembras y machos en el olfatómetro, y que además estimularon la antena de individuos de ambos sexos; y los volátiles producidos por el macho resultaron ser atractivos para las hembras y produjeron una fuerte respuesta electroantenográfica en la antena de la hembra. Los resultados sugieren que el comportamiento olfativo de *S. breve* se encuentra modulado por cairomonas provenientes de plantas de cacao y por una feromonas secretada por el macho de este insecto en su estado adulto.

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Various species of Coleoptera such as Scolytidae, Curculionidae, Dermestidae, Nitidulidae, Scarabeidae, Dynastidae, and Cerambycidae have been reported as pests (Rochat et al. 1991, 2000a,b; Finnegan & Chambers 1993; Jaffe et al. 1993; Fettöther et al. 1995; Malosse et al. 1995;

Cosse & Bartelt 2000). *Steirastoma breve* (Sulzer) (Coleoptera: Cerambycidae), known as the “cocoa beetle”, is one of the main pests in cocoa (*Theobroma cacao*, Sterculiaceae) plantations in many countries, and its presence has been reported in South America from Argentina to Venezuela, in

addition to some Caribbean islands such as Trinidad, Grenada, Martinique, Puerto Rico, and Jamaica among others (Entwistle 1972; Sánchez & Capriles 1979). According to the literature on *S. breve*, the egg phase lasts an average of 4.2 days, the larval phase 54.9 days, the pupal phase 10.9 days, and the adult phase 35 days for males and 69 days for females (Mendes & García 1984).

Adults feed on epidermal tissues of the main stem and branches of young plants, producing a characteristic gnawed area, which produces damage to the floral clusters due to the fact that flowers grow in tight clusters on the stem and branches. Moreover, female adults lay eggs inside slits cut into the bark with their mandibles. Usually, both sexes attack young trees aged from six months to four years (Entwistle 1972; Sánchez & Capriles 1979).

Larvae cause severe damage in stem and branches. After eclosion, larvae bore into the bark where they feed on the cambial tissues and the bark itself. First, larvae make a round chamber, progressively enlarging and elongating it until it forms a tunnel or irregular spiral-like galleries. This results in a ringed stem or ringed branch. Depending on the age and location of the damage, these events can kill the apical area. If the main stem is attacked, they can quickly kill the entire plant (Entwistle 1972; Sánchez & Capriles 1979).

Because the use of insecticides has progressively decreased, it is very important to search for alternative pest control methods that are safe for the environment and highly efficient in the management of *S. breve* populations. In most cases, ethological control has been applied successfully (Nakamuta et al. 1997; Howse et al. 1998; Seybold et al. 2000). This entails previous study of olfactory behavior, as well as the identification and evaluation of the chemical compounds involved in insect communication (Hernández et al. 1992; Howse et al. 1998). An olfactometer has been used to study the olfactory behavior of many coleopteran pests (Rochat et al. 1991; Jaffe et al. 1993; Cerdá et al. 1996, 1999). Electroantennography (EAG) is a technique that has been used for the analysis of biologically active compounds (Marion-Poll & Thiéry 1996). In Coleoptera, EAG and gas chromatography coupled with electroantennographic detection (GC-EAD) have been used to identify the chemical structure and stereochemistry of the sex pheromone of another cerambicid *Anaglyptus subsulcatus* Nakamuta et al. (1994). Since nothing is known about the olfactory behavior of *S. breve* towards odors derived from its host plant or from odors emitted by adult insects, the objectives of this study are evaluation of olfactory behavior and electroantennographic responses of *S. breve* when stimulated by odors derived from cocoa plant tissues and by volatiles produced by males, females, and *n*-hexane extracts of insect body parts.

## MATERIALS AND METHODS

### Collection of Insects and Cocoa Plant Branches

Insects and cocoa plant branches (CPB) were collected at experimental cocoa plantations of the Instituto Nacional de Investigaciones Agrícolas (INIA), located in Campo Central and Padrón, in Municipio Acevedo, Miranda State, Venezuela. Adult insects used in all experiments were collected directly from the field and then placed individually in plastic containers (7 cm high × 11 cm Ø), transported to the laboratory in a thermal container, and kept under controlled laboratory conditions. Insects were fed with pieces of CPB for 12 to 16 h before olfactometric and electroantennographic experiments were performed. The pieces of CPB were collected from 2- to 4-month old EEM-003 and Ocumare 61 cocoa plant cultivars.

### Olfactometric Experiments

Olfactometric bioassays were carried out at Laboratory of Entomology INIA-Miranda, Caucagua, in a two-choice olfactometer (Cerdá et al. 1996) with some modifications. Males and females were evaluated alternately with a minimum of 25 individuals of each sex. Odor sources used to stimulate the insects were adult females, adult males, apical chopped pieces of cocoa branches (2 to 4 months old) from EEM-003 selection, and combinations of the above totaling seven different odor sources. These were: (1) males, (2) females, (3) cocoa plant branches (CPB), (4) females + males, (5) females + CPB, (6) males + CPB, and (7) air. Each individual was placed in a chamber located in the olfactometer for 3 min before the evaluation of insect responses, in order to get them used to the system. After that period, the chamber door was opened and a small fan with a 200-ml/min flow located at the back of the olfactometer chamber was immediately switched on in order to disperse odors inside the system. Insect behavior was observed for 15 min with (1) quantification of individuals reaching either odor source, and (2) quantification of inactive or undecided individuals recorded. Each tested individual was considered as a replicate. At the end of each experiment, all remaining cue odors were removed by applying 70% v/v ethanol, and then circulating a hot air stream with a hair dryer through the device for approximately 10 min. All bioassays were performed from 8:00 to 12:00 and 13:00 to 15:00, and mean values of temperature and relative humidity were recorded during each experiment. Experimental data were analyzed by the binomial test (Wiedenhofer 1993) after comparison of the frequencies of individuals selecting one odor source vs. the other.

### Insect Extracts

Extracts of body parts from 25 males and 25 females of *S. breve* were prepared by placing each individual for 5 min in a refrigerator at 5°C in order to diminish insect activity. Then, each was dissected in a Petri dish without any fluid into three parts: head, prothorax, and pterothorax + abdomen. Each group of body parts was immediately placed in a 5-ml clean glass vial, containing 3 ml of *n*-hexane (HPLC grade), and extracted during a 48-h period. Then, the supernatant was removed with a Pasteur pipette and placed in a 4-ml clean glass vial. The extracts were concentrated to approximately 50 µl by a gentle nitrogen stream, and kept at -5°C until electroantennographic experiments were conducted.

### Electroantennographic Experiments

EAG experiments were performed in the Laboratorio de Comportamiento, Universidad Simón Bolívar. EAG responses of *S. breve* males and females were evaluated while being stimulated by (1) pieces of CPB of Ocumare 61 clone (four pieces 5 cm long × 1 cm Ø), (2) males (4 individuals), (3) females (4 individuals), (4) 1 µl of *n*-hexane extract of body parts (head, prothorax, pterothorax + abdomen), or (5) *n*-hexane. In experiments 1-3, the odor source was introduced into a glass chamber where the stimulus source was placed; a system to produce a wet air current, calibrated to 300 ml/min flow, and a stimulus controller (Syntech® model CS-05, Hilversum, The Netherlands), was adjusted to a time pulse of 0.5 s and 500 ml/min flow. In experiments 4 and 5 (*n*-hexane extracts and control), stimuli were released from Pasteur pipettes containing a piece of filter paper previously impregnated with 1 µl of each extract or solvent after the solvent had been allowed to evaporate. The puff was delivered into the continuous air stream, after placing the pipette tip into the hole of the tube carrying the air stream. The antennal responses were amplified and recorded with a Syntech data acquisition controller and software.

Male or female antennae were excised and fixed between silver-gold electrodes with two droplets of an electrically conductive gel (Spectra 360® electrode gel, Parker, Orange, NJ) applied to the electrodes. Each stimulus was tested in 10 replicate experiments in which the antenna received three stimulus pulses at 3-min intervals. For quantification of EAG amplitudes (mV) we only considered the first pulse applied in each replicate. Antennal responses (mV) from males and females were compared by means of the Mann-Whitney *U*-Test.

### RESULTS

The responses of *S. breve* in olfactometric bioassays when stimulated with various odor

sources are shown in Table 1. Only three sets of experiments showed statistically significant differences. For example, when CPB vs. male volatiles were tested as odor sources, 55% of the insects showed a statistically significant orientation towards CPB while 29% responded to male odors, with 16% remaining undecided or inactive. There was a clear preference of *S. breve* towards CPB (67%,  $P < 0.05$  Binomial test) versus a clean air stimulus.

When results from orientation of both sexes towards female vs. male odors are compared, we observed that 67% of the evaluated females oriented toward male odors (Binomial test,  $P < 0.05$ ). Male to male, male to female, and female to female orientations were not statistically different. The treatments with other odor source combinations ( $\text{♀} + \text{CPB}$ ,  $\text{♂} + \text{CPB}$ ,  $\text{♂} + \text{♀}$ ) did not show significant differences (Binomial test,  $P > 0.05$ ) (Table 1).

Table 2 shows the results of EAG experiments when male and female antennae were stimulated with female odors, male odors, CPB, and clean wet air. Female antennae produced significantly higher amplitudes (Mann-Whitney *U*-test,  $P < 0.05$ ) than males, and gave stronger responses when stimulated with male odors. Male and female electric potential average responses were similar when exposed to CPB odors. Table 2 shows that male and female antennae stimulated with clean wet air showed slight variations in electrical potentials (mV). EAG responses of female antennae towards male + CPB odor produced a larger antennal depolarization that was significant compared to male antennae (Mann-Whitney *U*-test,  $P < 0.05$ ) (Table 2).

Additional EAG results obtained from stimulating male and female antennae with *n*-hexane extracts of body parts are shown in Table 3. Female antennal responses were stronger than males, especially when male prothorax extract was applied as stimulus. Female antennal responses were higher (Mann-Whitney *U*-test,  $P < 0.05$ ) when they were exposed to male extracts. On the other hand, these results also show that female head extract produced a stronger signal from male antennae, even though they are both of low intensity, and that prothorax extract generated a significant, slightly higher electroantennographic response from female antennae.

### DISCUSSION

The results from olfactometric and EAG experiments suggest that *S. breve* olfactory behavior is highly influenced by odors emitted by cocoa plants (kairomones) and also quite possibly by a sex pheromone. The fact that during olfactometric tests *S. breve* female and male individuals were attracted by CPB volatiles, and that additionally these plant odors produced a significant electrical potential deflection (mV) in antennae of

TABLE 1. RESPONSES OF *S. BREVE* ADULTS IN OLFACTOMETRIC BIOASSAYS STIMULATED BY VARIOUS ODOR SIGNALS.

Odor sources	Frequencies		
	♂	♀	Total
Air vs. Air	Air	9	6
	Air	8	5
	Not decided	5	4
	Total	22	15
CPB vs. Air	CPB	17	13
	Air	6	5
	Not decided	2	1
	Total	25	20
CPB vs. ♂	CPB	17	15
	♂	10	7
	Not decided	3	6
	Total	30	28
CPB vs. ♀	CPB	10	13
	♀	16	4
	Not decided	6	11
	Total	32	28
♂ vs. ♀	♂	20	20*
	♀	15	6
	Not decided	6	4
	Total	41	30
♂ vs. ♂ + ♀	♂	12	9
	♂ + ♀	14	9
	Not decided	6	10
	Total	32	28
♂ + ♀ vs. ♀	♂ + ♀	7	12
	♀	9	12
	Not decided	9	1
	Total	25	25

\*Statistically significant differences, Binomial test,  $P < 0.05$ .

both sexes of *S. breve*, suggests that volatile compounds emitted by cocoa plant tissues are used by the insects as cues to locate cocoa plants as their

hosts. After the insect arrives, the host could be a site for feeding, mating, and oviposition, as has been reported for other coleopteran pests of the

TABLE 2. ELECTROANTENNOGRAPHIC RESPONSES OF MALES AND FEMALES OF *S. BREVE*, WHEN STIMULATED WITH AIR, VOLATILE COMPOUNDS EMITTED BY INSECTS, AND BY COCOA PLANT BRANCHES ( $N = 10$  REPLICATES).

Treatments	EAG responses from males (mV)		EAG responses from females (mV)
	Average $\pm$ SD	Average $\pm$ SD	Average $\pm$ SD
Volatile compounds emitted by:			
♀	0.7 $\pm$ 0.1		1.2 $\pm$ 0.3*
♂	0.2 $\pm$ 0.1		7.5 $\pm$ 0.4*
CPB	1.4 $\pm$ 0.1		1.6 $\pm$ 0.3
♀ + CPB	0.6 $\pm$ 0.1		0.5 $\pm$ 0.1
♂ + CPB	1.5 $\pm$ 0.2		3.4 $\pm$ 0.3
♂ + ♀	1.3 $\pm$ 0.2		1.3 $\pm$ 0.2
Air	0.1 $\pm$ 0.0		0.1 $\pm$ 0.0

\*Statistically significant differences (Mann-Whitney  $U$ -Test,  $P < 0.05$ ) when column values are compared.

TABLE 3. ELECTROANTENNOGRAPHIC RESPONSES FROM MALE AND FEMALE ANTENNAE OF *S. breve* WHEN STIMULATED WITH *n*-HEXANE EXTRACTS OF BODY PARTS FROM *S. breve* (*N* = 10 REPLICATES).

Treatments <i>n</i> -hexane extracts of:	EAG response from males (mV)		EAG response from females (mV)
	Average ± SD	Average ± SD	Average ± SD
Head ♂	0.4 ± 0.1		6.0 ± 0.8*
Prothorax ♂	0.5 ± 0.2		15.5 ± 3.0*
Pterothorax + abdomen of ♂	1.3 ± 0.3		6.5 ± 1.0*
Head ♀	1.0 ± 0.2		0.5 ± 0.1*
Prothorax ♀	1.2 ± 0.3		2.1 ± 0.3*
Pterothorax + abdomen of ♀	0.6 ± 0.1		0.8 ± 0.5
<i>n</i> -hexane	0.2 ± 0.3		0.1 ± 0.0

\*Statistically significant differences (Mann-Whitney *U*-test, *P* < 0.05) when the columns are compared.

Curculionidae and Cerambycidae families (Jaffe et al. 1993; Hanks 1999). The presence of a characteristic superficially gnawed area over the attacked cocoa plant cortex is evidence that *S. breve* use plants as a substrate for feeding.

In another Cerambycidae, *Anaglyptus subfasciatus*, the existence of a male-produced sex pheromone was first suspected by the fact that females were attracted to males in wind tunnel bioassays (Nakamuta et al. 1994). This was later confirmed by Leal et al. (1995), who identified the pheromone components, but Nakamuta et al. (1997) showed that a blend of host plant volatiles and the male sex pheromone used as baits in yellow water traps were more attractive than sex pheromone or host attractant alone. In some other Cerambycidae species, the existence of sex pheromones in males and also in females is confirmed, along with their host plant relationships (Schröder et al. 1994; Fettköther et al. 1995; Bento et al. 1993; Fukaya et al. 1996).

The presence of a male sex pheromone in *S. breve* is suggested by olfactometric test results which indicate that females are significantly more attracted to males. In addition, female antennae showed a very strong deflection in EAG experiments only when stimulated by male odors. Female antennae generated strong amplitude signals with all *n*-hexane male extracts. Male prothorax extract was the source that caused the strongest response.

Sources for pheromone production in Coleoptera vary according to the kind of tissues involved. In *Carpophilus freemani* (Nitidulidae) the pheromone gland has been located in the abdomen (Dowd & Bartelt 1993). However, in other coleopterans the gland is located in the prothorax. In the case of *Anthonomus grandis* Boheman (Curculionidae), the aggregation pheromone is produced in the fat bodies associated with the digestive tract (Wiygul et al. 1982), and in *Rhynchophorus palmarum* L. (Curculionidae), the pheromone glands are located in the male prothorax (Sánchez et al. 1996). In the cerambycid

*Hylotrupes bajulus* the pheromone is produced in a gland situated in the male prothorax (Fettköther et al. 1995). According to the strong evidence provided by the olfactometric and electroantennographic experiments of the present study, it is highly probable that the *S. breve* pheromone production system is also located in the prothorax. That each of the *n*-hexane body extracts elicited EAG responses implies that the *S. breve* digestive system could be associated with pheromone dispersion, as has been reported for other coleopteran insects. Therefore, once male insects arrive at a plant having been attracted by volatile host compounds, it is very probable that males start releasing a pheromone in order to enhance female searching behavior for mating.

In conclusion, the results of the present study when combined with the findings reported in the literature indicate that the chemical communication system and olfactory behavior of *S. breve* is probably similar to that described for the cerambycid *A. subfasciatus*. However, it is necessary to continue research, currently in progress, in order to identify the chemical compounds involved in the *S. breve* communication system. This may enable their use as safe tools for the control of this important neotropical pest.

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## POTENTIAL IMPACT OF LADY BEETLES ON *DIAPHORINA CITRI* (HOMOPTERA: PSYLLIDAE) IN PUERTO RICO

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### ABSTRACT

The first detections of Asian citrus psyllid, *Diaphorina citri* Kuwayama, in North America occurred almost simultaneously in Florida and in the Caribbean (Guadeloupe). Damaging populations on citrus have been reported in Florida but not in Puerto Rico where the psyllid was first detected in 2001, having probably arrived with its parasitoid, *Tamarixia radiata* Waterston. In an effort to identify additional sources of mortality, the relative abundance of coccinellid species was estimated on 180 citrus trees from early April to early July 2003. The most abundant species were *Coelophora inaequalis* F. (38.8%) and *Cyclonedda sanguinea limbifer* L. (31.3%), and the least abundant were *Cladis nitidula* F. (5.9%), *Coleomegilla innonata* Mulsant (4.8%), *Chilocorus cacti* L. (2.1%), *Scymnus* sp. (5.9%), *Hippodamia convergens* Guerin (2.4%), and *Cryptolaemus montrouzieri* Mulsant (8.8%). These eight species were collected from citrus production areas in Puerto Rico for a laboratory study of feeding behavior. All eight consumed *D. citri* nymphs, with *C. innonata* consuming psyllids at a greater rate than *C. cacti* or *Scymnus* sp. Choice tests showed that *C. inaequalis* and *C. s. limbifer* preferred brown citrus aphid (BCA) *Toxoptera citricida* to *D. citri* as prey, whereas *C. nitidula* and *C. cacti* (both Chilocorini) preferred *D. citri*. Our results suggest that coccinellid species could play an important role as predators of the psyllid in Puerto Rico and contribute to its natural control.

**Key Words:** Asian citrus psyllid, Coccinellidae, predator, relative abundance, feeding behavior, natural control

### RESUMEN

La primera identificación en Norte América del psílido asiático del cítrico, *Diaphorina citri* Kuwayama, se produjo casi simultáneamente en Florida y Guadeloupe. Se habían detectado daños en cítrico en Florida pero no en Puerto Rico, donde el psílido fue descubierto por primera vez en el 2001, llegando posiblemente junto con su parasitoide *Tamarixia radiata* Waterston. En un intento por encontrar posibles fuentes adicionales de mortalidad para este insecto plaga, se estimó la abundancia relativa de especies de coccinélidos en 180 árboles de cítrico desde principios de Abril hasta principios de Julio del 2003. Las especies más abundantes fueron *Coelophora inaequalis* F. (38.8%) y *Cyclonedda sanguinea limbifer* L. (31.3%), y las menos abundantes fueron *Cladis nitidula* F. (5.9%), *Coleomegilla innonata* Mulsant (4.8%), *Chilocorus cacti* L. (2.1%), *Scymnus* sp. (5.9%), *Hippodamia convergens* Guerin (2.4%) y *Cryptolaemus montrouzieri* Mulsant (8.8%). Estas ocho especies fueron capturadas de áreas de producción cítrica de Puerto Rico para el estudio en laboratorio de su comportamiento alimenticio. Las ocho especies consumieron ninfas *D. citri*, pero *C. innonata* consumieron un mayor número de ninfas por hora comparado a *C. cacti* o *Scymnus* sp. Los ensayos de elección de presa mostraron que *C. inaequalis* y *C. s. limbifer* prefirieron como presa al áfido negro del cítrico (BCA) *Toxoptera citricida* en lugar de *D. citri*, mientras que *C. coeruleus* y *C. cacti* (ambos Chilocorini) prefirieron como presa a *D. citri*. Nuestros resultados sugieren que estas especies de coccinélidos podrían jugar un papel importante como depredadores del psílido en Puerto Rico y contribuir a su control natural.

Translation provided by the authors.

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama, was first described from Taiwan in 1907. It has been present in Brazil since the 1940s (Lima 1942) but was not detected in Florida until 1998 (Halbert et al. 1998) and in Texas

until September 2001 (French et al. 2001). In the Caribbean, the psyllid was first reported from Guadeloupe (Etienne et al. 1998) and subsequently detected in Puerto Rico in May, 2001 (Halbert & Nuñez 2004; P. Stansly, unpublished

data). *Diaphorina citri* spread rapidly throughout commercial citrus areas in Florida and heavy populations were observed, especially in the south. In contrast, infestations in Puerto Rico and Texas are typically light (unpublished data and V. French, pers. comm.).

*Diaphorina citri* feeds and reproduces on citrus and additional hosts belonging to the subfamily Aurantioidea of the family Rutaceae including jasmin orange, *Murraya paniculata* L., which is widely grown in Florida, Puerto Rico, and elsewhere as an ornamental plant. Feeding damage on citrus by large psyllid populations causes shoot distortion and abscission of the growing terminals. The psyllid also can vector the bacterium *Candidatus Liberibacter* spp., causal agent of citrus greening or 'huanglongbing' (Xu et al. 1988a). Citrus greening is considered to be the most serious disease of citrus in Asia; it has recently been reported from Brazil (Coletta-Filho et al. 2004), and poses a serious threat to citrus in Florida and Puerto Rico.

Two parasitoids have been imported and released in North America for control of *D. citri*, the endoparasitic encyrtid *Diaphorencyrtus aligarhensis* (Shafee, Alam & Agarwal) and the ectoparasitic euphorid *Tamarixia radiata* (Waterson). *Tamarixia radiata* established itself in Florida and apparently arrived spontaneously in Puerto Rico (unpublished data) and Texas (French et al. 2001).

Predators in the family Coccinellidae (Coleoptera) have been shown to be responsible for a considerable degree of natural control in Florida (Michaud 2004). Michaud (2001) also presented field data indicating that *Olla v-nigrum* Mulsant and *Harmonia axyridis* (Pallas), coccinellid species capable of feeding and reproducing on *D. citri*, increased in relative abundance in psyllid-infested citrus groves. The following study was undertaken to provide baseline data on relative abundance of coccinellid species in Puerto Rican citrus groves and to evaluate the ability of the most abundant species to feed on *D. citri*.

## MATERIALS AND METHODS

### Relative Abundance of Coccinellids on Citrus

Observations were carried out from early April to early July 2003, in citrus groves at the Agricultural Experimental Station in Adjuntas (18°10'N, 66°29'W, 457 m). The citrus groves of Adjuntas are well established and cover a significant proportion of both sides of the valley in which the experimental station is located. Because of the altitude and cooler weather, new growth in citrus is largely restricted to the main flush in the first half of the year. This is the time that population outbreaks of herbivorous insects such as aphids occur, with the subsequent increase of lady beetle numbers. The sampling period was designed to encompass this period of greater lady beetle activ-

ity. Thirty citrus trees were randomly selected and inspected between 8.00 a.m. and 12.00 a.m. every other week for a total of 180 trees, based on direct counts of all coccinellid adults, larvae, or pupae encountered. The relative abundance of each species was calculated as a proportion of the total coccinellids counted.

### Prey Acceptability and Prey Preference

Psyllid nymphs used in the experiments were collected from a greenhouse colony established from field-collected individuals and reared on *Murraya panniculata* at the Río Piedras Agricultural Experiment Station. Nymphs of brown citrus aphid (BCA) *Toxoptera citricida* (Kirkaldy) were collected from infested citrus flushes at the Adjuntas Agricultural Experimental Station and used directly in the experiments. Coccinellid adults of the various species were collected from citrus groves at the Adjuntas Agricultural Experimental Station in Puerto Rico during the period from April to June, 2003 and maintained on *Epeorus kuehniella* Keller (Lepidoptera: Pyralidae) eggs at Adjuntas until needed. *Chilocorus cacti*, *Cladis nitidula*, *Cryptolaemus montrouzieri*, and *Scymnus* sp. could not be reared on *E. kuehniella* eggs, and so were collected directly from the field when needed for experiments.

The following eight species of coccinellids were tested for prey acceptance and prey preference in choice and no-choice tests: *Cyclonedaa s. limbifer* L., *Coelophora inaequalis* F., *Cladis nitidula* F., *Chilocorus cacti* L., *Coleomegilla innonata* Mulsant, *Cryptolaemus montrouzieri* Mulsant, *Hippodamia convergens* Guerin, and *Scymnus* sp. Individual adult coccinellids were starved for 24 h and then confined in Petri dishes (5 cm diam.) with one of the following prey configurations: 10 psyllid nymphs only, 10 aphid nymphs only, or a combination of 5 psyllid nymphs and 5 aphid nymphs. The life stages of the two prey species were chosen to be of similar size (usually third-instar). Petri dishes were lined with white paper to assist with the visual assessment of feeding events. An experiment was judged to have been completed in the choice tests once the adult coccinellid had consumed all of one or the other prey species in the Petri dish. In the no-choice tests, the experiments were terminated after 7 h. Ten replicates of the tests were carried out and each adult coccinellid was tested only once. All experiments were carried out under controlled conditions at  $25 \pm 1^\circ\text{C}$  and  $75 \pm 10\%$  RH.

### Analysis

For determining the differences in coccinellid abundance, the square root transformation was used and then Tukey's multiple comparison test applied in Proc GLM in SAS (1999). For the feed-

ing data comparisons in the combined host experiments, normality was examined by the Shapiro-Wilk test and plot functions of Proc Capability (SAS 1999). The paired analysis was conducted based on the signed rank test in the same SAS procedure. For the consumption rate comparisons in the single host experiments, normality was determined by Proc Capability. Tukey's multiple comparison test in Proc GLM was then used to compare coccinellid consumption.

## RESULTS

### Relative Abundance of Coccinellids

A total of eight species of coccinellids were found in citrus groves of the Adjuntas experimental research station during the course of the survey; *C. inaequalis*, *C. s. limbifer*, *C. innonata*, *C. cacti*, *C. nitidula*, *Scymnus* sp., *H. convergens*, and *C. montrouzieri*. The number of coccinellids observed was relatively constant over the 3-month study period (Fig. 1). *Coelophora inaequalis* and *Cyclonedaa s. limbifer* were the most common species found over the entire study period with the remaining species found on a regular basis, but in much lower numbers (Fig. 2).

Additionally, two species of Hymenoptera parasitized the coccinellids. *Homalotylus* sp. near *terminalis* Say (Hymenoptera: Encyrtidae) emerged from a *C. s. limbifer* pupa, while *Oomyzus* sp. near *scapus* Thomson (Hymenoptera: Eulophidae) emerged from *C. montrouzieri* (larva) and *C. s. limbifer* (pupa) (determinations by M. W. Gates, pers. comm.).

### Prey Acceptability

All eight coccinellid species in the no-choice tests fed on both host species with no rejection of any offered prey type, although variation in the quantity and rate of prey consumption was observed. During the allotted interval there were few differences in relative amounts of prey consumed in no-choice tests, with the possible exception of *C. nitidula*, which ate twice as many psyllids as aphids (Table 1). *Hippodamia convergens* demonstrated the highest rate of aphid consumption whereas *C. cacti*, *C. nitidula*, and *Scymnus* sp. showed the lowest rate (Table 2). The highest rate of psyllid consumption was observed with *C. innonata*, although not significantly different from the rates of all others except *C. cacti* and *Scymnus* sp. (Table 3).

### Prey Preference

Two of the coccinellid species examined, *C. s. limbifer* and *C. inaequalis*, showed a strong preference for the brown citrus aphid, while *C. nitidula*, *C. cacti*, *C. innonata*, and *C. montrouzieri* showed significant preference for the Asian citrus psyllid (Table 4). The remaining two species, *H. convergens* and *Scymnus* sp. showed no preference for either prey species.

## DISCUSSION

Of the seven species of Coccinellidae listed by Michaud (2004) as common in Florida citrus, only the exotic *Coelophora inaequalis* also was found

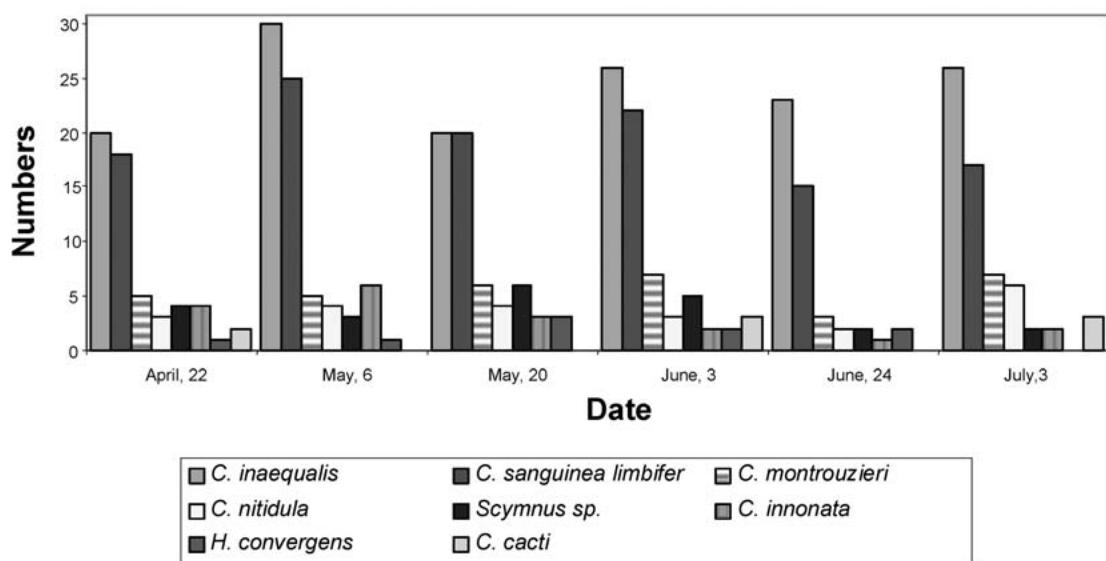


Fig. 1. Abundance of 8 coccinellid species during the 3-month sampling period in 2003 at the Adjuntas Agricultural Experimental Station in Puerto Rico.

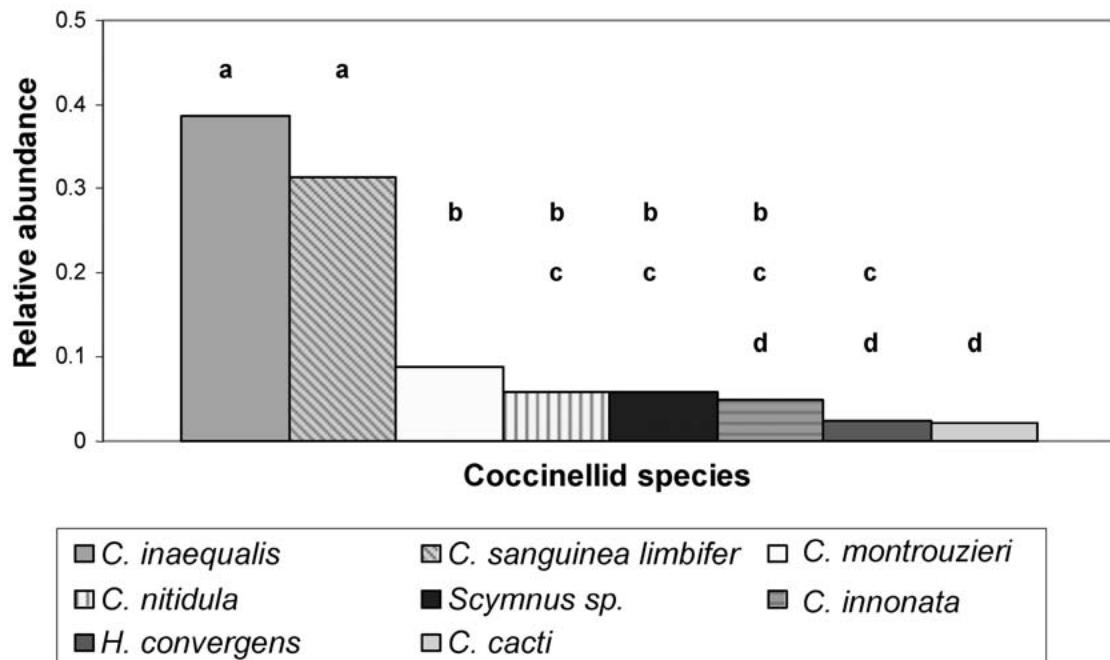


Fig. 2. Relative abundance of the coccinellid species caught at the Adjuntas Agricultural Experimental Station in Puerto Rico.

in this survey. However, *C. s. limbifer* was considered by Gordon (1985) as a subspecies of *C. sanguinea*, the most commonly encountered ladybeetle in Florida citrus groves prior to the invasion of *Harmonia axyridis* Pallas (Muma 1953; Michaud 2001; Michaud 2002). *Cyclonedada limbifer* and *C. inaequalis*, both aphid-feeders, were the two most abundant species encountered in this study and also the most abundant reported from Puerto Rican citrus groves by Michaud and Browning (1999). The predominance of aphid feeders followed by scale feeders are characteristics shared by the coccinellid fauna in citrus groves of Florida and Puerto Rico.

TABLE 1. MEAN PERCENTAGE  $\pm$  SD OF 10 PREY ITEMS CONSUMED IN NO CHOICE TESTS IN 7 H.

Species	Host species	
	BCA % eaten	ACP % eaten
<i>H. convergens</i>	94 $\pm$ 7%	80 $\pm$ 23%
<i>C. inaequalis</i>	84 $\pm$ 12%	83 $\pm$ 16%
<i>C. s. limbifer</i>	60 $\pm$ 32%	60 $\pm$ 13%
<i>C. nitidula</i>	58 $\pm$ 26%	54 $\pm$ 21%
<i>C. montrouzieri</i>	30 $\pm$ 13%	65 $\pm$ 25%
<i>C. cacti</i>	46 $\pm$ 21%	47 $\pm$ 21%
<i>Scymnus sp.</i>	36 $\pm$ 9%	47 $\pm$ 8%
	16 $\pm$ 5%	16 $\pm$ 9%

Our results showed that all ladybeetle species, with the exception of *C. nitidula*, consumed similar quantities of either host species presented separately. *Cladis nitidula* consumed more psyllids and also was the species showing greatest preference for *D. citri* when offered a choice between prey. Thus, choice and no-choice experiments were consistent in indicating *C. nitidula*'s preference for *D. citri* over *T. citricida*. A preference for *T. citricida* over *D. citri* was strongly expressed by *Cyclonedada s. limbifer*, and to a lesser extent by *Coelophora inaequalis*, the two most abundant ladybeetles in this study, although both species consumed equal amounts of both prey when given no choice. Michaud & Olsen (2004) found that *Cyclonedada sanguinea* also fed on *D. citri* as both larva and adult, but that larval development time was almost doubled compared to a diet of *E. kuehniella* and that female *C. sanguinea* stopped ovipositing following transferal to the *D. citri* diet. Further studies would be necessary see whether a *D. citri* diet negatively impacts the larval and reproductive performance of *Cyclonedada s. limbifer* as it does *C. sanguinea*.

Prey suitability for Coccinellidae is a complex issue that goes beyond the scope of prey acceptance and prey preference studied here, and generalizations such as "aphid feeders" and "scale feeders" are overly simplistic (Hodek 1996). Nevertheless, the species demonstrating the greatest degree of acceptance and preference for BCA,

TABLE 2. MEAN  $\pm$  SD CONSUMPTION RATES OF COCCINELLIDS ON THE BROWN CITRUS APHID IN NO CHOICE TESTS.

Species	Mean # of BCA consumed/h	Standard deviation	Tukey's comparison		
<i>H. convergens</i>	1.54	0.64	a		
<i>C. innonata</i>	1.09	0.42	a	b	
<i>C. s. limbifer</i>	0.93	0.43		b	c
<i>C. inaequalis</i>	0.78	0.40		b	c
<i>C. montrouzieri</i>	0.53	0.39		b	d
<i>C. cacti</i>	0.37	0.27		c	d
<i>C. nitidula</i>	0.30	0.26			d
<i>Scymnus</i> sp.	0.23	0.08			d

Note: different letters denote a significant difference at  $P = 0.05$ .

such as *C. s. limbifer*, *C. inaequalis*, and *H. convergens* were not surprisingly those considered to be aphid feeders. Those that preferred and/or most readily accepted ACP or showed no clear tendencies were either known to feed principally on other prey such as Diaspididae (*C. cacti*), and Pseudococcidae (*C. montrouzieri*), or had feeding habits that were largely undocumented (*C. nitidula*, *Scymnus* sp.).

*Diaphorina citri* was rarely encountered in Adjuntas during the course of this study, and ACP populations have remained generally low in Puerto Rico on both orange jasmine and citrus. Coccinellids typically respond to dense prey popu-

lations whereas parasitoids with narrow host ranges such as *T. radiata* are expected to track their host population at low densities. It is difficult to ascertain, in retrospect, why ACP never achieved high infestations in Puerto Rico as it has Florida; however, preliminary evidence suggests that *T. radiata* may be responsible for holding the psyllid in check in Puerto Rico (R. Pluke, unpublished data). In any case, the coccinellid guild present in Puerto Rican citrus, with its demonstrated ability to consume ACP and its similar mix of species in regard to feeding habits to the coccinellid guild in Florida, would likely respond positively to any future increase in psyllid numbers.

TABLE 3. MEAN  $\pm$  SD CONSUMPTION RATES OF COCCINELLIDS ON THE ASIAN CITRUS PSYLLID IN NO CHOICE TESTS.

Species	Mean # of ACP consumed/h	Standard deviation	Tukey's comparison		
<i>C. innonata</i>	1.21	0.18	a		
<i>H. convergens</i>	1.14	0.33	a	b	
<i>C. nitidula</i>	1.14	0.74	a	b	
<i>C. s. limbifer</i>	0.88	0.55	a	b	
<i>C. inaequalis</i>	0.76	0.60	a	b	c
<i>C. montrouzieri</i>	0.67	0.30	a	b	c
<i>C. cacti</i>	0.50	0.32		b	c
<i>Scymnus</i> sp.	0.16	0.15			c

Note: different letters denote a significant difference at  $P = 0.05$ .

TABLE 4. MEAN PERCENTAGE (OF 5 HOST INDIVIDUALS) EATEN BY COCCINELLIDS IN CHOICE TESTS.

Species	Prey species		Preference	<i>P</i> value
	BCA % eaten	ACP % eaten		
<i>C. s. limbifer</i>	64	4	Aphid**	0.004
<i>C. inaequalis</i>	78	28	Aphid**	0.016
<i>C. nitidula</i>	8	76	Psyllid**	0.002
<i>C. cacti</i>	30	80	Psyllid**	0.031
<i>C. innonata</i>	46	72	Psyllid**	0.031
<i>C. montrouzieri</i>	32	68	Psyllid*	0.055
<i>H. convergens</i>	60	58	N/A	0.711
<i>Scymnus</i> sp.	31	31	N/A	1.000

Note: \*\*significant at  $P \leq 0.05$ , \* $P$  significant at  $P \leq 0.1$ .

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## CONTINENTAL COMPARISONS OF THE INTERACTION BETWEEN CLIMATE AND THE HERBIVOROUS MITE, *FLORACARUS PERREPAE* (ACARI: ERIOPHYIDAE)

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### ABSTRACT

The Old World climbing fern, *Lygodium microphyllum*, is an invasive weed in the Florida Everglades and the leaf roll galling mite, *Floracarus perrepae*, is a proposed biological control agent. Field studies were conducted for one to two years at sites in its native range in Australia, New Caledonia, and India to evaluate the effect of climate on *F. perrepae*. Monthly counts of the proportion of *L. microphyllum* subpinnae (leaflets) with leaf roll galls were used to measure the incidence of damage caused by *F. perrepae*. Between sites the most significant weather variable was rainfall 14 to 28 days prior to sampling, with higher levels having a depressive effect on the incidence of leaf rolls. Within sites the mean maximum temperature was the only significant weather variable, showing a decrease in the incidence of leaf rolls above 27°C, and it was predicted that no leaf rolls would form above 35°C. The weather parameters in Homestead, Florida for 2002 were within the range of those evaluated in the eight native range field sites. Thus, we do not predict that climate will prevent the establishment of this biological control agent for *L. microphyllum* in southern Florida.

**Key Words:** climate, biological control of weeds, ferns, Florida Everglades, New Caledonia, India

### RESUMEN

El helecho trepador del viejo mundo, *Lygodium microphyllum*, es una maleza invasora en los Everglades de Florida y el ácaro que causa la agalla de enrollamiento de las hojas, *Floracarus perrepae*, es el agente de control biológico propuesto. Estudios de campo fueron llevados a cabo de uno a dos años en sitios localizados en su área nativa en Australia, Nuevo Caledonia, y India para evaluar el efecto del clima sobre *F. perrepae*. Se usaron los conteos mensuales de la proporción de subpinnae (hojuelas) de *L. microphyllum* con agallas de hojas enrolladas para medir la incidencia del daño causado por *F. perrepae*. Entre los sitios, la variable más significativa del clima fue la lluvia 14 a 28 días antes del muestreo, con los niveles más altos causando un efecto negativo sobre la incidencia de las hojas enrolladas. En el mismo sitio el promedio de la temperatura máxima fue la única variable del clima significativa, demostrando una baja de la incidencia del enrollamiento de las hojas a las temperaturas mayor de 27°C, y se predijo que ninguna hoja enrollada se formará a temperaturas mayores de 35°C. Los parámetros del clima en Homestead, Florida para el año 2002 estaban en el rango de los parámetros evaluados en el campo de los ocho sitios estudiados en el área nativo del ácaro. Por eso, nosotros no estimamos que el clima pueda prevenir el establecimiento de este agente de control biológico para *L. microphyllum* en el sur de Florida.

*Lygodium microphyllum* (Cav.) R. Br. (Lygodiaceae, Pteridophyta), Old World climbing fern, is native to the wet tropics and subtropics of Africa, Australasia, Asia, and Oceania (Pemberton 1998), and is an aggressive invasive weed of moist habi-

tats in southern Florida with the potential to spread into Central and South America (Pemberton & Ferriter 1998; Goolsby 2004). A biological control program was initiated in 1997 and surveys for potential agents were conducted in Australia

and South Asia (Goolsby et al. 2003). The eriophyid mite, *Floracarus perrepae* Knihinicki and Boczek, was the most widely distributed with several geographically specific genotypes identified (Goolsby et al. 2003; Goolsby et al. 2004a). Throughout its native distribution in Australia and Asia, *F. perrepae* causes significant damage to *L. microphyllum*. Feeding by the adults and immatures causes formation of leaf roll galls, leading to necrosis and premature defoliation of *L. microphyllum* pinnae, and the gradual debilitation of the plant (Goolsby et al. 2003; Freeman et al. 2005; Ozman & Goolsby 2005). Based on its narrow host-range (Goolsby et al. in press), and significant impact on *L. microphyllum* (Goolsby et al. 2004b), *F. perrepae* was prioritized for evaluation as a biological control agent (Goolsby & Pemberton 2005).

Ozman & Goolsby (2005) documented the biology and seasonal phenology of *F. perrepae* in Southeast Queensland, Australia, and found that it was active year round, with populations peaking when temperatures were cool and soil moisture levels were highest. In these studies, the proportion of mite infested, or incidence of rolled subpinnae, was measured monthly at four locations over a two-year period. The incidence of leaf rolls was used as an indicator of the mite's impact on the fern. Because the effect of weather was the most significant factor in the phenology of the mite in Australia, we replicated this study in India and New Caledonia to determine if the genotypes from these locations were affected in the same manner. The Indian and New Caledonian *F. perrepae* genotypes were known to have unique biological differences, in that they were co-adapted to the local genotype of the fern (Goolsby et al. unpublished data), but little was known about their possible adaptations to climate. Therefore, we used the incidence of the proportion of mite induced leaf rolls (or plant damage) to evaluate the seasonal phenology of *F. perrepae* in other parts of its native range that were climatically different to Southeast Queensland.

## MATERIALS AND METHODS

### Study Sites

The initial baseline studies were conducted in Southeast Queensland (QLD) from February 2001 to March 2003 on Bribie Island; at Gallagher's Point ( $27^{\circ}01.17'S$ ,  $153^{\circ}06.53'E$ ) and McMahon Rd. ( $27^{\circ}04.33'S$ ,  $153^{\circ}10.55'E$ ) and at Logan; Carbrook Creek ( $27^{\circ}41.30'S$ ,  $153^{\circ}16.23'E$ ) and Lagoon Rd. ( $27^{\circ}40.01'S$ ,  $153^{\circ}16.03'E$ ). Samples were collected monthly from February 2001 to February 2003. These sites are seasonally inundated with fresh water and *L. microphyllum* grows as a climbing vine on paper bark trees, *Melaleuca quinquenervia* (Cav.) St. Blake. The site at Carbrook Creek is different in that *L. microphyllum* grows under

a closed canopy of *M. quinquenervia* in deep shade as compared to the other sites with open canopies. The climate in southeast Queensland is subtropical. Rain falls mainly in the summer months, which are hot and humid. Winters are cool and dry with an average yearly temperature of  $20.6^{\circ}\text{C}$  and average rainfall of 1393 and 1256 mm at Bribie Island and Logan, respectively.

Sites in India were located in southern Tamil Nadu at Thomaiyarpuram, Nagercoil ( $8^{\circ}19.00'N$ ,  $77^{\circ}25.70'E$ ) and in Kerala near Ithikkara River, Quilon ( $8^{\circ}51.95'N$ ,  $76^{\circ}41.79'E$ ). Samples were collected monthly from July 2002 to August 2003. At the Nagercoil site *L. microphyllum* was growing under a canopy of coconut palms in deep shade. The Quilon site was exposed to full sun, with the fern growing in a ditch up a roadside embankment. The climate in southern India is monsoonal with heavy rainfall from April to August and hot, humid summers. The average yearly temperature and rainfall for Nagercoil and Quilon are  $30.0$  and  $27.5^{\circ}\text{C}$ , and 905 and 2932 mm, respectively. Sites in New Caledonia were located in Province Sud near Noumea at la Coulee ( $22^{\circ}14.09'S$ ,  $166^{\circ}34.75'E$ ) and Yaté ( $22^{\circ}06.36'S$ ,  $166^{\circ}56.08'E$ ). Samples were collected monthly from May 2002 to November 2003. At both sites *L. microphyllum* grows in partial shade with *M. quinquenervia*. The climate in New Caledonia is subtropical with rain evenly spaced throughout the year with an average yearly rainfall of 1106 mm. The average yearly temperature is  $23.4^{\circ}\text{C}$ .

We obtained the weather data from the closest available weather station for each location, daily maximum and minimum temperature, RH at 9 a.m. and 3 p.m. and rainfall. From these the daily soil moisture index (between 0 and 1 at soil saturation) was calculated based on a simple model developed by Fitzpatrick and Nix (1969). Yearly rainfall averages for India and New Caledonia were obtained from CLIMEX 2 (Sutherst et al. 2004) and the Queensland Department of Natural Resources for sites in Australia (Queensland 2004). For comparison of the native range to the proposed area of introduction for *F. perrepae*, we used weather data from 2002 for the Florida Automated Weather Network (Florida 2004), for Homestead, Florida, USA, which is located near Everglades National Park.

### Sampling Methods

*Lygodium microphyllum* grows as a twining vine, and each shoot or rachis is a true leaf, consisting of pinnae (leaflets) and subpinnae (subleaflets). Vines were typically found climbing up the trunks of trees, reaching up to 10 m with many yellowish-green, fertile or sterile pinnae branching off the main stem. Each pinna consists of 6-12 paired subpinnae, which are the smallest leaf unit. Fertile subpinnae are fringed with lobes

of sporangia, with sterile subpinnae having a smooth outer margin. *Floracarus perrepae* feed on and cause leaf roll galls on the fertile subpinnae, but they did not prefer this leaf form. Therefore, the field phenological studies were based on counts of sterile subpinnae. Each month 50 newly expanded sterile pinnae were collected at each site along a transect and returned to the laboratory for counting. At each site the numbers of infested and uninfested subpinnae were counted for each pinna. This count provided a measure of the incidence of infested subpinnae (leaf rolls), or total mite damage, at each location.

#### Statistical Analyses

Based on previous field research, it was determined that leaf rolls were the result of *F. perrepae* feeding activity from between 28 and 14 days before field collection (Goolsby et al. 2004; Ozman & Goolsby, in press). Therefore mean values of weather variables for this two-week period were used for the analyses. An analysis of variance was performed on the proportional incidence of leaf rolls to determine which of the weather variables accounted for the most variation between and within sites. Sites were assigned a value of 1 if the *L. microphyllum* grew under a canopy of full shade, with a value of 0 for sites with partial shade or full sun.

#### RESULTS

The incidence of leaf rolls was observed monthly for periods between one and two years at eight sites, two in India, two in New Caledonia, and four in Australia. The mean value of the proportional incidence of leaf rolls at each site is shown in the Table 1, together with mean

weather variables daily maximum and minimum temperature, rainfall, soil moisture, and shade.

An analysis of variance was performed on the proportion of leaf rolls to determine which of the abiotic variables accounted for the most variation between and within sites. The rainfall of the previous two-week period, averaged over each site, fitted as a quadratic equation explained 90% of the variation between the 8 sites ( $P < 0.001$ , linear regression coefficient ( $b_1$ ) = -0.00323, SE 0.00031 and quadratic regression coefficient ( $b_2$ ) = 0.0000485, SE = 0.000009,  $F_{2,4} = 22.71$ ) (Fig. 1). Shade explained a further 5% of the between site variation but only at ( $P < 0.07$ ) with leaf rolls from shaded locations (0.048 SE 0.019,  $F_{1,4} = 3.22$ ) higher than unshaded. The shape of the quadratic for rainfall indicated that rainfall decreased the incidence of leaf rolls steadily as it increased. However, after 72 mm of rainfall for a two-week period, the predicted proportional incidence of leaf rolls remained fairly constant as rainfall increased. Temperature, relative humidity, and soil moisture were not significant in the analysis between sites.

Within sites (after adjusting for mean site differences) the mean maximum temperature fitted as a quadratic explained the most variation in the incidence of leaf rolls ( $P < 0.001$ ,  $b_1 = 0.1598$ , SE 0.0532,  $b_2 = -0.00319$  SE = 0.00099,  $F_{2,188} = 7.95$ ) (Fig. 2). From the shape of the fitted curve, maximum temperature appeared to decrease the incidence of leaf rolls as the temperature rose above 27°C, but below the prediction was fairly constant. At mean maximum of above 35°C no curls were predicted. There was no interaction between the variables and sites; hence, all sites had the same response to the weather variables. Relative humidity, rainfall, and soil moisture were not significant within sites.

TABLE 1. MEAN PROPORTION OF *FLORACARUS PERREPÆ* INDUCED LEAF ROLL GALLS ON *LYGODIUM MICROPHYLLUM* WITH SELECTED WEATHER VARIABLES FOR EACH FIELD SITE.

Site	No. obs.	Mean <sup>a</sup> proportion leaf rolls ± SE	Mean <sup>b</sup> max. temp. °C	Mean <sup>b</sup> min. temp. °C	Mean <sup>c</sup> rain mm.	Soil <sup>d</sup> moisture	Shade <sup>e</sup>
Quilon, India	12	0.222 ± 0.051 ab	32.4	23.5	60.1	0.63	0
Nagercoil, India	13	0.346 ± 0.065 bed	32.7	23.8	37.1	0.59	1
La Coulee, New Caledonia	20	0.157 ± 0.036 a	28.1	17.3	70.0	0.74	0
Yate, New Caledonia	20	0.170 ± 0.028 a	26.9	19.6	117.0	0.85	0
Carbrook Creek, Australia	24	0.358 ± 0.022 cd	26.0	15.4	34.0	0.61	1
Lagoon Rd., Australia	24	0.289 ± 0.035 bc	26.0	15.4	33.5	0.66	0
Gallagher's Pt., Australia	24	0.329 ± 0.029 bcd	25.7	16.3	30.0	0.65	0
McMahon Rd., Australia	12	0.432 ± 0.053 d	25.5	16.0	18.5	0.65	0
Homestead, Florida	12	n/a	29.1	18.4	89.6	0.80	n/a

<sup>a</sup>Means in the column followed by the same letter are not significantly different ( $P < 0.05$ ) from transformed data analysis.

<sup>b</sup>Mean of the means for daily maximum/minimum temperatures 28–14 days prior to each sampling date.

<sup>c</sup>Mean of the sum of rainfall for 28–14 days prior to each sampling date.

<sup>d</sup>A value of 1 indicates soil saturation.

<sup>e</sup>Zero indicates full sun, with 1 being partial or full shade at the site.

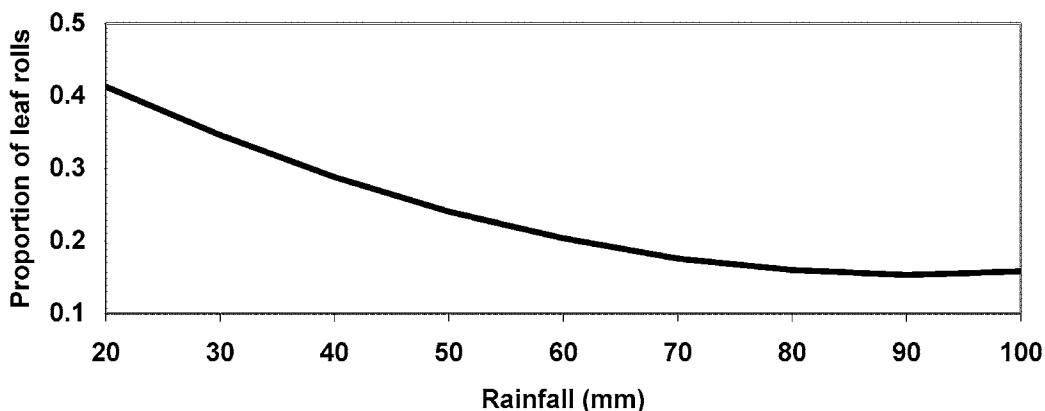


Fig. 1. Estimated effect of rainfall on annual proportion of uninfested subpinnae and mite induced leaf rolls (between sites). A higher proportion indicates a higher level of plant damage.

The weather data from Homestead, FL was calculated for 12 hypothetical monthly sample dates in 2002. The mean rainfall of the 12 two-week totals was 90 mm with the mean maximum temperatures of the two-week sampling periods in a range between 23.1 and 32.4°C (Table 1).

#### DISCUSSION

*Floracarus perrepae* is widely distributed in Australia and Asia across a range of tropical and subtropical climates (Goolsby et al. 2003). Within this distribution are several location specific genotypes that are adapted to their corresponding genotypes of their host, *L. microphyllum* (Goolsby et al. 2004a; Goolsby et al., unpublished data).

From these studies, it appears that the specific genotypes represented in southern Australia, New Caledonia, and India responded similarly to the weather variables in their environments, since there was no interaction with sites in the within site analysis.

The most significant weather variable between sites was rainfall. Jeppson et al. (1975) reported similar effects from high rainfall during the rainy season of Asiatic monsoon climates, which caused major reductions in mite populations such as the tea spider mite, *Oligonychus coffeae* (Nietner). In our studies, high amounts of rainfall 28-14 days months prior to the sampling date had a negative impact on the incidence of leaf rolls, which is an indicator of the local population density. Persis-

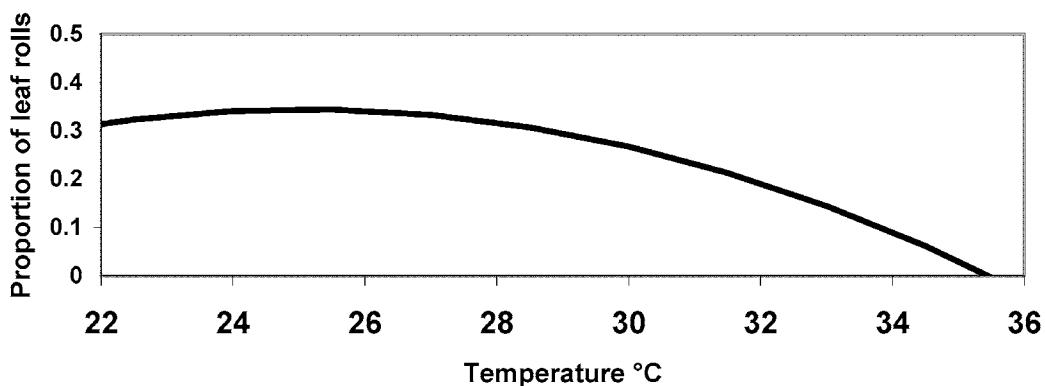


Fig. 2. Estimated effect of mean maximum temperature on proportion uninfested subpinnae and mite induced leaf rolls (within sites). A higher proportion indicates a higher level of plant damage.

tent and heavy rainfall during this time period most likely dislodged the dispersing *F. perrepae* as they attempted to settle and induce leaf rolls. This may explain why the sites in Quilon, India and New Caledonia had the lowest mean proportional incidence of leaf rolls. Quilon receives a double rainy period during the monsoon, and heavy persistent rain is common. In addition, the Quilon site has high rainfall, is in the open, and exposed to the direct effect of rain. In contrast, the site in Nagercoil received less rain, the *L. microphyllum* stand was under a sheltering canopy of palms, and the incidence of leaf rolls galls was higher, within the range of values for the Australian sites. In New Caledonia, high rainfall during 2002-03, including that associated with cyclone Erica, (March 2003) appears to have been responsible for the lower incidence of leaf rolls. Additionally, both sites in New Caledonia are partially exposed with *L. microphyllum* growing under a broken canopy of *M. quinquenervia*, thus allowing for the full impact of rainfall.

The higher average proportional incidence of leaf rolls at the Australian field sites also may be due to the drought conditions, which have affected eastern Australia since 2001 (Queensland 2004). *Floracarus perrepae* at these field sites have been exposed to fewer episodes of high rainfall, and mortality of dispersing females may have been lower. Thus, the higher proportional incidence of leaf rolls. The coconut mite, *Aceria guerrenoris* Keifer (Acar: Eriophyidae), appears to be affected similarly across its known distribution where it reaches higher densities in areas with drier climates (Moore & Howard 1996). The study by Ozman & Goolsby (2005) found populations of *F. perrepae* peaked when temperatures were cool and soil moisture levels were highest, which appears to contradict the findings of this study. However, the results from the continental comparisons of climatic effects further clarify the differences between the effects of soil moisture and rainfall on *F. perrepae* populations. Our results suggest that *F. perrepae* performs best when soil moisture is near saturation (promoting growth of *L. microphyllum*) and there are few periods of high intensity rainfall, which interfere with dispersal.

The mean rainfall for Homestead, Florida in 2002 was higher than that for any station measured in Australia. One could predict that populations of *F. perrepae* in the latter localities, would be similar to those in New Caledonia, where the mite caused considerable damage to *L. microphyllum*. The rainfall effect appears to have a limit as shown in Figure 1, in which the model predicts that effect will remain constant above 72 mm for a two-week period. This prediction is corroborated by the data from New Caledonia and India, which showed that high rainfall reduced the incidence of leaf rolls but the populations remained stable.

High temperatures also had a significant negative impact on *F. perrepae* populations within sites. The model predicts that the proportional incidence of leaf rolls will decrease as the mean maximum temperature rises above 27°C with no leaf roll formation above 35°C. None of the sites in the native range experienced mean maximum temperatures for a two-week period above 35°C. Similarly, in Homestead, Florida during 2002 the highest means for the summer months ranged between 31 and 32°C, respectively. High temperatures are important to consider for rearing and release of *F. perrepae*. Summer greenhouse temperatures can often reach mean maximum temperatures above 35°C. Optimum rearing environments should be maintained at a mean maximum temperature of 27°C. Field colonization of *F. perrepae* in Florida would be the most difficult in the summer months when temperatures and rainfall are the highest.

In conclusion, the genotypes of *F. perrepae* evaluated in this study from its native range in Australia, New Caledonia, and India were all negatively impacted by high rainfall and temperatures during leaf roll formation. Conversely, populations of *F. perrepae* reach their highest levels when temperatures are cool and episodes of high intensity rainfall are few. The climate of southern Florida falls within the parameters experienced in the native range of *F. perrepae*. Therefore, we do not predict that climate will prevent the establishment of this biological control agent for *L. microphyllum*.

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## TRAP-NESTING *ANCISTROCERUS SIKHIMENSIS* (HYMENOPTERA: EUMENIDAE) IN NEPAL: NEST STRUCTURE AND ASSOCIATES (HYMENOPTERA: CHRYSIDIDAE; ACARINA: SAPROGLYPHIDAE)

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### ABSTRACT

The contents of 21 trap-nests located in Sagarmatha National Park, Nepal, in 2002 and 2003 revealed interesting aspects of the biology of *Ancistrocerus sikhimensis* Bingham (Hymenoptera: Eumenidae). The nests included 2-7 brood cells separated by mud partitions. The dimensions of these structures seem to increase from the first cell to the last one constructed by the wasp. Females always used all the available space of the trap-nests, and the variability in the number of cells per nest essentially depended on their different dimensions. All the emerged adults were females, and we suspect that this species is bivoltine, with a highly shifted sex ratio between the two generations. In 2002, the parasite *Chrysis* sp. aff. *coelestina* Klug, recorded for the first time on this host, was responsible for a rate of parasitism per nest of 0%-100%, with an average of 41.65%. A second cuckoo wasp, *Chrysis violenta ultramonticola* Linsenmaier, emerged from one nest in 2003. Most *A. sikhimensis* females housed, mainly on the abdomen, hypopi of the mite *Vespacarus* sp., which is known to be involved in other wasp-mite associations. Unlike other mite-symbiotic eumenid wasps, *A. sikhimensis* does not present an acarinarium on its body to house the mites.

**Key Words:** trap-nest, solitary wasp, parasitism, mutualism, Himalaya, Hymenoptera, Eumenidae, *Ancistrocerus sikhimensis*

### RESUMEN

El estudio de los contenidos de 21 nidos trampa, localizados en el Parque Nacional de Sagarmatha, Nepal, ha dado a conocer aspectos interesantes de la biología de *Ancistrocerus sikhimensis* Bingham (Hymenoptera: Eumenidae). Los nidos contenían entre 2 y 7 celdas de cría, separadas por tabiques de barro, cuyas dimensiones—tanto de las celdas como de los tabiques—aumentaban según iban siendo construidas. En todos los casos las hembras construyeron celdas en todo el espacio disponible del nido, condicionando las dimensiones de éste el número de celdas por nido. Solo hembras emergieron de los nidos trampa, lo cual nos lleva a sospechar que esta es una especie bivoltina, con un sex-ratio diferente entre las dos generaciones. En el año 2002 el parásito *Chrysis* sp. aff. *coelestina* Klug, colectado por primera vez de este hospedador, fue el responsable de un alto parasitismo ( $\bar{x}$  (por nido) = 41.65%). En el año 2003 otro crisídido, *Chrysis violenta ultramonticola* Linsenmaier, emergió de un nido. Muchas hembras de *A. sikhimensis* albergaban, principalmente en el gáster, ácaros del género *Vespacarus* en el estado de hypopus, revelando una asociación entre estos artrópodos. A diferencia de otros euménidos simbiontes con ácaros, *A. sikhimensis* no presenta un acarinarium donde albergar a los ácaros.

Translation provided by the authors.

Most vespoid wasps of the family Eumenidae nest individually. Females provision their brood with paralyzed larvae of Lepidoptera or Coleoptera, and no overlapping of generations exist (Iwata 1976; Krombein 1978; Bohart et al. 1982; Cowan 1991). They are mass-provisioners and prey are rapidly brought to the nest after the oviposition. Nests are built with materials such as mud or

chewed leaves, dug into the soil, or built in pre-existing cavities (Iwata 1976), and include several cells. When the wasp has collected enough food for one larva, she seals the brood cell and starts to work on a new one (Krombein 1967; Cowan 1991).

Females of the widely distributed genus *Ancistrocerus* Wesmael nest in pre-existing cavities, modifying them with mud (Berland 1928; Nielsen

1932; Blüthgen 1943, 1961; Bonelli 1969; Richards 1980), however *Ancistrocerus oviventris* Wesmael typically builds aerial mud nests (Berland 1928). The cavity adopted by the wasp may be a tube in the hollowed pith of a twig, an old insect bore-hole in rotten wood, or many other kinds of holes (Blüthgen 1943; Kurzenko 1981). Most species separate the cells with simple mud cell partitions, but in a few species, such as *Ancistrocerus antilope* Panzer, *A. parietinus* L., and *A. ichneumonideus* Ratzeburg, cells (located in the cavity) are entirely made of mud (Blüthgen 1943). This opportunistic nesting habit has allowed researchers to use trap-nests in order to study many aspects of the biology of these wasps (Krombein 1967; Jacob-Remacle 1976; Wearing & Harris 1999). *Ancistrocerus* females provision their nests with caterpillars of several families of Lepidoptera (Iwata 1976; Harris 1994). Some species show considerable seasonal variations in the sex-ratio (typically male-biased in winter and female-biased in summer) (Fye 1965; Longair 1981).

This paper offers basic information about the nest structure and the brood sex ratio of *Ancistrocerus sikhimensis* Bingham. Data come from a study carried out by placing a number of wood trap-nests in Sagarmatha National Park, Nepal. We also report data on three organisms associated with *A. sikhimensis* nests, two cuckoo wasps (Chrysididae), and a saprogllyphid mite. Cuckoo wasps are very common parasites of eumenids (Alfken 1914; Nielsen 1932; Bonelli 1969). Associations between saprogllyphid mites and solitary wasps are known for species belonging to different families, but in particular for Eumenidae (Cooreman & Crèvecoeur 1948; Krombein 1961, 1967; Mostafa 1970; Pekkarinen & Hulden 1991). This mutualistic relationship is complex and often includes venereal transmission of the mites through wasp genital chambers (Cooper 1955), or sometimes alternating parasitic and saprophagous phases of the mite on the host body (Okabe & Makino 2003).

## MATERIALS AND METHODS

### Study Area

The study was carried out in Sagarmatha National Park ( $27^{\circ}45' \text{--} 28^{\circ}07' \text{N}$ ,  $86^{\circ}28' \text{--} 87^{\circ}07' \text{E}$ ; Solu-Khumbu district, Nepal). The average temperatures, measured during the study periods, were  $12.9^{\circ}\text{C}$  in 2002 and  $13.4^{\circ}\text{C}$  in 2003. In the study area, precipitation is concentrated in the monsoon season, lasting from the end of May to the end of September. In 2002 rain occurred, mostly in June, during 32% of the whole observation period (43 days) and in 2003 it rained every day (100%, 22 days).

### Trap-nests

Trap-nests of different sizes were made using wood of *Abies alba* (according to Krombein, 1967). The blocks ( $2 \times 2 \text{ cm}$  square section) were perforated to provide a suitable space where wasps could establish their nests. Seven different hole sizes were provided, with diameter ranging from 3 to 10 mm and tunnel length from 55 to 90 mm. After removal, the trap nest holes closed with mud were protected with a net or an adhesive tape, to avoid flights from each trap.

The trap-nests were later reopened by sawing along the longitudinal side and the collected material was preserved in 70% ethanol for determination. Head width of all specimens of *A. sikhimensis* and its chrysidiid parasite collected in 2002 were measured to the nearest 0.1 mm.

### Collecting Sites

Seventy-one artificial nests were placed in 2002 and forty-six in 2003. In 2002 the nests, divided into 9 groups, were placed at two different sites from May 9th to June 20th and reopened on September 10th. Site 1 was a garden in Namche Bazar, 3450 m, on a south-facing slope. Site 2 was an open area outside of Kangyuma, 3700 m, on a north-facing slope. Two groups were placed at site 1, composed of 9 (1a) and 8 (1b) blocks. Block 1a was placed at 1 m<sup>x</sup> under the roof of a house, while block 1b was placed at 2 m<sup>x</sup> between stones in a wall in the proximity of a garden in bloom. At site 2, there were seven groups (2a-g). Block 2a was on a *Rhododendron arboreum* tree in bloom, and blocks 2b-c were in a stone wall in an open area near a wood of birches (*Betula utilis*) and rhododendron trees. Blocks 2d-g were under the roof of an isolated house. Block 2a was set at 1.5 m high, 2b-c at 1 m, and blocks 2d-g at 2 m above the ground. The nests that were not covered by roofs were protected from the rain with transparent plastic sheets.

In 2003, nests were placed (June 22nd) only at site 2, in the same location of groups d-g of the previous year, i.e., under the roof of a house, 2 m above the ground. On July 14th nests were removed and reopened the following spring (2 April 2004).

## RESULTS

### Nest Structure

In 2002, seven holes from site 2 were colonized (Table 1). We found two nesting species: *A. sikhimensis* and *Ancistrocerus* sp. (gr. *parietinus*) (Hymenoptera: Eumenidae). Six out of 7 nests were colonized by *A. sikhimensis*. Since they were all closed by a final plug of mud, they should be considered as completed nests. The number of cells

TABLE 1. NEST STRUCTURE, BROOD CELL CONTENTS, AND REPRODUCTIVE SUCCESS OF COLONIZED NESTS AT KANGYUMA IN 2002.

Nest	Ø hole (mm)	Tunnel length (mm)	N cells	N ♀♀/nest	N parasites	Rate of parasitism by <i>Chrysis</i> sp. aff. <i>Coelestina</i>
894	4	68	2	1*	1	50.0
829	10	90	5	0	2	100.0
879	6	80	4	4	0	0.0
762	6	80	7	7	0	0.0
750	6	80	6	4	2	33.3
844	6	80	3	1	2**	66.6
775	6	80	1	0	1	100.0

\*Larva (undetermined sex); \*\*1 cocoon. Nest 775 was colonized by *Ancistrocerus* sp., the other nests by *A. sikhimensis*. Determination was based upon new born emergence, but female owners of nests 894 and 775 were captured in the field.

per nest was very variable ( $\bar{x} = 4.14 \pm 1.87$ ; range = 2–7), but this value does not seem to be related to the length of the cavity (Table 1). Apart from the vestibular cells (*sensu* Krombein, 1967), in all the other cells we found a wasp or a parasite at the stage of adult, pupa, or larva. All the eumenid individuals collected were females ( $n = 16$ ), such that for each nest ( $n = 4$ ), the sex-ratio was 0 ♂♂: 1 ♀♀ (Table 1). Head width of females ranged from 2.55 mm to 3.00 mm ( $\bar{x} = 2.76 \pm 0.14$ ;  $n = 16$ ). In two nests no vestibular cells were observed. No empty cells (*sensu* Krombein 1967) were found. The 4 (out of 5) empty cells found in nest 829 were probably used by the females as brood cells, but some eggs or the larvae at very immature stages were already dead from unknown causes. One additional nest (775) was colonized by another species of *Ancistrocerus*.

In all trap-nests the wasps used all the available space and the differences in the number of cells, for nests with equal burrow length, depended on the variable dimensions of the cells and/or the cell mud partitions. On average, both the cell lengths and the cell partition thicknesses were correlated with their order of construction (Table 2) (Spearman rank correlation;  $r_{\text{cells}} = 0.92$ ,  $r_{\text{cell partitions}} = 0.98$ ;  $n = 8$ ;  $P < 0.05$ ): vestibular cells and closing plugs were on average larger than the subsequent cell/cell partition, and the first cell/cell partitions (built by the wasp at the bottom of the nest) were the smallest ones.

In 2003, 14 active nests of *A. sikhimensis* were observed in the field. Only two of them were closed by the wasps, and they contained only larvae. In 8 nests we found adults of *A. sikhimensis*, sometimes associated with larvae. All the adults were females. In 6 other nests (included the two sealed ones) we found only dead larvae. In 4 nests we found also dry Lepidoptera larvae. In 2003, the observation of the activity of *A. sikhimensis* over six days, for a total of 10 h, gave the following results: (a) the wasps left the nest without any orientation flight (100%;  $n = 36$ ); (b) a collected prey was a larva of Lepidoptera (Glyptopterigidae); (c) the duration of provisioning flights ( $\bar{x} = 486 \text{ sec} \pm 524$ ) was not different from that of non-provisioning ones ( $\bar{x} = 704 \text{ sec} \pm 729$ ; Mann-Whitney Test;  $n_{1_{\text{pf}}} = 8$ ;  $n_{2_{\text{app}}} = 20$ ; n.s.); d) the time spent in the nest after a provisioning flight ( $\bar{x} = 347 \text{ sec} \pm 880$ ) was not different from that spent inside the nest after a non-provisioning flight ( $\bar{x} = 295 \text{ sec} \pm 405$ ; Mann-Whitney Test;  $n_{1_{\text{pf}}} = 11$ ;  $n_{2_{\text{npf}}} = 21$ ; n.s.).

#### Associates

Nest associates include Hymenoptera Chrysidae, and Acarina Saproglyphidae. Cuckoo wasps belong to two species, *Chrysis* sp. aff. *coelestina* Klug (found in 2002) and *Chrysis violenta ultramonticola* Linsenmaier (3 specimens found in 2003 in one nest containing even one specimen of *A. sikhimensis*). The rate of parasitism due to

TABLE 2. LENGTH (MM) OF CELLS AND CELL PARTITIONS IN *A. SIKHIMENSIS* NESTS (2002).

	Cp	Vc	7°P	7°C	6°P	6°C	5°P	5°C	4°P	4°C	3°P	3°C	2°P	2°C	1°P	1°C
$\bar{x}$	2.8	26.0	2.3	15.7	2.0	12.5	2.0	10.4	1.5	9.0	1.3	10.0	1.0	10.0	1	8
SD	1.3	14.5	1.5	7.1	0.9	4.9	0.7	3.2	0.6	2.9	0.6	2.0	0.0	0.0	—	—
$n$	5	5	4	6	6	6	5	5	4	4	3	3	2	2	1	1
Range	1-4	15-24	1-4	6-26	1-3	5-19	1-3	6-15	1-2	5-12	1-2	8-12	—	—	—	—

Cp = Closing plug; Vc = Vestibular cell; P = cell partition; C = brood cell.

*Chrysis* sp. aff. *coelestina*, calculated for *A. sikimensis*, ranged from 0% to 100%, and in general was high ( $\bar{x} = 41.65\%$ ;  $n = 6$ ) (Table 1). All specimens were females. Head width ranged from 2.00 mm to 2.65 mm ( $\bar{x} = 2.35 \pm 0.20$ ;  $n = 7$ ). On 15 out of 17 *A. sikimensis* females collected from the 2002 nests we found individuals of the mite *Vespacarus* sp. (Acarina: Saprolyphidae). The mites were located mainly on the dorsal side of the gaster of the wasps, between tergites I and II. Six individuals had a few mites on other parts of the body (eyes, wings, legs, neck, and thorax). All the mites were at the hypopi stage, the typical resting stage that these arachnids have evolved to face adverse conditions and as an efficient dispersal phase (Walter & Proctor 1999). The number of mites per wasp varied from 2 to 97, but these values should be considered underestimated because of the difficulty involved in counting all the specimens deeper in the abdominal tergites. We did not observe mites on the females emerging from the 2003 nests.

## DISCUSSION

### Nest Structure

As many other *Ancistrocerus* species, including the closely related *Ancistrocerus parietum* L. (Nielsen 1932; Krombein 1967), *A. sikimensis* nests in pre-existing cavities. Although we did not find empty cells (*sensu* Krombein 1967), they have been reported in some species of *Ancistrocerus*, and they are probably constructed to better defend the brood from parasites (e.g. Krombein 1967). A shifted sex-ratio, female-biased in summer, has been recorded for other species of *Ancistrocerus*, probably to face the mortality due to cold winter climatic conditions, as assumed by other authors (Fye 1965; Longair 1981). In fact we do not know the influence of monsoon in sex allocation in this wasp. Moreover, considering a partial bivoltinism model (Seger 1983), we could expect that males live longer than females (males mate with females of their own and next generation), resulting in a female-bias. More data should be obtained to clarify sex-ratio dynamics in this species.

### Associates

Bonelli (1969) recorded *Chrysis ruddii* Shuckard for *A. oviventris*; and Alfken (1914), Van Lith (1953), Nielsen (1932) and Micheli (1930) recorded *Chrysis ignita* L. for *A. parietinus*, *A. antilope*, *Ancistrocerus nigricornis* Curtis, and *A. oviventris*. *Chrysis coeruleans* Fabricius and *Chrysis nitidula* Fabricius were recorded as parasites of *A. antilope* and *Ancistrocerus catskill* *catskill* (Saussure) (Cooper 1953; Hobbs et al. 1961; Medler 1964; Fye 1965; Krombein 1967). *Chrysis inflata* Aaron was found as a parasite of *Ancistro-*

*cerus durangoensis* Cameron and *Ancistrocerus tuberculiceps* *tuberculiceps* (Saussure) (Krombein 1967). *Chrysis* sp. aff. *coelestina* and *Chrysis violenta ultramonticola* are the first parasites recorded for *A. sikimensis*.

Mutualistic associations between saprolyphid mites and solitary wasps are known for different species: e.g., *Vidia concellaria* Cooreman and *Cerceris arenaria* L. (Crabronidae), *Vidia cooremani* Thomas and *Ectemnius* sp. (Crabronidae), several *Vidia* Oudemans, *Macroharpa* Mostafa, *Zethacarus* Mostafa, *Calvolia* Oudemans species and *Zethus* spp. (Eumenidae) (Cooreman & Crèvecoeur 1948; Baker 1964; Mostafa 1970). Okabe & Makino (2003) reported an association between *Kurosaia jiju* Okabe & O'Conner and *Anterhynchium flavomarginatum mikado* (Smith), where mites display an alternation of parasitic and saprophagous phases during their life cycle on the host. In many cases the transmission of mites from one individual to another is known to be venereal, because hypopi enter the genital chamber of the female host during copulation of the wasps (Cooper 1955; Okabe & Makino 2003).

For the genus *Ancistrocerus*, associations are known between some species with *Kennethiella trisetosa* (Cooreman) and *Ensliniella trisetosa* Vitz. (Cooper 1955; Baker & Cunliffe 1960; Krombein 1961, 1967; Cowan 1984). The genus *Vespacarus* Baker, as far as we know, is associated only with *Ancistrocerus catskill* *catskill* and *Ancistrocerus tigris tigris* (Saussure) (now *Ancistrocerus adiabatus* (Saussure)) (Krombein 1967; *Vespacarus tigris* Baker and Cunliffe). Contrary to what we observed in our wasp-mite association, in most *Parancistrocerus* species the hypopi of *Vespacarus* are located in an acarinarium between tergites I and II of the abdomen (Krombein 1967). *Ancistrocerus adiabatus* does not have a true acarinarium and hypopi simply cluster in transversal series under some posterior abdominal tergites (Krombein 1967). This species seems to provide an intermediate step toward species with a true acarinarium. This structure evolved in some hymenopteran species that have mutualistic relationships with saprolyphid mites (Bequaert 1918; Giordani Soika 1985), possibly to increase the number of host mites or, maybe, to keep them in a fixed position of the body. However, the maximum (underestimated) number of 97 mites that we have obtained from a single wasp is close to the maximum load of mites (118) found by Krombein (1967) in the acarinarium of *Stenodynerus* (*Parancistrocerus*) *f. fulvipes* (Saussure). Clusters of *Kennethiella trisetosa* have been found on different parts of the body of *A. antilope*, such as the right side of the propodeum, the thorax, or on the back of the propodeum (Cooper 1955; Cowan 1984). Mites have been also found exclusively on the ventral surface of the thoracic segments of the host, although rarely on the head or the lateral

sides of the thorax (Okabe & Makino 2003). This would confirm the notion that different species of mite choose a specific part of the host body. On the other hand it seems that acarinaria of different wasp species, whenever they exist as in *Parancistrocerus*, are slightly differently shaped, possibly related to the specific mites they must house (Krombein, 1967). We collected specimens of *Vespacarus* sp. on females, but most studies have reported the presence of mites typically only on males (Cooper 1955; Krombein 1961; Pekkarinen & Huldén 1991). It has been proposed (Cooper 1955) that hypopi may be unable to infest the females' bodies because the female larvae eat the adult mites, while the male larvae allow the mites to live on them. Our observation demonstrate that this is not the only possible conclusion, and that probably other factors affect the survival of the mites in the wasp's nest cells of both sexes. In any case, owing to the absence of *A. sikhimensis* males in our nests, we do not know the extent of their infestation by part of *Vespacarus*.

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## A NEW SPECIES OF FLIGHTLESS PYGMY MOLE CRICKET FROM A FLORIDA SAND RIDGE (ORTHOPTERA: TRIDACTYLIDAE)

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### ABSTRACT

A new species of Tridactylidae, *Ellipes eisneri*, is described from the Brooksville Ridge in Florida. This is the second species of flightless tridactylid that is known to inhabit deep, well drained sand formations in Florida. It is associated with an algal layer occurring a few mm beneath the surface. When not feeding near the surface, *E. eisneri* retreats down vertical burrows. The range of the species is not well known, but it could be restricted to the Brooksville Ridge. The habits of this species are convergent with those of the previously described *Neotridactylus archboldi* Deyrup & Eisner. There is a good chance that there are additional species of upland pygmy mole crickets in other sandy areas of southeastern North America.

**Key Words:** *Ellipes*, *Neotridactylus*, Florida endemics, soil crusts, Brooksville Ridge, pygmy mole cricket

### RESUMEN

Se describe una nueva especie de Tridactylidae, *Ellipes eisneri*, del Brooksville Ridge en el estado de Florida. Este es la segunda especie conocida de un tridactílido no volante que habita las formaciones de arena profunda y con amplio drenaje en Florida. Es asociada con la capa de algas que ocurre unos pocos mm debajo de la superficie. Cuando no esta alimentandose sobre la superficie, *E. eisneri* regresa por los tuneles verticales. No se conoce bien el rango geográfico de esta especie, pero puede estar restringido al Brooksville Ridge. Los hábitos de esta especie están convergentes con los de *Neotridactylus archboldi* Deyrup & Eisner, una especie descrita anteriormente. Hay una buena posibilidad de que existen otras especies de tridactílidos en las áreas arenosas del sureste de América del Norte.

The pygmy mole crickets (Tridactylidae) are small (usually 12 mm long or less) burrowers, usually found in sandy habitats. They superficially resemble miniature versions of mole crickets (Gryllotalpidae), but the two groups differ in many ways, and are not at all closely related. Pygmy mole crickets are not members of the Ensifera, the lineage that includes the crickets (Gryllidae) and katydids (Tettigoniidae), but rather an early offshoot of the Caelifera, the lineage that includes the grasshoppers (Acrididae) and pygmy grouse locusts (Tetrigidae) (Blackith 1987). Blackith (1987) suggests that the antiquity of both the Tridactylidae and the Tetrigidae is correlated with their specialization as algal feeders, since algae are an ancient and conservative group that appear to present special challenges. Some of these challenges might be nutritional deficiency, defensive compounds, the problem of harvesting sufficient quantities of algae, and the tendency of algae to dry up and disappear in non-saturated habitats. The latter problem generally restricts pygmy mole crickets to wet habitats, such as rain forests, swamps and marshes, and the edges of streams, lakes, or standing water. Most species have well developed wings that allow individuals to disperse if their habitat becomes too dry. Species that live around water usually have enlarged

setae modified as flattened "swimming plates" on their hind tibiae, or dense rows of swimming hairs on the enlarged hind tibial spurs.

In the sandy uplands of Florida evolution has allowed pygmy mole crickets to colonize habitats that are dry for much of the year. These habitats are Florida scrub, high pine, and scrubby flatwoods, all of which were primevally maintained by fires started by lightning. These fires in the natural ecosystems appear to have had frequencies of roughly 5-50 years. For more detail on these fire-structured habitats, see Myers & Ewel (1990). A consequence of these relatively frequent fires is that there are usually patches of bare sand here and there among the trees, shrubs, and herbaceous vegetation. Light easily penetrates the upper layers of the silica sand of these bare patches. At a depth of 3-4 mm there may be a subsurface soil crust, composed of algae, cyanobacteria, lichens, fungi, mosses, and bacteria (Hawkes & Flechter 2002). In upland habitats of the Lake Wales Ridge of peninsular Florida, this crust sustains a species of pygmy mole cricket, *Neotridactylus archboldi* Deyrup & Eisner (Deyrup & Eisner 1996). The gut of *N. archboldi* has been shown to contain filamentous algae (Deyrup & Eisner 1996). This species is flightless; its migrations are short and vertical: down into the sand during dry periods, up to the

soil crust after a series of rains. *Neotridactylus archboldi* was found on the Lake Wales Ridge, a fossil sand dune area extending down the center of Florida through Lake, Polk, and Highlands Counties. It seemed reasonable to expect that other sandy uplands would also have pygmy mole crickets. Moreover, there are examples of endemic flightless insects restricted to ridges in peninsular Florida (Deyrup 1990, 1996), and it seemed possible that various isolated upland areas might have their own species of pygmy mole crickets. My search for more pygmy mole crickets has not been very intensive, and has sometimes been thwarted by a series of droughts that have kept the mole crickets deep underground. Most of the specimens that I have seen from sites off the Lake Wales Ridge resemble *N. archboldi*, but it is always possible that these are cryptic species. I did find, however, a series of specimens that clearly represent an undescribed species on the Brooksville Ridge, a large ridge that extends north and south through Hernando, Pasco, Citrus, and Levy Counties. This species belongs to the genus *Ellipes* rather than *Neotridactylus*.

A key to the three New World genera of Tridactylidae is provided by Gunther (1975). *Ellipes* is characterized by the extreme reduction of the hind tarsi, which are represented by a minute flap concealed between the huge hind tibial spurs (Fig. 2A). *Ellipes* also lacks the prosternal spur found in *Neotridactylus*.

#### *Ellipes eisneri*, new species

Description: Holotype male. Measurements in mm: length of head and body: 3.30; length of pronotum: 0.75; width of pronotum: 1.02; width of head across eyes: 0.75; length of hind femur: 1.92; length of hind tibia: 1.25; length of forewing: 0.72. Coloration: background color of a fresh specimen pale salmon; head blackish brown with narrow cream line along inner orbits and coronal and frontal sutures; antennae cream, terminal segments tinged with brown; pronotum with an irregular median light brown transverse band, interrupted medially; front legs pale cream, almost white; middle legs cream with dark markings as in Fig. 1; hind femora pale salmon, with a small basal brown chevron, a median transverse brown band, a brown dorsal subapical wedge, apical crescents of hind femora reddish brown, hind tibiae and tarsi cream; wings blackish brown basally, color more dilute apically, anal area pale; tergites 5-8 with irregular brown median spots; basal segment of cerci black, apical segment white; ventral cercus-like organ (*Paraproctfortsatz* of Gunther 1977) white.

Structural character states: antennae 10-segmented; front tibia with 4 teeth (Fig. 2B); swimming plates of hind tibiae absent; hind tibial spurs with dorsal rows of well-separated fine

hairs, not dense rows of flattened hairs (Fig. 2A); scraper present on the underside of the forewing, forewing abbreviated, hind wings absent.

Locality of holotype male (as on specimen label, except for abbreviations): FLORIDA: Citrus County; near Inverness; Withlacoochee Forest, Citrus Area; Forest Road 13, 1.3 mi. south of State Road 44; sandhill habitat with bare sand; dug from vertical burrow. 3 April 1995. M. Deyrup, collector.

Deposition of holotype male: Florida State Collection of Arthropods, Gainesville, FL. Description: Allotype female. Measurements in mm: length of head and body: 3.55; length of pronotum: 0.87; width of pronotum: 1.15; width of head across eyes: 0.87; Length of hind femur: 2.05; length of hind tibia: 1.47; length of forewing: 0.75. Coloration: as in holotype, except: pronotal brown band not interrupted medially; middle femora with a dark stripe connecting the median and sub-apical bands, delimiting an irregular pale rectangle; apical crescents of hind femora pale brown, lighter than femoral maculations; hind tibiae pale brown, pale at base. Structural characters: similar to male, including 10 segmented antennae, except stridulatory apparatus absent on wing.

Locality, site, date, collector of allotype female same as holotype male.

Deposition of allotype female: same as for holotype male.

Paratypes: 4 males, 3 females: same locality, site, date, collector as holotype; dry pinned specimens. 2 males, 10 females: FLORIDA: Citrus County; "Pine Oak Estates;" State Road 488, 3.7 mi. south of junction with U.S. 41; sandhill area with bare sand. 4 April 1996. M. Deyrup, collector. Specimens individually in vials of alcohol.

Deposition of paratypes: 1 male, 1 female (dry): Florida State Collection of Arthropods, Gainesville, FL. 1 male, 1 female (dry): Philadelphia Academy of Sciences, Pennsylvania. Remaining type material temporarily deposited in the arthropod collection of the Archbold Biological Station, Lake Placid, FL.

Diagnosis: Differs from other known *Ellipes* in being flightless (forewings abbreviated, hind wings absent), its pale salmon and brown coloration, and its occurrence in xeric habitats. The antennae have 10 segments in both sexes.

Etymology: This new species is named in honor of Dr. Thomas Eisner, in gratitude for his many studies on the natural history of Florida arthropods, including another species of pygmy mole cricket, *Neotridactylus archboldi*. The scientific work of Tom Eisner is remarkable for its scope, creativity, and sheer volume, but beyond the science he has always cheerfully confessed that a large part of his inspiration comes from aesthetics: the beauty of arthropods, the elegance of their design. It is hoped that he will find *Ellipes eisneri* a beautiful insect.

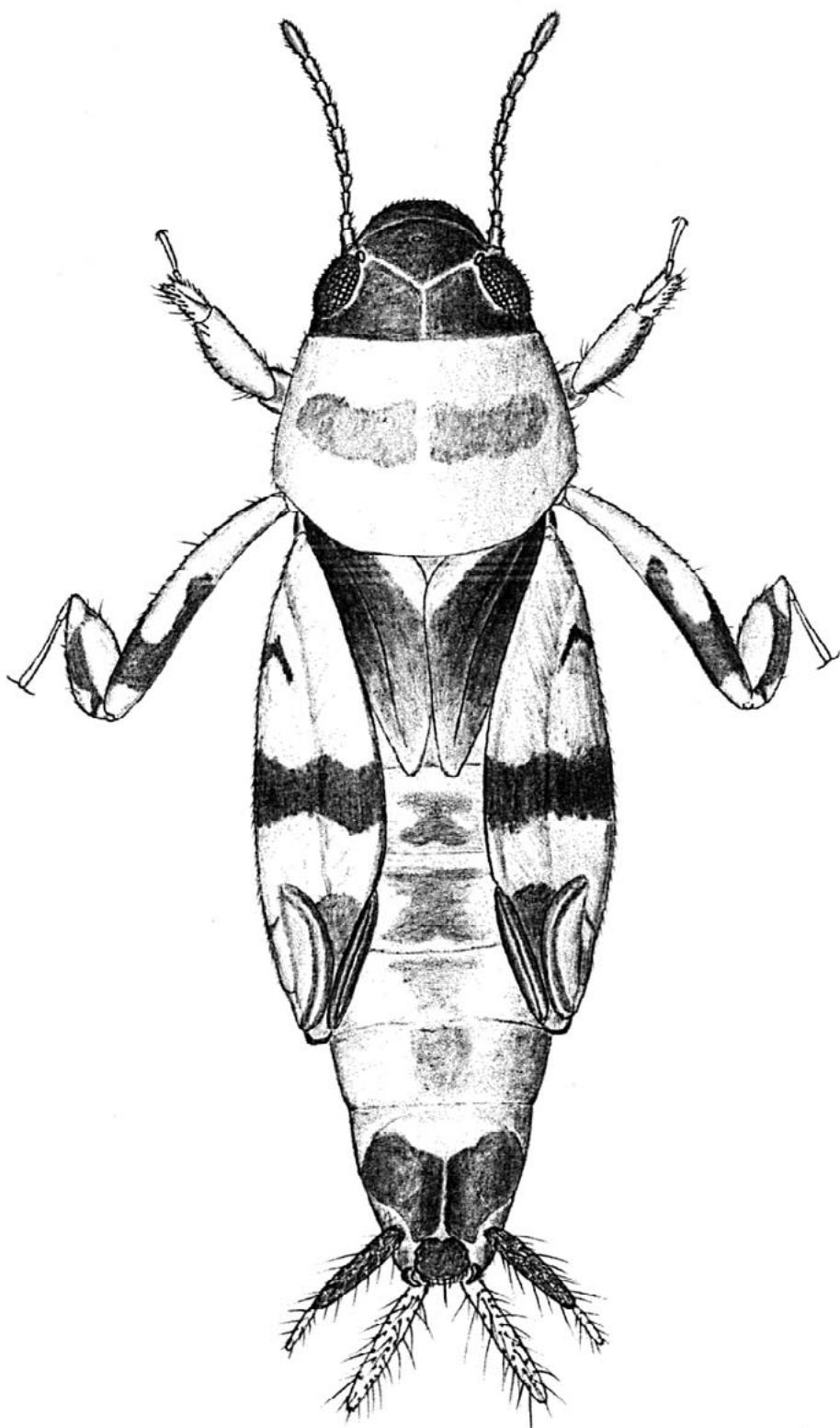


Fig. 1. *Ellipes eisneri*, new species, male, dorsal habitus. Markings matched to live specimen. Extent of maculae on pronotum and tergites highly variable in this species; in some specimens they are lacking.

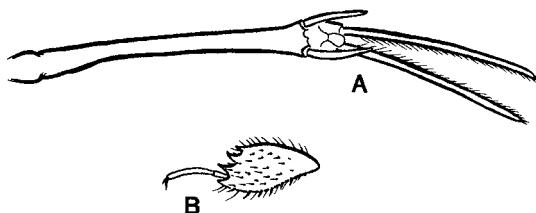


Fig. 2. *Ellipes eisneri*, new species. A. Dorsal view of right metatibia; small oval between the apical tibial spurs is the vestigial metatarsus. B. Lateral external view of left protibia and protarsus.

In keeping with the long tradition of Blatchley (1920), Helfer (1953) and Capinera et al. (2001), I am assigning an informal common name to *Ellipes eisneri*, "Eisner's Pygmy Mole Cricket." Not all orthopterists retain the old name, "pygmy mole crickets" for the Tridactylidae, because these insects are not crickets. Some papers (e.g., Gunther 1985) use the name "pygmy mole grasshoppers." There is, however, no rule that vernacular names must be phylogenetically correct, rather than being based on correlates of habitus or ecology, as in the "naked mole rats." Even from a phyletic standpoint, it is a dubious advance to put "grass" in the name of a lineage whose evolutionary and trophic divergence may well antedate the grasses. There is also the name "false mole crickets" (Naskrecki 2001), but this remains ambiguous (a false mole cricket could still be a cricket), and is annoyingly negative.

**Habitat:** All specimens were found in open sandhill habitats with a sparse cover of grasses, especially *Aristida beyrichiana* Trin. & Rupr., and various herbs: *Pityopsis graminifolia* (Michx.) Nutt., *Polygonella robusta* (Small) Horton, *Paronychia* sp., and *Baldina angustifolia* (Pursh) Robinson. There were scattered trees of *Pinus palustris* Mill., *Quercus laevis* Walt., *Q. incana* Bartr. and small clonal groups of *Q. geminata* Small. The openings where pygmy mole crickets occurred had a dark gray soil crust a few millimeters below the surface. The sand was a yellow entisol with no visible horizon in the top 25 cm. The specimens from the type locality (Withlacoochee State Forest) were collected from Candler fine sand, according to maps of the Citrus County soil survey (Pilny et al. 1988). This is an excessively drained, nutrient-poor, moderately to strongly acid, fine sand occurring on sites where the water table is at least 2 m below the surface throughout the year; the surface layer is dark grayish brown, the subsurface layer pale brown or yellowish brown (Pilny et al. 1988). The paratypes from the Pine-Oak Estates site were in an area mapped as Astatula fine sand, whose characteristics are very similar to Candler fine sand, but the subsurface layer tends to be yellow or orangish yellow (Pilny

et al. 1988). Attention to soil type, even among soils that are almost exclusively sand, may be important for understanding the distribution of burrowing arthropods. Halloran et al. (2000) provide evidence that Astatula fine sand is less stable than Satellite sand for burrow construction by *Geolycosa* spiders and *Myrmeleon* antlions.

#### COMMENTS

The discovery of this new species hints that there may be additional species of upland pygmy mole crickets still to be found. *Ellipes eisneri* and *Neotridactylus archboldi* are a pair of species that must have evolved from flying, semiaquatic lineages in their respective genera. This seems likely because all the genera of Tridactylidae around the world, not just *Ellipes* and *Neotridactylus*, are composed of species that can fly and occur in wet areas, so these are probably characteristics of early Tridactylidae before the appearance of the various synapomorphies that define the genera. Now that we know of two lineages that have made the transition to exploiting soil crusts of sandy uplands, it seems possible that there are additional lineages that have made this same transition, especially since one of the associated adaptive character states (loss of flying ability) tends to isolate lineages.

Several areas in the southeastern U.S. have ancient sand deposits with commonly occurring patches of open sand. Any of these areas might have concealed pygmy mole crickets. The insects can be found by searching for their trails just after a rain, as described by Deyrup & Eisner (1996). Most specimens of *E. eisneri* were obtained a few days after a rain, based on a more difficult clue: the small dark tumulus of subsurface sand left by the insect as it retreats down a vertical burrow after the surface sand dries out. If the sand is dry near the surface, but damp a few centimeters down, it may be possible to lure pygmy mole crickets up to the surface by watering with a watering can. A gallon of water on a square meter works well for *N. archboldi*, the subsurface foraging trails appearing in an hour or so after watering. Because the main obstacle to finding upland tridactylids is the need to be at the right place at the right time, surveys for new species or new locality records would be good projects for local naturalists or students in an ecology class. Even if no pygmy mole crickets are found, the time spent searching for them might not be wasted. There are many other interesting arthropods burrowing just below the surface of the sand, such as blind, flightless scarabs of the genus *Geopsammodius* (formerly placed in the genus *Psammodius*) (Deyrup & Woodruff 1991).

The geographic range of *E. eisneri* is unknown, but it has not yet been found co-occurring with *N. archboldi*, or specimens that appear to be

*N. archboldi*, on the Lake Wales Ridge, the Northern Mount Dora Ridge, the Atlantic Coastal Ridge, and the Trail Ridge. It is possible that *E. eisneri* is restricted to the Brooksville Ridge, which has an endemic grasshopper, *Melanoplus withlacoocheensis* Squitier & Deyrup (Squitier et al. 1998), a genetically distinct population of the gopher tortoise (Osentoski & Lamb 1995), and a morphologically distinguishable population of a species of ant, *Dorymyrmex elegans* (Trager). It would be premature to worry about the conservation status of *E. eisneri*, but the fact remains that there is only one protected site known for the species, and that site is only protected as long as management of the Withlacoochee State Forest continues to hold to its current enlightened goal of maintaining natural habitats and natural biological diversity in the forest. It would be appropriate, therefore, to sample more widely on the Brooksville Ridge during a period of wet weather and map the range of *E. eisneri* to determine whether it is a rare or endangered species.

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## NECTAR SOURCES FOR *LARRA BICOLOR* (HYMENOPTERA: SPHECIDAE), A PARASITOID OF *SCAPTERISCUS* MOLE CRICKETS (ORTHOPTERA: GRYLLOTALPIDAE), IN NORTHERN FLORIDA

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### ABSTRACT

*Larra bicolor* (F.) (Hymenoptera: Sphecidae) is an introduced biological control agent of pest *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae) in northern Florida. The pests are of southern South American origin. *Larra bicolor* is widespread in South America; the imported stock is from Bolivia. Its adults seem to require nectar sources. In South America, Puerto Rico (where it was also introduced from Brazil), and southern Florida (a separate introduction from Puerto Rico), the neotropical wildflower *Spermacoce verticillata* L. (Rubiaceae) has been observed to be a favored nectar source. In northern Florida (29°N) this wildflower is uncommon, freezes to the ground at first winter frost, and does not flower again until April-May. Nevertheless, where it has been planted in northern Florida, the wasps feed on it throughout the warmer months. Wasps were observed to feed at nectaries of 10 other plant species in northern Florida. Four of these other plants were compared experimentally with *S. verticillata*, but all received fewer visits from the wasps. Known disadvantages to the use of *S. verticillata* to augment *L. bicolor* are that it is not native to Florida, and that it grows vigorously in full sun when its roots are not immersed in water. It has been reported as a minor weed in southern Florida. However, it is the best alternative to attract *L. bicolor* to places where mole cricket control is needed.

**Key Words:** Nectar-feeding, nectar source, biocontrol, wasp-gardening, butterfly-gardening, turfgrass, *Larra bicolor*, mole crickets

### RESUMEN

*Larra bicolor* (F.) (Hymenoptera: Sphecidae) es un agente de control biológico de grillotopos del género *Scapteriscus* (Orthoptera: Gryllotalpidae) en el norte de la Florida. Esta plaga es originaria de Sur América. *Larra bicolor* se encuentra en varias partes de Sur América; las avispas que se encuentran al norte de la Florida se importaron desde Bolivia. En Puerto Rico (la cual se introdujo desde Brasil), y en el sur de la Florida (introducida desde Puerto Rico), *Spermacoce verticillata* L. (Rubiaceae), una planta silvestre neotropical ha sido la principal fuente de néctar para adultos de esta avispa. En el norte de la Florida (29°N) esta flor es comúnmente encontrada, se congela en el invierno con la primera helada y comienza a florecer nuevamente en abril o mayo. No obstante, la avispa se alimenta de esta flor en el norte de la Florida durante los meses calidos. La avispa fue observada alimentándose de los nectarios de otras 10 especies de plantas en el norte de la Florida. Cuatro de estas plantas fueron experimentalmente comparadas con *S. verticillata*, pero todas recibieron menos visitas de esta avispa. Algunos argumentos en contra del uso de *S. verticillata* para aumentar las poblaciones de *L. bicolor* son: la planta no es nativa a la Florida, crece vigorosamente bajo exposición total al sol siempre y cuando las raíces no se encuentren bajo el agua y se ha reportado como una maleza menor en el sur de la Florida. Sin embargo, esta planta es la mejor alternativa para atraer *L. bicolor* a lugares donde el control de grillotopos es necesario.

Translation provided by the authors.

*Larra bicolor* (F.) is a koinobiont ectoparasitoid of *Scapteriscus* spp. mole crickets in its native range in South America (Menke 1992). In 1936-1938 stock was imported from Belém, Pará, Brazil, to Puerto Rico, and established as a classical biological control agent of *S. didactylus* (Latrelle) (Wolcott 1938, 1941a). In 1981, stock was imported from Puerto Rico by J. A. Reinert and established at Ft. Lauderdale, Florida, as a classical biological control agent of *S. abbreviatus* Scudder, *S. borellii* Giglio-Tos, and *S. vicinus*

Scudder, all pests of South American origin (Sailer 1985). Stock of the same species was imported in 1988-89 from Santa Cruz, Bolivia, released and became established in and near Gainesville in northern Florida (Frank et al. 1995). The population established at the first Ft. Lauderdale site spread no more than 3 km, and attempts to redistribute it failed (Castner 1988). The stock established at Gainesville spread naturally, and has now been recorded in many counties in northern Florida, to a distance of >220 km

NW and S (J.H. Frank, unpublished). It was assumed simply that stock obtained from Bolivia was a more cold-hardy biotype of *L. bicolor* (Frank et al. 1995) because Menke (1992) could not distinguish them at the species level from the Belém/ Puerto Rico stock by morphological methods. The possibility of cryptic species has not yet been investigated. However, Menke (1992) observed and illustrated what he believed to be intraspecific variation in punctuation of the head capsule of the adults of *L. bicolor* from Belém/Puerto Rico and those from Bolivia. The *L. bicolor* established in northern Florida has the punctuation of the latter stock (Frank et al. 1995).

Because of its assumed cold-hardiness, it seems likely that the Bolivian stock will spread far more widely in northern Florida. Knowledge of the nectar sources of *L. bicolor* is needed to devise methods of improving the rate of spread both state-wide and locally. Encouraging the establishment of plants that serve as nectar sources (wasp-gardening) could be used to enhance wasp populations, as has been done to manage other biological control agents (Jervis 1988; Jervis & Kidd 1996).

Wolcott (1941a) collected *L. bicolor* adults from flowers of *Spermacoce verticillata* L. (Rubiaceae) in Belém, and imported them into Puerto Rico, where this plant also grows (Liogier 1980). Wolcott (1941b) considered *S. spermacoce* essential to the survival of *L. bicolor* in Puerto Rico. In Florida, all sites where *L. bicolor* was released were prepared in advance with plantings of *S. verticillata*, which was already widespread in southern Florida, but very sparse farther north, reported only in Alachua and St. Johns counties (Wunderlin 1979, 1998). Wolcott (1941b) also mentioned *Hyptis atrorubens* Poit. (Lamiaceae) as a nectar source for *L. bicolor* in Brazil and Puerto Rico. This plant is not established in Florida (Wunderlin 1998). In southern Florida, Castner (1988) observed that *S. verticillata* outperformed various native and ornamental plants in attracting adult *L. bicolor* of the Belém/Puerto Rico stock.

This paper reports research to explore several questions regarding nectar sources of *L. bicolor* in northern Florida. Does this plant have weedy characteristics? What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida? What other plant species may be used to encourage local buildup of *L. bicolor* populations? How does *L. bicolor* access nectar from *S. verticillata*, and can it do the same from other plant species?

Wunderlin (1998) states that *S. verticillata* is not native to Florida, but is native to the Neotropical region, including Cuba, Haiti, Jamaica, Puerto Rico, and the Bahamas. It was not detected as established in Florida until the 1960s (B. F. Hansen, pers. comm.).

In southern Florida *S. verticillata* flowers all year (Bryan Steinberg, pers. comm.). However, in

northern Florida it does not flower all year; in Gainesville (29°N) it freezes to the ground at the first frost (typically in early December), and does not flower again until late April or early May of the following year (JHF, observations). This limits its availability as a nectar source in northern Florida. The limitation is of little consequence for *Larra bicolor*, whose pupae diapause underground in winter, and whose adults have been observed to be killed by frost (Cabrera-Mireles 2000).

*Spermacoce verticillata* contains a low level (0.2%) of alkaloids which would be toxic if present in higher concentrations, but it serves as a non-preferred forage plant for cattle (Francis 2002 and references therein).

## MATERIALS AND METHODS

### Movement of Plants and Seeds (Weediness)

In 1990, one of us (JHF) planted a plot of *S. verticillata* plants (obtained from roadside waste land in Miami) in the grounds of the Entomology & Nematology Department, University of Florida and, in 1997-1998 planted five other plots (progeny of the first plot) on University of Florida property in the Gainesville area. These five were all planted by the same method; each had 25-26 plants installed in a single line, on 60 cm centers through a 2.6 × 16 m sheet of black polyethylene, 0.15 mm thick. Plantings were variously destroyed by prolonged flooding, maintenance crews, or a construction crew, so not all were constantly available. In 2000, a seventh was planted without mulch and in several rows by USDA collaborators at the USDA, Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) garden. The seven plots were installed to allow study of the seasonality of the plant and the wasp in northern Florida, as well as to harvest wasps for distribution to distant localities. Collaborators were recruited to monitor for presence of wasps in distant counties in 2002 and 2003, and we supplied them with some of the plants. We know of no easier way of observing and collecting the wasps than their attraction to *S. verticillata* flowers, although they can be collected at traps baited with phenylacetaldehyde (Meagher & Frank 1998).

What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida?

Three of the *Spermacoce verticillata* plots installed in 1997-1998 in the Gainesville, FL area were used for this 2001 study. They were at the Beef Cattle Research Unit, the Horse Teaching Unit, and the Fisheries and Aquatic Sciences Department. A fourth, at the USDA-CMAVE garden had many more plants, in several rows, without plastic mulch. The plots constantly (during the

warmer daylight hours) had feeding adult *L. bicolor* in September–November 2001.

Once every two weeks in September–November 2001, one of us (HAA) walked transects in the four cardinal and four secondary compass directions away from those four plots, until he was impeded by structures (buildings, fences, roads) or water bodies. Plants on which adult *L. bicolor* were seen feeding were identified and recorded. No attempt was made to analyze frequency of wasp-feeding observations because the observations were not random. The sole purpose was to compile a list of the plant species other than *S. verticillata* on which one or more *L. bicolor* adults were observed feeding in areas where *S. verticillata* maintained a wasp population.

What plant species may be used to encourage local buildup of *L. bicolor* populations?

Sites used were the Beef Cattle Research Unit, the G. C. Horn Turfgrass Research Laboratory, the Plant Sciences Unit at Citra, and the USDA-CMAVE garden. Four of the plant species identified as providing nectar to *L. bicolor* (*Conoclinium coelestinum* (L.) DC, *Elephantopus elatus* Bertol., *Passiflora coccinea* Aubl., and *Solidago fistulosa* Mill., see below) were available from local nurseries, and 32 of each were purchased in pots. Plants of *S. verticillata* were already in culture. All were planted in a completely randomized block design with four blocks. Each block was adjacent to one of the existing plots of *S. verticillata* to ensure that *L. bicolor* adults were present. The plants were removed from pots, planted through cuts made through a sheet of black polyethylene in the required block design, and watered daily for 5 days. Each plant was again watered once each 15 d with about 0.4 L of a 0.4% N10-P52-K10 fertilizer solution/suspension to promote flowering. Each of the blocks had five treatments (plant species), with eight plants per treatment, with each treatment in two lines of four. The separation between plants was 0.6 m within each treatment, and between treatments was 2.4 m, giving a block size of 92.8 m<sup>2</sup>. Observations were made weekly between late July and early November 2002. Repetitions were at 10, 11, and 12 AM (GMT-5). Data recorded were the total number of adult *L. bicolor* observed, when about 20 s were spent examining each plant. The routine used was for one observer (HAA) to move left to right and clockwise among the treatments, beginning with the plant in the southwest corner of the treatment and of the block. This was a repeated measures experiment with a completely randomized block design. Analysis was made by a  $\chi^2$  pairwise comparison with the least square means (LSM) procedure with one degree of freedom. The data were adjusted to a Poisson distribution for analysis in the SAS (2000) program.

This experimental design was discussed with several researchers before it was put into operation. All recognized that each plant species has a different floral size and architecture, that the number of flowers produced by each plant varies in time, and perhaps nectar production varies within each plant. However, it was the time spent by *L. bicolor* at each plant that was to be compared, so it was not appropriate to try to control for interplant species differences that were inherent in the comparison—they are not flaws in the design. Our methods are described so that readers may accept the results or reject them, or repeat them.

How does *L. bicolor* access nectar from *S. verticillata* and other plants?

Adult *L. bicolor* were observed feeding at nectaries in the field. For 40 flowers of each of the four species (*C. coelestinum*, *E. elatus*, *S. fistulosa*, and *S. verticillata*) having floral nectaries, the distance from the rim of the corolla to the nectaries was measured in the laboratory under a dissecting microscope, as was the length of the glossa of 20 adult male and 20 female wasps.

## RESULTS

### Movement of Seeds and Plants (Weediness)

An unrestrained plot of *S. verticillata*, planted in 1990 on the grounds of the Entomology/Nematology Department, University of Florida, Gainesville, Florida by the end of 2003 had produced infrequent seedlings in adjacent, occasionally-mowed Bahiagrass turf, <1 m to the east, 2 m to the north, 2 m to the south, and  $\approx$  25 m to the southwest. Mowing of adjacent turf was by rotary mower, which, we suspect (1) prevented nearby seedlings from flowering and (2) dispersed seeds around a corner of a building only in a southwest direction and to a much greater distance. In other words, a plot of the plant produced seedlings to a distance of up to 2 m in 13 years, but use of a rotary mower discharged a few seeds up to  $\approx$  25 m in one direction, which was presumably due to the track of the mowing crew. Later plots were established in 1997–1998 through  $\approx 2.6 \times 16$  m sheets of black polyethylene, whose original purpose was to allow establishment of the wildflower without competition from other plants. At the Beef Cattle Research Unit, after almost six years (fall 2003), there was only one seedling plant outside (by 5 cm) the confines of the original plot (outside the edge of the now-damaged plastic mulch). Mowing was done by a tractor-drawn reel mower, which may not have dispersed seeds. At the Horse Teaching Unit (after six years), there was spread by about 1 m to the south in places, but this probably was due to partial redistribution of the plot by a bulldozer moving it from its original line and destroying the

plastic mulch. Evidently *S. verticillata* is not highly 'invasive.' Seedlings it produces in adjacent turf may be controlled by occasional mowing. These are appropriate characteristics for a nectar-source plant for a beneficial insect: it may spread, and, once established, it does not demand constant care for its survival. Furthermore, *S. verticillata* plants installed in 2000 at Tifton, Georgia, were killed outright, by inadvertent application of glyphosate (Roundup®) (W. G. Hudson, pers. comm.), suggesting that the plant is easily controlled by application of this chemical herbicide. In many places, its vigorous growth is desirable.

What other plant species provide useful nectar sources for *L. bicolor* and might these further its range expansion throughout Florida?

*Larra* adults were observed feeding at nectaries of 10 species of plants in addition to *S. verticillata* (Table 1). The number of Florida counties shown by Wunderlin & Hansen (2003) to be occupied by the plant in question is also shown in Table 1.

What other plant species may be used to encourage local buildup of *L. bicolor* populations?

Results of the experiment are shown in Table 2 and Fig. 1. It is clear that *L. bicolor* adults spent much more time at *S. verticillata* plants than at any of the other four tested. We here assume this was due to its superiority as a nectar source. There were significant differences in all pairwise comparisons between plant species except between *P. coccinea* and *S. fistulosa* (where  $P = 0.7232$ ).

How does *L. bicolor* access nectar from *S. verticillata* and other plants?

The length of the glossa in relation to the floral depth is shown in Fig. 2. There was no difference

between length of the glossa of males and females ( $F = 0.20$ ;  $df = 1,19$ ;  $P = 0.6631$ ). For two of the plant species, *S. fistulosa* ( $F = 80.54$ ;  $df = 1,39$ ;  $P < 0.001$ ), *C. coelestinum* ( $F = 81.86$ ;  $df = 1,39$ ;  $P < 0.0001$ ), the floral depth is less than the length of the glossa. In *P. coccinea*, the principal nectaries are extrafloral, and the measurement is irrelevant. The floral depth of *S. verticillata* matches the length of the glossa ( $F = 1.46$ ;  $df = 1, 39$ ;  $P = 0.2341$ ). The floral depth of *E. elatus* seems too great to allow access by the wasp to nectaries ( $F = 498.02$ ;  $df = 1,39$ ;  $P < 0.0001$ ). However, wasps were observed to extend mandibles, push the head into the flower, move the head from side to side, and thus access nectaries with the glossa. The petals are loosened from the corolla and typically fall as the wasp removes its head or leaves the flower.

#### CONCLUSION AND DISCUSSION

Although *S. verticillata* is not native to Florida, it is now widely distributed in the south of the peninsula (Table 1). Its floral nectaries are highly attractive to adult *Larra bicolor* wasps. It flowers, and presumably provides nectar, throughout the year in southern Florida, and for at least seven months of the year near Gainesville (29°N) in northern Florida. No other plant has yet been shown to rival it in Florida or Puerto Rico as an attractant for these wasps. It has potential for use in wasp-gardening, in which it is planted in plots intended to enhance local populations of *Larra bicolor* wasps to help control pest mole crickets. Its planting in areas not yet occupied by the wasp will pave the way for arrival, establishment, and beneficial effects of the wasp. Areas not yet occupied by the wasp are most likely (a) most of southern Florida, in part of which *S. verticillata* already is widespread, and (b) most of the Florida panhandle. Beneficial effects of this wasp may also be experienced in southern Georgia.

TABLE 1. LIST OF PLANTS ON WHICH *LARRA BICOLOR* ADULTS WERE OBSERVED FEEDING IN THE GAINESVILLE, FLORIDA, AREA IN 2001.

Species	Family	Status	Distribution in Florida <sup>1</sup>
<i>Aralia spinosa</i> L.	Araliaceae	native	30 counties
<i>Conoclinium coelestinum</i> (L.) DC	Asteraceae	native	58 counties
<i>Elaphanthopus elatus</i> Bertol.	Asteraceae	native	58 counties
<i>Heliotropum angiospermum</i> Murray	Boraginaceae	native	18 counties
<i>Heliotropum curassavicum</i> L.	Boraginaceae	not native	17 counties
<i>Lobularia maritima</i> (L.) Desv.	Brassicaceae	not native	3 counties
<i>Melilotus albus</i> Medik	Brassicaceae	not native	40 counties
<i>Passiflora coccinea</i> Aubl. <sup>2</sup>	Passifloraceae	not native	3 counties
<i>Richardia brasiliensis</i> Gomes	Rubiaceae	not native	51 counties
<i>Solidago fistulosa</i> Mill.	Asteraceae	native	55 counties
<i>Spermacoce verticillata</i> L.	Rubiaceae	not native	12 counties

<sup>1</sup>From Wunderlin & Hansen 2003.

<sup>2</sup>Observation by Craig Welch, graduate student, Entomology/Nematology Dept., University of Florida. It has extra-floral nectaries on which *L. bicolor* feeds.

TABLE 2. RESULTS OF PAIRWISE COMPARISONS WITH THE  $\chi^2$  TEST TO SHOW FREQUENCY OF FEEDING BY ADULT *L. BICOLOR* AT NECTARIES OF THE FIVE PLANT SPECIES.

Plant species 1	Plant species 2	df	$\chi^2$	Pr > $\chi^2$
<i>C. coelestinum</i>	<i>E. elatus</i>	1,326	30.14	<0.0001
<i>C. coelestinum</i>	<i>P. coccinea</i>	1,326	13.48	0.0002
<i>C. coelestinum</i>	<i>S. fistulosa</i>	1,326	21.57	<0.0001
<i>C. coelestinum</i>	<i>S. verticillata</i>	1,326	178.13	<0.0001
<i>E. elatus</i>	<i>P. coccinea</i>	1,326	8.95	0.0028
<i>E. elatus</i>	<i>S. fistulosa</i>	1,326	8.20	0.0042
<i>E. elatus</i>	<i>S. verticillata</i>	1,326	98.40	<0.0001
<i>P. coccinea</i>	<i>S. fistulosa</i>	1,326	0.13	0.7232
<i>P. coccinea</i>	<i>S. verticillata</i>	1,326	122.45	<0.0001
<i>S. fistulosa</i>	<i>S. verticillata</i>	1,326	169.96	<0.0001

Vernacular names assigned to *S. verticillata* include 'whitehead broom' (Murphy et al. 1998, said to have been assigned by the Weed Science Society of America) and 'shrubby false buttonweed' assigned by Wunderlin (1998). The Puerto Rican common name *botón blanco* (= white button) was used by Francis (2002). The native *Spermacoce assurgens* Ruiz and Pavon ('bushy buttonweed') and non-native *S. verticillata* ('whitehead broom') are reported as weeds in turfgrass in the southern USA (Murphy et al. 1998). No golf course superintendent, extension agent, or rancher with whom we spoke recognized either of these two names (nor did they recognize the name shrubby false buttonweed). However, that publication alerts us to the 'weediness', somewhere, of *S. verticillata*.

We tried to find a native plant in northern Florida as attractive as *S. verticillata* to the wasp. This was done by searching the vicinity of established plots of *S. verticillata* for evidence of feeding on other plants, and then by experimental evaluation of relative attractiveness. We did not test the plants (mostly non-native) on which the native wasp *Larra analis* (F.) was reported by

Smith (1935) to feed in Louisiana. That wasp attacks only the native mole cricket *Neocurtilla hexadactyla* (Perty). Further tests should be made of a wider range of plants, including those on which *L. analis* has been observed to feed, others on which *L. bicolor* has been observed to feed (Table 1), and native Florida species of *Spermacoce*.

Butterfly-gardeners routinely promote some non-native weedy plants such as *Buddleia* and *Lantana* species as nectar sources for butterflies, as well as others (*Asclepias*, *Aristolochia*, etc.) for host plants to draw interesting butterfly species. The crops protected are *Cynodon dactylon* (L.). Pers. and hybrids with *C. transvaalensis* Burtt-Davey (Bermudagrass, the major turfgrass in southern Florida), *Paspalum notatum* Fluegge (Bahiagrass, the major pasturegrass in Florida, and also used widely as a turfgrass), and numerous kinds of vegetable seedlings, none of which is native to Florida. We suggest that using a small percentage of the area of these crop plants to grow *S. verticillata*, another non-native plant, is much more sensible than using broad-spectrum chemical pesticides as the sole means of control of non-native pest mole crickets.

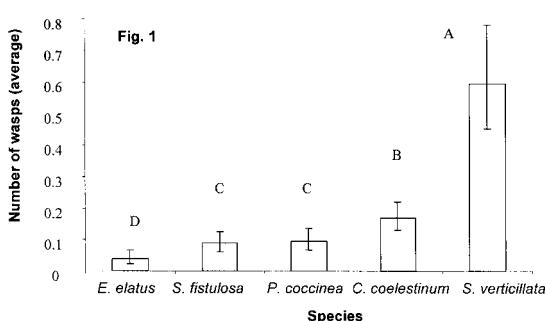


Fig. 1. Mean comparison for plant selection test made by *Larra bicolor* in the field. Data represent the average number of wasps per treatment per sampling  $\pm$  SE. Bars with different letters are significantly different ( $P \leq 0.005$ ) according to chi-square pairwise comparisons under Poisson distribution.

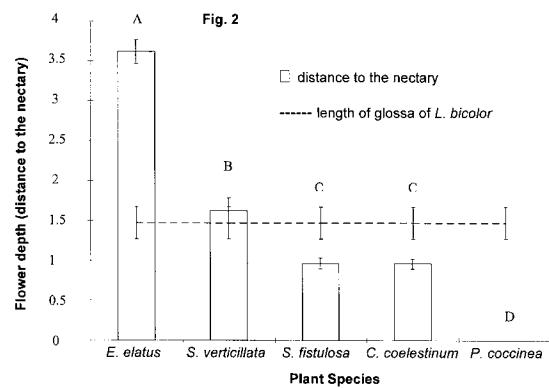


Fig. 2. Mean comparisons of floral depth (distance to the nectaries) and the length of *L. bicolor*'s glossa. Data with the same letter do not differ ( $\alpha = 0.05$ ) according to Duncan's test. Nectaries of *P. coccinea* are extra-floral.

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## POPULATION DYNAMICS OF THE COTTON APHID, *APHIS GOSSYPII* (HOMOPTERA: APHIDIDAE), ON STRAWBERRIES GROWN UNDER PROTECTED STRUCTURE

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### ABSTRACT

A well developed management plan is in place for control of pests such as the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), in field and greenhouse grown strawberry, *Fragaria ananassa* Duchesne; however, an integrated pest management approach to control the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), is not available. In order to initiate an effective program for the cotton aphid, the population dynamics of the aphid and the effectiveness of the pink spotted lady beetle, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), to control aphids were studied on greenhouse strawberry. Results from this experiment established peaks of aphid infestation throughout the growing season and location of different cotton aphid life forms on the plant. The greatest positive response of the pink spotted lady beetle to cotton aphid occurred at high prey density. This characteristic indicates that the pink spotted lady beetle may be a good candidate for augmentative biological control of cotton aphid on strawberry in the greenhouse. This study provides a basis for developing a biological control of cotton aphid component for an integrated strawberry pest management program.

**Key Words:** *Aphis gossypii*, biological control, *Coleomegilla maculata*, *Fragaria*, greenhouse, integrated pest management, pests, pink spotted lady beetle, strawberry

### RESUMEN

Existen programas de manejo establecidos para el control de la araña roja, *Tetranychus urticae* Koch (Acari: Tetranychidae) en el cultivo de la fresa, *Fragaria ananassa* Duchesne, producida en el campo e invernadero; sin embargo, un manejo integral para el control del pulgón del algodón, *Aphis gossypii* Glover (Homoptera: Aphididae), en el mismo cultivo, todavía no está disponible. A fin de establecer la efectividad de programas de control del pulgón del algodón, la dinámica poblacional del pulgón y la efectividad del predador, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), fue estudiada en el invernadero. Resultados de este experimento demostraron los picos de infestación durante el desarrollo del cultivo y la localización de las diferentes formas del pulgón en la planta. El predador responde mejor cuando la población de pulgones es más densa. Esta característica da la indicación del potencial del predador como agente controlador de pulgón. Estos estudios proveen las bases de control biológico que han de implementarse en un programa sostenido del pulgón en el cultivo de la fresa.

Translation provided by the authors.

The strawberry, *Fragaria ananassa* Duchesne, is a high value crop commercially produced in California, Florida, Michigan, North Carolina, New York, Ohio, Oregon, Pennsylvania, Washington, and Wisconsin (Sorensen et al. 1997). Florida ranks second in harvested area, total yield, and production after California (USDA-FAD 2001-2002). The Florida strawberry industry produces during the months of November through March in the field and high-quality production during these months is the key to maintaining profitability. The Florida strawberry industry seeks to remain competitive during this small window of opportunity when market prices are high and

the volume from California is low (NASS-USDA 2003). One alternative to increase strawberry profitability is through greenhouse production. Growing strawberries under protective culture has become a viable alternative for strawberry producers worldwide because of the elimination of soil fumigation, the reduction of fungal and bacterial diseases, and the reduction of water usage. At present, the area of strawberries grown under protected cultivation in Florida is less than 1 ha (NASS-USDA 2003); however, this is expected to increase as growers look for new alternatives to enhance early production (Paranjpe 2004).

Mites are the most important pest of field and greenhouse strawberries, with the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), a potential pest wherever strawberries are produced (Oatman & McMurtry 1966; Howard et al. 1985; Price & Kring 1991). The twospotted spider mite has been successfully controlled in the field in some areas of Florida by the introduction of *Phytoseiulus persimilis* Athias-Henriot (Acari: Phytoseiidae) onto the strawberry crop when about 5–10% of the strawberry leaflets have been infested with one or more mites (Van de Vrie & Price 1994). In the greenhouse, the use of *Neoseiulus californicus* McGregor to control twospotted spider mites has been very successful (unpublished data).

Although aphids are not a major problem on field strawberries (Mosser & Nesheim 2003), in greenhouse strawberries, the cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), can be a serious problem (Leclant & Deguine 1994). This small, soft-bodied insect feeds on the underside of leaves sucking out plant sap. The cotton aphid varies in color and size (Watt & Hales 1996); spring populations can be darker and may be twice the size of "yellow dwarfs" generally present in the summer (Nevo & Coll 2001). High populations of aphids can reduce the vigor of the plant, making it susceptible to other pests. The honeydew that aphids excrete reduces fruit quality because of the development of a black sooty mold on the substrate. Moreover, this sooty mold reduces photosynthate production and otherwise reduces the quality of the plant causing considerable economic injury. Natural enemies are important in control and regulation of the cotton aphid. Any factor reducing parasitoids, predators or other biological control agent could result in economic damage to the crop (Kaplan & Eubanks 2002). Natural enemies effective against the cotton aphid include lady beetles *Coccinella septempunctata* L. and *Hippodamia convergens* Guérin-Ménéville, the green lacewing *Chrysoperla carnea* Stephens, and wasps *Lysiphlebus testaceipes* (Cress) and *Aphidius colemani* L. (Howard et al. 1985; Van Driesche & Bellows 1996; Kaplan & Eubanks 2002). The pink spotted lady beetle, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae), also is known to feed on the cotton aphid (Rondon et al. 2004); however, the role of the pink spotted lady beetle in the strawberry ecosystem is relatively unknown. This polyphagous predator is abundant in herbaceous crops such as maize *Zea mays* L., alfalfa *Medicago sativa* L., and potato *Solanum tuberosum* L. where the lady beetle feeds on various prey (Gordon 1985; Krafus & Obrycki 2000).

This 2-year study was initiated to monitor the population dynamics of natural late-fall and early-spring infestations of the cotton aphid on strawberries grown in a greenhouse and to evaluate the effectiveness of pink spotted lady beetle third instars to control aphids.

## MATERIALS AND METHODS

### Strawberry Production

Strawberry plants were produced at the University of Florida (UF), Horticultural Sciences Department, Institute of Food and Agriculture Sciences in Gainesville, FL, following the protocol of Paranjpe et al. (2003). The following exceptions were made; after cutting the runners from mother plants, 'Sweet Charlie' strawberry plugs were grown in the greenhouse using a low mist fertigation system (water plus fertilizer). Four trays of 80 plugs per tray were set on a 1 Peat:1 Perlite mix media. Plugs were exposed to a 2-week chilling period (4.0–6.0°C) in the growth chamber before transplanting at 25.0 ± 2.0 and 10.0 ± 2.0°C day and night temperature, respectively, with a 9-h photoperiod. After the chilling period, plugs were transplanted to 2-liter plastic pots in soilless medium (2 Peat:1 Perlite mix). Forty rows of four pots per row were arranged on top of an 8-m long metal bench. Monitoring started when four fully developed leaves appeared. A weekly rotation of Quadris® 2.08F (azoxystrobin at 275 g active ingredient/ha) and Nova® 40W (myclobutanil at 142 g active ingredient/ha) sprays were made as necessary for preventing powdery mildew, the main disease in strawberry greenhouse production.

### Aphid Sampling Methods

The seasonal population dynamics of the cotton aphid was monitored in strawberries grown in a greenhouse at the UF. Aphid populations were monitored twice weekly from January to May during 2002 and 2003. The average temperature in the greenhouse during this experiment was 22 and 16°C, day and night, respectively. The experimental design was a randomized complete block with four replications. Each block consisted of 20 plants from which five plants per replication were randomly selected. Six rows of strawberries separated each block. The total numbers of apterous and alate adults and nymphs were counted *in situ* from the developing bud and from the middle strawberry leaflet (sampling unit) of one plant with the aid of 5× and 14× lenses. The 5× lens was used to locate the aphids, and the 14× to separate life forms. Nymphs and "dwarf" forms were discriminated from the adults based on the short cauda plate present at the tip of the abdomen in the immature stages as compared with a long cauda present in the adult form (Blackman & Eastop 2000). No insecticide was used at any time during the investigation and the reduction of aphid population was caused by natural "overex-

ploitation" of the habitat (number of aphids/leaflet). The elimination of old strawberries leaflets was the only measure used as cultural control. *Aphis forbesi* (Weed), the strawberry root aphid, and other pests were physically removed from leaves and buds to avoid any effect on the study.

#### Caged Greenhouse Trial

An experiment was conducted to examine the effectiveness of the pink spotted lady beetle third instar as a predator of the cotton aphid. The pink spotted lady beetle was obtained from Entomos LLC (Gainesville, FL 32608), a local insect supplier during the first year of the experiment. The second year, the pink spotted lady beetle came from our own colony. Lady beetles were reared following proprietary Entomos protocol.

In a greenhouse, five clean strawberry plants (one plant per pot) were placed in a 1-m<sup>3</sup> nylon covered cage. The five strawberry plants were infested with adult aphids on a marked leaf. Three cages of plants were infested with five aphids per plant (low infestation), three cages were infested with ten aphids per plant (medium infestation), and three cages were infested with 15 aphids per plant (high infestation). To diminish the possibility of the dispersal of the aphids, only three stems per plant were kept. Based on previous observations, three stems per plant allow the sustainability of a strawberry plant. After 1 week, the number of aphids on the labeled compound leaf per plant was counted. After counting was completed, one, three, or five third instars of the pink spotted lady beetle were released into the cages. The number of aphids consumed on the marked leaf was counted weekly for 4 weeks. The experiment was repeated three times on a split block design in time. Parasitized aphids and other pests were physically removed from the strawberry plants.

#### Data Analysis

The general linear model (GLM) procedure was used to construct analysis of variance (ANOVA) for mean number of nymphs, apterous, and alate adult aphids each year (SAS Institute 2000).

Means of the proportions of prey consumed by the pink spotted lady beetle third instar were compared and separated by the least significant difference (LSD) test ( $P = 0.05$ ).

#### RESULTS

##### Population Dynamics of Aphids

In 2002, overall mean numbers of nymphs observed on leaves were greater than number of nymphs observed on emerging buds ( $F = 26.34; df = 3, 12; P > 0.001$ ) (Table 1). Nymph densities on the bud were greatest on 15 March ( $16.75 \pm 5.46$ ) and then gradually decreased towards the end of the sampling period (Fig. 1A). Two peak populations were observed on leaves on 25 February ( $15.95 \pm 4.33$ ) and on 15 March ( $14.5 \pm 3.81$ ) (Fig. 1A). Overall numbers of apterous adults on the bud were greater than number of adults on the leaves ( $F = 18.34; df = 3, 12; P > 0.001$ ) (Table 1). Adult density on the bud was greatest on 25 February ( $24.55 \pm 5.32$ ) and on 15 March ( $39.65 \pm 7.23$ ) and then density gradually decreased towards the end of the sampling period (Fig. 1B). Two peaks were observed on leaves on 25 February ( $19.50 \pm 7.23$ ) and on 15 March ( $17.55 \pm 4.41$ ) (Fig. 1B). Overall numbers of alate adults on the bud were greater than numbers of alate adults on leaves ( $F = 14.34; df = 3, 12; P > 0.001$ ) (Table 1). On the bud, numbers of alate adults were greatest on 11 March ( $0.2 \pm 0.1$ ) (Fig. 1C). Two peaks were observed on 11 March ( $3.00 \pm 1.18$ ) and 8 and 15 April ( $0.5 \pm 0.2$  and  $0.5 \pm 0.1$ , respectively) (Fig. 1C). Overall combined numbers of aphids (nymphs, adults and alate adults) on the bud were greater than combined number of aphids on the leaves ( $F = 12.34; df = 3, 12; P > 0.001$ ) (Table 1). On the bud, combined numbers of aphids were highest on 15 February ( $24.65 \pm 9.87$ ) and on 15 March ( $56.40 \pm 11.35$ ) (Fig. 1D). Two peaks also were observed on leaves on 25 February ( $35.50 \pm 9.85$ ) and 15 March ( $32.15 \pm 8.15$ ) (Fig. 1D).

In 2003, overall mean numbers of nymphs observed on leaves were greater than number of aphids observed on the emerging bud ( $F = 23.18; df = 3, 12; P > 0.068$ ). Nymph densities on the bud

TABLE 1. OVERALL MEAN NUMBER ( $\pm$  SE) OF NYMPHS, APTEROUS, AND ALATE ADULTS, AND COMBINED NUMBER OF COTTON APHIDS INFESTING STRAWBERRIES GROWN UNDER PROTECTED CULTIVATION, GAINESVILLE, FL.

Life form	2002		2003	
	Leaflet	Developing buds	Leaflet	Developing buds
Nymphs	$5.09 \pm 1.43$	$3.94 \pm 1.25$	$4.30 \pm 1.35$	$3.07 \pm 1.27$
Apterous adult	$6.62 \pm 1.56$	$8.29 \pm 1.54$	$6.67 \pm 1.13$	$9.26 \pm 2.35$
Alate adult	$0.03 \pm 0.01$	$0.29 \pm 0.11$	$0.46 \pm 0.23$	$0.03 \pm 0.01$
Combined (nymphs and apterous and alate adults)	$11.90 \pm 3.11$	$12.26 \pm 2.80$	$11.23 \pm 2.89$	$12.36 \pm 3.63$

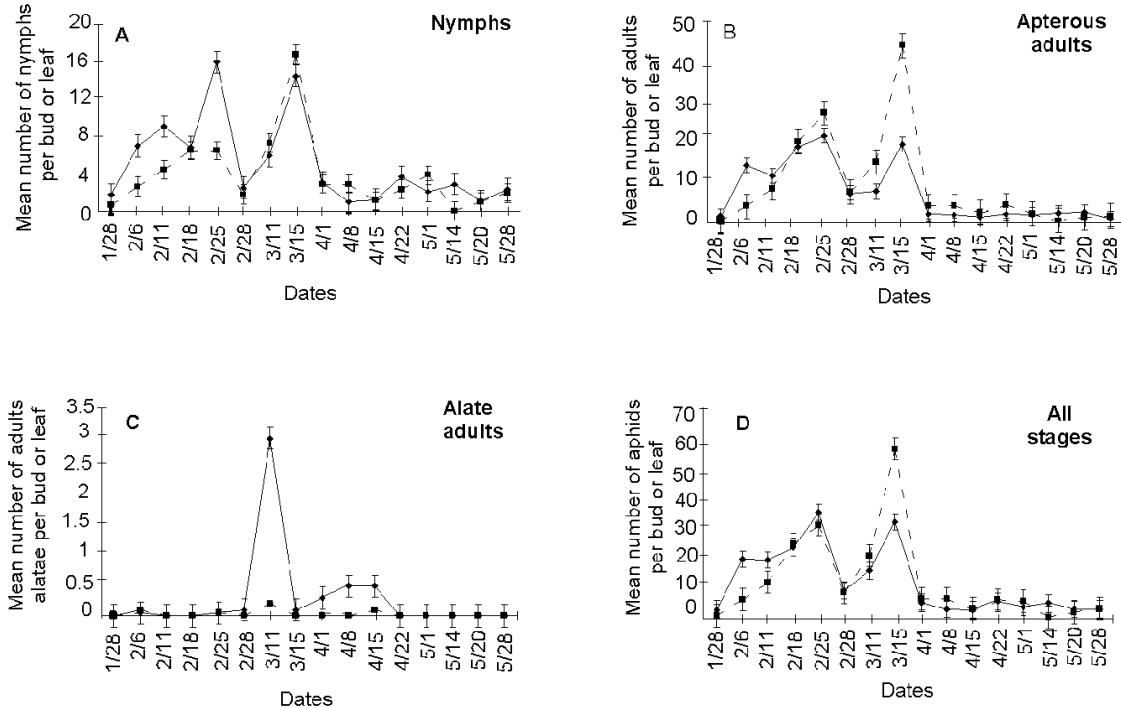


Fig. 1. Population dynamics of nymphs (A), apterous adults (B), alate adults (C), and total (D) of the cotton aphid on strawberries grown in a greenhouse during 2002 in Gainesville, FL. Legend: ——□—— bud ——◆—— leaf.

were greatest on 16 March ( $8.45 \pm 1.46$ ) and then gradually decreased towards the end of the sampling period (Fig. 2A). Two peaks were observed on leaves on 9 February ( $7.85 \pm 2.31$ ) and on 16 March ( $10.16 \pm 3.2$ ) (Fig. 2A). Overall numbers of apterous adults on the bud were greater than on the leaves ( $F = 15.14$ ;  $df = 3, 12$ ;  $P > 0.069$ ). Adult densities on the bud were greatest on 15 February ( $24.18 \pm 2.65$ ) and on 1 March ( $24.96 \pm 3.26$ ) and then gradually decreased towards the end of the sampling period (Fig. 2B). One peak was observed on leaves 16 March ( $21.16 \pm 3.86$ ) (Fig. 2B). Overall numbers of alate adults on the leaves were greater than on buds ( $F = 8.37$ ;  $df = 3, 12$ ;  $P > 0.071$ ). In the bud, numbers of alate adults were low throughout the experiment while in the leaves, it reached its highest on 9 March ( $3.68 \pm 0.9$ ) (Fig. 2C). Overall combined numbers of aphids on the bud were greater than on the leaves ( $F = 9.15$ ;  $df = 3, 12$ ;  $P > 0.079$ ). On the bud, combined numbers of aphids were greatest on 15 February ( $33.16 \pm 2.89$ ) (Fig. 2D). One peak was observed on leaves on 16 March ( $37.16 \pm 3.46$ ) (Fig. 2D).

#### Response of the Pink Spotted Lady Beetle Third Instar to Different Aphid Densities

One week after five aphids were released per plant, there was an average of  $59.9 \pm 12.6$  aphids per strawberry leaf. One third instar pink spotted

lady beetle reduced 53.8, 36.2, 27.2, and 20.6% of the aphid population after 1, 2, 3, and 4 weeks, respectively ( $F = 13.57$ ;  $df = 2, 5$ ;  $P > 0.001$ ) (Fig. 3A). Three predators reduced 97, 28.6, 15, and 13.2% of the aphid population 1, 2, 3, and 4 weeks, respectively, after being released ( $F = 11.14$ ;  $df = 2, 5$ ;  $P > 0.078$ ) (Fig. 3A). Five predators reduced 29, 5, 2.2, and 2.2% of the aphid population 1, 2, 3, and 4 weeks, respectively, after being released ( $F = 3.47$ ;  $df = 2, 5$ ;  $P > 0.090$ ) (Fig. 3A).

One week after ten aphids were released per plant, there was an average of  $77.7 \pm 21.6$  aphids per strawberry leaf. One third instar pink spotted lady beetle reduced 70.2, 11, 8 and 6.2% of the aphid population after 1, 2, 3, and 4 weeks, respectively ( $F = 21.14$ ;  $df = 2, 5$ ;  $P > 0.001$ ) (Fig. 3B). Three predators reduced 87.6, 12.6, 11 and 8% of the aphid population 1, 2, 3, and 4 weeks, respectively, after being released ( $F = 18.53$ ;  $df = 2, 5$ ;  $P > 0.001$ ) (Fig. 3B). Five predators reduced 75.2, 22.8, 8.6, and 4.2% of the aphid population 1, 2, 3, and 4 weeks, respectively, after being released ( $F = 15.10$ ;  $df = 2, 5$ ;  $P > 0.004$ ) (Fig. 3B).

One week after 15 aphids were released per plant, there was an average of  $167.3 \pm 28.4$  aphids per strawberry leaf. One third instar pink spotted lady beetle reduced 96.3, 46.2, 46.3 and 39.0% of the aphid population after 1, 2, 3, and 4 weeks, respectively ( $F = 9.60$ ;  $df = 2, 5$ ;  $P > 0.001$ ) (Fig. 3C). Three predators reduced 98.2, 87.3, 35.4, and

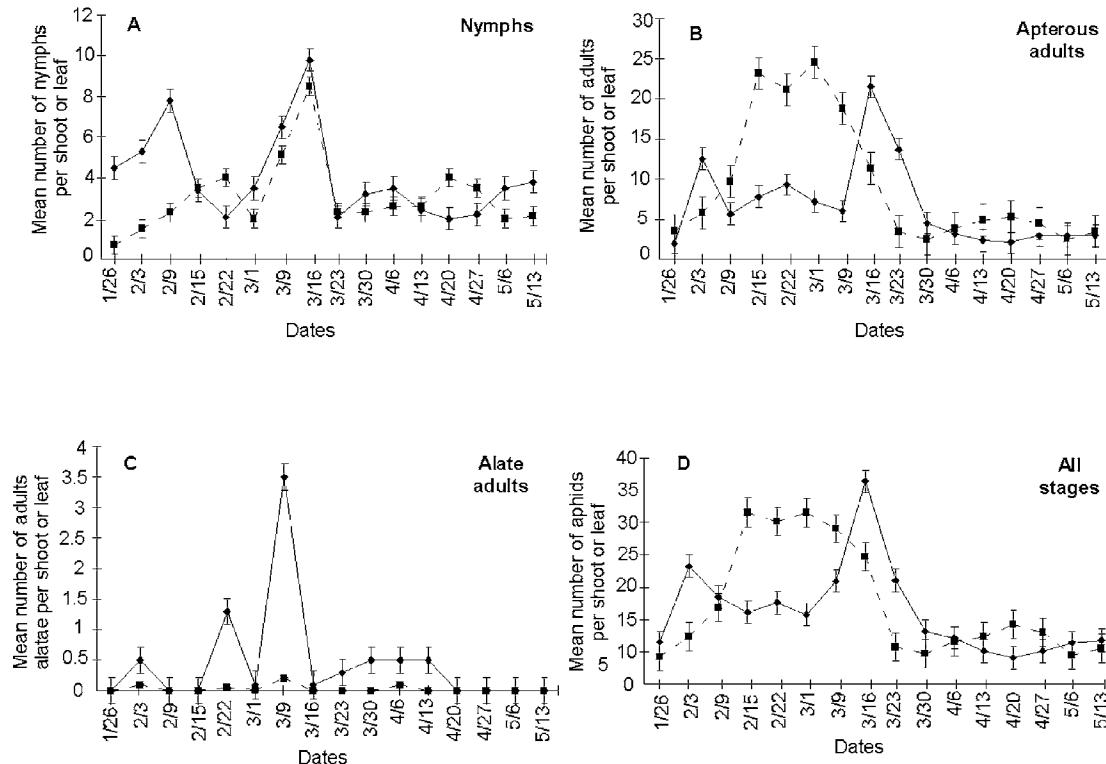


Fig. 2. Population dynamics of nymphs (A), apterous adults (B), alate adults (C), and total (D) of the cotton aphid on strawberries grown in a greenhouse during 2003 in Gainesville, FL. Legend: — bud — leaf.

23.4% of the aphid population 1, 2, 3, and 4 weeks, respectively after being released ( $F = 7.34$ ;  $df = 2, 5$ ;  $P > 0.001$ ) (Fig. 3C). Five predators reduced 98.2, 96.4, 57.3, and 22.4% of the aphid population 1, 2, 3, and 4 weeks, respectively, after being released ( $F = 11.75$ ;  $df = 2, 5$ ;  $P > 0.011$ ) (Fig. 3C).

#### DISCUSSION

This study of the population dynamics of the cotton aphid on strawberries grown under protected cultivation established a basis for the development of future cotton aphid management. This study gave us an insight into when to expect possible pest outbreaks and the best time to apply control measures. However, multi-year data will be needed in order to establish a useful extension of this prediction. The seasonal peaks and distribution of different life forms (stages) of the pest within plants were successfully established.

In general, the cotton aphid was more abundant from mid-February to late-March, in a greenhouse located in Gainesville, FL. During those months, temperature in the greenhouse averaged 22 and 16°C, day and night, respectively, which is favorable for aphid development and reproduction (Leclant & Deguine 1994). Several studies already have been conducted regarding

the effect of the temperature on development, survivorship, and reproduction on different aphid species (Campbell et al. 1974; Aalbersberg 1987; Wang & Tsai 2000). Moreover, studies by Campbell et al. (1974) have indicated that peaks on aphid populations were positively correlated with moderate increases in temperatures.

Determining the location of the pest within the plant is important in order to establish the most effective control method. Different cotton aphid life forms predominated at different plant locations. In both years, nymphs were more frequently found on leaves than on the developing buds but apterous adults predominated on the buds. Alate adults were rare throughout the experiment and their presence in either developing buds or leaves was inconsistent in both years. The hypothesis as to why a specific life form prefers a specific site within the plant would be speculative at this point. Although in both years we found similar patterns, sugar content of the plant and specific nutritional requirements of each stage should have been taken.

The greatest positive response of the pink spotted lady beetle to cotton aphid occurred when the prey was most dense. This is a characteristic of an efficient predator and indicated that the pink spotted lady beetle might be a good candi-

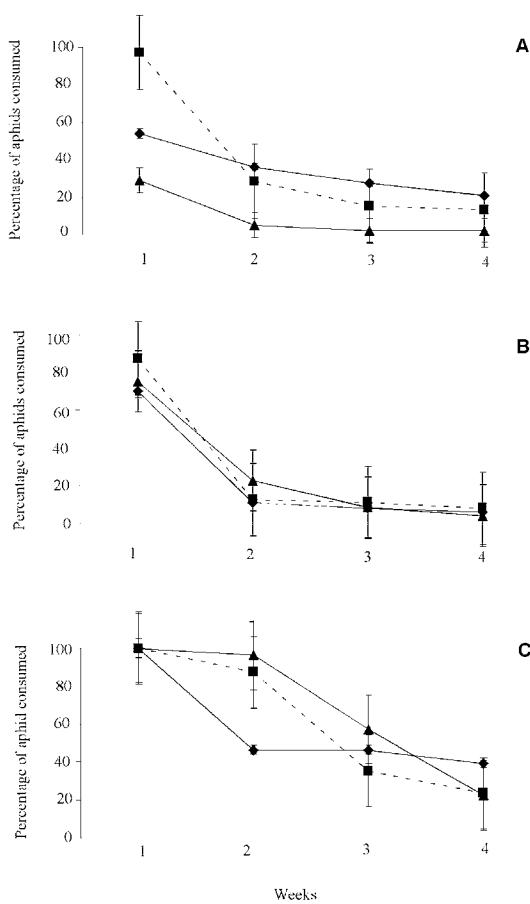


Fig. 3. Third instar pink spotted lady beetle response to low (A), medium (B), and high (C) densities of the cotton aphid. Legend: —◆— 1 predator; - - -■- 3 predators; —▲— 5 predators.

date for biological control of cotton aphid on strawberries. This is not the case for some other lady beetle species, in which their distribution did not always correspond with that of other aphid species (Park & Obrycki 2004). Studies by Rondon et al. (2004) have determined the benefits of using lady beetles to control cotton aphids and twospotted spider mites. In a series of laboratory studies, they determined that approximately 80% of the prey offered was consumed by the pink spotted lady beetle only after 2 h of being exposed to the prey. The ability of lady beetles to seek aphids was evident during the cage greenhouse trial (personal observation).

These studies have increased the understanding of the relationship among the strawberry, its cotton aphid pest and an important biological control agent. They provide a basis for developing a biological control of cotton aphid component to a comprehensive integrated program of greenhouse strawberry pest management.

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## ERADICATION OF THE LITTLE FIRE ANT, *WASMANNIA AUROPUNCTATA* (HYMENOPTERA: FORMICIDAE), FROM MARCHENA ISLAND, GALÁPAGOS: ON THE EDGE OF SUCCESS?

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### ABSTRACT

The development of effective techniques to eradicate populations of invasive ant species is crucial to the conservation of native biodiversity. An intensive program was initiated in 2001 to eradicate the invasive little fire ant, *Wasmannia auropunctata* (Roger) from ~21 ha on Marchena Island in the Galápagos Archipelago. Linear transects, approximately 10 m apart, were cut through the vegetation of the infested area and a buffer zone of 6 ha. Amdro® (Hydramethylnon) was applied manually up to three times in the treatment area at three-month intervals between March and October 2001. To date, five follow-up monitoring surveys have placed sticks painted with peanut butter in a grid 3-4 m apart. Two small populations (0.1% of the area originally occupied by *W. auropunctata*) were detected in April and October 2002 and were subsequently treated with Amdro®. No *W. auropunctata* ants were found in May 2003 and April 2004. Five nocturnal surveys carried out in the immediate area of introduction of *W. auropunctata* did not detect any individuals. Monitoring surveys will continue for an additional two years to ensure eradication of any remaining populations and verify the success of this program. This paper discusses the procedures used to kill *W. auropunctata* and monitor the efficacy of the eradication methods, the program's costs, and its applicability to other island ecosystems.

Key Words: Amdro®, ant control, dispersal, costs, invasive ants, monitoring

### RESUMEN

El desarrollo de técnicas efectivas para erradicar poblaciones de hormigas invasoras es esencial para la conservación de la biodiversidad nativa. Un programa intensivo fue iniciado en 2001 para erradicar la hormiga colorada invasora *Wasmannia auropunctata* (Roger), de un área de ~21 ha en la Isla Marchena, Galápagos. Transectos lineales de aproximadamente 10 m entre cada uno, fueron hechos dentro de la vegetación del área infestada y en una zona de amortiguamiento de 6 ha. Amdro® (Hydramethylnon) fue aplicado manualmente hasta tres veces en el área de tratamiento a intervalos de tres meses entre marzo y octubre 2001. Hasta la fecha, se ha realizado cinco monitoreos para evaluar la eficacia del programa de erradicación colocando palitos pintados con mantequilla de maní en cuadrículas de 3-4 m. En abril y octubre 2002 se detectaron dos poblaciones pequeñas (0.1% del área ocupada originalmente por *W. auropunctata*) las cuales fueron tratadas con Amdro®. No se encontró a *W. auropunctata* en Mayo 2003 y Abril 2004. Tampoco se encontró a la hormiga de fuego en cinco monitoreos nocturnos realizados en la zona de introducción de la hormiga. Los monitoreos continuarán por dos años adicionales para asegurar que no existen parches de hormigas y para verificar el éxito del programa. En este artículo se discute los procedimientos para erradicar *W. auropunctata* y para evaluar la eficacia de los métodos utilizados, los costos del programa y su aplicabilidad en otros ecosistemas isleños.

Translation provided by the authors.

Ants are highly successful invaders of both islands and continents (McGlynn 1999). Eradication of recently introduced populations is fundamental to preventing their dispersal and subsequent impacts on native biodiversity. Eradication is especially important for areas of high conservation value where the loss of endemic fauna, in particular invertebrates, is at risk. The probability of successful eradication decreases with in-

creased distribution and when eradication is not feasible, control techniques are costly and time consuming. Moreover, they are often ineffective in preventing species from spreading through natural mechanisms, and most importantly with the aid of humans (Suarez et al. 2001). Historically, eradication has involved small, recently introduced populations at ports of entry. Techniques for eradication at a larger scale are still in the

early stages of development and there have been few success stories: the removal of *Wasmannia auropunctata* (Roger) from 3 ha on Santa Fe Island in the Galàpagos (Abedrabbo 1994) is an example. Also, early results suggest that infestations of the ants *Pheidole megacephala* F. (up to 10 ha) and *Solenopsis geminata* (F.) (up to 3 ha) in Kakadu National Park, Australia may have been eradicated (Hoffman & O'Connor 2004). Yet, because of their unusual social organization and reproductive strategies (Passera 1994; Tsutsui & Suarez 2003), some species of ants are good candidates for eradication.

The little fire ant, *Wasmannia auropunctata* has been listed as one of the 100 worst invaders in the world by the Invasive Species Specialist Group of The World Conservation Union (IUCN) (Lowe et al. 2002). It is easily transported on fruits and vegetables, and growing trade between countries has facilitated this Neotropical insect's colonization in many parts of the world. In the last 25 years, at least seven Pacific island groups including Hawaii and recently Tahiti have been successfully colonized by *W. auropunctata* (Wetterer & Porter 2003; E. Loeve, Fenua Animalia, Tahiti, pers. comm.). Attributes that make *W. auropunctata* a successful invader include its adaptability to a wide range of habitats, polyphagous feeding habits, high interspecific aggression, and lack of intraspecific aggression which leads to unicoloniality (Ulloa-Chacón & Cherix 1990; Le Breton et al. 2004). Colonies are polygynous (Hölldobler & Wilson 1977), increasing the likelihood that small numbers of ants that are split off from the colony or are transported by man are able to found a new colony.

Introduced into the Galàpagos archipelago between 35 and 70 years ago, *W. auropunctata* has colonized eight large islands: Santa Cruz, San Cristóbal, Isabela, Floreana, Santiago, Santa Fe, Pinzón, and Marchena (Silberglied 1972; Lubin 1984; Abedrabbo 1994). It also has been found recently on some of the smaller islands: Champion, Mao, Cousins, Albany, and Eden (C. E. C., unpubl. data). The ants were most likely transported between the inhabited islands on plants, food, and in soil. The uninhabited islands, on the other hand, are less frequently visited and then only by scientists and park rangers, and illegally by fisherman. Ants may have been transported in camping provisions and equipment or may have arrived on vegetation rafts.

Known locally as the "hormiga colorada", *W. auropunctata* has had a wide-ranging impact on biodiversity in the Galàpagos, in particular to native invertebrates (Clark et al. 1982; Lubin 1984; Roque-Albelo et al. 2000; Mieles 2002). It also negatively affects the nesting activities and young of reptiles and birds and its painful sting makes it a significant pest to farmers and conservation workers (Lubin 1985; Roque-Albelo et al.

2000; C. E. C., unpubl. data). Additionally, *W. auropunctata* aids the build up and spread of populations of the cottony cushion scale (*Icerya purchasi* Maskell). Honeydew produced by this scale insect is exchanged for transportation and protection from predators (Causton 2001).

Mitigation of the impacts of *W. auropunctata* has been recognized as a priority for conservation organizations in Galàpagos. On the larger islands the little fire ant is now distributed over thousands of hectares and is beyond the means of current methods of control. However, eradication programs are expected to be more successful on the smaller islands or areas that have been recently colonized where distributions are less than a few dozen hectares. This was demonstrated with the successful removal of *W. auropunctata* from Santa Fe Island (Abedrabbo 1994). Eradication was also considered feasible for the recently invaded Marchena Island, a near pristine island in the northern part of the Archipelago (Roque-Albelo et al. 2000).

*Wasmannia auropunctata* was first discovered in 1988 at a campsite on Playa Negra, a large black sand beach on the southwestern side of Marchena Island (Fig. 1) (Roque-Albelo et al. 2000). In 1992, the area infested by *W. auropunctata* was estimated at 0.5 ha (Fig. 2a, b) and a control program was initiated by the Galàpagos National Park Service (GNPS) and the Charles Darwin Research Station (CDRS) adopting the methodology previously used to eradicate *W. auropunctata* from Santa Fe Island (Abedrabbo 1994). Between 1993 and 1996, three attempts were made to eradicate *W. auropunctata* with Amdro® (Zuñiga 1994; Roque-Albelo et al. 2000). Follow up surveys indicated that the poison bait applications were only partially successful (Fig. 2a, b), probably because populations were missed and the area of infestation was underestimated. In 1996, *W. auropunctata* still occupied 1.5 ha, but the eradication program was suspended due to lack of funding. By 1998, an El Niño year, the area had increased to 17 ha (Fig. 2a, b) (Roque-Albelo et al. 2000). High precipitation rates during El Niño may have accounted for a rise in ant numbers. Lubin (1984, 1985), measured *W. auropunctata* spread at a rate of 170m/year in Santa Cruz Island, increasing to 500 m in El Niño years. Nevertheless, it is highly unlikely that El Niño was solely responsible for such dramatic population growth on Marchena, further suggesting that earlier assessments had missed some populations. Two years later in 2000, the infested area was estimated at 24 ha (Roque-Albelo et al. 2000). This proved to be an overestimate as later calculations showed the actual infested area to be 19.3 ha; an increase of approximately 2.3 ha. from 1998.

What was evident from surveys carried out post 1996 was that the distribution of *W. auropunctata* in Marchena was still expanding and

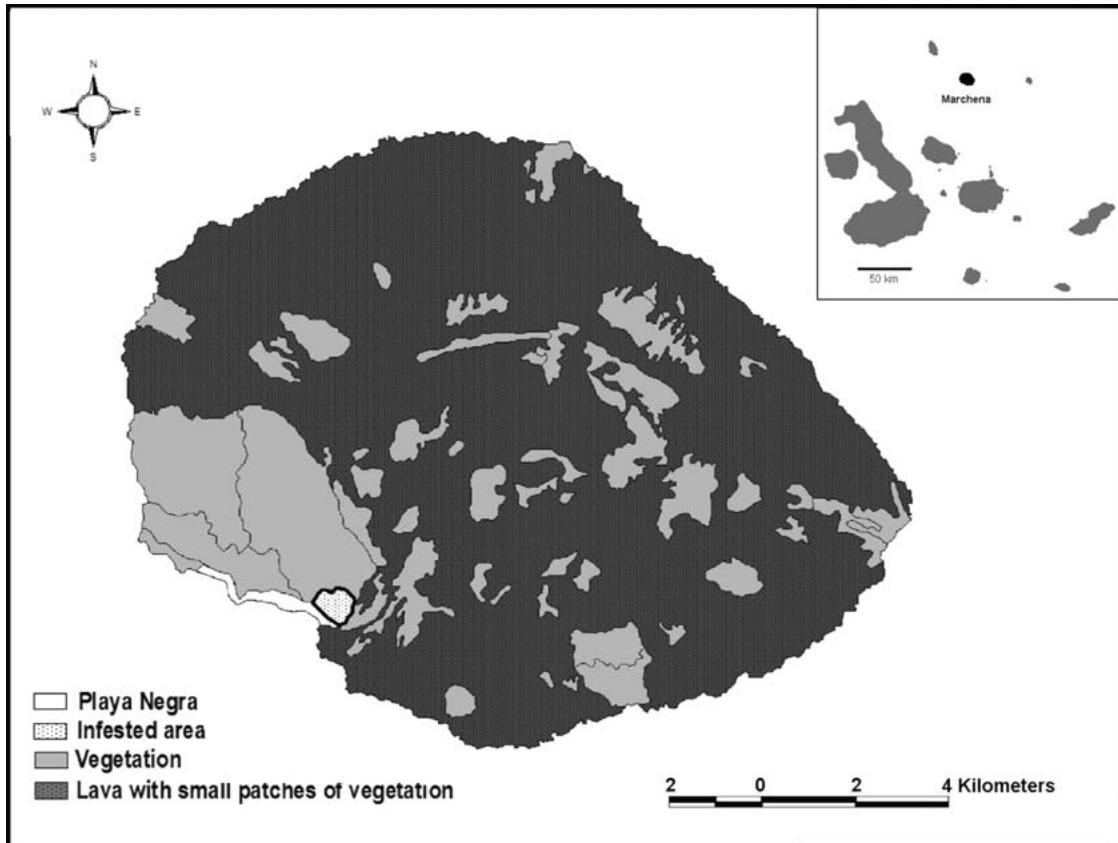


Fig. 1. Marchena Island ( $130 \text{ km}^2$ , inset shows location within the Galápagos Archipelago) and location of *W. auropunctata* infestation in 2001 (~20.5 ha).

that there was a striking contrast between the composition of ant communities in habitats where *W. auropunctata* was present and areas that had not been invaded (Roque-Albelo et al. 2000). *Wasmannia auropunctata* typically infested only vegetated areas and in Marchena, vegetation covers only 25% of the total area of the island ( $130 \text{ km}^2$ ). If *W. auropunctata* continued to spread at the same rate, it could eliminate many of the native invertebrate species that occupy these habitats, especially those that are localized in distribution.

Paradoxically, the reproductive strategies that make *W. auropunctata* a successful colonizer and enable it to expand its distribution also facilitate the success of any program aimed at reducing population numbers. This is primarily because new colonies are typically formed by budding (Hölldobler & Wilson 1977), which restricts the dispersal capacity of *W. auropunctata* and contains it to areas immediately adjacent to existing colonies. As a consequence, eradication was still thought to be possible and in 2001 a program was initiated to eradicate *W. auropunctata* from Marchena Island. This paper evaluates the methods used in the current eradication program and

discusses their applicability to other areas of conservation value.

## MATERIALS AND METHODS

### Description of Area Infested by *W. auropunctata*

Colonies of *W. auropunctata* were found between 0–50 m elevation. Marchena Island is arid and the infested area was principally covered by areas of dry eroded soil and fresh lava fields. Vegetation was dense in parts, particularly in the rainy season, and was composed of dry forest dominated by *Bursera graveolens* (HBK) Trian. and Planch., *Croton scouleri* Hook. f., *Waltheria ovata* Cav., *Lantana peduncularis* Andersss., *Opuntia helleri* K. Scum., and *Castela galapageia* Hook. f. (Hamman 1981). In the Galápagos, January to May is the warm/wet season with occasional rain and is followed by a cooler/dry season from May to December with little or no rain and lower temperatures. Annual meteorological records do not exist for this island. Day time temperatures recorded during field trips from 2001 to 2004 ranged from  $24^\circ\text{C}$  to  $44^\circ\text{C}$  with a relative humidity of between 52 and 65%.

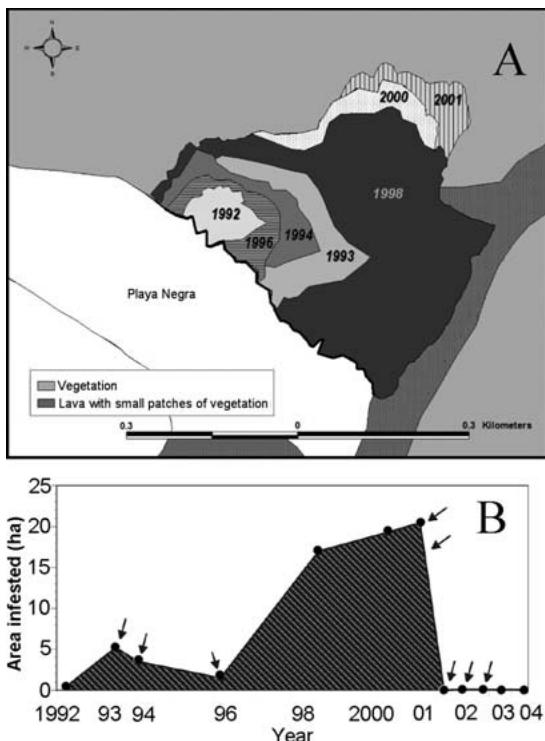


Fig. 2. A) Expansion of *W. auropunctata* at Playa Negra in Marchena Island, 1992-2001. B) Change in size of infested area. Arrows indicate applications of poison bait (Amdro®; see text for details).

#### Calculating the Size of the Treatment Area

In March 2001, 50 m longitudinal transects were cut outwards from the perimeter established in 2000 (Fig. 3) at 20 m intervals. Hot dogs (~5 mm thick, made of beef) were placed on the lower ends of 30-cm wire flags that were placed in the ground at 5-m intervals along these transects. Baits were checked after 45 min. In the event that *W. auropunctata* was recorded, transects were extended and additional baits placed at 5-m intervals until ants had not been detected for 50 m. The perimeter of the treatment area was established at least 50 m from the last infested point found in each transect to create a buffer zone between the infested and *W. auropunctata*-free areas (Fig. 3). The perimeter was tracked with a handheld GPS unit and the size of the area calculated with ArcView GIS (Version 3.2a, Environmental System Research Institute 1999). The area infested by *W. auropunctata* was estimated to be 20.5 ha. *Wasmannia auropunctata* was found up to 75 m away from where it was recorded in 2000. Including the buffer zone (6.1 ha), the area in which poison was applied and monitoring was conducted was estimated to be 26.6 ha. These measurements are two-dimensional and did not consider the topography of the area.

#### Preparation of Treatment Area

To enable the homogenous application of poison and facilitate monitoring, the treatment area was divided into five sectors (A, B, C, D, and I) based on the old perimeters and natural divisions provided by the terrain. In each sector 1.5-m wide longitudinal transects were cut with machetes through the vegetation at approximately 10-m intervals. By 2003, a total of 352 longitudinal transects had been cut in the treatment area ranging between 58 and 289 m in length (Fig. 3). Short latitudinal transects were cut in areas of especially dense vegetation. The sectors and transects were mapped by tracking with GPS units.

#### Control Techniques

Amdro® (Hydramethylnon with soybean oil, 0.88% active ingredient), a product developed for *Solenopsis* fire ants was used (Collins et al. 1992). This insecticide was the most attractive to *W. auropunctata* of four fire-ant products tested by Williams & Whelan (1992) and was also used to successfully control it on Santa Fe Island (Abedrabbo 1994). Amdro® was considered to be a minimum risk to non-targets because of its low toxicity to vertebrates, because it cannot be absorbed through the insect cuticle, and because it is not known to accumulate in the environment (Vander Meer et al. 1982; Extension Toxicology Network 1996; Bacev 2000). Some scavenging arthropods and arthropod predators, in particular ants, were expected to feed on the bait, but any localized non-target impacts that might occur would be negligible following re-colonization of the treatment area by invertebrates. However, the disadvantages of using this toxic bait are that it decomposes quickly and cannot be applied during or soon after rainfall (Vander Meer et al. 1982). Before each trip to Marchena, Amdro® was sampled at random and tested to ensure that it was still attractive to the *W. auropunctata*.

Amdro® was applied after 15.00 h to reduce exposure to sunlight. The bait was hand broadcast by groups of field assistants walking parallel to each other along adjacent transects. An average of 4.9 kg of Amdro® per hectare was applied to all sectors in the treatment area in March and June 2001 (Table 1). This was over double the quantity recommended by specialists (2.2 kg/ha) (D. Williams, University of Florida, Gainesville, pers. comm.), but was considered necessary because of the hilly terrain and the presence of caves and dense vegetation. Amdro® was only applied to sectors A and B in October 2001 after *W. auropunctata* had not been detected by the monitoring program in Sectors C, D and I in June and October 2001. In April and October 2002, the application of Amdro® was restricted to areas where small populations of *W. auropunctata* were found (Table 1).

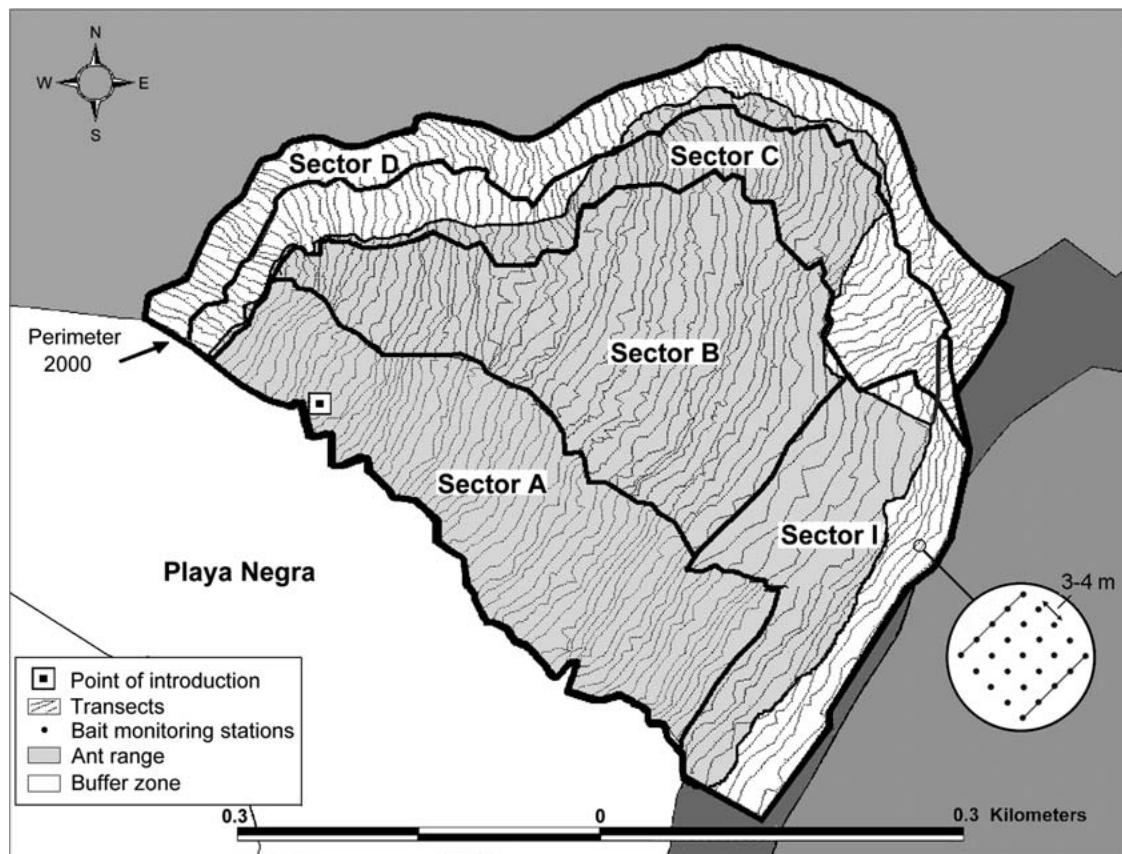


Fig. 3. Treatment area for eradicating *W. auropunctata* (Sector A: 6.8 ha, 65 transects; Sector B: 7.5 ha, 62 transects; Sector C: 4.4 ha, 103; Sector D: 3.9 ha, 107 transects; Sector I: 4.1 ha, 15 transects). The inset is a closer view of the 3-4 m grid of bait monitoring stations.

#### Monitoring the Effectiveness of Amdro® Applications

The intensity of monitoring increased as Amdro® applications decreased. In June 2001, three months after the first application, the primary objective was to detect any further spread of *W. auropunctata* in outlying Sectors C and D (Fig. 3). Hot dog baits were placed every 5 m in alternate transects and techniques were similar to those used to calculate the size of the treatment area in March 2001. Six months after the first application of Amdro® (October 2001), a more intensive monitoring program was begun placing peanut butter baits in grids 3-4 m apart (Fig. 3). In October 2001, the grid system was applied to sectors C, D, and part of I (bait stations were placed at 3-4 m intervals only along the length of the transects in the remaining sectors). For the last four trips (April and October 2002, May 2003, and April 2004), all sectors of the treatment area were monitored with a 3-4 m grid of bait stations. Additionally, in the area of introduction of *W. auropunctata* (Sector A, Fig. 3) and in the areas where small populations were found in April and Octo-

ber 2002, the distance between bait stations was reduced to every 1 m.

Peanut butter baits were used instead of hot dog baits because of the high proportion of hot dog baits that were eaten by lizards and hermit crabs on the first survey, and because peanut butter baits were easier to use in large numbers. The baits consisted of a wooden kebab stick (30 cm long) painted with a fluorescent marker on one end. Peanut butter was applied to the unpainted end from midway down. The pointed end of the stick was placed firmly in the ground to avoid removal by lizards and doves. Monitoring activities took place between 05:40-10:30 and 15:00-18:00 h and were not carried out on rainy days or during hours of intense sunlight when ants are less abundant. Bait stations were placed every 3-4 m along each of the longitudinal transects in the treatment area. Additional bait stations were placed every 3-4 m to the left and to the right of each of these bait stations until the bait stations on the adjacent transect were reached, thus forming a grid of 3-4 m squares (Fig. 3). The number of bait stations placed on each trip is shown in Table

TABLE 1. QUANTITY OF AMDRO® (KG) APPLIED TO TREATMENT AREA.

Date	Sectors	Area treated (ha)	Amdro (kg)	kg/ha
Mar 01	A, B, C, D, I	26.6	130	4.9
Jun 01	A, B, C, D, I	26.6	134	5.0
Oct 01	A, B	14.3	60	4.2
Apr 02 <sup>a</sup>	Part of A and I	3.4	27	7.8
Oct 02 <sup>a</sup>	Part of A, all of I	10.9	45	4.2
Total			396	

<sup>a</sup>Amdro® applied in response to finding colony fragments of *W. auropunctata*.

2. To ensure that the entire area was covered by bait stations, 4–5 groups of field workers worked parallel to each other along adjacent transects. Bait was left for one hour after which it was inspected for ants. Field workers were trained to identify and record *W. auropunctata* and the three most common ant species that they might encounter: *Tapinoma melanocephalum* (Fabricius), *Cardiocondyla emeryi* Forel and *Monomorium floridola* (Jerdon). When a field worker believed that they had detected *W. auropunctata*, the ants were collected and the site was marked. Bait sticks were counted at the end of each transect to check that all bait stations had been collected.

#### Nocturnal Monitoring

Nocturnal monitoring was carried out because it is possible that *W. auropunctata* resorts to feeding more actively in the night when diurnal temperatures are high and humidity is low (Meier 1994). Because time was limited, we surveyed only where *W. auropunctata* had initially been introduced in Sector A (Fig. 3). Bait stations were laid out on one night of each trip between 20:00 and 21:00 h. In October 2001, hot dog baits were placed every 10 m for 100 m along three transects with 30 m between transects. The number of transects was intensified on subsequent trips and peanut butter baits were used. Baits were placed at 5-m intervals along the first 50 m of 10 transects in April 2002, and along 42 transects in

October 2002, May 2003, and April 2004 (Table 2). In all cases transects commenced at the beach. Additionally, baits were laid out every 1 m in the areas where colony fragments were found on previous trips.

#### Estimation of Colony Fragment Size

To determine the size of the remnant colonies discovered in April and October 2002, we placed peanut butter sticks in a grid with 1-m intervals centered on the bait station where *W. auropunctata* was detected. Baits were checked after an hour and in the event that ants were found, the flags were left in place and the grid amplified until ants had not been observed for 10 m in each direction.

## RESULTS

#### *Wasmannia auropunctata* numbers

*Wasmannia auropunctata* was not detected at 700 non-toxic bait stations in Sectors C and D three months after the onset of the eradication program in June 2001 (Table 2). After two applications of Amdro® (October 2001), *W. auropunctata* was not recorded at 11,058 bait stations placed in all sectors of the treatment area. In April 2002, one year after the first toxic bait application *W. auropunctata* was recorded at three of 33,638 non-toxic bait stations (Table 2). A pop-

TABLE 2. MONITORING EFFORT IN THE TREATMENT AREA (26.6 HA) TO DETECT THE PRESENCE OF *W. AUROPUNCTATA* AND OTHER ANT SPECIES (CALCULATED NUMBER OF STATIONS REQUIRED FOR COMPLETE COVERAGE OF A TWO-DIMENSIONAL AREA WITH 3 M BETWEEN POINTS WAS 36,012).

	Monitoring dates					
	Jun 01	Oct 01	Apr 02	Oct 02	May 03	Apr 04
Man hours (in the field)	~392	~432	504	743	698	735
Total number of diurnal bait stations	700	11,058	33,638	36,251	44,142	40,100
Total number of nocturnal bait stations	—	33	110	570	780	780
Stations with <i>W. auropunctata</i>	0	0	3	2	0	0
Stations with other ant species	—	897	35	6,530	3,408	10,812

ulation of ants was located in a dry streambed in Sector I. Along a 1-m grid of non-toxic baits the infestation size was estimated to be at least 87 m<sup>2</sup> and measured about 6 m by 18 m. *Wasmannia auropunctata* was also found on two out of 36,251 non-toxic bait stations in October 2002 in Sector A (Table 2). With baits set in a 1-m grid, the colony was estimated to measure 99 m<sup>2</sup> in an irregular patch up to 10 m wide and 21 m long. After discovery of these populations, Amdro® was applied to the infested areas and the surrounding non-infested areas (Table 1). *Wasmannia auropunctata* was not registered in these areas on subsequent trips. In the last two monitoring surveys in May 2003 and April 2004, *W. auropunctata* was not detected at 44,142 and 40,100 bait stations, respectively. *Wasmannia auropunctata* was not recorded at any of the non-toxic bait stations placed at night during the monitoring surveys (Table 2).

Based on an equidistant point method on Arcview, the number of non-toxic bait stations placed in the last four monitoring surveys was calculated to be similar to or higher than the number of stations required for complete coverage with 3 m between points on a two-dimensional plane (Table 2).

#### Presence of Other Ant Species

*Tapinoma melanocephalum*, *C. emeryi*, and *M. floridana* were recorded from the non-toxic bait stations in varying intensities on all four monitoring trips. In October 2001 these ant species were present in approximately 8% of the total number of non-toxic bait stations, whereas in April and October 2002 ants occupied 0.1% and 18% of the stations, respectively. In May 2003 and April 2004, these ant species were again found in 7.7% and 27% of the stations (Table 2). It is possible that some of the identifications of *C. emeryi* may have been *Cardiocondyla nuda* (Mayr), as these two species are visually similar.

## DISCUSSION

#### Efficacy of Chemical Applications

To our knowledge, this is the largest eradication program that has been attempted for *W. auropunctata*. Monitoring results suggest that the application of Amdro® along a series of closely cut linear transects is an effective means of reducing *W. auropunctata* populations rapidly. Following three applications of poison bait over a 9-month period we have detected only two small patches of ants in approximately 0.1% of the area originally infested by *W. auropunctata*. A larger number of nest remnants was expected, especially given the difficult terrain. Negative results with intensive monitoring techniques in Sectors C and D suggests that *W. auropunctata* spread was contained

and that ants may have been eradicated from this area after two applications of Amdro®. Nevertheless, as intensive monitoring was only carried out in all sectors beginning with the third application, we cannot make any determinations about the effectiveness of each individual application in eradicating ants from the entire infested area. It may have been that there were only a few survivors after the first application. However, because funding was limited and we wanted to guarantee that the populations were hit hard, additional bait applications and land clearing activities were given priority over monitoring surveys.

The apparent effectiveness of the chemical applications may have been augmented by the effect of extended dry periods following the first bait application in March 2001. The lower elevations of Marchena Island are typically very arid during the dry season (May to December) and there was very little green vegetation on subsequent trips in June and October 2001. *Wasmannia auropunctata* prefers moist habitats and only dominates arid zones when temperature and humidity are high (Clark et al. 1982; Lubin 1984, 1985). These dry conditions probably inhibited nest-founding activities in any nests that were not destroyed by the first chemical application. Both *W. auropunctata* density and the production of sexuals appear to be influenced by humidity, and decreases in both occur in the drier months (Clark et al. 1982; Ulloa-Chacón 1990). Furthermore, ant nests are typically found above ground (Lubin 1984, 1985; Ulloa-Chacón & Cherix 1990; Ambrecht & Ulloa-Chacón 2003) and are susceptible to drying out when humid nesting sites are less abundant (Lubin 1984).

During the monitoring surveys, two small populations of *W. auropunctata* were discovered. These may have been missed by the chemical applications because of the hilly and volcanic terrain, particularly at the beginning when the methodology was still being worked out and the distance between transects was larger. This is the most likely explanation for the small population discovered in Sector I in April 2002 where Amdro® was only applied twice. However, it is less likely that the population discovered in Sector A in October 2002 was missed, as Amdro® was applied three times in this area. The topography of the land may have influenced the success rate of some of the applications, while some poison bait may have been deactivated by high temperatures during shipment to the Galápagos. Intensive monitoring along a 3-4 m grid was only initiated in these sectors in April 2002 and may explain why these populations were not detected earlier. It is also possible that nests that were partially hit by the Amdro® applications in 2001 may have taken some time to build up population numbers and initiate foraging activities. For example, in New Zealand non-toxic baits did not attract the Argentine ant, *Linepithema humile* (Mayr) nine months

after treatment with toxic bait although searching revealed their presence (Harris et al. 2002).

Conversely, at least three sympatric ant species (*T. melanocephalum*, *C. emeryi*, and *M. floridana*) were collected from the non-toxic baits following the application of Amdro®. These introduced species were present in the *W. auropunctata* infested area before treatment began (Roque-Albelo et al. 2000; Mieles 2002) suggesting that they either were not affected as much by the toxic bait or had re-invaded rapidly after treatment. Fluctuations in ant numbers corresponded to patterns observed in non-infested areas during the same period and appear to be related to climate (Mieles 2002).

#### Effectiveness of Monitoring

The monitoring techniques used during this program should have been sufficient to detect the presence of *W. auropunctata*. Baits were made up of peanut butter, which has been shown to be highly attractive to *W. auropunctata* in the laboratory and in the field (Williams & Whelan 1992). Studies on the foraging behavior of *W. auropunctata* suggest that if ants were present in the area they would have been attracted to the non-toxic baits under most climatic conditions (including strong wind, heavy rain, and full sunlight) and at all times of the day, although ant numbers may vary (Clark et al. 1982; Meier 1994; Delsinne et al. 2001). The distance between bait stations should have permitted ants to reach the baits within an hour. *Wasemannia auropunctata* typically makes superficial nests under most environmental conditions (Lubin 1984, 1985; Ulloa-Chacón & Cherix 1990; Ambrecht & Ulloa-Chacón 2003). Although little is known about the foraging distances of *W. auropunctata*, workers have been observed to forage up to 2 m high in trees (de la Vega 1994; Meier 1994). With a mean foraging speed of between 15–18 cm/min (Meier 1994), and assuming that ants could detect baits up to ~2.1 m away (radius of the circle defined by the grid size), ants should have been recruited to the baits within an hour. Extended drought periods, however, are associated with lower ant abundance (Clark et al. 1982; Ulloa-Chacón 1990) and have been known to cause hypogaeic nesting in other parts of the Galápagos (Abedrabbo 1994; Meier 1994). Thus, some nests may not have been able to locate the bait within an hour under these conditions. Although, we did not find any nests below the ground in Marchena, we have restricted our monitoring efforts in the last two years to the end of the wet season when surviving colonies are expanding and food is in demand.

Populations that have been reduced by toxic baits also may be slow in reacting to the non-toxic bait stations. When peanut butter bait stations were set 3–4 m apart, *W. auropunctata* was de-

tected at only three bait stations in April 2002 and two bait stations in October 2002. Yet, on both occasions, the area occupied by *W. auropunctata* proved to be larger (87 m<sup>2</sup> and 99 m<sup>2</sup>, respectively), as was discovered when the distance between bait stations was reduced to 1 m. Approximately 13 bait stations should have picked up *W. auropunctata* at 3-m intervals. This may be because the populations were small and workers took longer to find and recruit to the baits at wider spacing.

Studies on the foraging behavior of *W. auropunctata* have not been repeated in different climatic conditions sufficiently to identify optimal conditions for monitoring. While it is likely that non-toxic bait stations were laid out when *W. auropunctata* was active, we suggest that repeated experimental trials be carried out with different population sizes (including those that have been partially hit by toxic bait applications) to determine foraging speed, distance, and peak foraging hours under all climatic conditions and that monitoring activities are modified accordingly. Nevertheless, provided that monitoring is maintained for several years it is likely that any surviving pockets of *W. auropunctata* should grow large enough to be detected at the level of intensity being employed in this study.

#### Could *W. auropunctata* Exist Outside the Containment Area?

Current evidence suggests that outlying populations are unlikely on Marchena unless independent introductions have occurred elsewhere on the island. *Wasemannia auropunctata* has not been collected from six batteries of pitfall traps randomly placed within a 500-m radius of the treatment area on six occasions between 2000 and 2004 (Mieles 2002; A. Mieles, CDRS, Galápagos, pers. comm.), nor has it been collected in surveys that have been initiated on other parts of the island (C. S., unpubl. data).

These findings seem to indicate that *W. auropunctata* has not used long distance dispersal as a means for spreading in Marchena. In areas where it has been introduced, *W. auropunctata* typically forms new colonies by budding, where inseminated queens are accompanied by workers on foot to a nearby site (e.g., Hölldobler & Wilson 1977; Lubin 1984). This leads to well-demarcated boundaries of infested versus non-infested areas as shown by Clark et al. (1982) and which were observed on Marchena Island. This dispersal strategy is corroborated by observations in the laboratory of intranidal mating and by the fact that queens were unable to establish new colonies in the absence of workers (Ulloa-Chacón 1990; Ulloa-Chacón & Cherix 1990). Furthermore, workers have been observed moving winged queens on Santa Cruz Island in the Galápagos (Clark et al. 1982), and until recently nuptial flights of *W. au-*

*ropunctata* had never been observed either in the field or laboratory (Spencer 1941; Sielberglied 1972; Lubin 1984; Ulloa-Chacón 1990). Mating flights have been reported, however, among *W. auropunctata* populations in Puerto Rico (Torres et al. 2001). It is evident that there are still many gaps in our knowledge of the population biology of *W. auropunctata*, highlighting the need for continued monitoring in Marchena and further studies in its native and introduced range to better understand the mechanisms used for colony reproduction.

#### Future Needs and Application to Other Island Ecosystems

Given the track record of *W. auropunctata*, it can only be a matter of time before it is introduced into other islands, especially in the Pacific. We strongly recommend that early warning systems are set up and that rapid response plans are available in the event that *W. auropunctata* is detected. We also recommend pre-approval of effective bait treatment because this can save months of delays if suitable treatment products are not currently registered for use.

Our results from Marchena indicate that these eradication techniques are effective for limiting the spread and possibly also for eradicating well established populations of *W. auropunctata* of up to at least 20 ha in size. Aerial application should be considered if the infestation is more than a few hectares and suitable aircraft are reasonably available. Amdro® is relatively safe to use in conservation areas but we recommend that toxicity studies be carried out on non-targets before applying the poison bait to areas where re-colonization of invertebrates from outlying areas is not possible. Caution should also be used in areas with water sources because of its toxicity to fish (Extension Toxicology Network 1996) and possible impact on aquatic invertebrates. Chemical applications are likely to be more successful at the beginning of the dry season when the reproductive potential of *W. auropunctata* is reduced and toxic bait applications are more effective. Post application surveys are crucial to the success of the program and are the only way to ensure that *W. auropunctata* has been eliminated. Surveys should be carried out only in the rainy season. Although labor intensive, there is no substitute for detailed mapping of the area with bait sticks. The smaller the grid size the greater the accuracy in evaluating the effectiveness of the poison bait applications.

Ultimately the intensity of the monitoring will depend upon financial and manpower constraints, but we believe that intense early monitoring may provide savings in the long run. To date, the program in Marchena has cost approximately \$212,736 US (this includes time spent preparing for the field trips, field and laboratory work, and

overhead). The cost for the purchase and shipping of Amdro® was approximately \$10,700. Assuming that no more ants are found on the next two monitoring trips, the total projected cost for removing *W. auropunctata* for each hectare of infested area is estimated at \$13,680. Personnel costs accounted for approximately 47% of the total spent on this program and can be reduced by using trained volunteers. Approximately 25% of these costs were for inter-island transport and surveys to evaluate the response of native invertebrate communities and may not be needed elsewhere. Additional studies on foraging behavior and the refinement of bait application and monitoring procedures should help us improve these techniques and make them less labor intensive and costly.

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## DYNAMICS OF A SUBTROPICAL POPULATION OF THE ZEBRA LONGWING BUTTERFLY *HELICONIUS CHARITHONIA* (NYMPHALIDAE)

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### ABSTRACT

We studied the population dynamics of the zebra longwing butterfly, *Heliconius charithonia* (Nymphalidae), in a 0.05 ha garden in Miami, Florida, for 2 years to answer the following questions: (1) How stable is a suburban, subtropical population of this widespread neotropical butterfly? (2) What are the major factors influencing its population dynamics? (3) What are the implications of adult mobility regarding gene flow within and between fragmented urban populations of this species? A mark-recapture study indicated that adult population size averaged 59 individuals (range: 9-115 individuals). Peak numbers occurred in mid-wet season (September) in 1996 and in the late dry and early wet seasons (April through June) in 1997. Fluctuations in size of the adult population paralleled changes in biomass of the larval food plant, *Passiflora incense*. Population sex ratio was consistently male-biased (68% males). Reproduction occurred year-round, and parasitism by a trichogrammatid wasp killed about 50% of *Heliconius* eggs throughout the year. Recruitment of adults from chrysalises produced in the garden and deaths, rather than immigration and emigration, accounted for most numerical changes. Both males and females apparently adjust their home range locations in response to changes in the biomass of *Passiflora* plants. Females search these plants for suitable oviposition sites, and males search them for female pupae (mates). In addition to relatively high mortality from egg parasitism, fluctuations in the number of suitable oviposition sites and amount of larval food limited recruitment into the adult butterfly population. High adult mobility probably results in substantial gene flow within and between populations of this butterfly in urban south Florida.

Key Words: population dynamics, food limitation, egg parasitism, *Heliconius charithonia*

### RESUMEN

Estudiamos la dinámica poblacional de la mariposa "cebra de alas largas", *Heliconius charithonia* (Nymphalidae), en un jardín de 0,05 ha en Miami, Florida, por dos años para responder a las siguientes preguntas: (1) ¿Qué tan estable es una población suburbana subtropical de esta mariposa neotropical ampliamente distribuida? (2) Cuáles son los principales factores que influencian su dinámica poblacional? (3) Cuáles son las implicaciones del movimiento de adultos en el flujo de genes dentro de y entre poblaciones urbanas fragmentadas? Un estudio de marca-recaptura indicó que el tamaño de la población adulta promedió 59 individuos (rango: 9-115 individuos), con picos en la mitad de la estación lluviosa (Septiembre) de 1996 y al final de la estación seca e inicio de la estación lluviosa (Abril a Junio) de 1997. Las fluctuaciones en el tamaño de la población adulta fueron paralelas a los cambios en biomasa de la planta huésped del estadio larval, *Passiflora incense*. La proporción de sexos en la población fue consistentemente sesgada hacia los machos (68% machos). La reproducción ocurrió durante todo el año, pero una especie de avispa tricogrammatidae parásita mató alrededor del 50% de los huevos de *H. charithonia* producidos en el año. Más que la inmigración y emigración, el reclutamiento de adultos, a partir de crisálidas producidas en el jardín, y la mortalidad controlaron el tamaño de la población. Tanto machos como hembras aparentemente modifican sus ámbitos hogareños en respuesta a cambios en la biomasa de las plantas *Passiflora*. Las hembras visitan estas plantas en búsqueda de sitios óptimos para la oviposición, mientras que los machos lo hacen para buscar pupas hembras (parejas para reproducción). Además de la relativa alta mortalidad causada por el parasitismo de los huevos, las fluctuaciones en el número de sitios óptimos para la oviposición y la cantidad de comida para las larvas limitaron el reclutamiento hacia la población adulta de mariposas. La alta movilidad de los adultos probablemente resulta en un flujo sustancial de genes dentro de y entre las poblaciones de esta mariposa en el sur de la Florida.

Translation provided by the authors.

Butterflies of the genus *Heliconius* are common and conspicuous members of forest habitats throughout the neotropics. Studies of their population dynamics indicate that *Heliconius* butterfly populations (i) occur in low population densities (<5 per ha), (ii) have biased sex ratios (usually towards males), (iii) reproduce year-round, (iv) have maximum adult lifespans ranging from 90 to 180 days, and (v) contain individuals that exhibit home range behavior (Ehrlich & Gilbert 1973; Cook et al. 1976; Saalfeld & Araujo 1981; Quintero 1988; Gilbert 1991; Ramos & Freitas 1999). Compared with most temperate butterflies, species of *Heliconius* are low density, long-lived insects with extended reproductive lifespans. The degree to which *Heliconius* populations are genetically open or closed has been controversial. Brazilian workers (e.g., Romanowski et al. 1985; Haag et al. 1993; Silva & Araujo 1994) have reported that populations of *Heliconius erato* are 'insular' and inbred whereas Mallet (1986) suggested, based on mark-recapture data, that individuals of *H. erato* in Costa Rica disperse considerable distances and are not likely to be inbred.

What are the structure and dynamics of *Heliconius* butterfly populations near the northern geographic limits of the group? As has been reported for some species of temperate butterflies (e.g., Thomas et al. 1994; Shreeve et al. 1996; VanStrien et al. 1997), are subtropical populations more variable in size than tropical populations? Are mortality rates higher and reproductive seasons shorter in northern populations? To answer these questions, we studied the dynamics of a population of the zebra longwing butterfly, *Heliconius charithonia* L. (Nymphalidae), for two years in Miami-Dade County, Florida. *H. charithonia* is widely distributed in the southeastern United States, the West Indies, and Central and northern South America (Opler & Krizek 1984). According to Gilbert (1991), this species is more common in disturbed than in undisturbed sites and prefers seasonal tropical sites to evergreen forests. *H. charithonia* is a phylogenetically advanced member of its genus the larvae of which are feeding specialists on non-woody, short-lived species of *Passiflora* (passion vines) found in successional habitats. Adults tend to collect smaller, less nutritious pollen grains than less-advanced heliconiines. Pupal mating occurs in this species, and females lay their eggs gregariously on fresh shoots. Larvae are non-aggressive and feed on older leaves only after fresh shoots are depleted.

#### MATERIALS AND METHODS

##### Study Site

This study was conducted for two years (mid-December 1995 to mid-December 1997) in a 0.05-ha. garden in suburban Miami, Florida ( $25^{\circ}49'N$ ,

$80^{\circ}17'W$ ). Most observations were made in a 15-m  $\times$  20-m section of the garden, which was planted in 'butterfly' plants, including *Pentas lanceolata*, *Stachytarpheta fruiticosa*, and *Hamelia patens*, that provided nectar and pollen for adults. One individual of *Passiflora incense*, a non-native (hybrid) larval food plant (Vanderplank 2000), was planted at the base of a clothes pole that served as an arbor in January 1995. By late 1995 rhizomes of this plant had spread over a relatively large portion of the 300 m<sup>2</sup> intensive study area, and new stems emerged continuously throughout the study. Prior to 1 August 1997, we did little to control the growth of *Passiflora* in the garden. By that date, however, vines had overgrown much of the study area, and we removed >95% of the *Passiflora* biomass. A native *Passiflora* (*P. suberosa*) occasionally 'volunteered' in the garden but was quickly eliminated by *Heliconius* herbivory.

The climate of Miami includes a 5-month dry season (November-April) and a 7-month wet season (May-October). About 75% of Miami's average annual rainfall of 1,420 mm falls during the wet season. Annual rainfall was above average in 1996 and 1997 and totaled 1,466 and 1,795 mm, respectively. During the study, lowest temperatures of 2-3°C occurred on one day each in January and February 1996 and January 1997, and highest temperatures of 34°C occurred in June 1996 and July and August 1997. In this study, we recognize two seasons per year: dry season (1 November-30 April) and wet season (1 May-31 October).

In addition to *H. charithonia*, two other species of heliconiid butterflies frequented the garden. The gulf fritillary, *Agraulis vanillae* (L.), was present in low numbers (but sometimes in equal abundance to that of the zebra longwing) during most of the study. Its larvae co-occurred with those of *H. charithonia* on *Passiflora* vines. Much less common than the gulf fritillary was the julia, *Dryas iulia* Clench, which apparently did not oviposit in the study area.

##### Methods

Size of the adult population feeding, ovipositing, and mating in the garden was estimated by mark-recapture methods. One morning each week, butterflies were captured with hand nets and released during a period of 1.5-2 h. Captured individuals were marked by writing a number on the outside surface of both hindwings with a Sharpie® Extra Fine Point permanent marker. At an individual's initial capture, we noted its sex, wing length, and wing wear condition. On subsequent captures, we recorded ID number and wing wear condition. We measured wing length to the nearest 0.5 mm with a plastic ruler as the distance from the bottom of the left hindwing to the tip of the left forewing. We scored wing wear condition in one of five categories: 0 = no wear (bright,

intense color), 1 = early wear (color less intense but still bright), 2 = medium wear (color faded but wings still opaque), 3 = extensive wear (color very faded and wings partially transparent), and 4 = extreme wear (little color, wings extensively damaged). Butterflies were retained for ca. 5 min at each capture. At approximately weekly intervals between 4 July 1996 and 24 March 1997, we counted the number of adults night-roosting on a *Citrus* tree in the garden after sunset.

We surveyed 2–10 major *Passiflora* stems, depending on availability, for heliconiid eggs, larvae, and chrysalises once each week. Throughout the study the “clothes pole” plant was the largest *Passiflora* stem and was always surveyed for immature life stages. Other stems  $\geq 1$  m in length were surveyed whenever they contained fresh growth. We could not distinguish between the eggs of *H. charithonia* and *A. vanillae*, so our egg counts include both species. Number of larvae in each of three size categories (<1/3 maximum length (= “small”), >1/3 but <1/2 maximum length (= “medium”), and >1/2 maximum length (= “large”) was recorded separately for both heliconiid species. Finally, we searched each plant for zebra longwing chrysalises and counted and marked each one with a small spot of fingernail polish to distinguish “new” from “old” chrysalises.

We estimated egg mortality due to parasitism by trichogrammatid wasps between 6 October 1996 and 21 November 1997 in two ways. First, every two weeks between 6 October 1996 and 10 May 1997 we removed 15 randomly chosen eggs from new leaves or stems and placed each one in a labeled 1.5 mL microcapillary tube. Eggs were examined daily and the fate of each egg (no larvae or parasites emerged, larva emerged, parasitic wasps emerged) was recorded for two weeks. Because all larvae emerging from eggs were those of *H. charithonia*, these mortality estimates are not confounded by the presence of *A. vanillae*. Second, six times in 1997 (twice in April and May, once in July and November) a series of 4–109 eggs on 1–14 plants was marked with a dot of ink on the leaf next to each egg. For the next 2–9 days the status of each egg was scored as “parasitized” (egg turned dark gray), hatched (egg shell empty), or “gone.” Number of small larvae of *H. charithonia* and condition of the leaf bearing the eggs (intact or chewed) also were recorded every day.

To estimate the amount of *Passiflora* biomass present in the study area each week, we took notes on the condition of each stem (i.e., each ramet) that we surveyed. Each stem was given one of the following “condition” scores: 0 = chewed back to ground level; 1 = new or regrowth  $\leq 1$  m long; 2 = a medium-sized, non-flowering plant; 3 = a large, flowering plant; and 4 = a very large, flowering plant. These scores represent the approximate greatest length (in m) of each stem. The sum of the scores of stems was multiplied by 2 to represent

the approximate biomass of *Passiflora* foliage (in  $m^2$ ) present in the intensive study area each week.

#### Population Estimation

Capture-recapture data were analyzed in a set of models implemented in program TMSURVIV; J. Hines, unpublished), a modification of program SURVIV (White 1983) developed to compute estimates under the transient models of Pradel et al. (1997). Transient models represent a generalization of the standard Cormack-Jolly-Seber model (Cormack 1964; Jolly 1965; Seber 1965) developed for open populations (i.e., populations that can experience gains and losses between sampling periods). The generalization basically involves the possibility of different survival probabilities for animals captured in any sampling period,  $i$ , depending on whether or not the animal has been captured previously (i.e., whether the animal is marked or unmarked).

The most general model contains three kinds of parameters indexed by both time (sampling period,  $i$ ) and sex ( $s$ ):

$\phi_i^{(s)}$  = the probability that a marked butterfly of sex  $s$  in the population at time  $i$  survives and is present in the area exposed to sampling efforts in period  $i+1$ ;

$\gamma_i^{(s)}$  = the ratio of survival probabilities ( $i$  to  $i+1$ ) of unmarked to marked butterflies of sex  $s$  (in a population with transient individuals, this ratio is the probability that an unmarked butterfly is a transient);

$p_i^{(s)}$  = the probability that a butterfly of sex  $s$ , in the population at time  $i$ , is captured at  $i$ .

The zebra longwing butterfly data set contained 97 sampling occasions, and the most general model thus contained a very large number of parameters. Several reduced-parameter models were created by eliminating one or more sources of variation in model parameters. For example, models with  $\gamma_i^{(s)} = 1$  incorporated the assumption of the standard Cormack-Jolly-Seber model that survival probability does not depend on mark status. Some models incorporated the assumption of parameters constant over time (e.g.,  $p_i^{(s)} = p^{(s)}$ ), and others assumed equality of parameters for males ( $m$ ) and females ( $f$ ) (e.g.,  $p_i^{(m)} = p_i^{(f)} = p_i$ ). Although most sampling was conducted on a weekly basis, this was not strictly true, and the time intervals separating successive sampling periods varied. For this reason, the time constraint,  $\phi_i^{(s)} = \phi^{(s)}$  makes little biological sense. However, the hypothesis of equal survival per unit time is reasonable, so we reparameterized survival in the model as  $\phi_i^{(s)} = (S_i^{(s)})^t$ , where  $t$  denoted the time period (in weeks) separating sampling periods  $i$  and  $i+1$ . Thus, we effectively scaled survival probability to a weekly time interval, and the hypothesis of

equal weekly survival over time,  $S_i^{(s)} = S^{(s)}$ , is biologically plausible.

We fit 14 different models to the data and followed the general suggestions of Lebreton et al. (1992) for model notation. Model  $(S_i^{(s)}, p_i^{(s)}, \gamma_i^{(s)})$ , the general model of Pradel et al. (1997), was the most general model in our model set. All three classes of parameter include the subscript  $i$ , denoting full time variation, and the superscript  $s$ , denoting sex-specificity of parameters. Absence of a superscript or subscript indicates that the source of variation is omitted from the model parameterization. For example, model  $(S^{(s)}, p^{(s)}, \gamma)$  includes no time-specificity for any parameter (denoted by absence of subscripts,  $i$ ), and no sex-specificity either for  $\gamma$ . We considered models that assumed equal survival probabilities for previously marked and unmarked butterflies (the Cormack-Jolly-Seber model assumption), and denoted this assumption as  $\gamma = 1$ . Thus, model  $(S, p, \gamma = 1)$  denotes a simple 2-parameter model in which survival and capture probability are constant over time and the same for males and females and survival is the same for marked and unmarked butterflies.

The software, TMSURVIV, provides maximum likelihood estimates of the parameters and associated estimates of variances and covariances under each model. Adequacy of fit of the most general model was judged using a parametric bootstrap approach. Under this approach, capture history data were simulated under the general model by treating the parameter estimates as true values. The general model was then fit to each simulated data set, and maximum likelihood estimates obtained. The standard  $G^2$  goodness-of-fit statistic was also computed for each simulated data set. We ran 100 simulations and compared the  $G^2$  statistic from the actual data analysis with the distribution of statistics from the simulated data sets to test for fit. In the event of poor model fit, we followed the approach recommended by White & Burnham (1999) of estimating a variance inflation factor,  $\hat{c}$ , as the ratio of the observed  $G^2$  to the mean of the  $G^2$  values from the simulations. In the event of poor model fit, variance estimates were obtained using a quasi-likelihood approach (e.g., Burnham et al. 1987; Lebreton et al. 1992) as the product  $\hat{c} \text{var}(\hat{\theta})$ , where  $\hat{\theta}$  is the maximum likelihood estimate of parameter  $\theta$  under the selected model,  $\text{var}(\hat{\theta})$  is the associated model-based variance estimate, and  $\hat{c}$  is the variance inflation factor associated with lack of model fit and estimated as described above.

Model selection was based on QAI<sub>C</sub> values, Akaike's Information Criterion adjusted for lack of fit (quasi-likelihood) and sample size (Burnham & Anderson 1998). AIC can be viewed as an optimization criterion useful in model selection (Akaike 1973; Burnham & Anderson 1998). The criterion places value on good fit of the model to the data and on describing the data with as few

parameters as possible (Burnham & Anderson 1992, 1998; Lebreton et al. 1992).

Population size was not a model parameter but was estimated using the numbers of butterflies caught at each period ( $n_i^{(m)} =$  number of males;  $n_i^{(f)} =$  number of females;  $n_i^{(m)} + n_i^{(f)} = n_i =$  number of butterflies of both sexes combined) in conjunction with the associated estimates of capture probability ( $p_i^{(s)}$ ). Specifically, population size and its associated variance were estimated as:

$$\hat{N}_i^{(s)} = \frac{n_i^{(s)}}{\hat{p}_i^{(s)}} \quad (1)$$

$$\text{var}(\hat{N}_i^{(s)}) = \frac{(n_i^{(s)})^2 \text{var}(\hat{p}_i^{(s)})}{(\hat{p}_i^{(s)})^4} + \frac{n_i^{(s)}(1 - \hat{p}_i^{(s)})}{(\hat{p}_i^{(s)})^2} \quad (2)$$

Confidence intervals for  $\hat{N}_i^{(s)}$  were approximated using the approach of Chao (1989; also used and recommended by Rexstad & Burnham (1991)). The estimation is based on the estimated number of butterflies not detected,  $\hat{f}_{0,i}^{(s)} = \hat{N}_i^{(s)} - n_i^{(s)}$ . The  $\ln(\hat{f}_{0,i}^{(s)})$  is treated as an approximately normal random variable, yielding the following 95% confidence interval,  $(n_i^{(s)} + \hat{f}_{0,i}^{(s)}/C, n_i^{(s)} + \hat{f}_{0,i}^{(s)}C)$ , where

$$C = \exp\left(1.96 \left[ \ln\left(1 + \frac{\text{var}(\hat{N}_i^{(s)})}{(\hat{f}_{0,i}^{(s)})^2}\right)\right]^{1/2}\right).$$

#### Statistical Analyses

We used one- and two-way ANOVAs to test for the effects of sex and season on individual and population variables and Pearson correlation analyses to assess relationships between rainfall and butterfly population variables and *Passiflora* biomass. Analyses were conducted with Statmost ver. 3.5 (Dataxiom Software, Inc., Los Angeles, CA) and Systat ver. 10 (SPSS, Inc., Chicago, IL). For population and weekly count data, we tested for effects of season using repeated measures (rm) ANOVAs with Type III sums of squares (Zar, 1999) using SPSS ver. 10.1.0 (SPSS 2000, SPSS, Inc., Chicago, IL). We used the Greenhouse-Geisser correction to adjust degrees of freedom whenever inequality of sphericity was rejected by Mauchly's test. We used the "Standard Tests" module in Eco-Sim (Gotelli & Entsminger 2003) to assess the relationship between weekly adult population size and *Passiflora* biomass. Unless otherwise noted, means  $\pm 1$  SE are reported throughout this paper.

#### RESULTS

##### Capture Statistics

In the 2 yrs, we marked a total of 1,476 adults, including 929 males (62.9%) and 547 females, and

recorded a total of 2,729 captures and recaptures. Capture statistics, including the number of captures per individual and the number of weeks between first and last capture for each individual by sex and season, are summarized in Fig. 1A,B. A two-way ANOVA indicated that number of captures per individual varied by sex and season (sex:  $F_{1,1470} = 7.43, P = 0.007$ ; season:  $F_{4,1470} = 23.55, P < 0.0001$ ; the interaction was also significant:  $F_{4,1470} = 4.79, P = 0.001$ ). Males were generally captured more times than females (grand means were  $2.08 \pm 0.06$  and  $1.82 \pm 0.08$  captures, respectively), and number of captures per individual in the 1996-97 dry season was about 50% higher than in other seasons (Fig. 1A). A two-way ANOVA revealed that the number of weeks between first and last capture also differed by sex and season (sex:  $F_{1,1471} = 8.64, P = 0.003$ ; season:  $F_{4,1471} = 20.91, P < 0.0001$ ; the interaction was not significant ( $P = 0.23$ )). Males were generally captured over a longer time period than females (grand means were  $2.05 \pm 0.05$  and  $1.81 \pm 0.07$  weeks, respectively), and individuals had longer capture periods in the first three seasons of this study than in the last two (Fig. 1B). Reanalysis of these data after data from the short dry season in late 1997 were eliminated produced similar results.

#### The Population Model

The  $G^2$  goodness-of-fit statistic for general model ( $S_i^{(s)}, p_i^{(s)}, \gamma_i^{(s)}$ ) was 692.83 and was larger than all 100 of the  $G^2$ 's resulting from the bootstrap simulations. Because of the lack of fit of the general model to the capture-recapture data, we computed the quasi-likelihood variance inflation factor as described above to obtain  $\hat{c} = 1.21$ . Models with the lowest QAIICc values were those with parameters constant over time and with no difference between survival of unmarked and marked butterflies ( $\gamma = 1$ ) (Table 1). There was very little basis for selecting among the first few models, as indicated by the small  $\Delta\text{QAIICc}$ s. Daily survival estimates based on model ( $S^{(s)}, p^{(s)}, \gamma = 1$ ) were  $0.944 \pm 0.0021$  for males and  $0.939 \pm 0.0032$  for females. Estimated capture probabilities under this model were  $0.479 \pm 0.0163$  for males and  $0.447 \pm 0.0231$  for females. Parameter estimates for males and females were very similar, providing little evidence for sex-specific differences in capture probabilities. In fact, the very simplest model ( $S, p, \gamma = 1$ ) with single survival and capture parameters that were constant over time and sex was among the most reasonable models for this data set. From this, we conclude that survival rates and capture probabilities likely did not vary with season or sex.

#### Adult Population Size and Sexual and Body Size Composition

Estimates of the number of adults foraging in or passing through the garden each week aver-

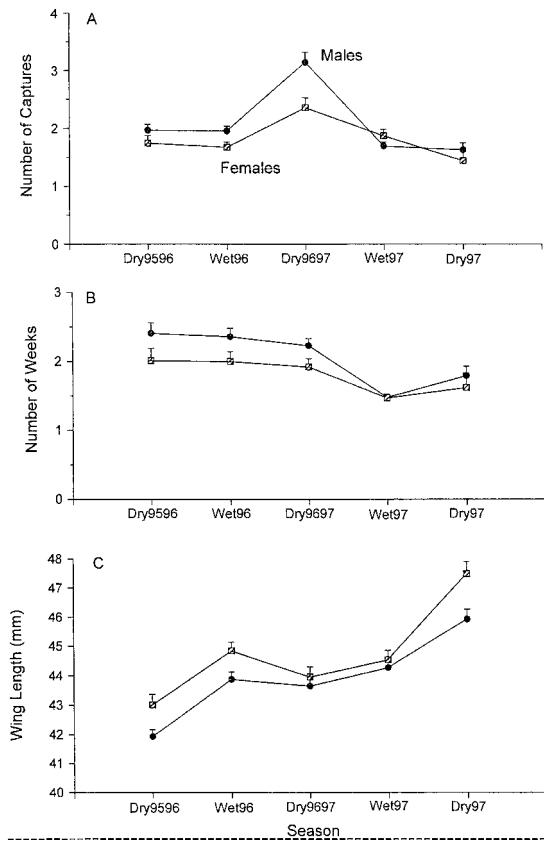


Fig. 1. Summary of the capture statistics for adults of *Heliconius charithonia*. A. Number of captures and recaptures per individual. B. Number of weeks between first and last capture records. C. Size of adults. Data are means  $\pm 1$  SE. Sample sizes range from 29-330.

aged  $58.9 \pm 2.9$  and ranged from a minimum of 9 (in August 1997) to a maximum of about 115 (in April 1997) (Fig. 2). In 1996 the population ranged between 20 and 70 individuals except for peaks of 85-100 individuals in the middle of the wet season (September) and early in the 1996-97 dry season (December). In 1997, the population steadily increased during the dry season and averaged over 80 individuals through July before declining rapidly. We removed most of the *Passiflora* in the garden after the decline began, and numbers slowly increased after 1 August 1997.

Size of the adult population fluctuated in parallel with changes in the biomass of *Passiflora* in the study area (Fig. 2). A simulation comprising 1,000 iterations indicated that the correlation between adult population size (Y) and *Passiflora* biomass (X) was significant ( $r_{\text{observed}} = 0.69; r_{\text{simulated}} = 0.00, P = 0.00$ ). One-way rm ANOVAs indicated that seasonal differences in weekly size of the butterfly population and *Passiflora* biomass were significant (adult population size: Greenhouse-

TABLE 1. MODEL SELECTION INFORMATION FOR MODELS FIT TO ZEBRA LONGWING BUTTERFLY CAPTURE-RECAPTURE DATA. QAICC IS CORRECTED QUASI-LIKELIHOOD AKAIKE'S INFORMATION CRITERIA.

Model <sup>a</sup>	No. parameters estimated	Log-likelihood	ΔQAICC
$S^{(s)}, p, \gamma = 1$	3	-1366.9	0.0
$S^{(s)}, p^{(s)}, \gamma = 1$	4	-1366.3	0.5
$S, p^{(s)}, \gamma = 1$	3	-1367.5	0.9
$S, p, \gamma = 1$	2	-1369.1	1.4
$S, p, \gamma$	3	-1368.6	3.4
$S^{(s)}, p^{(s)}, \gamma^{(s)}$	6	-1365.8	4.5
$S, p_i, \gamma = 1$	97	-1258.7	17.1
$S^{(s)}, p_i(s), \gamma = 1$	194	-1177.4	99.2

<sup>a</sup>Abbreviations:  $S$  = probability that a marked butterfly survives from sampling time  $i$  to  $i + 1$ ,  $p$  = probability that a butterfly that is in the population at time  $i$  is captured at  $i$ , and  $\gamma$  = the ratio of survival probabilities (from  $i$  to  $i + 1$ ) of unmarked to marked butterflies,  $s$  = sex.

Geisser-corrected  $F_{1,6,26,4} = 5.90$ ,  $P = 0.012$ ; *Passiflora* biomass: Greenhouse-Geisser-corrected  $F_{1,5,28,6} = 13.54$ ,  $P < 0.001$ ; data from the abbreviated 1997 dry season not included in these analyses). The adult butterfly population averaged 36% larger in 1997 than in 1996, and *Passiflora* bio-

mass was 48% lower during the 1995–96 dry season than in the next three seasons (Fig. 3A).

Neither size of the adult butterfly population nor *Passiflora* biomass appeared to respond to rainfall seasonality. Correlations between weekly estimates of both adult population size and pas-

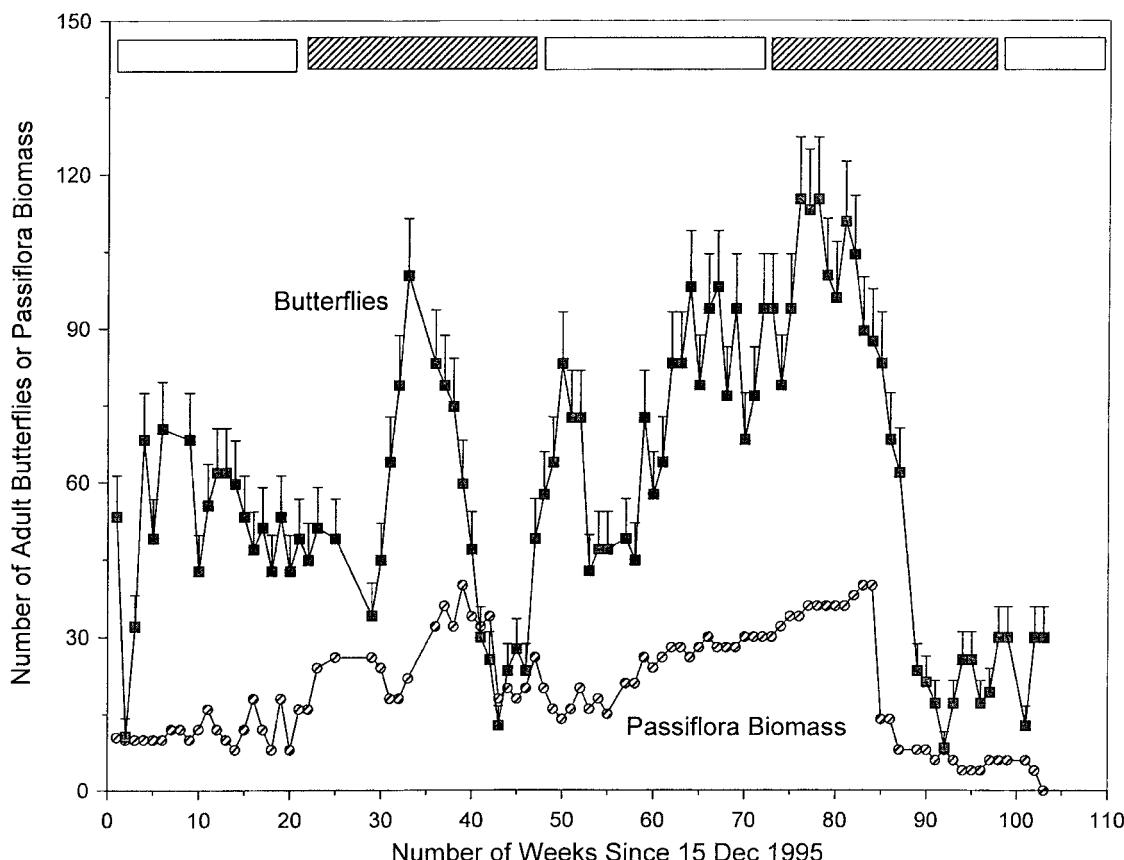


Fig. 2. Weekly estimates of the adult population size (+ 1 SE) of *Heliconius charithonia* and the biomass (in  $m^2$ ) of the larval host plant *Passiflora incense* from 15 December 1995 to 15 December 1997. The upper rectangles indicate dry seasons (open) and wet seasons (hatched).

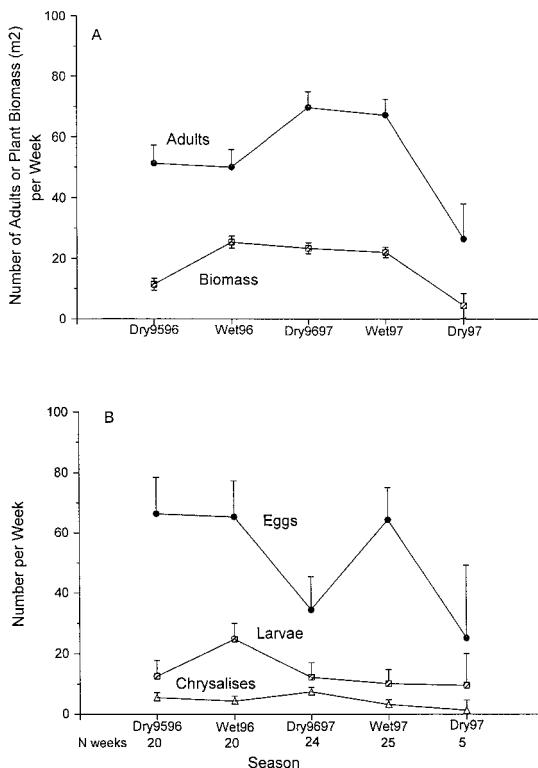


Fig. 3. Seasonal summaries of (A) weekly estimates of the adult butterfly population size and biomass of its larval host plant, *Passiflora incense*, and (B) weekly numbers of butterfly eggs, larvae, and chrysalises. Data are means + 1 SE. Sample sizes are indicated as N weeks.

sion vine biomass and rainfall were nonsignificant (Bonferroni-corrected  $P_s \geq 0.28$ ). Similar results were obtained when rainfall records were lagged by one and two weeks ( $P_s \geq 0.26$ ).

Between 4 July 1996 and 24 March 1997, an average of  $20.3 \pm 2.6$  (range = 0-46;  $n = 25$  counts) adults night-roosted in the garden. The number of night-roosting adults was positively correlated with adult population size (Pearson's  $r = 0.75$ ,  $df = 23$ ,  $P < 0.001$ ). At times, most of the adults foraging in the garden night-roosted there.

In most weeks the adult sex ratio was male-biased and averaged  $65.6 \pm 1.2\%$  males overall ( $n = 94$  weeks). A one-way rm ANOVA indicated that sex ratio varied among seasons ( $F_{3,57} = 2.95$ ,  $P = 0.04$ ; arcsine squareroot-transformed data and the short 1997 dry season not included). Sex ratio was higher in the dry season of 1996-97 ( $71.7 \pm 2.1\%$  males) than in the wet season of 1997 ( $60.3 \pm 2.7\%$  males). Thirty of 72 adults (41.7%) that we sexed at eclosure in the garden were males. Sex ratios of marked and enclosed adults differed significantly ( $\chi^2 = 12.3$ ,  $df = 1$ ,  $P < 0.001$ ).

A two-way ANOVA indicated that the size (wing length) of adults varied by sex and season

(sex:  $F_{1,1445} = 12.50$ ,  $P < 0.001$ ; season:  $F_{4,1445} = 24.49$ ,  $P < 0.001$ ). Except in the wet season of 1997, females were larger than males, and butterflies of both sexes were about 10% larger in late 1997 than in early 1996 (Fig. 1C).

#### Adult Survivorship and Wing Wear

Turnover rate of adults in this population was relatively high. Most adults were recaptured for periods of less than three weeks (Fig. 1B), and maximum capture periods were 9-10 weeks in both sexes. Most adults (86%,  $n = 1,504$ ) were recently eclosed (condition score 0) at first capture; individuals occurring in condition score categories 0 or 1 accounted for 98% of all first captures. In 1996, we tallied the wing wear condition of 57 and 30 recently eclosed males and females, respectively, that were captured  $\geq 3$  times. Rate of wing wear was high in both sexes. Most individuals had worn to very worn wings 6-8 weeks after first capture.

Results of the mark-recapture analysis also indicated that adult turnover rate was high. Adult males and females had daily survival probabilities of about 0.94, which represents a weekly survival probability of about 0.65. At this rate, adults had a probability of 0.031 and 0.013 of being alive eight and ten weeks after eclosion, respectively.

#### Egg and Larval Surveys

Eggs and larvae were found in most weeks, indicating that reproduction occurs year-round in south Florida (Fig. 4). The number of eggs counted each week averaged  $55.3 \pm 5.6$  (range: 0-326) and exhibited no significant seasonal variation (one-way rm ANOVA:  $F_{3,57} = 2.36$ ,  $P = 0.08$ ; only the first four seasons included in this analysis) (Fig. 3B). The number of zebra longwing butterfly larvae (of all sizes) counted per week averaged  $14.3 \pm 2.4$  (range: 0-167) and also exhibited no significant seasonal variation (Greenhouse-Geisser-corrected one-way rm ANOVA:  $F_{1,7,31,7} = 1.55$ ,  $P = 0.23$ ) (Fig. 3B). Finally, number of zebra longwing butterfly chrysalises counted per week averaged  $5.0 \pm 0.78$  (range: 0-38) and did not vary seasonally (Greenhouse-Geisser-corrected one-way rm ANOVA:  $F_{1,6,30,2} = 1.90$ ,  $P = 0.17$ ) (Fig. 3B).

Only the number of eggs per week was significantly correlated with estimated adult population size (Pearson's  $r = 0.32$ ,  $df = 90$ , Bonferroni-corrected  $P = 0.037$ ). Total number of *H. charithonia* larvae per week but not chrysalises was significantly correlated with number of eggs (Pearson's  $r = 0.36$ ,  $df = 92$ , Bonferroni-corrected  $P = 0.011$ ). When we applied a 3-week time lag (the average egg-to-chrysalis duration) to the egg data, however, number of chrysalises was significantly correlated with number of eggs (Pearson's  $r = 0.25$ ,  $df = 89$ , Bonferroni-corrected  $P = 0.018$ ). Finally,

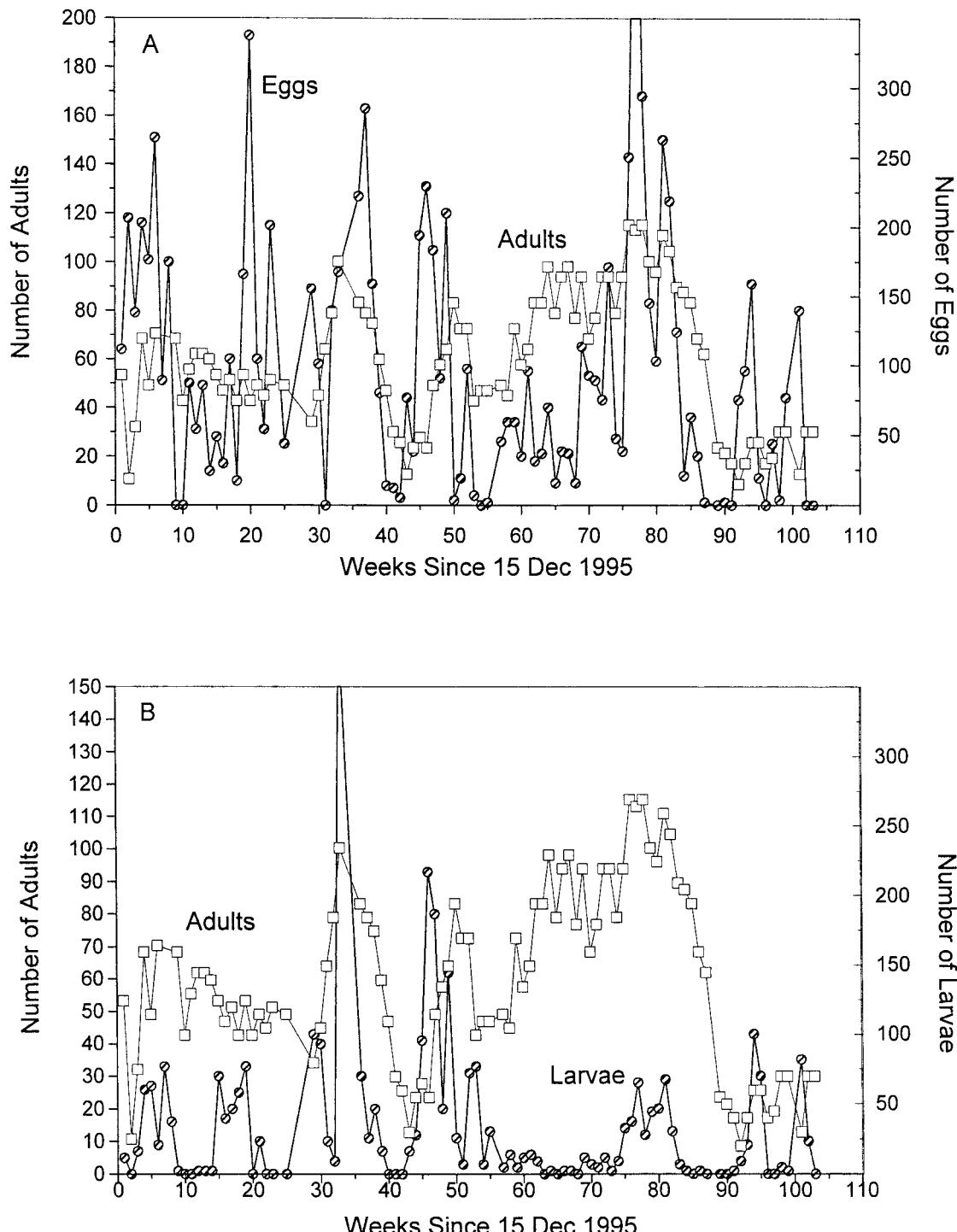


Fig. 4. Weekly counts of eggs (A) and larvae (B) of *Heliconius charithonia* between 15 December 1995 and 15 December 1997. Estimated weekly adult population size is included in each panel.

weekly numbers of eggs, larvae, and chrysalises were not correlated with weekly *Passiflora* biomass (Bonferroni-corrected  $P_s \geq 0.18$ ).

Two species of heliconiid larvae co-occurred on the *Passiflora* vines between weeks 30 and 85 (from July 1996 to August 1997). During this pe-

riod, number of zebra longwing butterfly larvae averaged  $17.0 \pm 3.9$  per week (range: 0-167), and number of gulf fritillary larvae averaged  $10.7 \pm 1.9$  (range: 0-55); these means do not differ significantly (paired t-test,  $t = 1.56$ ,  $df = 52$ ,  $P = 0.12$ ). Numbers of these two larvae were not correlated (Pearson's  $r = 0.17$ ,  $df = 51$ ,  $P = 0.21$ ), and hence the larvae did not appear to be interacting in an antagonistic (i.e., competitive or predator-prey) fashion.

#### Egg Parasitism

Egg survival was low (about 14% based on ratios of eggs to small larvae over all seasons), and a major reason for this was parasitism by an unidentified trichogrammatid wasp. We determined percent parasitism of zebra longwing butterfly eggs by this wasp on 22 occasions between 6 October 1996 (week 43) and 25 November 1997 (week 101). Percent parasitism averaged  $53.0 \pm 5.0\%$  (range: 0-100%). Parasitized eggs produced an average of  $6.6 \pm 0.6$  wasps ( $n = 34$ ; range: 1-14). There appeared to be no seasonal pattern to wasp parasitism.

#### Rates of Population Increase and Decrease

Changes in population size result from the interaction of four factors: births, deaths, immigration, and emigration. To what extent are the population increases and decreases we documented in this study (Fig. 2) the result of demographic (i.e., births, deaths) rather than behavioral (i.e., immigration, emigration) factors? To answer this question, we analyzed events occurring during three increase episodes (weeks 29-33, 46-50, and 58-64; Fig. 2) and three decrease episodes (weeks 33-43, 50-53, and 81-92). For the increase epi-

sodes, we determined the relative contribution of "births" (i.e., eclosion of new adults) to the overall population increase by comparing the number of chrysalises produced during each episode to the estimated number of individuals added to the population. To avoid re-counting the same chrysalises, we counted only "new" (= unmarked) chrysalises that we found beginning and ending one week before the increase period began and ended. For the decrease episodes, we compared the number of individuals that were "lost" from the population with the number of expected losses based on a weekly survival rate of 0.65.

Results of these calculations (Table 2) indicated that local births and deaths likely accounted for most of the changes in population size during these episodes. Eclosion of new adults accounted for about 90% of the population increases, and expected adult deaths accounted for virtually 100% of the population decreases. Absolute values of increase ( $\approx 13.5$  adults/wk) and decrease ( $\approx 10.5$  adults/wk) were similar during these six episodes (Fig. 2).

#### DISCUSSION

Our results indicate that the size of a suburban, subtropical population of *H. charithonia* ranged from about 10-115 adults during a two-year period. Weekly variation in adult population size was moderate (coefficient of variation = 0.47), and changes in the size of this population closely mirrored changes in the biomass of the larval host plant *Passiflora incense*. Neither adult population size nor passion vine biomass appeared to be influenced by weekly rainfall. Reproduction occurred year-round, and although the sex ratio at eclosion was female-

TABLE 2. ESTIMATES OF THE CONTRIBUTION OF LOCAL BIRTHS AND DEATHS TO POPULATION INCREASES AND DECREASES, RESPECTIVELY, IN THE ZEBRA LONGWING BUTTERFLY.

#### A. Population increases

Weeks ( <i>n</i> )	$\Delta n$	<i>n</i> Chrysalises	<i>n</i> Chrysal./ $\Delta n$
29-33 (4)	+66.2	57	0.86
46-50 (4)	+59.7	96	$1.61 \approx 1.00$
58-64 (6)	+53.3	39	0.73
		Mean	$\approx 0.90$

#### B. Population decreases

Weeks ( <i>n</i> )	$\Delta n$	Expected $\Delta n^a$	Expected/observed
33-43 (10)	-87.5	-85.5	0.98
50-53 (3)	-40.5	-60.4	1.49
81-92 (11)	-102.4	-107.8	1.02
		Mean	$\approx 1.00$

<sup>a</sup>Calculated as  $n_1 - (n_1 * 0.65^n)$ , where  $n_1$  is the number of individuals in week 1 of the episode, 0.65 is the weekly adult survival rate, and *n* is number of weeks in the episode.

biased, sex ratio of the adult population was strongly male-biased. Rates of wing wear in adults were high, and maximum adult longevity was 9-10 weeks. Given that the egg-to-adult period lasts 30 days in this species in the subtropics (Quintero 1988), maximum lifespan of *H. charithonia* in south Florida is 13-14 weeks (91-98 days). Most adults, however, live less than one month, so average lifespan is less than eight weeks (<56 days).

The best predictor of adult population size in this study was biomass of the larval host plant, *Passiflora incense*. It appeared that both males and females were tracking biomass of this plant, but for different reasons. Females track passion vine biomass in searching for oviposition sites, whereas males track this biomass looking for female chrysalises (mates). Of the two sexes, males appeared to be more sedentary, as indicated by their higher number of recaptures and the longer time between first and last capture. Many males virtually 'camped out' in the garden waiting for female chrysalises to become sexually receptive. Receptive females were quickly 'swarmed' by several males, one of which ultimately mated with her (THF, pers. obs.). Females, in contrast, appeared to be "trap-lining" and passed through our study site en route to other oviposition or feeding sites. While in the garden, they laid one or more eggs on new growth on several stems.

The shift from a female-biased sex ratio at eclosion to a male-biased sex ratio in the local population suggests that adult females are more mobile than males. As in most birds (Greenwood 1980), females appear to be the dispersing sex in *H. charithonia*. Many males apparently stay near their natal sites to search for mates whereas females disperse some distance away from their natal sites before establishing a home range. Because of high female mobility, however, males are not likely to mate with close relatives, and rates of inbreeding are likely to be low. This mobility also likely results in substantial gene flow among zebra longwing butterfly subpopulations.

In support of these predictions, Kronforst & Fleming (2001) reported very low levels of inbreeding (Wright's fixation index  $F_{is} = -0.027$ , a slight excess of heterozygotes) and low population genetic subdivision (Wright's fixation index  $F_{st} = 0.003$ ) in *H. charithonia* over a wide area in Miami-Dade County. These results call into question the suggestion (e.g., Haag et al. 1993) that *Heliconius* populations have an island-like structure and are highly inbred.

Although the dynamics of all populations are influenced by abiotic and biotic factors, biotic factors are likely to most strongly influence the population dynamics of *H. charithonia* in south Florida. This conclusion is based on the absence of a correlation between rainfall and adult population size and *Passiflora* biomass as well as the absence of a strong seasonal effect on numbers of eggs, lar-

vae, chrysalises, and rates of egg parasitism. Butterly reproduction, egg parasitism, and *Passiflora* growth occurred year-round and were not strongly seasonal. *H. charithonia* in south Florida is clearly behaving like a tropical butterfly.

Two biotic factors, host plant biomass, especially the availability of growing shoot tips, and egg parasitism, appeared to affect the dynamics of this population. Although numbers of eggs and larvae showed no strong seasonal trends, their numbers were highly variable from week to week. Coefficients of variation of weekly number of eggs, total larvae, and chrysalises, respectively, were 0.99, 1.64, and 1.52 compared to a value of 0.47 for number of adults. Variation in egg number, in part, reflected variation in the availability of fresh growing tips which was determined, in turn, by caterpillar herbivory. At times, herbivory eliminated the growing tips from most stems, which resulted in few eggs being laid. Egg parasitism by trichogrammatid wasps also eliminated an average of 50% of potential *Heliconius* larvae. Parasitism was especially high during weeks 61-69 (early February-mid-April 1997) when it averaged 75%. During that period, few larvae were produced, and *Passiflora* biomass began to steadily increase. Despite low larval numbers during this period, adult butterfly numbers were high. This was the only time during the study that immigration, rather than in situ recruitment, appeared to be responsible for high adult population numbers.

Many aspects of the population biology of *H. charithonia* in south Florida are similar to those of tropical *Heliconius* populations. Similarities include low, relatively stable population densities, biased adult sex ratios, year-round reproduction, and the importance of larval host plants in determining the distributions and densities of adult butterflies (e.g., Ehrlich & Gilbert 1973; Cook et al. 1976; Quintero 1988; Gilbert 1991; Ramos & Freitas 1999). Coefficients of variation of population size of *H. charithonia* in Costa Rica and Puerto Rico ranged from 0.34 to 1.05 (based on data in Cook et al. 1976; Quintero 1988) compared with 0.47 in this study; CV of mean population size in *Heliconius erato* in southern Brazil was 0.73 (Ramos & Freitas 1999). As in this study, Quintero (1988) found no correlation between population size and rainfall in Puerto Rico. Under normal conditions, the population density of *H. charithonia* in urban Miami is low, on the order of a few individuals per hectare in forested areas (Kronforst & Fleming 2001). This low density undoubtedly reflects the low density and biomass of *Passiflora* in these areas. Average lifespan was also similar in populations of *H. charithonia* in south Florida, Puerto Rico, and Costa Rica. Compared with *H. ethilla* the maximum lifespan of which in Trinidad is about 180 days and *H. erato* in southern Brazil with a maximum lifespan of about 150 days, *H. charithonia* is a short-lived butterfly. Maximum

longevities are 90–116 days, and average adult lifespans are less than one month after eclosion.

In conclusion, populations trends in *H. charithonia* in south Florida do not appear to differ qualitatively or quantitatively from those at lower latitudes. The mild climate of subtropical Miami permits this butterfly to act as though it is still in the tropics. These results contrast with those reported for butterflies in England, where marginal populations of some species fluctuate much more strongly than central populations (Thomas et al. 1994; Shreeve et al. 1996). Studies of the population dynamics of *H. charithonia* closer to its northern geographic limits (e.g., in northern Florida) are needed to determine whether population sizes are more variable and more strongly influenced by abiotic factors than in south Florida. Such studies in *H. erato* in subtropical Brazil indicate that climate-related (either cold temperatures or drought) extinctions can occur in *Heliconius* butterflies at the southern limits of their distribution (Saalfeld & Araujo 1981).

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## VOLATILE COMPOUNDS RELEASED BY DISTURBED FEMALES OF *CEPHALONOMIA STEPHANODERIS* (HYMENOPTERA: BETHYLIDAE): A PARASITOID OF THE COFFEE BERRY BORER *HYPOTHENEMUS HAMPEI* (COLEOPTERA: SCOLYTIDAE)

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### ABSTRACT

Volatile compounds released by disturbed females of the bethylid wasp *Cephalonomia stephanoderis* Betrem were collected and analyzed by gas chromatography-mass spectrometry. The origin of volatiles and their behavioral effects on conspecifics were also investigated. The source of the volatile compounds was found to be the head, and more specifically, the mandibular glands. These glands contain skatole as the main volatile component. Behavioral bioassays demonstrated that extracts of parasitoid heads and synthetic skatole evoked the same alarm behavior in this species. The possible function of this chemical is discussed.

**Key Words:** *Cephalonomia stephanoderis*, *Hypothenemus hampei*, alarm pheromone, skatole, biological control

### RESUMEN

Los compuestos volátiles liberados por hembras molestadas del parasitoide betílico *Cephalonomia stephanoderis* Betrem fueron colectadas y analizadas por cromatografía de gases y espectrometría de masas. El origen de los volátiles y su efecto comportamental en los parasitoides conespecíficos fueron también investigados. La fuente de los compuestos volátiles fue localizada en la cabeza, y más específicamente, en las glándulas mandibulares. Estas glándulas contienen skatol como el principal componente volátil. Los bioensayos comportamentales demostraron que los extractos de cabezas del parasitido y skatol sintético provocaron el mismo comportamiento de alarma en esta especie. Se discute la posible función de este compuesto químico.

Translation provided by the authors.

The bethylid wasp *Cephalonomia stephanoderis* Betrem (Hymenoptera: Bethylidae) is an ectoparasitoid of larvae and pupae of the coffee berry borer, *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), which is the most important pest of coffee worldwide (Barrera et al. 1990; Murphy & Moore 1990). *Cephalonomia stephanoderis* is native to Central West Africa and has been introduced to various coffee-producing countries (Murphy & Moore 1990). Adults of *C. stephanoderis* emit a strong odor when they are disturbed or transported to be released in field (Gómez 2005). This odor can be detected by the human nose (Infante et al. 2001). A number of parasitic wasps are known to emit more or less pungent odors (Townes 1939). The function of these odors in wasp behavior remains largely unknown; in some cases they have been thought to have a defensive role (Buckingham 1975) or to play an important role in courtship (Williams et al. 1988). However, only one *Cephalonomia* species, *C. gal-*

*licola* (Ashmead), a cosmopolitan ectoparasitoid of anobiid beetles, has been reported to release an odor when squashed by forceps (Kuwahara 1984). The odor originated from the head, and the chemical identified was skatole (3-methylindole). Infante et al. (2001) suggested that a similar secretion could be released by *C. stephanoderis*, but no studies have been carried out to identify the chemicals released by this species.

We describe here behavioral evaluation, origin, and identification of the volatile compounds emitted by adult female of *C. stephanoderis* when disturbed.

### MATERIALS AND METHODS

#### Biological Material and Experimental Conditions

Adult *C. stephanoderis* were obtained from the laboratory colony maintained at El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico. Al-

though both sexes emit the odor (J. Gómez unpublished), females were chosen because of their importance as biological control agents and because the sex ratio is markedly biased in favor of females (Barrera 1994). The colony was established with insects collected from coffee plantations near Tapachula in 1999 and reared as described by Barrera et al. (1991). Bioassays were conducted in a room at  $24 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  relative humidity and lit with red light (10 lux). Parasitoids used in the bioassays were collected from adult emergence jars on the day of the tests and placed in the bioassay room 3 h before testing.

#### Headspace Bioassay

Two groups of 20 *C. stephanoderis* females were placed in a separate glass vial (50 mm high  $\times$  20 mm diameter) and a plastic vial (70 mm high  $\times$  20 mm diameter) connected to each other by a plastic tube (Fig. 1). The first group of insects was strongly

shaken for 1 min. Preliminary observations showed that during this time the insects released the odor. The second group of insects was not disturbed. A disposable syringe was used to inject 35 ml of clean air into the glass vial in order to blow the volatiles through the tube on to undisturbed insects. Test insects were observed for any change in their behavior for 10 min after the influx of air. Alarm behavior was considered to happen if insects showed movements such as agitated running or attempts to take flight. No change in behavior was considered a lack of observable response. Clean air and the odor from undisturbed insects were used as controls. Six replicates per treatment were performed.

#### Bioassays with Extracts and Synthetic Skatole

Four different extracts were made with 20 and 40 heads, 40 thoraces, and 40 abdomens of *C. stephanoderis* females. Heads, thoraces, and abdomens were macerated in 200  $\mu\text{l}$  of hexane. For eval-

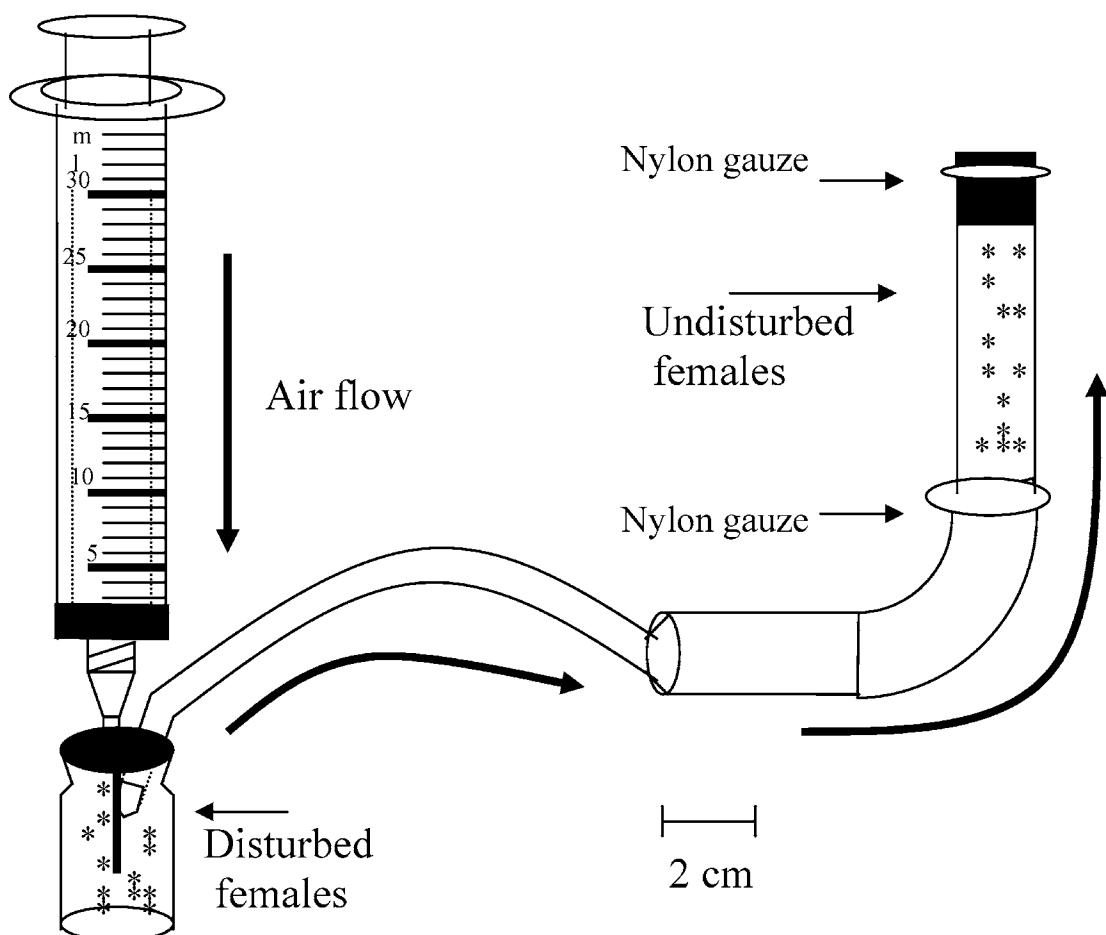


Fig. 1. Design of olfactometer used to determine the response of *C. stephanoderis* females to volatiles from disturbed and undisturbed insects and clean air.

uating the biological activity of these extracts, 10  $\mu$ l of the chosen extract (equivalent to one or two heads, two thoraces, or two abdomens depending on the extract used) was applied to a piece (1 cm<sup>2</sup>) of Whatman No. 2 filter paper. The solvent was allowed to evaporate for 1 min and then the piece of filter paper was placed in a Petri dish (10 cm diameter). An upside down glass tube (70 mm high  $\times$  15 mm diameter) containing 20 undisturbed female parasitoids (prepared 2 h prior to the test) was placed over the filter paper. An alarm response was noted if the insects exhibited any movement. The number of insects showing alarm behavior was counted every 5 min for 40 min. In other experiments, several doses (0.1, 0.5, 1, 10, and 100 ng) of synthetic skatole in hexane were tested in the bioassay described above. Hexane alone was used as control in all experiments. Ten replicates were performed for all treatments. Skatole (98%) was obtained from Sigma Chemical, Co. (Toluca, Mexico).

#### Solid Phase Micro-Extraction (SPME)

SPME was conducted with a holder and a 100- $\mu$ m poly-(dimethylsiloxane)-coated fiber which were obtained from SUPELCO (Toluca, Mexico). Twenty *C. stephanoderis* females were placed inside a glass vial (7.5 mm high  $\times$  1.5 cm diameter) with a foam cap. Sampling was performed by inserting the SPME needle, through the foam cap, into the headspace of the glass vial. Volatiles were allowed to be adsorbed onto the fiber for 5 min. Subsequently, it was removed from the vial and volatiles desorbed inside the heated injection port of a gas chromatograph for 5 min.

#### Solid Sampling Preparation for Chemical Analysis

Females of *C. stephanoderis* were killed by placing them in a refrigerator for 24 h. Dissections were made under a binocular microscope with fine forceps and entomological pins. Three heads, thoraces, and abdomens, and ten mandibles with the gland attached were dissected in distilled water and placed in thin-walled soft glass tubes previously sealed at one end; the open end was then sealed in a micro-flame for analysis by coupled gas chromatography-mass spectrometry (Morgan 1990). In addition, three whole insects were analyzed by this technique.

#### Chemical Analysis

Gas chromatography-mass spectrometry (GC-MS) was conducted with a Varian Star 3400 CX gas chromatograph linked to a Varian Saturn 4D mass spectrometer. The samples were analyzed in a fused silica column (30 m  $\times$  0.25 mm) coated with poly-(5-diphenyl-95%-dimethylsiloxane) programmed from 50°C to 250°C at 15°C/min. The flow rate of helium through the column was main-

tained at 1 ml/min. The injector port temperature was held at 200°C. The glass capillaries containing either the glands, heads, abdomens, or thoraces were directly inserted into the injection area and heated and crushed as described by Morgan (1990).

#### Scanning Electron Microscopy

Mandibular glands of *C. stephanoderis* females were washed and fixed in a solution of 3% glutaraldehyde in phosphate buffer (0.1 M, pH 7.2). Glands were washed twice for 5 min with distilled water, and then passed through increasing grades of ethanol, from 10% to absolute ethanol, 30 min each. Finally they were dried to critical point of CO<sub>2</sub>, mounted in aluminum stubs and sputter coated with gold-palladium (Dykstra 1993). The samples were examined and photographed in a Topcon SM-510 electron microscope operated at 5 kV.

#### Statistical Analysis

Results were subjected to one-way analysis of variance (ANOVA) (SPSS for Windows 8.0. SPSS Inc.), except the data for insect response to head extracts and skatole over time were analyzed by repeated measures ANOVA. When *F* values were significant, means were compared by Tukey's test at  $\alpha = 0.05$ .

## RESULTS

#### Headspace Bioassay

Undisturbed *C. stephanoderis* females showed a significant response to volatiles from disturbed females compared with the response to volatiles from undisturbed females and clean air ( $F = 85.5$ ;  $df = 2, 15$ ;  $P < 0.001$ ). From a total of 120 insects tested, 81 females were affected by the odor from disturbed females; the rest (39) remained stationary. In contrast, most of the individuals remained stationary when clean air and air from undisturbed insects (111 out of 120 in each treatment) was passed over the parasitoids.

#### Female Response to Extracts

Females showed a significant alarm response (e.g., agitated running) to head extracts, but not to thorax extracts or abdomen extracts or hexane control ( $F = 90.7$ ;  $df = 4, 45$ ;  $P < 0.001$ ) (Fig. 2). Of the total of females tested, 64% were disturbed when a one-head equivalent extract was introduced on the filter paper, whereas 80% were disturbed when a two-head equivalent extract was used.

#### Chemical Analysis

The SPME and GC-MS analysis of the parasitoid volatiles showed that agitated *C. stephanoderis* females released at least two compounds (Fig.

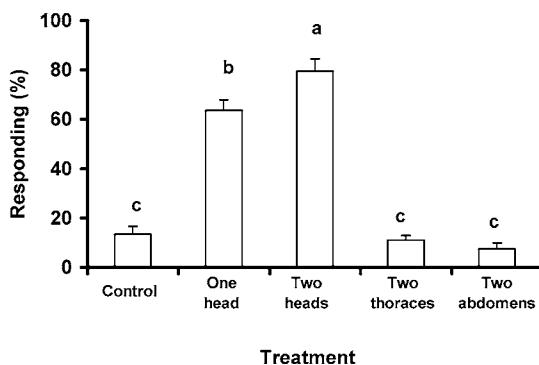


Fig. 2. Percentage of *C. stephanoderis* females that responded to extracts containing equivalent of one or two heads, two thoraces, or two abdomens. Vertical bars indicate the standard error of the mean. Different letters over bars indicate that means are significantly different (ANOVA, followed by Tukey test,  $P < 0.05$ )

3a). Compound 1 was identified as skatole (3-methylindole) by comparison of retention time and mass spectrum with that of the synthetic standard. Compound 2 with a Kovat's Index of 15.95 showed mass spectrum fragment ions at  $m/z$  55 (80%), 69 (70%), 83 (25%), 97 (100%), and 111 (25%). This mass spectrum resembled that of a branched alkene. Undisturbed insects did not release skatole or compound 2 (data not shown). Solid sampling analysis of heads of *C. stephanoderis* showed that the volatiles contained a mixture of hydrocarbons and nitrogen compounds (Fig. 3b). The compounds detected were skatole, unidentified compound 2, and other nitrogen compounds which were tentatively identified by mass spectral matching to a library data base (NIST 2002) as (3) uric acid, (4) dl-alanyl-l-leucine, (5) hexahydro-3-[2-methylpropyl]pyrrolo [1, 2-a] pyrazine-1, 4-dione and (7) oleamide. Compounds 6, 8, 9, 10, 11, 12, 13, 14, 15, and 16, which were common in all solid samples were cuticular in origin as confirmed by analysis of a small fragment of cuticle. They have mass spectra typical of hydrocarbons, which have been identified previously from *C. stephanoderis* by Howard & Infante (1996). The analysis of the mandibular glands showed that one of the components of the glands was skatole with traces of all the other compounds found in the head (Fig. 3c). Analysis of thoraces and abdomens showed that these contained the compounds 3, 4, 5, and 7, and hydrocarbons but not skatole or unidentified compound 2 (Figs. 4d and 4e), confirming that compounds 1 and 2 are found specifically in the head. Skatole content in the head varied from 0.4 to 1.0 ng ( $n = 6$ ;  $0.5 \pm 0.1$  SE).

#### Female Response to Synthetic Skatole

The alarm behavior of *C. stephanoderis* was significantly influenced by the dose of synthetic

skatole ( $F = 5.0$ ;  $df = 4, 45$ ;  $P = 0.002$ ). Multiple comparisons indicated that the dose of 1 ng skatole elicited significantly larger alarm behavior compared with those evoked by the doses of 0.1 and 100 ng of this compound. The alarm responses elicited by the doses of 0.5 and 10 ng of skatole were intermediate between and not significantly different from those evoked by the doses of 1, 0.1, 100 ng of this compound (Fig. 4).

The wasp response to head extracts and skatole over time revealed that the type of chemical stimuli used did not influence differently the alarm behavior of *C. stephanoderis* ( $F = 1.23$ ;  $df = 2, 27$ ;  $P = 0.307$ ). In contrast, time affected the wasp response to the chemical stimulus ( $F = 6.34$ ;  $df = 7, 189$ ;  $P < 0.001$ ). In the three treatments, the parasitoids started to respond soon after the samples were delivered to the vial reaching the highest response at 15–20 min (35 min in the case of skatole), and after this time the insect response gradually declined (Fig. 5). The chemical stimulus  $\times$  time interaction term was not significant ( $F = 1.24$ ;  $df = 14, 189$ ;  $P = 0.25$ ).

#### Scanning Electron Microscopy

The microphotograph showed that the mandibular gland is connected to the base of the mandible (Fig. 6a). The gland is comparable in size to the mandible. A close-up shows that the gland consists of a series of tubular structures attached to the mandible (Fig. 6b).

#### DISCUSSION

The alarm behavior of *C. stephanoderis* was observed in individuals that were exposed to the headspace volatiles collected from disturbed females. No such behavior was observed in wasps exposed to the volatiles collected similarly from undisturbed females. SPME and GC-MS analysis showed that skatole was the main volatile compound emitted by disturbed females. The presence of skatole has been reported in two species of Neuroptera (Blum & Wallace 1973), one species of Diptera (Blum 1981), one species of Coleoptera (Burger et al. 2002) and several species of Hymenoptera (Law et al. 1965; Smith & Roubick 1983; Kuwahara 1984; Keegans et al. 1993; Billen et al. 1998). For instance, skatole was found in the ant *Pheidole fallax* Mayr, although its function was not determined (Law et al. 1965). This compound is released from the abdomen of the army ant, *Labidus praedator* (Smith) and functions as a trail pheromone (Keegans et al. 1993). The mandibular gland of a leptanillidan ant, *Leptanilla* sp. contains a large amount of skatole that is released as an alarm pheromone (Billen et al. 1998). Skatole is present in the mandibular gland of a stingless bee, *Melipona interrupta triplaridis* Schwarz as a component of the alarm pheromone (Smith &

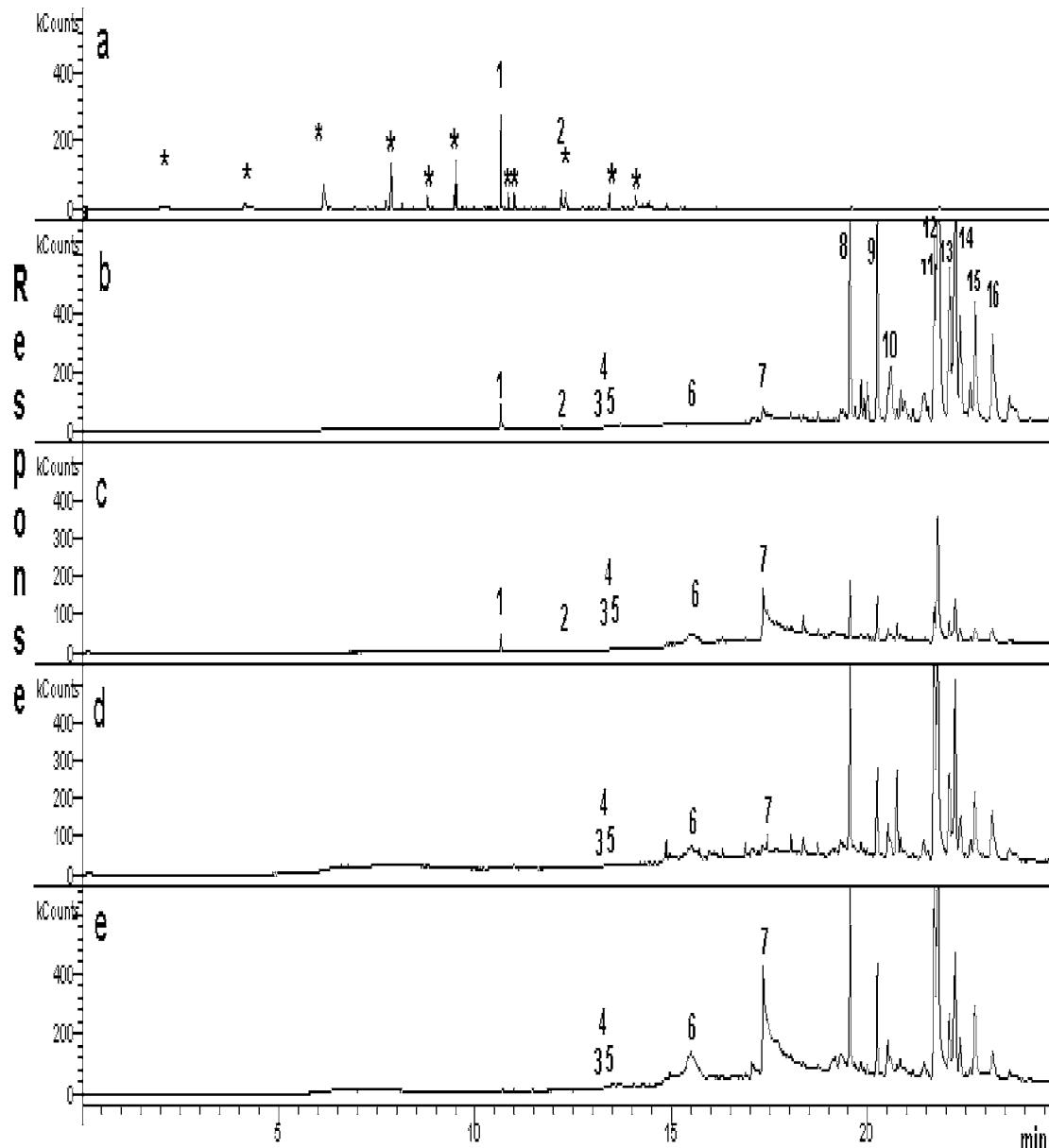


Fig. 3. Representative chromatograms of compounds released by disturbed females collected by SPME (a); and solid sampling analysis of crushed heads (b), mandibular glands (c), abdomens (d), and thoraces (e) of *C. stephanoderis* females. Skatole (1), unidentified (2), uric acid (3), dl-Alanyl-1-leucine (4) hexahydro-3-[2-methylpropyl]-pyrrole [1,2-a] pyrazine-1, 4-dione (5), oleamide (7) and hydrocarbons (6), (8), (9), (10), (11), (12), (13), (14), (15) and (16). \*Contaminants from the solid phase fiber.

Roubik 1983). Kuwahara (1984) detected skatole in the head of *C. gallicola*, another bethylid wasp, and proposed a function as an allomone.

Female parasitoids of *C. stephanoderis* showed the highest alarm response to one and two-head equivalent extracts and 1 ng of skatole. However, response was reduced to higher doses of skatole,

which may indicate that higher doses of skatole may disrupt the effective alarm communication between parasitoids, as has been found in aphids (El-Agamy & Haynes 1992). A high dose of (*E*)- $\beta$ -farnesene, the aphid alarm pheromone, produced a rapid sensory habituation of aphids to this compound (Calebrese & Sorenson 1978). In our study,

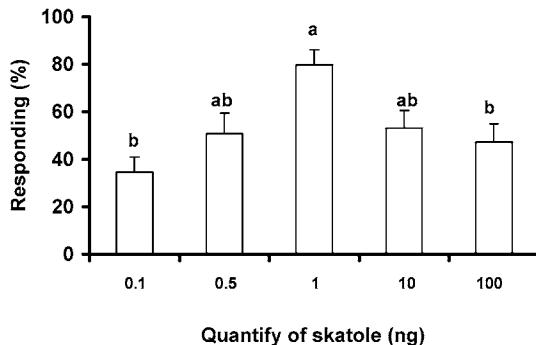


Fig. 4. Percentage of *C. stephanoderis* females that responded to different quantities of skatole. Vertical bars indicate the standard error of the mean. Different letters over bars indicate that means are significantly different (ANOVA, followed by Tukey test,  $P < 0.05$ ).

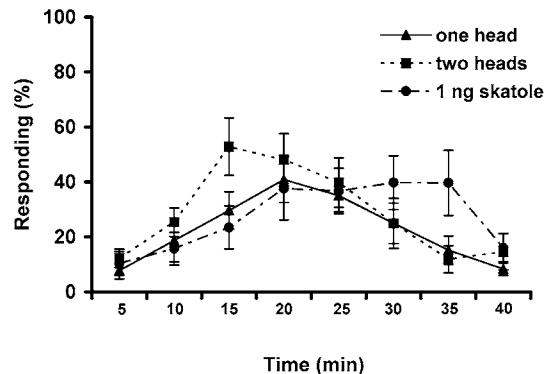


Fig. 5. Percentage of *C. stephanoderis* females that responded to one or two-head equivalent extracts and skatole (1 ng), at different times of observation. Vertical bars indicate the standard error of the mean.

the stimulatory effect of head extracts or skatole was short-lived (<35 min); therefore the decrease in the alarm response of *C. stephanoderis* may be due to sensory adaptation or habituation.

The odor released by agitated adults of *C. stephanoderis* may have multiple functions as reported for other insects (e.g., Blatt et al. 1998; Staples et al. 2002; Wardle et al. 2003). In this study, we analyzed only females, but preliminary studies have indicated that male parasitoids also release the same compounds (unpublished data). The fact that both sexes produce the same components in the secretion suggests that they do not function as sexual pheromones. Generally, defensive secretions are released by both sexes as has been demonstrated for bugs (Leal et al. 1994), thrips (Teerling et al. 1991), and cockroaches (Farine et al. 2002). For example, the defensive secretions of the glandular pouches of the adults of both sexes of cockroaches *Therea petiveriana*

(L.) contain volatile compounds that function as an alarm pheromone for adults (Farine et al. 2002). A potential function for skatole in *C. stephanoderis* is as an alarm pheromone causing dispersal. *Cephalonomia stephanoderis* adults are found in groups of sisters and brothers inside coffee berries after they emerge from the cocoon and they remain together 4–5 days to mate before they disperse (Barrera et al. 1989). A pheromone could promote the dispersion of the parasitoids after mating. A prerequisite to the evolution of alarm pheromones is the evolution of sociality (Nault & Phelan 1984). Another possible function of skatole in *C. stephanoderis* is as an epideictic pheromone, promoting spacing in the natural habitat (Haynes & Birch 1985). *Hypothenemus hampei*, the host of *C. stephanoderis*, reproduces inside coffee fruits, which may represent a limited resource for both coffee berry borer and parasitoids, thus an epideictic pheromone could well be ad-

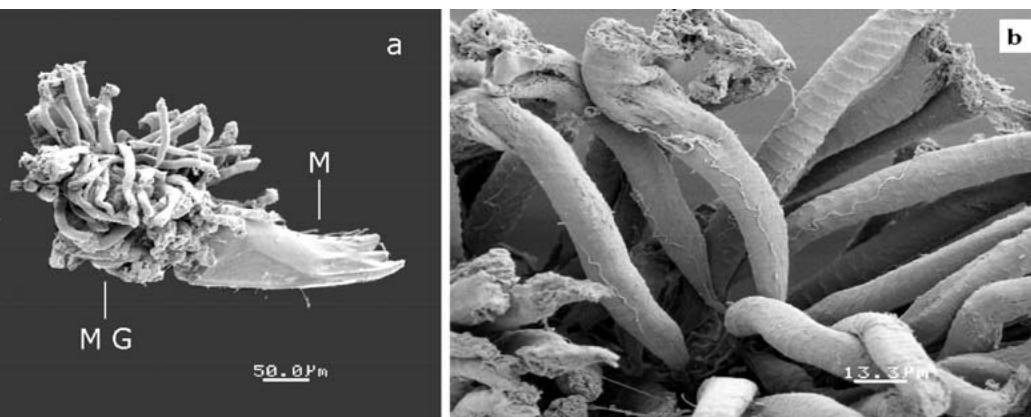


Fig. 6. Scanning photomicrograph of the mandibular gland attached to the mandibula of *C. stephanoderis*, M = mandible; MG = mandibular gland (a), Close up of the tubular structure of the gland (b).

aptative for individuals to reduce competition for resources. Barrera et al. (1994) presented evidence of a marking pheromone in *C. stephanoderis* to avoid use of hosts previously parasitized, but this possible pheromone seems to act over short distances compared to the odor released by agitated adults. A third possible function is that the odor is released in direct response to threats. Several species of spiders and ants have been reported to attack to *C. stephanoderis* as well as a bethylid wasp, *Prorops nasuta* Waterston in the coffee plantations of Mexico (Henaut et al. 2001; Infante et al. 2003). Some ant species occasionally forage inside coffee fruits and prey upon immature and adult *P. nasuta* and possibly *C. stephanoderis* (Infante et al. 2003). The hyperparasitoid *Alloxysta brevis* (Thompson) applies defensive compounds stored in mandibular gland reservoirs against attacking ants and other generalist predators like spiders (Hubner & Dettner 2000). Finally, the odor could mediate the interactions between *C. stephanoderis* and two other species of bethylid parasitoids of the coffee berry borer, *P. nasuta* and *C. hyalinipennis* Ashmead. Female parasitoids actively defend parasitized hosts and their progeny when intruders attempt to take possession of these resources (Pérez-Lachaud et al. 2002). Thus, it is possible that the odor of *C. stephanoderis* could function as an alarm pheromone, epideictic pheromone, or allomone depending on the specific situation. For example, the same compounds that function as an alarm pheromone in the bedbug, *Cimex lectularius* L., also serve a defensive role, as they can effectively repel *Monormorium pharaonis* (L.), a natural enemy of bedbugs (Levinson et al. 1974). These glandular secretions also make bedbugs distasteful to bat species which are predators to *C. lectularius*. It is believed that this defensive role may have been the primary function of the secretion and the alarm response of conspecifics evolved secondarily (Nault & Phelan 1984).

Our microphotographs of the mandibular gland of female *C. stephanoderis* show that the gland is not typical of Hymenoptera, which have been shown to contain a reservoir in the form of a sac (e.g., Cruz-Landim 1990; Mayhe & Caetano 1994). The mandibular gland secretion in *C. stephanoderis* presumably is produced by the tubular structures attached to the mandible.

In conclusion, this study showed that the behavior of female wasps of *C. stephanoderis* was affected by the introduction of volatiles released from disturbed conspecific females. The source of the volatile compounds was found to be the head, and more specifically, the mandibular glands. These glands contain skatole as the main volatile component. Behavioral bioassays demonstrated that extracts of parasitoid heads and synthetic skatole evoked the same alarm behavior in this species. An unidentified compound 2 released by

agitated females should be properly identified and its biological activity evaluated alone and in combination with skatole. Other unidentified compounds found in the heads and mandibular glands beside skatole may not be involved in the alarm behavior of *C. stephanoderis* because they were not detected in the headspace volatile analysis.

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## DESCRIPTION OF THE MATURE LARVA OF *ANCISTROCERUS SIKHIMENSIS* (HYMENOPTERA: EUMENIDAE)

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### ABSTRACT

The mature larva of *A. sikhimensis* is described, illustrated, and compared with that of other species of the genus so far described. The final-instar of this species and the different species recently described, or redescribed, in this genus are *A. kitcheneri*, *A. longispinosus*, and *A. trifasciatus*, and all can be differentiated on the basis of the following characters: (a) the development of antennae; (b) the development of the atrium with respect to the subatrium; (c) the number of the sensilla of the labrum and galea, and (d) the presence/absence of spinules and papillae on the labium.

**Key Words:** Hymenoptera, Eumenidae, *Ancistrocerus*, mature larva, Nepal

### RESUMEN

Se describe, y compara con las ya descritas del género, la larva madura de *Ancistrocerus sikhimensis*. Los caracteres que permiten distinguir las larvas maduras, recientemente descritas o redescritas, del género *Ancistrocerus*: *A. kitcheneri*, *A. longispinosus*, y *A. trifasciatus*, radican en: (a) desarrollo de las antenas y del atrium con respecto al subatrium; (b) número de sensillas del labrum y galeas, y (c) presencia/ausencia de espínulas y papilas en el labium.

Translation provided by the authors.

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Most of the taxonomy of eumenid wasps is based on external adult morphology, and relatively little attention has been paid to interspecific differences in larval characters, even though they could be useful. In this respect, of 2500-3500 species described (Yamane 1990), larval morphology is only known for 42 (Tormos et al. 1998). Within this set, only five species of the genus *Ancistrocerus* Wesmael, 1836, have been described: *A. trifasciatus* (Müller, 1776) (Enslin 1921; Jørgensen 1942; Tormos et al. 1998); *A. oviventris* (Wesmael, 1836) (Micheli 1930); *A. gazella* (Panzer, 1793) (Grandi 1961), *A. kitcheneri* (Dusmet, 1917) and *A. longispinosus* (de Saussure, 1885) (Tormos et al. 1998). This study addresses the larval morphology of *A. sikhimensis* Bingham, 1898, obtained during a study on the fauna of rubicolous species of Nepal.

### MATERIALS AND METHODS

The methods employed to prepare larval specimens as well as the terminology for larval morphology and the format used in the descriptions follow Evans (1987) and Sime and Wahl (1998). The following abbreviations are employed: d = di-

ameter, h = height, l = length, w = width. The material is deposited at the "Torres-Sala" Entomological Foundation (Valencia, Spain).

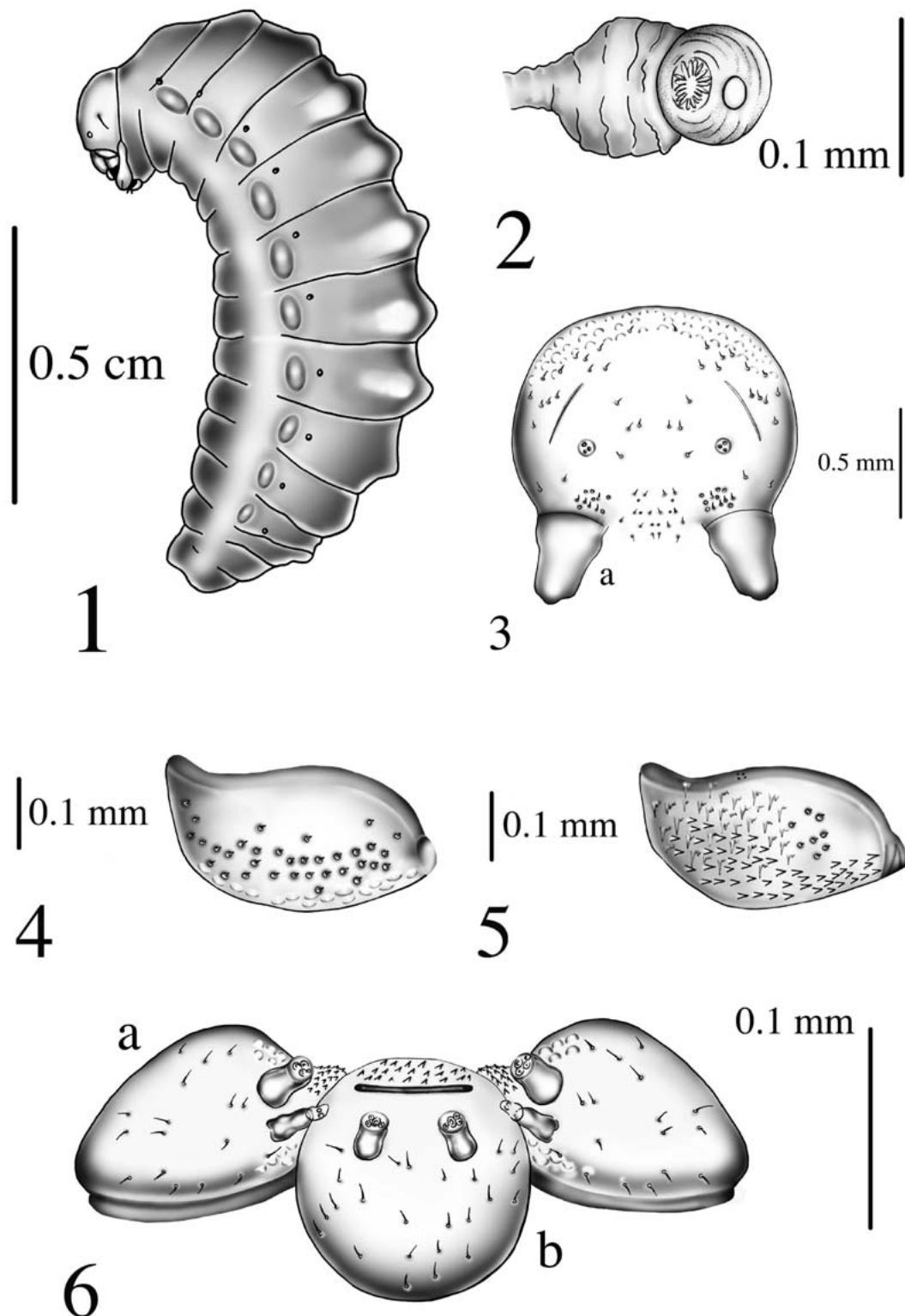
The description is based on four mature larvae obtained by R. Boesi in Nepal in 2003. Absolute measurements, except for the body width and length, are based on data for the one specimen.

### DESCRIPTION OF MATURE LARVA

*A. sikhimensis* Bingham, 1898 (Figs. 1-6)

Body (Fig. 1) ( $l = 11.7\text{-}12.6$  mm, maximum  $w = 2.1\text{-}2.3$  mm) robust; first five abdominal segments divided into two annulets by a transverse crease. Anus terminal, in central position, as a transverse slit. Pleural lobes developed. Integument with scanty and disperse setae ( $l = 9\text{-}11 \mu\text{m}$ ) and punctures. Spiracles (Fig. 2) with walls of atrium with ridges and asperities; opening into subatrium spinulose; subatrium ( $d = 84 \mu\text{m}$ ) as wide as atrium ( $d = 81 \mu\text{m}$ ).

Cranium (Fig. 3) ( $w = 1.4 \mu\text{m}$ ,  $h$  (exclusive of labrum) =  $1.1 \mu\text{m}$ ) with sparse setae ( $l = 9\text{-}12 \mu\text{m}$ ) and punctures. Coronal suture absent and parietal bands present. Antennae ( $d = 65 \mu\text{m}$ ) almost



Figs. 1-6. Mature larva of *Ancistrocerus sikhimensis*: (1) Body, lateral view; (2) Anterior thoracic spiracle (side view) (atrium, subatrium and tracheal trunk); (3) Cranium (frontal view); (3 a) mandible; (4) Labrum; (5) Epipharynx; (6 a) Maxilla; (6 b) Labium.

flat, circular, with 3 sensilla. Clypeus with setae ( $l = 4 \mu\text{m}$ ) and punctures. Labrum (Fig. 4) ( $w = 685 \mu\text{m}$ ) emarginate, with around 68 conical sensilla ( $w = 8 \mu\text{m}$ ). Epipharynx (Fig. 5) spinulose, with 16 sensilla ( $d = 2 \mu\text{m}$ ).

Mouthparts. Mandible (Fig. 3a) ( $l = 410 \mu\text{m}$ ,  $w = 250 \mu\text{m}$ ) weakly tridentate. Maxilla (Fig. 6a) ( $w = 292 \mu\text{m}$ ) spinulose on the lacinial area and with 16 setae ( $l = 5-8 \mu\text{m}$ ) on external part. Maxillary palpus conical ( $h = 87 \mu\text{m}$ ,  $w = 45 \mu\text{m}$ ) with 4 protuberant apical sensilla ( $w = 3-6 \mu\text{m}$ ); galea ( $l = 130 \mu\text{m}$ ,  $w = 50 \mu\text{m}$ ) long, attenuated at apex, with 2 apical sensilla. Labium (Fig. 6b) ( $w = 375 \mu\text{m}$ ) spinulose dorsally to salivary orifice; labial palpus ( $l = 80 \mu\text{m}$ ,  $w = 60 \mu\text{m}$ ) with 4 apical sensilla ( $w = 3-6 \mu\text{m}$ ); prementum with setae ( $l = 12-15 \mu\text{m}$ ); salivary orifice a transverse slit ( $w = 130 \mu\text{m}$ ).

## DISCUSSION

The present description of the morphology of the mature larva of *A. sikhimensis*, together with previous descriptions carried out by our team or some of its members (Tormos et al. 1998), show that the mature larvae of *Ancistrocerus* are very similar, differing in (a) the presence/absence of the coronal suture and setae of the labrum; (b) more or less developed parietal bands; (c) the number and arrangement of the sensilla of the epipharynx, and (d) the development of apical sensilla of the galeae (Table 1). Additionally,

TABLE 1. CHARACTERS USED IN THE DISCUSSION OF THE MATURE LARVAE OF *ANCISTROCERUS*.

	1	2	3	4	5	6	7
<i>A. gazella</i>	A	A	B	A	A	B	A
<i>A. kitcheneri</i>	A	B	A	B	B	B	A
<i>A. longispinosus</i>	A	B	B	B	B	B	B
<i>A. oviventris</i>	B	B	B	A	A	A	B
<i>A. sikhimensis</i>	B	A	A	B	A	B	A
<i>A. trifasciatus</i>	A	A	A	A	B	B	A

Coronal suture: (1) present (A); absent (B). Epipharynx: (2) with 16 sensilla (A); with 8 to 10-12 sensilla (B). Galea: (3) longer (higher) than the maxillary palpus (A); as long as or shorter than the maxillary palpus (B). Labrum: (4) with setae (A); without setae (B). Maxillae: (5) with more than 6 setae on the external margin (A); with 6 setae on the external margin (B). Maxillary palpus: (6) with 6 sensilla at apex (A); with 4 sensilla at apex (B). Parietal bands: (7) well developed (A); very weakly developed (practically absent) (B).

other differences can be observed between *A. sikhimensis* and *A. kitcheneri*, *A. longispinosus*, and *A. trifasciatus*, recently described or re-described, by the authors. These differences are (a) the development of antennae; (b) the development of the atrium with respect to the subatrium; (c) the number of the sensilla of the labrum and galea, and (d) the presence/absence of spinules and papillae on the labium.

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## A LABORATORY METHOD FOR REARING *CATOLACCUS HUNTERI* (HYMENOPTERA: PTEROMALIDAE), A PARASITOID OF THE PEPPER WEEVIL (COLEOPTERA: CURCULIONIDAE)

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The pepper weevil, *Anthonomus eugenii* Cano, is a serious pest of cultivated *Capsicum* spp. peppers in the southern United States, Hawaii, Mexico, Guatemala, Honduras, Costa Rica, and Puerto Rico (Schuster et al. 1996). Eggs are deposited in flower buds and fruit, where larvae and pupae complete their development. Infested buds and fruit often abscise, but larvae and pupae can complete development if fallen buds and fruit do not desiccate. Yield losses can reach 90% in Florida, if the weevil is not controlled (Schuster & Everett 1982). Broad spectrum insecticides have been used most often to manage the pest but may lead to unintended consequences, including insecticide resistance and outbreaks of non-target pests. Biological control could be an alternative or adjunct to insecticides in managing the pepper weevil.

At least three species of predators and seven species of parasitoids have been reported to attack the pepper weevil (Riley & King 1994). The most abundant parasitoid recovered from the pepper weevil in Florida was *Catolaccus hunteri* Crawford (Hymenoptera: Pteromalidae) (Riley & Schuster 1992). While natural enemies generally are regarded as contributing little to control of the pest (Elmore & Campbell 1954), 50% parasitism of pepper weevil larvae by *C. hunteri* was observed in fallen jalapeno buds and over 20% parasitism in fallen bell pepper buds (Schuster et al. 1988). Augmentative releases of *C. hunteri* on alternative host plants during the summer off-season and on pepper at the initiation of flowering have resulted in reduced or delayed damage by weevil larvae (Schuster unpublished data). Because *C. hunteri* has shown potential for bio-control of the pepper weevil, a method of rearing the parasitoid in the laboratory is needed.

A commercial diet for rearing pepper weevil larvae is available (Bio-Serv, Entomology Division, Frenchtown, NJ); however, the diet was not used due to low egg hatch (Toapanta 2001). Because rearing the pepper weevil in pepper fruit is time and space consuming, an alternative host was sought. The cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae), was shown to be a suitable facultative host for rearing *Catolaccus grandis* (Burks) (Rojas et al. 1998), a closely related parasitoid of the boll weevil, *A. grandis* Boheman. The *C. maculatus* larvae were encapsulated in Parafilm® (Pechiney

Plastic Packaging, Inc., Menasha, WI) for presentation to parasitoid adults. This method was developed for exposing *A. grandis* larvae to ovipositing *C. grandis* (Cate 1987) and was mechanized for mass production (Roberson & Harsh 1993). Methods also were developed for producing *C. maculatus* larvae in pieces of garbanzo beans (chick peas), *Cicer arietinum* L. (Leyva et al. 2002). The pieces were not large enough for larvae to complete their development within, thus forcing the larvae to exit the bean pieces. The larvae then were easier to collect prior to encapsulation. This method had been used successfully to rear *C. hunteri* in the laboratory. Life history parameters including pre-oviposition period, oviposition period, adult longevity, fecundity, and egg to adult development period of *C. hunteri* on *A. eugenii* were found to be the same whether the parasitoid had originally been reared on either *C. maculatus* or *A. eugenii* (Seal et al. 2002). Collecting larvae and encapsulating them in Parafilm represents extra investments in time and equipment. Therefore, a method was developed for rearing *C. hunteri* on *C. maculatus* larvae directly in garbanzo beans.

Two colonies of *C. maculatus* were maintained in a room at a temperature of about 27°C, relative humidity of about 60% and a photoperiod of 14L:10D. The colonies were maintained on black-eyed peas, *Vigna unguiculata* (L.) Walp., and on garbanzo beans. The black-eyed peas were used to maintain the colony of *C. maculatus* and the garbanzo beans were used for exposing *C. maculatus* larvae to the *C. hunteri* parasitoid.

Three times a week, six narrow-mouth 800-ml "Mason" glass jars (Ball Corporation, Muncie, IN) were filled with 300 g of black-eyed peas each. About 100 *C. maculatus* adults were collected with an aspirator connected to a vacuum pump and were deposited in each jar, which then was sealed with a screen, filter disc, and metal ring. These jars were stored upright. A new generation of bruchid adults emerged about every 30 d.

Three times a week, ca. 400 *C. maculatus* adults were collected with an aspirator and put into each of ten 800-ml glass jars that contained 300 g each of garbanzo beans. The *C. maculatus* adults were removed 48 h later by placing the beans and bruchids on a metal sieve placed in the large opening of a 25-cm diam galvanized funnel,

the narrow end of which was attached to a wet dry vacuum cleaner. The vacuum was operated until all *C. maculatus* adults were drawn through the sieve. The beans then were returned to the jars, which were laid on their sides. In about 21 d, the hatching larvae were 4<sup>th</sup> instars, the lifestage used previously for parasitism (Rodriguez-Leyva et al. 2000). The larvae form pupation cells and chew an emergence hole, leaving only the integument of the bean. These opaque "windows" can be seen readily and aid in the selection of beans with 3<sup>rd</sup> instars present. These jars were moved to the *C. hunteri* rearing room, which was maintained under the same conditions as the *C. maculatus* rearing room.

The beans were placed in trays (9 × 8 × 2 cm) with 115–125 beans in each tray. The trays were plastic strawberry baskets with the sides trimmed to 2 cm high (Fig. 1a). Corks were glued to the bottoms of the trays to elevate them, thus allowing more accessibility of the female parasitoids to the beans on the bottoms of the trays. Ev-

ery Monday, Tuesday, and Wednesday, two trays were placed in each of two oviposition containers consisting of No. 6 (2.8 liter) plastic jars (Newell Rubbermaid Co., Wooster, OH) laid on their sides (Fig. 1b). Water was provided by inserting two water-filled, cotton-plugged 1-dram vials through two 1.3-cm diameter holes in the upper surface of each container. A cloth sleeve was attached to the mouth of each container and was sealed with a rubber band when not in use. Drops of honey were placed on the inside top of the containers to provide food and were replenished when consumed by the parasitoids. About 50 female and 50 male parasitoids were introduced into each oviposition container. The trays in the containers were changed three times a week for 26 days, at which time the oviposition containers were disassembled and cleaned for re-use.

The beans that had been exposed 2–3 days to parasitoids were placed in No. 3, 2.4-liter rectangular, plastic containers (Newell Rubbermaid Co., Wooster, OH) with screen covered square holes cut in the lid to allow ventilation but prevent escape of emerging *C. maculatus* adults. The beans were divided into three containers and each container was placed individually in Plexiglas® (Atofina Chemicals, Inc., Philadelphia, PA) incubation cages (30.5 × 30.5 × 30.5 cm) with a cloth sleeve on one end. Two sides of the cage were covered with organdy fabric to allow ventilation.

After about 7 d, adult parasitoids began to emerge and were collected with a vacuum pump aspirator. The garbanzo beans were sifted to remove *C. maculatus* adults. The beans were then placed on a wax paper-lined fiberglass lunchroom tray (45 × 35 cm), one layer deep and the trays were placed on the shelves of an emergence box (Fig. 2a). The emergence boxes were constructed of wood and had 4–8 shelves with individual, sealable doors for each shelf. The shelves did not extend to the back of the emergence box and the bean-filled trays were not placed on the shelf all the way to the back. Thus, an open space was created at the back of the box from the bottom to the top. At the top of this open space, a hole (5 × 20 cm) was cut and covered with metal window screen that allowed passage of *C. hunteri* but prevented that of the *C. maculatus* adults. A Plexiglas collection chamber (32 × 32 × 21 cm) (Fig. 2b) was attached to the top of the emergence box over the screen-covered slot. The sides of the Plexiglas box had cloth sleeves installed, allowing access for collecting parasitoid adults with a vacuum pump aspirator. Two water-filled, cotton-plugged vials were placed in the bottom of the Plexiglas box and honey was streaked on the inside of the top and front. Both were replenished as needed. Trays were replaced within the emergence box every 23 days as new parasitoid-exposed, *C. maculatus*-infested beans were added. Once a week, the Plexiglas box was thoroughly cleaned with

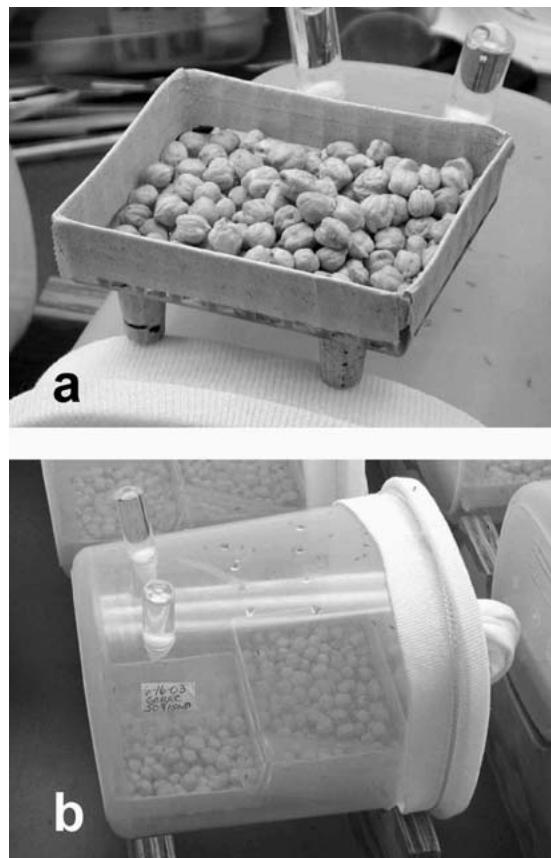


Fig. 1. Strawberry basket (a) for exposing garbanzo beans with 4<sup>th</sup> instar *Callosobruchus maculatus* in oviposition containers to adult *Catolaccus hunteri* (b) in the laboratory.

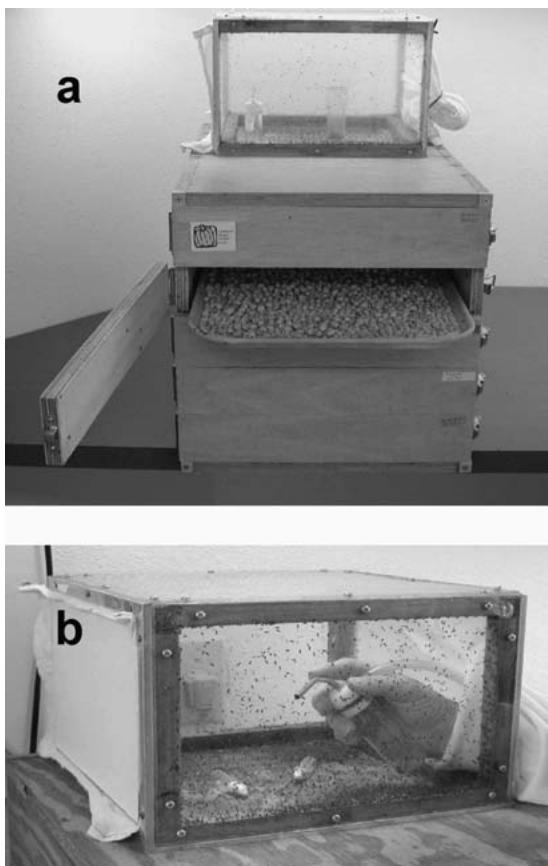


Fig. 2. Emergence box (a) with tray of garbanzo beans infested with 3<sup>rd</sup> instar *Callosobruchus maculatus* that have been exposed to adult *Catolaccus hunteri*. Aspirating *C. hunteri* adults from the Plexiglas® collection chamber (b).

Kimwipes® tissue (Kimberly-Clark Corp., Roswell, GA) moistened with water.

Approximately 18,000 parasitoids were produced weekly by these rearing methods with 26 oviposition cages. Start-up costs include about \$133 for 180 "Mason" jars for rearing the *C. maculatus*; about \$150 for a humidifier to maintain RH at 60% in the *C. maculatus* rearing room; about \$315 for 26 *C. hunteri* oviposition cages including plastic jars, vials, cotton balls, honey, plastic berry baskets, corks, fabric (also used for incubation cages), twine rope, and rubber bands; about \$80 for each of 9 *C. hunteri* larval incubation cages; and about \$155 for labor and supplies to build each of three adult emergence cages. About 22 h/wk were required in the maintenance of both the *C. maculatus* and *C. hunteri* colonies. About 4 kg of black-eyed peas and 6 kg of garbanzo beans were used each week. At \$8/h for labor and \$1.22/kg for the peas and \$0.90/kg for beans, the estimated recurring cost of production was about \$186/wk.

Anecdotal observations have indicated that bi-weekly releases of 1,500 *C. hunteri* along one edge of pepper fields of different sizes during the summer and fall fallow season resulted in reduced infestations of the pepper weevil on pepper during the following spring season. In addition, experimental evidence on an organic farm indicated that weekly releases of the parasitoid at about 7,400/ha delayed the pepper weevil infestation (Schuster, unpublished data). In experimental plots, weekly releases of 1,500 *C. hunteri* in nightshade during the fallow, off-season followed by weekly releases at 7,400/ha in adjacent pepper in the spring resulted in 65-75% fewer pepper fruit infested by the pepper weevil.

It is estimated that for an organic grower with a 1-ha block to make releases of 1,500 *C. hunteri* adults every 2 wk for 32 wks (16 releases during the fallow off-season) would cost about \$250 in recurring expenses. To add additional releases of 7,400/ha would cost about another \$76/wk during the early pepper season. Neither of these cost estimates includes the cost of labor to release the parasitoid adults. The estimated cost for fallow season releases is probably cost effective but the in-season costs may be prohibitive; however, in discussions with organic producers, this latter cost may not be prohibitive in light of few effective alternatives for managing the pepper weevil. The current rate of parasitism in the *C. maculatus* host is about 40%. If the rate of parasitism could be increased without increasing production costs, the cost for releases of *C. hunteri* for managing the pepper weevil during the spring season could become more cost effective.

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#### SUMMARY

Methodology was developed to rear *Catolaccus hunteri* Crawford, a parasitoid of the pepper weevil (*Anthonomus eugenii* Cano), on an alternative host, the cowpea weevil (*Callosobruchus maculatus* F.) in temperature controlled rooms at 27°C, 60% relative humidity and 14L:10D photoperiod. Black-eyed peas, *Vigna unguiculata* (L.) Walp., were used to maintain a colony of *C. maculatus*, and garbanzo beans, *Cicer arietinum* L., were used to expose the *C. maculatus* larvae to *C. hunteri* females. About 250 garbanzo beans containing 4<sup>th</sup> instar *C. maculatus* were exposed 48 to 72 h to 50 female and 50 male *C. hunteri*. Parasitoid-exposed beans were held for about 7 days and were placed into emergence boxes with screened-covered slots, which retained *C. maculatus* adults in the box but allowed *C. hunteri* adults to pass into a Plexiglas collection chamber. With an invest-

ment of about 22 h/wk, about 18,000 parasitoids can be reared weekly at an estimated recurring cost of \$186/wk for labor and supplies. Start-up costs for rearing containers and a rearing room humidifier totaled about \$1,800.

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## PATHOGENICITY OF *METARHIZIUM ANISOPliae* VAR. *ACRIDUM* TO THE FALSE SPIDER MITE *BREVIPALPUS PHoenicis* (ACARI: TENUIPALPIDAE)

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The flat mite, *Brevipalpus phoenicis* (Geijkes), is a polyphagous pest found in many subtropical and tropical regions of the world (Childers et al. 2003). *Brevipalpus phoenicis* is recognized as the most economically harmful virus vector species in citrus areas where *Citrus leprosis* Virus (CiLV) has been reported. So far, chemical sprays have been the main approach adopted to control the mite in citrus (Rodrigues & Machado 2003). Otherwise, fungal pathogens are frequently found causing diseases and epizootics in mite populations (Alves 1998; Van Der Geest et al. 2000). Recently, the potential to control *Brevipalpus* populations by spraying with the fungus *Hirsutella thompsonii* Fisher was suggested (Rosas-Acevedo & Sampedro 2000; Rossi 2002). However, the development of *H. thompsonii* as a bio-acaricide has been hampered by difficulties in mass producing aerial conidia. In contrast, the entomopathogenic fungus *Metarhizium anisopliae* var. *acridum* Driver & Milner is easily produced in large scale and has been developed as a mycoinsecticide in several countries (Magalhães et al. 2000; Lomer et al. 2001). This fungus is tolerant of high temperatures, an important characteristic for pathogens developed for tropical agroecosystems. We studied the pathogenicity of *M. anisopliae* var. *acridum* against *B. phoenicis* to evaluate its potential use as an acaricide.

Mites used in this study were 5-15 days old adult females derived from clonal lineage established from a single female isolated from a mite colony collected from citrus in Plant City, Florida (Rodrigues et al. 2004). The fungus assayed was the isolate CG423 of *M. anisopliae* var. *acridum*, produced on SDAY medium, harvested, and dried according to Magalhães & Boucias (2004). The viability of the conidia used in the bioassays was determined by plate assay to be >95%. The bioassay unit was a 90-mm diameter Petri dish containing a 60-mm diameter plate glued to the bottom. A 1.5-cm diameter circular leaf arena clipped from *Ligustrum lucidum* Aitton was placed over a layer of cotton wool which was saturated with water. Twenty mites were transferred to the leaf arena. Two h later the

pathogen was applied at a concentration of  $10^8$  conidia/ml in a 200- $\mu$ l suspension applied by air flow with a pressure of 150 psi with the aid of a spray tower (Pereira 1991). Mite survival was monitored daily and infection confirmed by examining the specimens with an epifluorescence microscope. Dead mites were stained with Calcofluor White (Sigma) (1 mg/ml) for 30 min and washed three times. To prevent rapid fading, the stained material was immersed in a mounting medium containing DABCO (Sigma) (100 mg), glycerine (30 ml) and Hepes (10 ml) for microscopic examination.

In preliminary experiments, the mites were sprayed with fungus + 0.5% Tween 20 (v/v) (polyoxyethylenesorbitan monolaurate, P-1379 (Sigma)) in deionized water, water + 0.5% Tween 20, and control (no treatment). Each treatment was repeated five times. Observations showed very low survival rates (<23%) 24 h after treating the mites with the fungus + Tween 20 but also with the Tween 20 alone. Otherwise, the mortality rates in the controls (water treatment) were insignificant, indicating acaricidal activity by the wetting agent. Therefore, we tested the direct mortality to *B. phoenicis* with 0.5, 0.05, 0.005, 0.0005, and 0.00005% of Tween 20 to determine a safe concentration for subsequent studies. The results from a linear regression confirmed that mite survival was dependent on the Tween 20 concentration ( $P < 0.001$ ) (Zar 1998). When Tween 20 was applied at a concentration 0.5%, survival at 24 h after inoculation was lower than 35%. In a separate experiment, similar mortality was observed when the leaf substrates were treated with Tween 20 prior to transferring the test mites (data not shown).

Survival of adult female mites treated with 0.00005% Tween 20 was not significantly different from the control and was therefore used in the fungal assays. The fungus was able to germinate and penetrate the treated adult female mites (Fig. 1). The SEM observations confirmed these findings. At 8 days post-treatment, the fungus caused 90% mortality (Fig. 2), where infection levels were confirmed by fluorescence microscopy.

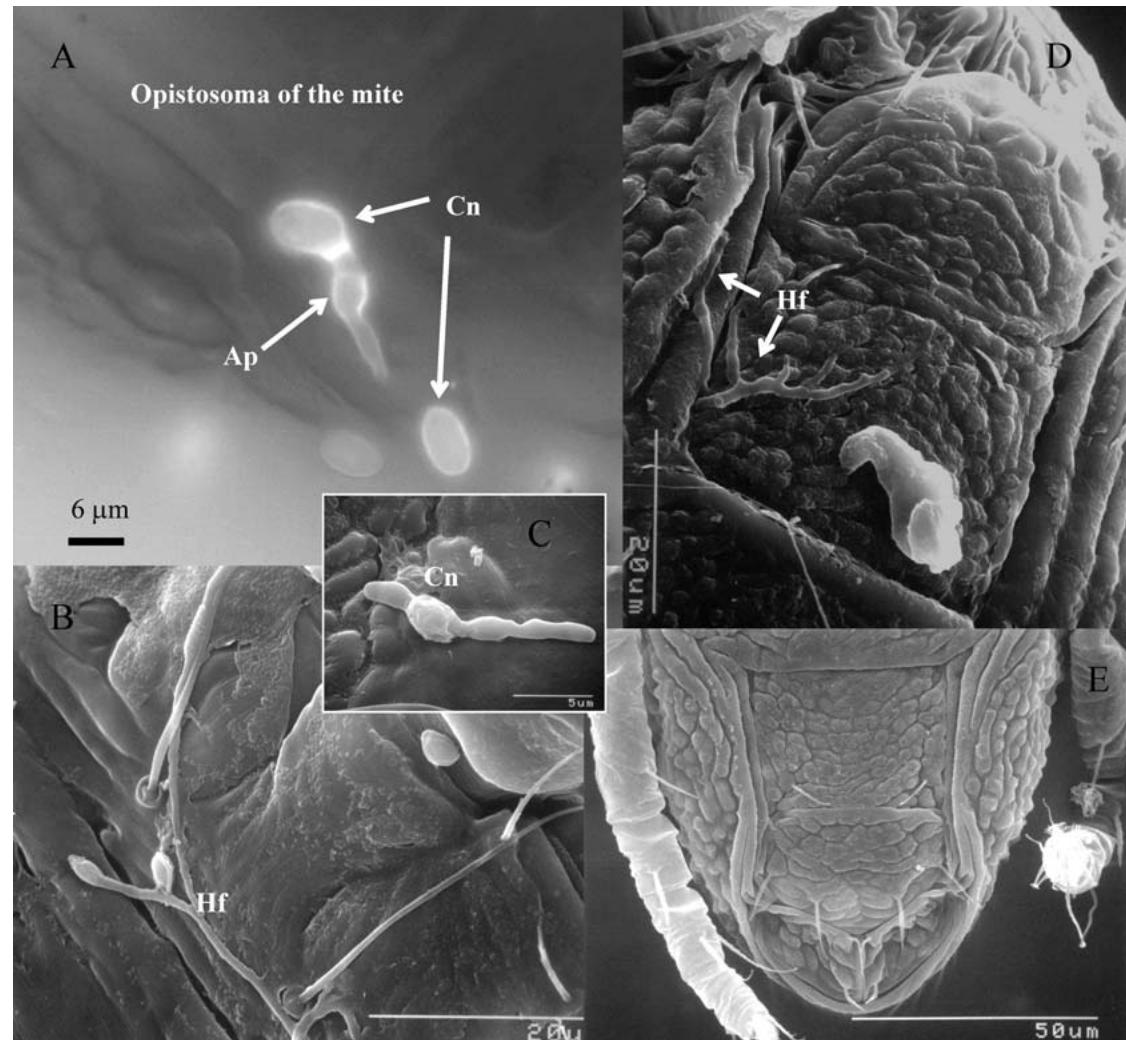


Fig. 1. (A) Microphotograph of *Metarhizium anisopliae* var. *acridum* infecting *Brevipalpus phoenicis* 3 days after inoculation by fluorescence microscopy. (B, C, and D) SEM of infection and colonization process. (E) Control sprayed with water. (Cn = Conidium, Ap = Appressorium, Hf = Hypha).

Fungal infection also reduced the production of eggs by *B. phoenicis* females in comparison to treatments that received only Tween 20 or water (Table 1). These results are important and support the possibility of exploiting *M. anisopliae* var. *acridum* as a biological acaricide against *B. phoenicis*. Additional research is needed to determine the acaricidal effects of Tween 20 and similar products for false spider mite control.

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#### SUMMARY

*Metarhizium anisopliae* var. *acridum* Driver & Milner isolate CG423 was demonstrated to be pathogenic to the false spider mite *Brevipalpus phoenicis* Geijskes (Acari: Tenuipalpidae). Effects on mite survival and egg production were assessed. The fungus was able to infect treated adult mites at least 4 days after inoculation and reached 90% mortality by the 8<sup>th</sup> day. We also demonstrated that Tween 20 shows acaricidal activity at low concentrations to *B. phoenicis*.

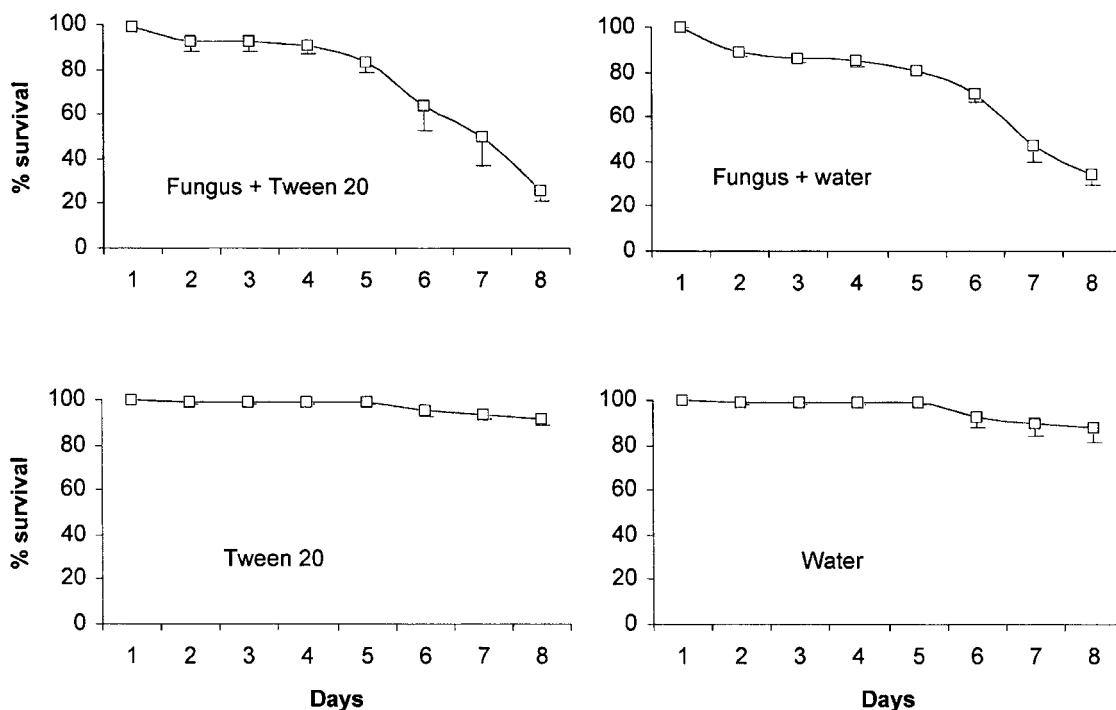


Fig. 2. Survival curves of *Brevipalpus phoenicis* females treated with *Metarhizium anisopliae* var. *acridum* suspended in Tween 20 and water. The fungus suspension was sprayed at concentration of  $10^8$  conidia/ml and 0.00005% Tween 20. Vertical bars = Standard Error.

TABLE 1. EGG PRODUCTION OF *Brevipalpus phoenicis* FEMALES BY THE 4<sup>TH</sup> DAY AFTER INOCULATION WITH *Metarhizium anisopliae* VAR. *acridum*. AVERAGE OF FIVE REPLICATES.

Treatment	Females alive	Eggs produced	Eggs/Female (S.E.)
Water (W)	19.8 ( $\pm 0.2$ ) a*	17.2 ( $\pm 4.1$ ) a*	0.87 ( $\pm 0.21$ ) a
Tween 20 (T)	18.6 ( $\pm 0.8$ ) a	9.64 ( $\pm 3.7$ ) ab	0.51 ( $\pm 0.20$ ) ab
Fungus + W	17.0 ( $\pm 0.4$ ) a	4.2 ( $\pm 0.2$ ) b	0.25 ( $\pm 0.01$ ) b
Fungus + T	18.2 ( $\pm 0.9$ ) a	3.8 ( $\pm 1.5$ ) b	0.20 ( $\pm 0.08$ ) b

\*Means followed by the same letter do not differ at  $P < 0.05$ ; Tukey ( $\alpha = 0.05$ ); S.E. = Standard Error.

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**THE FEMALE OF *OXYBLEPTES MERIDIONALIS***  
**(COLEOPTERA: STAPHYLINIDAE: STAPHYLININAE)**  
**AND RANGE EXTENSION FOR *OXYBLEPTES***

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Smetana (1982) described a new genus (*Oxybleptes*) to include four species of Xantholinini from America north of Mexico. One species, *O. davisi* (Notman), was previously known from the District of Columbia, New Jersey, and New York. The remaining three species (*O. kiteleyi* Smetana, *O. hatchi* Smetana, and *O. pusio* Smetana) were described as new. None of these species was reported to occur farther south than North Carolina.

In 1984, J. H. Frank submitted to Ales Smetana (Canadian National Collection, Ottawa, Ontario) North American specimens of Xantholininae that either could not be identified using Smetana's (1982) keys, or gave evidence of range extensions. Thereafter, Smetana (1988) described a new species (*O. meridionalis* Smetana) based upon five specimens. One paratype was retained by the Canadian National Collection (CNC), the holotype and one paratype were in 1987 deposited in the Florida State Collection of Arthropods (FSCA), and two paratypes were retained in the collection of J. H. Frank. All were from the grounds of what is now called Florida Medical Entomology Laboratory (of the University of Florida), at Vero Beach, Indian River County, Florida, a location a few hundred meters west of the Indian River (intra-coastal waterway) on Florida's Atlantic coast, and at  $\approx 27^{\circ}36'N$ . Four were collected in August 1973 and one in May 1976, in oak-palm hammock. The collection method was unusual: Frank had been operating 5-gallon (~20-liter) green plastic water-filled tubs to attract ovipositing *Culex* mosquitoes. Four *Oxybleptes* specimens were collected drowned or drowning from the surface of the water in the tubs, and one alive from the conical lid with which half of the tubs at any time were fitted. This lid was of light-reflecting sheet aluminum. The attractant (if there really was an attractant) for the beetles may have been the reflectance of the water surface or the aluminum. Although Frank routinely ran an ultraviolet light trap, and later a Malaise trap, at this and other nearby locations, no specimens of *Oxybleptes* were caught in those traps. The habitat and behavior of these curious little beetles remained unresolved. All five specimens collected were males. Smetana (1988), therefore, was able to describe only the male of this species, and he found it to be bicolored, like that of *O. davisi* and contrasting with males of the three other known species.

In 2002, J. L. Foltz and D. T. Almquist operated various kinds of traps for insects at Bee Island in the Myakka River State Park,  $27^{\circ}15.12'N$ ,

$82^{\circ}15.09'W$ . Most of the park is in Sarasota County, Florida, but Bee Island is in Manatee County. This park is within 25 km of Florida's Gulf of Mexico coast. In November 2002, Almquist asked Frank to identify staphylinid specimens collected in pine flatwoods at Bee Island. Frank recognized some of them as being *O. meridionalis*, explained their apparent rarity, and urged Almquist to collect more, alive if possible for behavioral study. The initial collection in the park was on 16 October, and included eight specimens (7 male, 1 female). The second collection was on 18 December and included 18 specimens (13 male, 5 female).

Again, the collection method was curious. All specimens were collected in water, with the October 2002 collection from the water-filled, white plastic top of a Lindgren funnel trap (Phero Tech, Inc., Delta, BC, Canada), and the December collection from plastic shoe boxes with a few cm of soapy water. The collections again suggested attraction to a reflective surface. All collections yielded a preponderance of males (for 1973-1976, 5:0 males: females, October 2002, 7:1, December 2002, 13:5) but the significance of this imbalance in sex ratio is unclear. Adult activity is known to occur in May, August, October, and December. Eggs and larvae were not obtained, nor was any behavioral information obtained except that Foltz remembers that the specimens collected on 18 December were trapped in the early afternoon, thus in daylight.

The female (Fig. 1), similar in size and coloration to male, is "bicolored", testaceous to rufotestaceous; the head and elytra are piceous to piceous black, and the abdominal apex darkened. The antenna of female with less swollen apical antennomere and with antennomere III distinctly shorter than II (Fig. 2a) cf. as long as II in male (Fig. 2b). The maxillary palpus of the female (Fig. 2c) has all palpomeres shorter and therefore relatively broader than in male (Fig. 2d). The meso- and metatarsus are shorter than the respective tibia (cf. as long in male), and tarsomeres II-IV are relatively shorter than in male. Such sexual dimorphism occurs also in other species of the genus (Smetana 1982). Female specimens will be deposited in FSCA and CNC together with additional male specimens.

Heretofore there is no record of any species of *Oxybleptes* other than *O. meridionalis* in Florida. Among staphylinids collected by C. W. O'Brien was one female specimen of *Oxybleptes* labeled USA, Florida, Franklin Co., 3 mi NW of Alligator Point, 17-IV-1974, Berlese funnel extract of pine litter, O'Brien and Marshall. This specimen is not

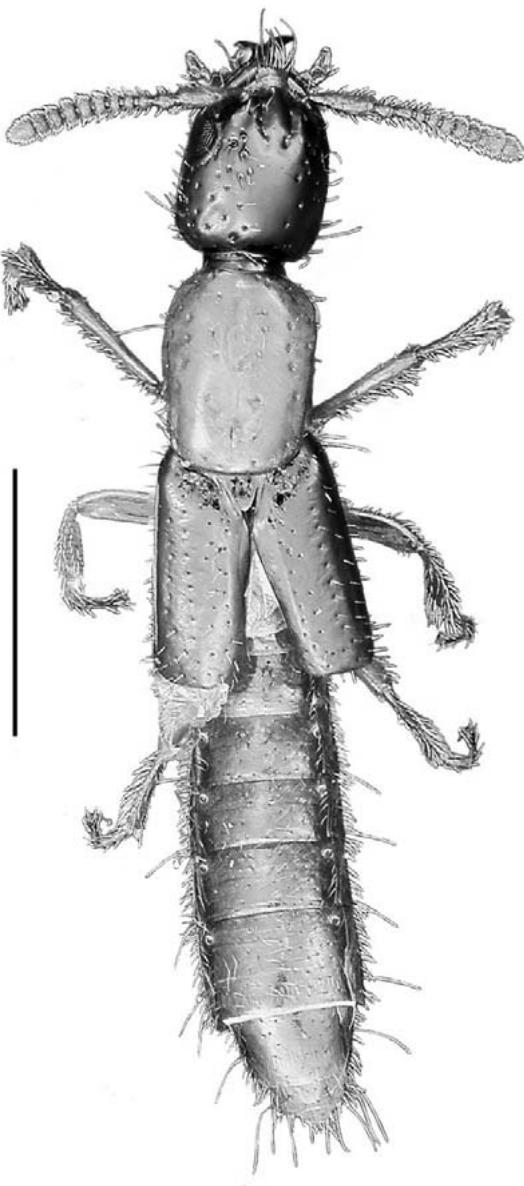


Fig. 1. Habitus of female *Oxybleptes meridionalis*. The scale line represents 1.0 mm.

distinctly bicolored, is considerably larger than the diminutive *O. meridionalis*, and most closely matches the description of *O. kiteleyi* by Smetana (1982). Thus, in the northwest of Florida, distant by about 380 km from the nearest known location for *O. meridionalis* and in a harsher climatic zone, seems to exist a population perhaps of *O. kiteleyi*. If this identification is correct, it would represent the southernmost record for *O. kiteleyi*, which until now had not been known south of North Carolina. Smetana (1982) noted the difficulty of distinguishing females of *O. kiteleyi* from those of

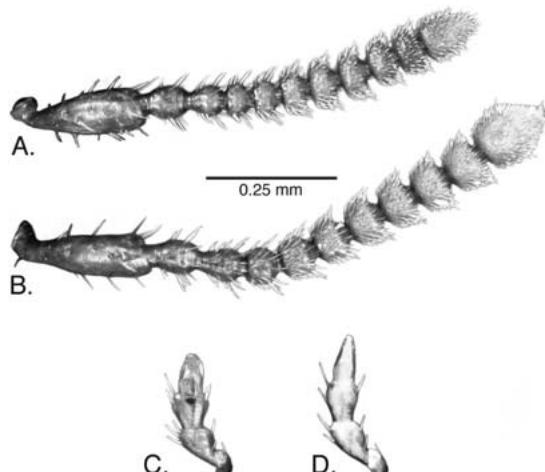


Fig. 2. *Oxybleptes meridionalis*, 2a. Antenna of female, 2b of male, 2c maxillary palpus of female, 2d of male. The scale line represents 0.25 mm.

*O. davisi*, so there is doubt in this determination, and it would be advantageous to examine male specimens from northwestern Florida.

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#### SUMMARY

*Oxybleptes meridionalis* Smetana (Staphylinidae: Staphylininae: Xantholinini) was previously known only from five males collected in 1973-1976 from Indian River County, Florida, at 27°36'N. It is here reported from Manatee County, on the other side of the Florida Peninsula, at 27°15'N. The new collections include six females together with 20 males. The female is colored similarly to the male, but differs in the structure of antennomeres III and XI, the maxillary palpi, and the meso- and metatarsi, which are here described. One female specimen of another species of *Oxybleptes*, of uncertain identity, was collected in Franklin County, Florida, some 380 km farther north.

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## DISTRIBUTION OF THE TERMITE GENUS *COPTOTERMES* (ISOPTERA: RHINOTERMITIDAE) IN FLORIDA

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Two non-endemic and highly destructive species of *Coptotermes* Wasmann occur in Florida. In 1980, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was first discovered in Florida infesting condominiums along the Atlantic Ocean and the Intracoastal Waterway in Broward County (Anonymous 1980; Koehler 1980). Later, *C. formosanus* was found in neighboring Dade Co. and in central Florida (Orange Co.) and in the western Panhandle (Escambia Co.) (Thompson 1985). A 1987 survey of structure-infesting termites of Florida (Scheffrahn et al. 1988) showed a low incidence of *C. formosanus* in urban areas of the peninsula compared with other pest species. Based on published reports and personal

communications but no voucher specimens, Woodson et al. (2001) added Okaloosa, Hillsborough, Santa Rosa, Palm Beach, Marin, Citrus, and Leon as additional Florida counties where *C. formosanus* is distributed.

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann), was first discovered in Florida in 1996 infesting a storefront in Miami (Su et al. 1997). Originally classified as *C. havilandi* Holmgren, this species was recently synonymized with *C. gestroi* by Kirton & Brown (2003). Since 1996, no additional reports have been published on the distribution of *C. gestroi* in Florida. *Coptotermes formosanus* and *C. gestroi* can be differentiated from each other by soldier (Scheffrahn et al.

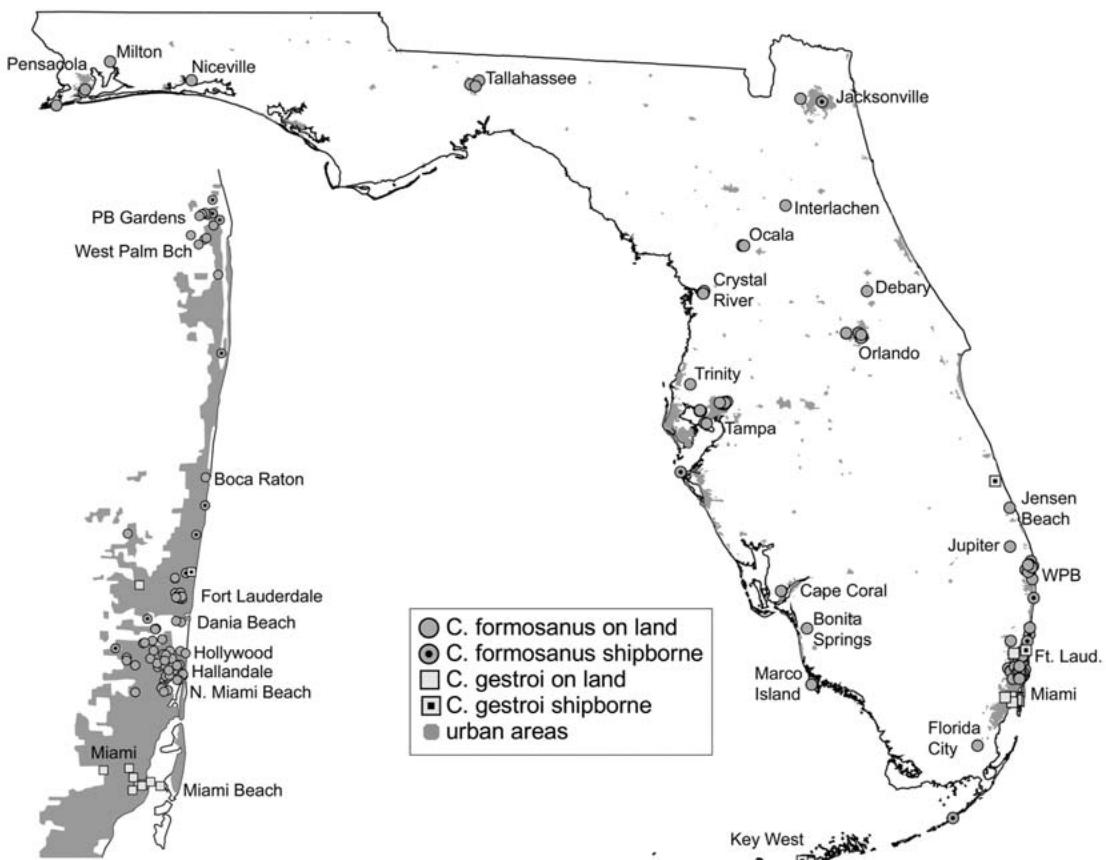


Fig. 1. Distribution of *Coptotermes formosanus* and *C. gestroi* in Florida. Inset on left shows greater detail of the southeast coast. Grey areas represent urbanized land zones.

1990) and imago (Scheffrahn & Su 2000) morphology. Both species separate into different clades when sequences of their respective mtDNA 16s RNA genes are compared (Scheffrahn et al. 2004).

Over the last 19 years, we have directly collected or have received more than 3,000 termite colony samples from throughout Florida including those of the two now well established *Coptotermes* species. We herein report on the current distribution of *Coptotermes* in Florida based on locality records of 168 *C. formosanus* and 35 *C. gestroi* samples. All samples are cataloged and housed in the University of Florida Termite Collection, Ft. Lauderdale Research and Education Center, Ft. Lauderdale, Florida.

The geographical distribution of *Coptotermes* in Florida, including incidences where these species have been collected aboard boats or ships, is mapped in Fig. 1. Most urban centers throughout Florida, with the exception of Pinellas Co. (St. Petersburg), now support populations of *C. formosanus*. *Coptotermes* localities in Florida are listed in Table 1 by county, city, and year of discovery. Including shipborne infestations, *C. formosanus* has been collected from 20 counties and 40 cities in Florida, while *C. gestroi* has been collected in 4 counties and 8 cities (Table 1). All populations of *C. gestroi* are currently restricted to tropical southeastern Florida. Dade and Broward Counties, Florida, are the only geographies worldwide where these two species have sympatric distributions. Both species are exclusively synanthropic in Florida and have only been collected in or within foraging distance of a structure.

The tendency of both *C. formosanus* and *C. gestroi* to colonize boats (<40-m-long) and ships (>100-m-long) likely contributed to the dispersal of these and other *Coptotermes* species from their other ranges to exotic localities, such as Florida (Scheffrahn et al. 2004). Colonies have been observed reaching maturity aboard watercraft and dispersal flights from watercraft could initiate land-based infestations near dockage.

One particular infestation is worth reporting here because it provides compelling evidence for shipboard establishment and movement of *Coptotermes* colonies over long distances. In January 2001, a 29 meter-long yacht docked off the Intra-coastal Waterway in Ft. Lauderdale was found to be infested with *C. gestroi*. Since 1993, the yacht's winter dockage was at the Turtle Cove Marina in Providenciales, Turks and Caicos Islands, British West Indies. Providenciales is some 930 km southeast of Ft. Lauderdale. *Coptotermes gestroi* was first collected in the Turks and Caicos at Turtle Cove in 1990 (R. Scheffrahn, unpubl.). It is very probable that during one or more preceding winters in Providenciales, lighting on the yacht attracted alates during a nocturnal dispersal flight in the Turtle Cove vicinity (*C. gestroi* dispersal flights in Florida and the West Indies occur

TABLE 1. DISTRIBUTION OF *COPTOTERMES* IN FLORIDA BY COUNTY, CITY, AND YEAR RECORDED.

County	City	Year
<i>Coptotermes formosanus</i>		
Broward	Dania Beach	2001
	Fort Lauderdale	
	Hallandale	1980
	Hillsborough Beach <sup>1</sup>	2000
	Hollywood	1994
	Lighthouse Point <sup>1</sup>	1998
	Pembroke Pines	2002
	Pompano Beach <sup>1</sup>	2001
	Wilton Manors	2002
Citrus	Crystal River	1999
Collier	Marco Island	2003
Dade	Florida City	2002
	Miami	2004
	North Miami Beach	1999
Duval	Jacksonville <sup>2</sup>	2004
Escambia	Pensacola	1985
	Warrington	1989
Hillsborough	Tampa	1996
	Temple Terrace	1991
Lee	Bonita Springs	2003
	Cape Coral	2004
Leon	Tallahassee	2000
Manatee	Holms Beach <sup>1</sup>	2002
Marion	Ocala	2003
Martin	Jensen Beach	1998
Monroe	Islamorada <sup>1</sup>	1986
Okaloosa	Niceville	2001
Orange	Orlando	1985
Palm Beach	Boca Raton	2003
	Hypoluxo <sup>1</sup>	1985
	Jupiter	1999
	Lake Park	2001
	North Palm Beach	1996
	Palm Beach Gardens	2000
	Riviera Beach	1999
	West Palm Beach	1999
Pasco	Trinity	2003
Putnam	Interlachen	2004
Santa Rosa	Milton	1991
Volusia	Debary	2003
<i>Coptotermes gestroi</i>		
Broward	Fort Lauderdale <sup>1</sup>	2001
	Lauderhill	2002
Dade	Hialeah	2004
	Miami	1996
	Miami Beach	2002
Monroe	Key West	1999
	Stock Island	2004
St. Lucie	Fort Pierce <sup>1</sup>	1991

<sup>1</sup>Collected from boat only.

<sup>2</sup>Collected previously from ship in 1999.

at night from January to March). Dealates then proceeded to colonize the boat. At least one colony became established on board and subsequently grew until the infestation was detected and the boat fumigated in Ft. Lauderdale before returning to Providenciales.

It is likely that shipboard infestations will continue to contribute to the intrastate spread of *Coptotermes* in Florida, where the overwhelming number of infestations are within one kilometer of marine boat dockage. Eighteen *C. formosanus* and 3 *C. gestroi* samples in our collection database were taken aboard boats and ships. In inland locations, such as the Orlando and Tallahassee areas, land-based commodities such as railroad ties and landscape materials harboring incipient colonies may have served as vehicles of introduction.

We thank the many pest control professionals and others that have collected and submitted samples that were included herein, including Paul Ban, Bob Benham, Ron Box, Lyle Buss, Gabe Centeno, Jim Chase, Mary Cohen, Bruce and Jeff Edwards, Louis Giaccone, Terry Harper, Jim Maler, John Mangold, Bruce Ryser, Jeff Stotts, and Mark Weinberg. William H. Kern Jr. and Brian J. Cabrera reviewed this contribution R-10474 of the University of Florida Agricultural Experiment Station Journal Series.

#### SUMMARY

Confirmed Florida localities of the Formosan subterranean termite, *Coptotermes formosanus*, and the Asian subterranean termite, *C. gestroi*, are reported. *Coptotermes formosanus* has been collected from 20 counties and 40 cities in Florida, while *C. gestroi* has been collected in 4 counties and 8 cities. Dispersal of both *Coptotermes* species have been facilitated by shipboard infestations and land-based commodities.

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## MANDIBULAR MORPHOLOGY OF SOME FLORIDIAN GRASSHOPPERS (ORTHOPTERA: ACRIDIDAE)

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The relationship between mouthpart structure and diet has been known for years. This connection between mouthpart morphology and specific food types is incredibly pronounced in the class Insecta (Snodgrass 1935). As insects have evolved and adapted to new food sources, their mouthparts have changed accordingly. This is an extremely important trait for evolutionary biologists (Brues 1939) as well as systematists (Mulkern 1967).

Isley (1944) was one of the first to study grasshopper mouthparts in detail. He described three groups of mandibles according to general structure and characteristic diet. These three groups, still used today, were graminivorous (grass-feeding type) with grinding molars and incisors typically fused into a scythe-like cutting edge, forbivorous (forb or broadleaf plant-feeding type) which have a molar region consisting of a depression surrounded by raised teeth and sharp interlocking incisor teeth, and herbivorous (mixed-feeding type) that have characteristics of both of the aforementioned groups. The original findings by Isley (1944) have since been proven to be widespread in grasshoppers. Additional studies have been conducted by Snodgrass (1928), Gangwere (1965, 1966), Gangwere et al. (1976), and Patterson (1984) in North America; Lieberman (1968) and Gangwere & Ronderos (1975) in South America; Williams (1954), Kaufmann (1965), and Gangwere & Morales (1973) in Europe; Gangwere & Spiller (1995) and Gangwere et al. (1998) in the Mediterranean islands; Feroz & Chaudhry (1975), Gapud (1968), and Kang et al. (1999) in Asia; and Chapman (1964) in Africa.

The relationship between grasshopper mouthparts and food is far from precise. Mulkern (1967) was convinced that only the grossest determinations could be made between mandibular structure and diet (i.e., graminivorous, forbivorous, and herbivorous). Occasionally, grasshoppers with forb-feeding mandibles regularly feed on grasses or vice versa (Chapman 1964). Nevertheless, there is some value in assessing mouthpart structure relative to predicting diet and habitat of grasshoppers, especially for the many rare or non-economic species that are unlikely to be studied in detail. Thus, the morphological characteristics and structural adaptations of the mouthparts of 36 of the 71 grasshoppers occurring in Florida were examined.

Grasshoppers were collected from various habitats throughout north-central Florida in 2001 and 2002. Thirty-six of the most common Floridian grasshopper species were identified with the taxonomic key found in Smith et al. (2004) and fro-

zen until examination. Mandibles were removed from thawed specimens by lifting the labrum and pulling out each mandible separately with forceps. Only young adults were used in an effort to avoid confusion of mandible type due to mandible erosion (Chapman 1964; Uvarov 1977). An example of moderate erosion can be seen in Figure 1 (I). This process was replicated with 10 individuals from each species. After air-drying, each mandible was glued to the head of a #3 or #2 insect pin, depending on its size, for easier manipulation, and examined microscopically.

We used Isley's (1944) description of mandible types and their adaptive functions to divide the mandibles into 3 major categories: forbivorous (forb-feeding), graminivorous (grass-feeding), and herbivorous (mixed-feeding).

Mandibles were lightly brushed with 80 percent ETOH and distilled water in an effort to remove most of the sand and debris adhering to the mouthparts. Photographs were taken with the Syncroscopy Auto-Montage system (University of Florida, Entomology and Nematology Dept.).

The mandible structure of 36 species of grasshopper, from five subfamilies (Acridinae, Cyrtacanthacridinae, Gomphocerinae, Oedipodinae, and Romaleinae), found in Florida was microscopically examined. These grasshoppers were collected from a variety of habitats including disturbed freshwater marsh, high pine, swamp, and oak hammocks. All grasshoppers had distinctive mouthparts that could be described as forbivorous (forb-feeding type), herbivorous (mixed-feeding type), or graminivorous (grass-feeding type) (Fig. 1, A-L). A list of each species studied and the mandible type is given in Table 1.

Of the subfamilies examined, the Cyrtacanthacridinae demonstrated the most diversity in mandible type; however, most of them displayed either herbivorous or forbivorous mandibles, indicating a tendency toward forb-feeding. These grasshoppers can be found in a wide range of habitats, usually in dense vegetation or woodland areas, and are quite active in both walking and flying. It is interesting to note that both the grasshoppers in this subfamily that did display graminivorous type mandibles (*L. marginicollis* and *S. vitreipennis*) also have extremely slender, elongated bodies and can be found on the edges of ponds or in freshwater marshes (Isley 1944; Squitter & Capinera 2002b; Smith & Capinera 2005). These grasshoppers typically grasp the stems of emergent grass or grass-like vegetation such as sedges or cattails, blending in almost perfectly.

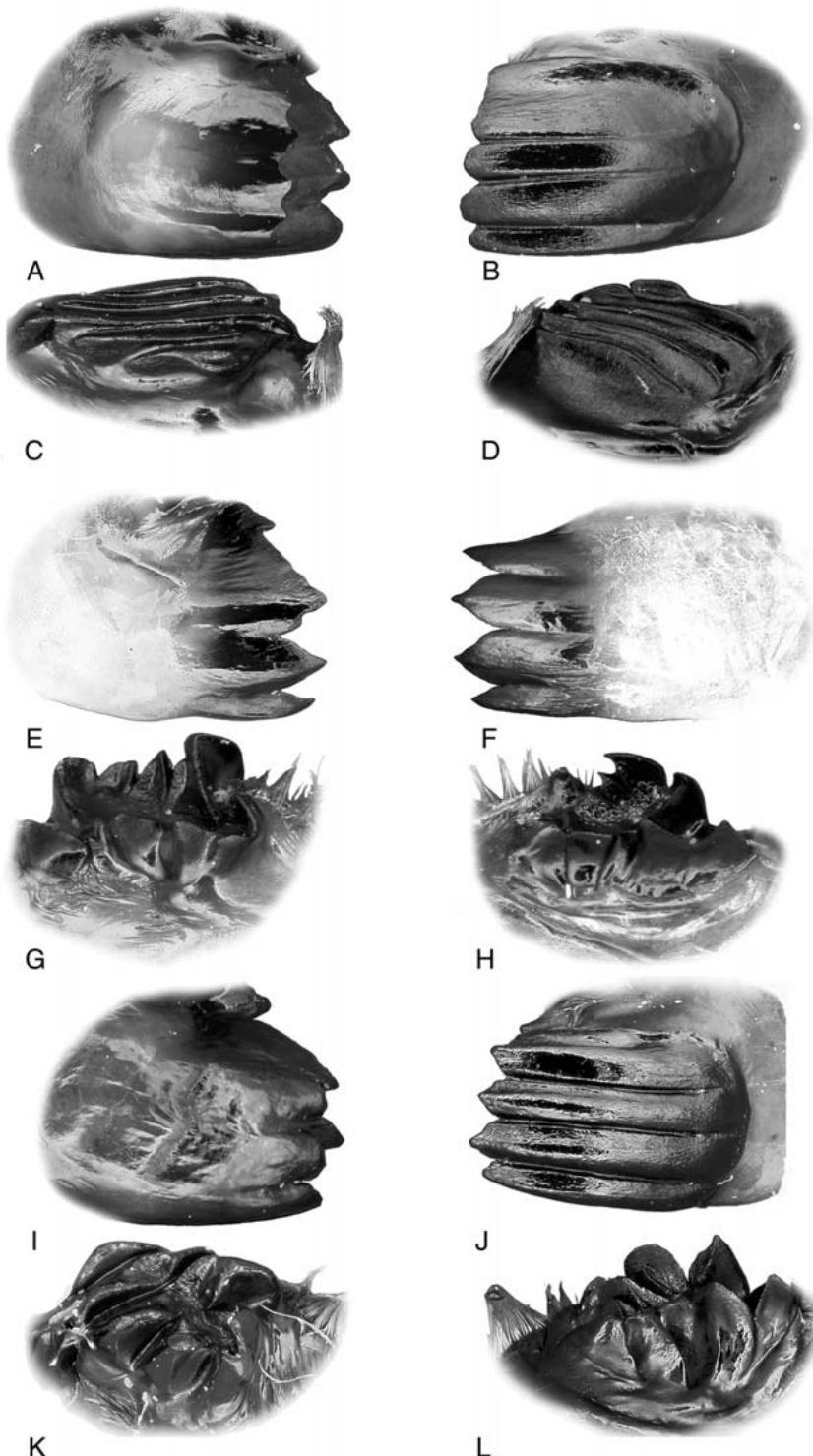


Fig. 1. Mandibles of *Amblytropidia mysteca*, a representative graminivorous species: right incisor region (A), left incisor region (B), right molar region (C), and left molar region (D); *Schistocerca ceratiola*, a representative forbivorous species: right incisor region (E), left incisor region (F), right molar region (G), and left molar region (H); and *Spharagemon cristatum*, a representative herbivorous species: right incisor region (I), left incisor region (J), right molar region (K), and left molar region (L).

TABLE 1. ACRIDID SPECIES EXAMINED AND THEIR MOUTHPART MORPHOLOGY.

Graminivorous type mandibles	Herbivorous type mandibles	Forbivorous type mandibles
Acridinae	Cyrtacanthacridinae	Cyrtacanthacridinae
<i>Metalepta brevicornis</i> (Johannson)	<i>Gymnosciertes pusillus</i> Scudder	<i>Aptenopedes aptera</i> Scudder
	<i>Melanoplus bispinosus</i> Scudder	<i>Aptenopedes sphenariooides</i> Scudder
Cyrtacanthacridinae	<i>Melanoplus sanguinipes</i> (Fabricius)	<i>Melanoplus keeleri</i> (Thomas)
<i>Leptysma marginicollis</i> (Serville)	<i>Schistocerca alutacea</i> (Harris)	<i>Melanoplus ordwayae</i> Deyrup
<i>Stenacris vitreipennis</i> (Marschall)	<i>Schistocerca americana</i> (Drury)	<i>Melanoplus propinquus</i> Scudder
Gomphocerinae	<i>Schistocerca obscura</i> (Fabricius)	<i>Melanoplus punctulatus</i> Scudder
<i>Achurum carinatum</i> (F. Walker)	<i>Schistocerca rubiginosa</i> (Scudder)	<i>Melanoplus querneus</i> Rehn & Hebard
<i>Amblytropidia mysteca</i> (Saussure)	Oedipodinae	<i>Melanoplus rotundipennis</i> Scudder
<i>Dichromorpha viridis</i> (Scudder)	<i>Chortophaga australior</i> (Rehn & Hebard)	<i>Paroxya atlantica</i> Scudder
<i>Eritettix obscurus</i> (Scudder)	<i>Spharagemon crepitans</i> (Saussure)	<i>Paroxya clavuliger</i> (Serville)
<i>Mermiria intertexta</i> Scudder	<i>Spharagemon cristatum</i> (Scudder)	<i>Schistocerca ceratiola</i> Hubbell & Walker
<i>Orphulella pelidna</i> (Burmeister)		<i>Schistocerca damnifica</i> (Saussure)
<i>Syrphula admirabilis</i> (Uhler)		
Oedipodinae		Romaleinae
<i>Arphia granulata</i> (Saussure)		<i>Romalea microptera</i> (Beauvois)
<i>Hippiscus ocelote</i> (Saussure)		
<i>Pardalophora phoenicoptera</i> (Burmeister)		

However, the overwhelming majority of these grasshoppers display either herbivorous or forbivorous mandibles (Isley 1944; Gangwere 1965, 1966; Squitier & Capinera 2002a).

The Oedipodinae were split between two mandible types: graminivorous and herbivorous. This signifies a more grass-dominated diet. However, these grasshoppers are much more divergent and some may be completely graminivorous or forbivorous. Most of the species in this subfamily were found on the ground in open areas on bare soil, rarely on plants or grasses. As a general rule, the Oedipodinae show the most mandible diversity of all the grasshopper subfamilies. Isley (1944), Gangwere (1966), and Kang et al. (1999) found a fairly even distribution of the three mouthpart types in this group.

The gomphocerinae all had graminivorous mandibles, indicating a consistent diet of grasses. These findings are reinforced by the preferred habitats of this subfamily, usually open grassy fields and pastures. Virtually all Gomphocerinae are graminivorous (Lockwood et al. 1994), or at least have graminivorous type mandibles. Occasionally a gomphocerine will display graminivorous type mandibles but feed entirely on forbs (Gangwere & Morales 1973); however, these are rare exceptions. In almost every study carried out on orthopteran mouthpart morphology, the Gomphocerinae display graminivorous type mandibles (Isley 1944; Gangwere 1965, 1966; Lockwood et al. 1994; Kang et al. 1999).

Due to only one representative species from both the subfamilies Acridinae and Romaleinae, determination of the mandibular morphology of

these subfamilies was limited. The Acridinae are typically considered to be grass-feeders, displaying the classic graminivorous type mandibles (Chapman 1964; Isley 1944). Very rarely a species in this subfamily will display herbivorous type mandibles (Chapman 1964). The Romaleinae are always forb feeders and always display forbivorous type mandibles (Isley 1944; Squitier & Capinera 2002a; Smith & Capinera 2005).

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## SUMMARY

Mouthpart consistency within subfamilies indicates that evolution is just as important as ecological factors in determining food plants; for most subfamilies there is a strong association with a particular form of vegetation. It is evident that the ability, or tendency, of grasshoppers to change hosts is partly limited by the structure of their mandibles. However, because there are exceptions to the strong association of cyrtacanthacridines with forbs, and gomphocerines with grasses, we see evidence that behavioral plasticity or ecological opportunism is present even in relatively primitive taxa such as Orthoptera.

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## HYPOXIA REDUCES REPRODUCTIVE SUSCEPTIBILITY OF PLUM CURCULIO (COLEOPTERA: CURCULIONIDAE) TO IONIZING RADIATION

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Ionizing irradiation is used in Florida and Hawaii to disinfest several fruits and sweetpotatoes of fruit flies (Diptera: Tephritidae) or other insects (Hallman 2004a). Importation of fruit irradiated against 11 fruit fly species and the mango seed weevil, *Cryptorhynchus mangiferae* (F.) (Coleoptera: Curculionidae), has been approved (APHIS 2002). The treatment shows promise for widespread implementation, as it is safe, broadly efficacious, accepted by consumers, cost-effective, may be applied after packing, and widely tolerated by fresh agricultural commodities (Hallman 2002).

A large body of research has determined minimum absorbed doses of ionizing radiation to prevent development or reproduction for many different species of arthropods <<http://www-infocris.iaea.org/ididas/start.htm>>. Some of this research has shown that hypoxia can reduce some of the detrimental effects of irradiation to insects used in sterile release programs. The effect of hypoxia on irradiation disinfection treatment efficacy has not been studied until recently, although some agricultural commodities are stored under hypoxic conditions, a strategy that is increasing in application. Hallman (2004a) found that while no oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), fifth instars developed to the adult stage when irradiated at 200 Gy in ambient atmosphere, 5.3% of those irradiated in hypoxic atmospheres developed to the adult. Hypoxia caused a small increase in the ability of apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae), third instars to emerge as adults after irradiation (Hallman 2004b).

Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), is native to the eastern Nearctic and is a quarantine pest of stone and pome fruits exported from the United States and Canada. Hallman (2003) determined that a minimum absorbed dose of 92 Gy (the maximum recorded dose when 80 Gy was sought) prevented reproduction of adults, the most radiotolerant stage of the insect. The objective of this research was to determine the effect of hypoxia on reproduction of the plum curculio.

Plum curculios originally collected near Gainesville, Florida, were obtained from a colony at the United States Department of Agriculture, Agricultural Research Service facility in Byron, Georgia. The insects were reared at about 25EC, 70% RH, 12: 12h (L:D), on immature apples that were picked when about 3 cm in diameter. Larvae emerging from the apples were placed on sterilized potting soil until adult emergence.

A radiation source of  $^{137}\text{Cs}$  (Husman Model 521A, Isomedix, Inc., Whippany, NJ) that delivered a gamma ray dose rate of about 40 Gy·min<sup>-1</sup> was used in this research. Routine dosimetry was done with radiochromic film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) and read with a spectrophotometer at 510 nm (Milton Roy Spectronic 401, Ivyland, PA).

Adult plum curculios were irradiated in ambient atmospheres and in atmospheres of mostly nitrogen. Cylinders (polyvinyl chloride, 37.5 cm inside length, 10 cm inside diameter) fitted on one end with a screw cap sealed with vacuum grease and on the other end with 2 brass, barbed-nipple compression hose fittings (25 mm long, 4-mm inside diameter) were constructed. Two-week-old plum curculio adults were placed inside the cylinder with a few immature apples, and the atmosphere was purged through the hose fittings with nitrogen at a pressure of about 3 kPa for 2 minutes 20, 16, and 2 h before irradiation with an absorbed dose of 40 Gy. After purging, the hose fittings were sealed with rubber septa and the cylinders held at about 24°C. About 1.5 h after irradiation, the cylinders were opened to return the insects to ambient atmosphere. There were 6 replicates of 300-600 each for adults irradiated in ambient or hypoxic atmospheres. Controls consisted of 6 replicates of 30-100 insects each in a cylinder under ambient atmosphere that were not irradiated.

After irradiation, adults were maintained on immature apples at about 25°C, and mortality was determined every week. The apples were replaced every week and maintained at 25°C for development of any immatures inside. Larvae emerging from apples were collected every 1-3 days and placed on potting soil for pupation and adult emergence. After larvae were no longer emerging, the apples were opened and any remaining insects collected. Analyses of variance were done with Prism 4 ([www.graphpad.com](http://www.graphpad.com)).

The mortality rate for the irradiated plum curculios was greater than that for the control until 15 weeks after irradiation when the rate for the control accelerated (Fig. 1). There was no significant difference among the treatments for time to reach 95% mortality (overall mean =  $27.4 \pm 2.6$  weeks,  $F = 1.56$ ,  $P = 0.26$ ,  $df: 2, 15$ ).

Reproduction under irradiation and hypoxia was increased by over 20-fold compared with irradiation in ambient atmosphere (Table 1). Reproduction was greater than the 34.4 Gy for 4th instars per female reported by Hallman (2003) for

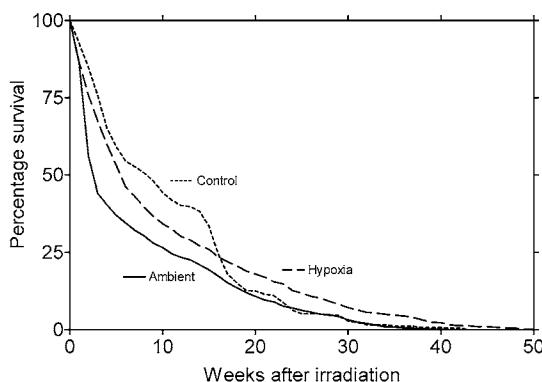


Fig. 1. Mortality rate for control and irradiated (40 Gy) plum curculios.

the control, but similar to that reported for 40 Gy under ambient atmosphere (0.31). Although few 4th instars were produced with irradiation under ambient conditions, significantly more of these became adults compared with the control and irradiation under hypoxia. Adults irradiated under ambient conditions did not live significantly less time than control or adults irradiated under hypoxia, but their reproductive period was shorter and peaked earlier.

Previous studies on the effect of hypoxia on irradiation phytosanitary treatment efficacy concluded that, although an effect could be observed, it did not necessarily threaten the ability of the treatment to prevent an infestation. Although 5.3% of irradiated (200 Gy) oriental fruit moth 5th instars developed to adults, they all died within 10 days of emergence without ovipositing, while non-irradiated controls lived up to 28 days and laid abundant eggs that hatched (Hallman 2004a). Even though apple maggots irradiated as 3rd instars in apples subject to hypoxia had an estimated increase in the dose required to prevent adult emergence of 17% compared with those irradiated in ambient atmosphere, no adults emerged

in large-scale testing (Hallman 2004b). In both of these cases the measure of efficacy was prevention of adult emergence, which allows for a greater margin of error than a treatment against adults where prevention of successful reproduction is the only viable efficacy standard. In a treatment designed to prevent adult emergence, even if some adults emerge, as long as they will not successfully reproduce, establishment of the pest in a new area is prevented. But in a pest where adults may be present, such as plum curculio, any failure in prevention of reproduction means that some individuals would be capable of a new infestation.

The difference in reproductive success between plum curculios irradiated under hypoxia and ambient atmosphere is approximately of the same order of magnitude as the difference between 20 and 40 Gy under ambient conditions (Hallman 2003). This may give a rough indication that hosts of plum curculio irradiated under low oxygen storage might require twice the dose as under ambient conditions, or about 180 Gy to prevent reproduction. This dose would need to be confirmed by large-scale testing before it could be used as a phytosanitary treatment. Until that is done, irradiation against plum curculio should not be used for fruits under low oxygen storage.

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## SUMMARY

Adult plum curculios irradiated in a hypoxic atmosphere accomplished by flushing a cylinder with nitrogen gas were more tolerant of ionizing radiation than plum curculios irradiated in ambient atmosphere. Some hosts of plum curculio,

TABLE 1. REPRODUCTION OF PLUM CURCULIO IRRADIATED WITH 40 Gy IN HYPOXIC AND AMBIENT ATMOSPHERES.<sup>a</sup>

Treatment	Mean 4th instars/female	4th instars developing to adult (%)	Week of peak reproduction post irradiation	Mean 4th instars/female during week of peak reproduction	Final week of reproduction	Final contiguous week of reproduction
Control	89.8 ± 10.1 a	42.5 ± 1.9 b	4.8 ± 0.79 a	18.4 ± 3.6 a	24.3 ± 3.1 a	17.3 ± 0.67 a
Hypoxia	5.9 ± 0.91 b	40.0 ± 3.7 b	5.8 ± 1.1 a	1.9 ± 0.30 b	28.8 ± 2.5 a	17.5 ± 1.4 a
Ambient	0.27 ± 0.12 c	64.7 ± 2.9 a	1.5 ± 0.34 b	0.18 ± 0.08 c	8.8 ± 1.5 b	2.8 ± 0.48 b
—Results of statistical analyses (degrees of freedom for all are 2, 15):						
F value	72.4	25.9	6.8	25.1	17.6	102
P value	0.0001	0.0001	0.014	0.0001	0.0005	0.0001

<sup>a</sup>Means followed by the same letter are not significantly different, Tukey's, 95% confidence.

such as apples, are stored under hypoxia. An irradiation quarantine treatment against plum curculio for apples stored in hypoxia would probably need to be greater than the 92 Gy determined to be efficacious in ambient atmosphere.

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MARINE PUDDLING IN *PAPILIO POLYTES* (LEPIDOPTERA: PAPILIONIDAE)M. POLA<sup>1,2</sup> AND M. GARCÍA-PARÍS<sup>3</sup><sup>1</sup>Marine Laboratory, University of Guam, UOG Station, Mangilao, GU 96913, USA<sup>2</sup>Permanent address: Departamento de Biología, Facultad de Ciencias del Mar y Ambientales Universidad de Cádiz, Polígono Río San Pedro s/n Ap. 40. 11510, Puerto Real, Cádiz, Spain<sup>3</sup>Museo Nacional de Ciencias Naturales, CSIC. José Gutiérrez Abascal, 2. 28006, Madrid, Spain

Smedly & Eisner (1995, 1996) stressed the difficulties that terrestrial herbivores, including Lepidoptera, suffer in order to fulfill their need for sodium, an essential ion plentiful in the seas, but in short supply in plants.

Adult Lepidoptera drink from puddles, edges of streams, carrion, and excreta from which they obtain sodium and proteins (Beck et al. 1999). Puddling behavior is typically, although not exclusively, carried on by males (Boggs et al. 1991; Sculley & Boggs 1996). It has been demonstrated that sodium acquisition from puddles enables males to provide mates with sodium, presumably via the spermatophore (Pivnik & McNeil 1987; Smedly &

Eisner 1996). Sodium intake by males affects their reproductive success, while the transfer of sodium from male to female enhances the reproductive successes of both females and eggs (Pivnik & McNeil 1987) since females subsequently transfer sodium to their eggs (Smedly & Eisner 1996).

Location of resources, which are usually rare and patchily distributed (i.e., puddles with the appropriate salt concentration), is not a simple matter for Lepidoptera, which might use both visual (Papilionidae, Pieridae) and olfactory stimuli (Lycaenidae, Nymphalidae) to locate them (Beck et al. 1999). The sea, an easy to locate source of sodium, is basically unexploited by butterflies.



Fig. 1. Reef shelf at Ipan (Guam) at low tide, showing four specimens of *Papilio polytes* scattered through the shelf on green algae mats.



Fig. 2. Specimen of *Papilio polytes* drinking seawater on Ipan reef shelf (Guam). Note the proboscis introduced into the water.

On August 28, 2004, on a sunny afternoon, we observed about 20 male specimens of *Papilio polytes* Linnaeus, 1758, drinking seawater at low tide, on the Ipan reef shelf on the southeast coast of the island of Guam (Micronesia, USA) (Fig. 1). The butterflies were extending their proboscis directly into the sea while standing on green algae floating mats or on exposed coral structures (Fig. 2), at a distance from the shore ranging from 0.3 to 15 m. This behavior was observed for about 1 h until the butterflies left. No other Lepidoptera were seen on the reef, but numerous specimens of *Euploea eunice* Quoy, 1815, were observed on the beach shrubs.

Sodium may be a rare resource on the oceanic island of Guam, as it is often the case in out-washed tropical soils (Ross & Dykes 1996). However, inland sodium resources are widespread enough to permit butterflies' persistence without drinking marine water. Seawater intake by *P. polytes* in Guam is not likely the only possibility for this butterfly to obtain minerals, but because of the number of specimens seen, it seems a favored option. Higher salt concentration as a result of evaporation during sunny low tide, and resting

places on the water, are factors that favored the presence of *P. polytes* in the shallow open waters of the reef shelf. The absence of these factors alone cannot explain why the sea is not more widely used by Lepidoptera as a source for sodium.

We hypothesize that water temperature is a critical factor diverting butterflies from seawater as a sodium source. Water temperature recorded at the sea reef platform at low tide reached 36 to 42°C on the surface, a temperature often reached in mud-puddles used by butterflies in tropical and Mediterranean regions. This hypothesis, together with differences in water availability (Launer et al. 1996), might explain the intraspecific regional differences found in the use of mud puddles, much less visited by the same or closely related species in the cooler climate of central Europe than in the dry steppe biomes of the Mediterranean region (Beck et al. 1999).

We thank the University of Guam Marine Lab friends and colleagues for help and assistance during our visit and especially to Elaine Pinder. MP's stay in Guam was supported by a short-term fellowship of the Ministerio de Educación, Cultura y Deportes of Spain.

## SUMMARY

Adult Lepidoptera rarely uses seawater as a source for sodium. We observed specimens of *Papilio polytes* drinking seawater on the coast of Guam. Based on our observations, we hypothesize that water temperature might play a key role while choosing among puddling sites.

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## DISPERSAL OF THE FIRE ANT DECAPITATING FLY, *PSEUDACTEON CURVATUS* (DIPTERA: PHORIDAE) IN NORTHEAST MISSISSIPPI

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*Pseudacteon curvatus* Borgmeier is one of three species of phorid decapitating flies currently approved for release in the U.S. for the suppression of the red, black, and hybrid imported fire ants, *Solenopsis invicta* Buren, *S. richteri* Forel, and *S. invicta* × *richteri*, respectively. Two biotypes of *P. curvatus* are established in the U.S. The Las Flores, Argentina biotype prefers black and hybrid imported fire ants (Porter & Briano 2000) and is established at sites in Alabama, Mississippi, and Tennessee (Graham et al. 2003; Thead et al. 2003; Vogt & Streett 2003; Vail et al. 2004; Ward et al. 2004). The Formosa, Argentina biotype prefers red imported fire ants and is established at sites in Florida (R. J. Vasquez & S. D. Porter, pers. comm.).

This study reports dispersal of flies of the Las Flores biotype, first released in spring, 2002 in a grazed pasture (Knox site) in Clay Co., MS (3.25 ha, 33°40'05.87"N, 88°34'48.02"W) (Fig. 1) (Vogt & Streett 2003). By Sept. 2002, flies had established on a mixture of black and hybrid imported fire ants and had spread up to 600 m from the original release site (Vogt & Streett 2003). Additional releases, with the same protocol, were made during spring 2002 and 2003 in a grazed pasture (Prima site) in Clay Co., MS, about 8.8

km and 149.7° SE of the Knox site (Fig. 1). Fly presence was confirmed at both sites during 2003 (J. T. V. & L. G. T., unpubl. data).

Observations were made outside the release sites on 23 dates from May-Sept. 2004, between 09:25 and 15:45 hours at 134 active fire ant mounds. Sampling areas were randomly selected and located on roadsides that were bordered by forests with overhanging vegetation or by grazed pastures. The presence of *P. curvatus* was determined by making a round depression (about 4-5 cm wide and 5-10 cm deep) in black and hybrid imported fire ant mounds. Hovering flies were counted within and around the depression. Ants were macerated and dropped into the depression to release semiochemicals that attract the flies (Porter et al. 2004). All sampled areas were geo-referenced. Mounds were observed for up to 35 min. If flies were found in an area, we moved and sampled farther from the release sites. An area was re-sampled later unless flies were found farther from the release sites along a similar compass bearing. Average air temperature during sampling was  $29.9 \pm 2.6^\circ\text{C}$  ( $\pm\text{SD}$ ), with a mean relative humidity of  $66.3 \pm 18.5\%$ , and a mean wind speed of  $1.2 \pm 1.45 \text{ km/h}$ .

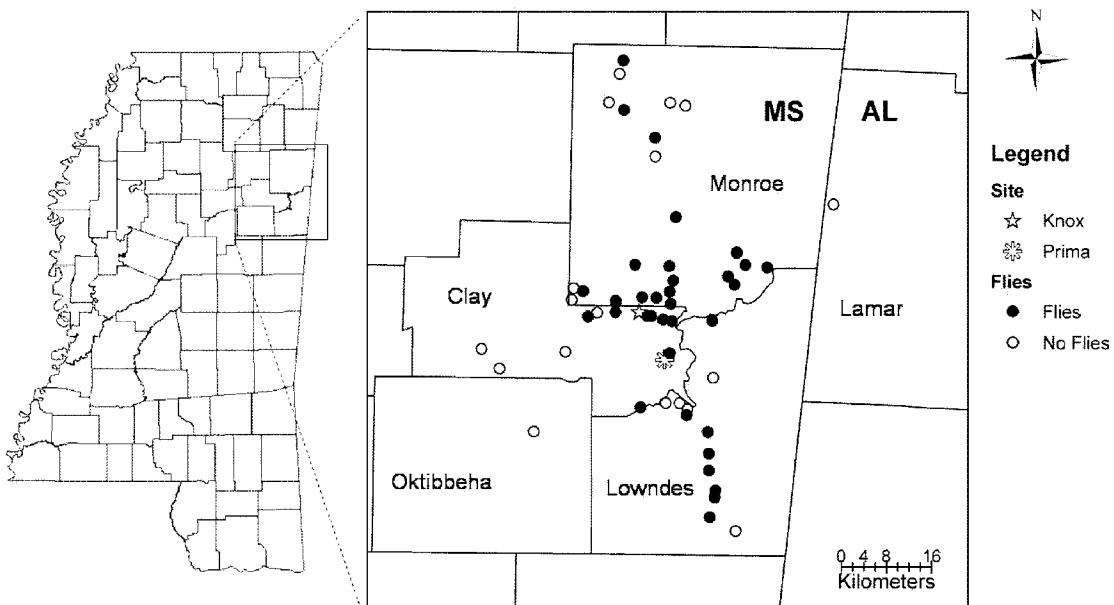


Fig. 1. Dispersal, as of 2004, of the decapitating fly *P. curvatus* from 2002 and 2003 releases at two sites in Clay Co., MS.

A total of 130 flies were recorded attacking ants in approximately 33% (44/134) of the mounds. Each of 44 mounds had from 1 to 14 flies with an average of  $3.0 \pm 2.7$  flies. Time of fly arrival at a mound ranged from about 10 sec to 20 min, averaging  $6.1 \pm 5.3$  min.

A modified electric livestock prod, TheBlueOne™ LMPlus®, (Hot-Shot Products Co., Inc., Savage, MN) was used randomly to shock the ants to provoke alarm pheromone release (Vander Meer et al. 2002; Barr 2004) in approximately 40% (53/134) of the mounds. Flies were attracted to ants in about 25% (13/53) of the stimulated mounds and approximately 38% (31/81) of unstimulated mounds. It took longer for flies to arrive at stimulated mounds ( $8.2 \pm 6.7$  min) than unstimulated mounds ( $5.1 \pm 4.3$  min). Unlike *P. tricuspis* (Barr 2004), the use of the electric livestock prod on ants did not appear to attract *P. curvatus* as quickly or at a greater rate.

Regression analysis showed no correlation ( $P > 0.05$ ) between time to fly arrival and temperature, relative humidity, wind speed or time of day. A significant correlation ( $P < 0.001$ ) between time to fly arrival and day sampled explained 61% of variance, but confounding of sampling farther from the release sites as the season progressed made the model ( $y = 2E - 10x^{4.43}$ ) unreliable. Therefore, a significant correlation ( $P < 0.001$ ) between time to fly arrival and distance from Knox site is shown (Fig. 2). It is likely that there were fewer flies at the more distant sites (Porter et al. 2004).

Fly dispersal from the Knox and Prima sites is illustrated in Fig. 1. By spring 2004, *P. curvatus* populations at the Knox and Prima sites had merged. By Sept. 2004, flies had spread over 44 km (356° NW), 37 km (162° SE), and 24 km (70°

NE) from the center of the Knox site. The outer boundaries of fly expansion to the north, south, and east of the release sites may have extended farther than we were able to observe in this study. Time restraints prevented sampling beyond Sept. 2004. Fly movement to the west of the release sites appeared to be slower than in other directions. By Aug. 2004, dispersal was about 11 km (291° NW) from the Knox site. No flies were found farther than 11 km west of the Knox site in Sept. 2004. Habitat variation or sampling effort may explain slower dispersal (Porter et al. 2004).

*P. curvatus* occupied an area that encompassed more than 2249 km<sup>2</sup> (>224,914 ha) by Sept. 2004 (Fig. 1). Dispersal was 11 to at least 44 km in 2½ years.

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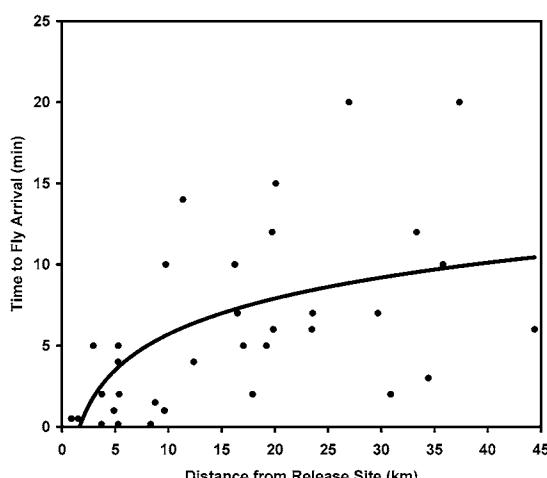
## SUMMARY

The fire ant decapitating fly, *Pseudacteon curvatus*, first released in Clay County Mississippi during spring 2002, occupied an area of over 2249 km<sup>2</sup> by Sept. 2004. Dispersal was at least 44 km in 2½ years.

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Fig. 2. Time to fly arrival at a fire ant mound as a function of distance from Knox release site. A logarithmic regression is plotted ( $y = 3.18 \ln(x) - 1.63$ ,  $R^2 = 0.30$ ,  $P < 0.001$ ).



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**A NEW HOST RECORD FOR THE EGG PARASITOID *PARACENTROBIA AMERICANA* (GIRAULT) (HYMENOPTERA: TRICHOGRAMMATIDAE) OF THE PROCONIINE SHARPSHOOTER *HOMALODISCA INSOLITA* (WALKER) (HEMIPTERA: CLYPEORRYNCHA: CICADELLIDAE)**

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Interest in the natural enemies of proconiine sharpshooters has increased since the introduction and establishment of the glassy-winged sharpshooter, *Homalodisca coagulata* (Say), in California. Previous surveys of egg parasitoids of proconiine sharpshooters from Florida indicated several species in the families Mymaridae and Trichogrammatidae (Hymenoptera) as the most common (Triapitsyn et al. 1998; Triapitsyn & Hoddle 2001). Presently, classical biocontrol efforts to manage introduced populations of *H. coagulata* in California rely exclusively on inundative releases of egg parasitoids of the genus *Gonatocerus*. Triapitsyn (2003) created a key to the genera of trichogrammatid parasitoids of proconiine sharpshooter eggs in the southeastern US that included an unidentified species of the genus *Paracentrobia* Howard. Poor condition of type specimens of *P. acuminata* (Ashmead), deposited in the National Museum of Natural History, Washington, D.C., did not allow then for a positive identification of the *Paracentrobia* sp. from Florida and Georgia as *P. acuminata* (Triapitsyn 2003). More recently, specimens of *Paracentrobia* obtained from egg masses of *Cuerna costalis* (F.) and *Homalodisca insolita* (Walker) collected from Byron and Centerville, Georgia were identified as *P. acuminata* (Hoddle & Triapitsyn 2004). Additionally, specimens listed as *P. acuminata*, collected previously from Monticello, Florida, and Fort Valley, Georgia were verified by J. George (pers. comm.). We have reared another species of *Paracentrobia*, *P. americana* (Girault), that has not previously been reported from the southeastern United States. This parasitoid attacked the eggs of *H. insolita* that were cultured in cages of Johnson grass, *Sorghum halepense* (L.) Persoon.

Notes on *Paracentrobia americana*. This species was described from Salt Lake City, UT where it was reported to parasitize the eggs of an undetermined leafhopper found on *Elymus* sp. (Girault 1917). Prior to this study, the species description included the only published host association and distribution records for *P. americana*.

Material Examined. *Paracentrobia americana*: USA, Florida, Gadsden Co., Quincy, 30-IX-2004, C. Tipping, numerous females and males (emerged from egg masses of a culture of *H. insolita* reared on *S. halepense*). All specimens exam-

ined for this record were tentatively determined by S. V. Triapitsyn and then confirmed by J. George (Department of Entomology, University of California, Riverside); voucher material was deposited at UCRC (Entomology Research Museum, University of California, Riverside).

Notes on the Host, *Homalodisca insolita*. Prior to 1944, this species had a reported distribution that included Mexico, Arizona, New Mexico, and Texas. It was subsequently discovered in Georgia (Kaloostian & Yeomans 1944). By the late 1950s, its distribution was reported to include Louisiana, Arkansas, Tennessee, Mississippi, Alabama, Florida, Georgia, South Carolina, and North Carolina (Pollard et al. 1959). It has been reported to feed on many plants but prefers grasses (Turner & Pollard 1959), including Texas millet (*Panicum texanum* Buckley), crab grasses (*Digitaria* spp.), and Johnson grass (*S. halepense* (L.) Persoon). In north Florida, reproductive plant hosts of *H. insolita* include Johnson grass and southern sand spur, *Cenchrus echinatus* L. (Tipping et al. 2004). The most common parasitoid of *H. insolita* eggs in Florida is *Acmonoplyne sema* Schaufuß (Hymenoptera: Mymaridae) (Triapitsyn et al. 2002).

*H. insolita* is a competent vector of *Xylella fastidiosa* bacterium, the causative agent of many plant diseases including phony peach, Pierce's disease of grape, and plum leaf scald (Turner & Pollard 1955). Many of the grass and weed species fed upon by *H. insolita* are reservoirs of *Xylella* bacteria. While *H. insolita* feeds primarily on herbaceous hosts, we have documented that it also feeds on economically important hardwood species that are susceptible to *X. fastidiosa* such as peach and plum (Mizell & French 1987). Potentially, the parasitoid *P. americana* may serve as a management tool in combating the spread of *X. fastidiosa* diseases.

We thank Jeremiah George for confirmation of our initial identification of *Paracentrobia americana*, and Gisette Seferina, Brent Brodbeck, and Peter Andersen for editorial suggestions on an earlier version of this manuscript. The California Department of Food and Agriculture as well as the University of California, Davis provided funding for this research. Contribution of the Florida Agricultural Experiment Station Journal Series No. R-10891.

## SUMMARY

The trichogrammatid wasp, *Paracentrobia americana* (Girault), was reared from egg masses of the leafhopper *Homalodisca insolita* (Walker) maintained in culture at the University of Florida North Florida Research and Education Center in Quincy, Florida. This discovery is a new host record for *P. americana*. Parasitized egg masses were found on Johnson grass, *Sorghum halepense* (L.) Persoon.

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## DEVELOPMENT OF RESISTANCE IN SOUTHERN CHINCH BUGS (HEMIPTERA: LYGAEIDAE) TO THE INSECTICIDE BIFENTHRIN

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St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze lawns are used throughout the southern United States for their climatic adaptation and their ability to tolerate full sun to moderate shade. The southern chinch bug, *Blissus insularis* Barber, is the plant's most damaging pest (Crocker 1993). The adaptability of this insect is shown by developing resistance to insecticides (Reinert & Porter 1983) and overcoming host plant resistance (Busey & Center 1987; Cherry & Nagata 1997).

Insecticide resistance in southern chinch bugs (SCB) was first noted in 1953 in Miami where Wolfenbarger (1953) showed poor control with chlordane. By 1958, Kerr and Robinson (1958) documented resistance to DDT at Sarasota, Florida. The chinch bugs had become resistant to parathion at Fort Lauderdale, Florida by 1960 (Kerr 1960). Chinch bug resistance to both chlorpyrifos and diazinon was confirmed in 1977 at Pompano Beach, Florida (Reinert & Niemczyk 1982). And, in 1983, Reinert and Porter (1983) reported a 9.2 fold level of resistance to the carbamate insecticide, propoxur by SCB. Hence, by 1983, SCB had shown some resistance to chlorinated hydrocarbon, organophosphate, and carbamate insecticides.

In recent years, synthetic pyrethroid insecticides have become increasingly used for SCB control in Florida. Bifenthrin is a synthetic pyrethroid compound used as a contact and stomach poison insecticide/acaricide (Thomson 1998). Bifenthrin has been and still is being used for SCB control in Florida. During 2003, instances of difficulty in controlling SCB with bifenthrin in Florida came to our attention. The objective of our study was to determine if southern chinch bugs had become resistant to bifenthrin, and if so, note possible trends in this resistance.

Chinch bugs were collected by vacuuming in lawns of infested St. Augustinegrass. After collection, the insects were stored at 18°C in buckets with St. Augustinegrass until used for testing. The insects were collected from 16 different urban areas throughout Florida except for extreme northern Florida where fewer chinch bugs are found and control problems were not brought to our attention. Eight of the populations came from locations where there was difficulty in controlling chinch bugs with bifenthrin and resistance was suspected. In contrast, eight populations were selected randomly as encountered in other areas with there being no knowledge of the insecticidal use history of the location or current efficacy of bifenthrin against the insects.

Methods for testing closely approximated methods of Reinert and Porter (1983) used earlier in toxicological tests against SCB. In the laboratory, serial dilutions of bifenthrin were made from Talstar Flowable 7.9% AI (FMC, Philadelphia, PA). Freshly harvested St. Augustinegrass stolons (ca 10 cm long) were dipped into dilutions and allowed to air dry. Stolons were placed individually into Petri dishes (15 cm diameter) containing moist filter paper to maintain high humidity. Twenty adult SCB were placed into each Petri dish and held 24 h at 28°C and 14 D/10 L. For each test, five to seven doses with a control were tested. Robertson et al. (1984) noted that a sample size of 120 appears to be the minimum necessary for reliable estimation for LC<sub>50</sub> estimation. Our sample sizes of adults tested ranged from 200 (10 doses) to 480 (24 doses) for each location to estimate LC<sub>50</sub> for that location. Different numbers of adults tested per location depended on availability of adults plus variability noted in testing. Since our own objective was to estimate LC<sub>50</sub> values, we selected doses expected to give 25 to 75% mortality for best LC<sub>50</sub> estimation as suggested by Robertson et al. (1984). Mortality is defined as virtually no movement by an adult during a 5 minute observation period through a 5× large magnifying lens. The no movement criterion was used to avoid ambiguities of comatose, unable to stand, moribund, etc. In a separate test, >95% of adults we classified as dead after insecticide exposure did not regain movement after 24 h and the <5% showed only small twitches. Hence we believe the 24 h holding period with no movement criterion was a good measure of mortality since adults classified as dead still appeared dead 24 h later. Lethal median concentrations (LC<sub>50</sub>) and slopes were calculated for each population by probit analysis on Log<sub>10</sub> dose (SAS 1996).

Toxicological data for the 16 chinch bug populations are shown in Table 1. Lowery and Smirle (2003) note that non-overlapping 95% confidence intervals show that LC<sub>50</sub> values are significantly different ( $P < 0.05$ ). A wide range of LC<sub>50</sub> values were observed in the 16 populations with many of the LC<sub>50</sub> values being significantly different. These data clearly show that SCB has now developed resistance to bifenthrin in some locations. Resistant populations were observed inland (Clermont) and on both the east coast (i.e., Daytona Beach) and west coast (i.e., Sarasota) of Florida. The resistant populations extended from as far south as Key Largo (25°15' latitude) to as far

TABLE 1. RESPONSES OF FLORIDA POPULATIONS OF SOUTHERN CHINCH BUGS TO BIFENTHRIN.<sup>a</sup>

Location	Adults tested	Slope $\pm$ SE <sup>b</sup>	LC <sub>50</sub> <sup>c</sup>	CI <sup>d</sup>		Resistance ratio <sup>e</sup>
				Lower	Upper	
<b>Control problem</b>						
Clermont	200	1.5 $\pm$ 0.4	78.7	41.1	426.8	34.2
Daytona Beach	220	0.9 $\pm$ 0.2	243.3	56.2	813.9	105.8
Key Largo	480	1.2 $\pm$ 0.2	148.2	105.1	240.1	64.4
Ormond Beach	220	0.7 $\pm$ 0.2	698.8	230.4	2,767.6	303.8
Palm Coast	240	0.5 $\pm$ 0.1	1,693.4	796.1	3,998.9	736.3
Palmetto	320	0.8 $\pm$ 0.2	493.6	159.3	3,088.7	214.6
Sarasota	220	1.3 $\pm$ 0.3	89.2	45.6	192.7	38.8
Spring Hill	240	0.7 $\pm$ 0.2	159.3	47.5	30,238.3	69.3
<b>Random</b>						
Belle Glade	300	1.3 $\pm$ 0.2	2.6	1.7	4.2	1.1
Gainesville	200	1.8 $\pm$ 0.2	10.6	7.3	14.2	4.6
Fort Pierce	240	1.3 $\pm$ 0.3	2.8	1.4	6.7	1.2
Kendall Lakes	240	1.0 $\pm$ 0.2	9.3	3.1	22.0	4.0
Lithia	240	1.6 $\pm$ 0.2	5.4	4.1	7.0	2.3
Orlando	220	1.3 $\pm$ 0.3	2.3	0.5	4.6	1.0
Royal Palm Beach	200	1.2 $\pm$ 0.2	7.3	4.4	10.7	3.2
Tamarac	220	0.8 $\pm$ 0.2	9.7	4.3	16.4	4.2

<sup>a</sup>24 h exposure at 28°C by the method of Reinert and Portier (1983).<sup>b</sup>Probit analysis with Log<sub>10</sub>(dose).<sup>c</sup>LC<sub>50</sub> = AI = ppm..<sup>d</sup>CI = 95% confidence interval<sup>e</sup>Resistance ratio = LC<sub>50</sub> of population/lowest LC<sub>50</sub> of any population.

north as Palm Coast (29°35' latitude). These data show that SCB populations resistant to bifenthrin already range throughout much of Florida.

The LC<sub>50</sub> data clearly fell into two distinct groups. Locations where control problems were encountered consistently had high LC<sub>50</sub> values. These data suggest the control problems at these locations were at least partly caused by resistance and not faulty application procedures, etc. In contrast, locations which were randomly selected consistently had low LC<sub>50</sub> values. It should be noted that LC<sub>50</sub> values of all random populations were significantly lower than LC<sub>50</sub> values of all control problem populations clearly showing a sharp delineation between the two groups. These latter data show most SCB populations in Florida are more susceptible to bifenthrin than the resistant populations encountered in control problem locations. The high variability in insecticide resistance we observed between populations of SCB within Florida is consistent with earlier reports (see Reinert (1982) for review; and Reinert and Portier (1983) for best example).

In summary, our study is the first to report on southern chinch bug resistance to a synthetic pyrethroid, bifenthrin. As noted earlier, various synthetic pyrethroids are commonly used for chinch bug control in Florida turf. Hence, the potential problem exists of increasing insecticide re-

sistance in southern chinch bugs to other synthetic pyrethroids.

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## SUMMARY

Synthetic pyrethroids are currently widely used for southern chinch bug control in Florida turf. Southern chinch bugs were tested from 16 locations in Florida to determine possible resistance to the synthetic pyrethroid, bifenthrin. This study is the first to show southern chinch bug resistance to a synthetic pyrethroid, bifenthrin.

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## RESPONSE OF *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) TO APPLICATION CONCENTRATIONS OF A PARTICLE FILM

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Kaolin-based particle films were developed for horticultural applications as an environmentally benign method to deter arthropod pests and plant diseases (Glenn et al. 1999). Since the commercialization of a wettable powder formulation (Surround® WP, Engelhard Corp., Iselin, NJ), this product has been examined for applications against pests of temperate fruit trees (Puterka et al. 2000; Knight et al. 2000; Unruh et al. 2000); cotton (Showler 2002); whitefly (Liang & Liu 2002); thrips (Kerns & Wright 2000); and other pests. The value of the film includes deterrence of feeding and oviposition, and beneficial effects on carbon assimilation, leaf temperature, and fruit yield (Glenn et al. 2001). Particle film technology seems especially well suited for use in areas of low rainfall where leaf residues of the product can be maintained without frequent re-application. Use of particle films in the humid subtropical environment of Florida citrus groves may be limited by removal of residues by seasonally heavy rain (Lapointe 2000).

In addition to deterring pests (Puterka et al. 2000; Unruh et al. 2000; Knight et al. 2000; Lapointe 2000), particle films have been shown to increase fruit tree productivity in semiarid and subhumid environments by reducing heat stress (Glenn et al. 2001). The humid subtropical environment experienced by citrus trees in Florida presents a different set of challenges for successful use of particle films. Periods of high rainfall are interspersed with dry periods accentuated by highly porous soils and intense solar radiation. Particle films, while effective in the laboratory (Lapointe 2000), may not adhere sufficiently to citrus leaves in the field in Florida to maintain deterrence to *Diaprepes* root weevils after heavy rains over the entire rainy season (May–October) when adults are active.

Prior to establishment of field trials (reported elsewhere), I investigated the feeding and oviposition response of *Diaprepes* root weevil to varying

concentrations of Surround WP. A hand-held sprayer was used to apply the product at three concentrations to bouquets of citrus leaves harvested from *Citrus macrophylla* Wester seedlings grown in a greenhouse. Methods were similar to those reported by Lapointe (2000). Foliage bouquets were sprayed to runoff with the manufacturer's recommended concentration ( $x = 3\% \text{ wt/vol}$ ),  $0.5x$ , or  $0.1x$ , or with water alone. Foliage was allowed to dry and then placed in screened cages ( $30 \times 30 \times 30 \text{ cm}$ ) containing 5 male and 5 female *Diaprepes* root weevils. Weevils were obtained from a colony maintained at the U.S. Horticultural Laboratory, Ft. Pierce, FL (for rearing conditions, see Lapointe & Shapiro 1999). Each of the 4 treatments was replicated 3 times for a total of 12 cages and 120 weevils. Cages were randomly arranged on open shelves in a temperature-controlled greenhouse. Each cage was provided with wax paper strips as substrates for oviposition (Wolcott 1933). Bouquets and wax paper strips were removed every 2 days until 17 days and examined for egg masses. Leaf area consumed was assessed by tracing the leaf notches. Tracings were digitally scanned and the resulting files were imported into an image analysis computer program as described by Lapointe (2000). Leaf area consumed and total number of eggs oviposited were analyzed by ANOVA with the type III sum of squares for cage as the error term. Means were compared by the Tukey honestly significant differences (HSD) test (Abacus Concepts 1996). Linear regression was used to calculate the deposition of particle film required to achieve 50% reduction in leaf consumption and oviposition.

The deterrent effect of Surround against feeding and oviposition fell off quickly as coverage was reduced in a greenhouse trial. Adult weevils fed untreated citrus leaves over 17 d consumed approximately 3 times as much leaf area as weevils

TABLE 1. MEAN ( $\pm$  SEM,  $n = 3$ ) LEAF AREA CONSUMED AND NUMBER OF EGGS PRODUCED OVER 17 DAYS BY *DIAPREPES* ROOT WEEVIL ADULTS FED CITRUS FOLIAGE SPRAYED WITH THE RECOMMENDED CONCENTRATION (1.0X) OF SURROUND® (3% WT/VOL), 0.5X, 0.1X, OR FOLIAGE SPRAYED WITH WATER (CONTROL).

	Leaf area consumed ( $\text{cm}^2$ )	% of control	Number of eggs laid	% of control
Control	236 $\pm$ 30 a		4,575 $\pm$ 255 a	
0.1x	207 $\pm$ 10 a	88	2,792 $\pm$ 168 ab	61
0.5x	145 $\pm$ 44 ab	61	1,263 $\pm$ 1,085 b	28
1.0x	83 $\pm$ 13 b	35	823 $\pm$ 288 b	18

Means within columns followed by the same letter do not differ ( $\alpha = 0.05$ , Tukey's HSD).

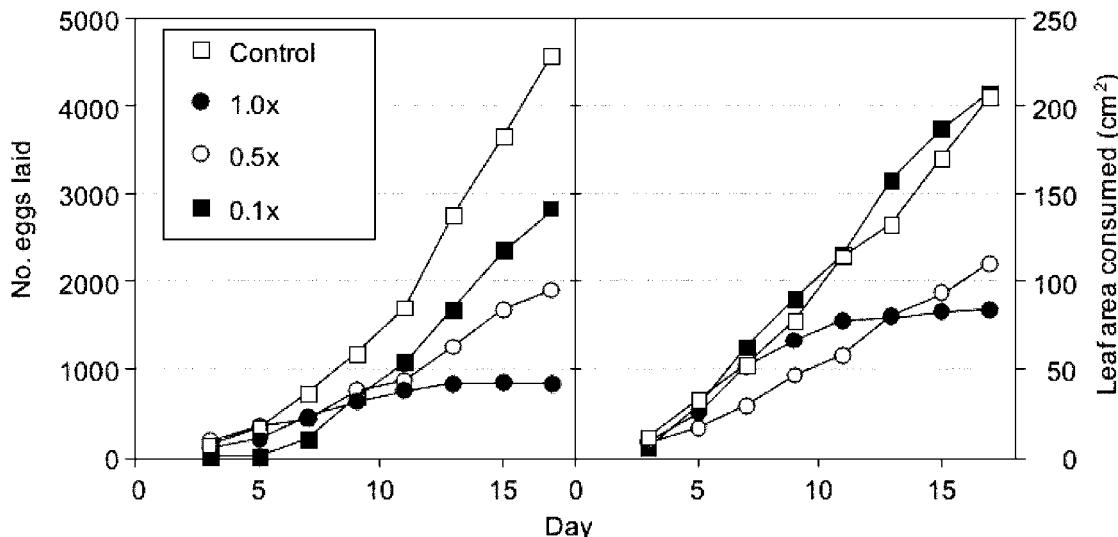


Fig. 1. Mean cumulative oviposition and leaf consumption by adult *Diaprepes* root weevils caged with untreated citrus leaves (control), or leaves treated with Surround® WP sprayed at one of three concentrations: 3% (1.0x), 1.5% (0.5x), or 0.3% (0.1x) wt/vol Surround/water.

fed citrus leaves treated with Surround at the recommended concentration (Table 1). By regressing percent reduction in leaf area consumed on concentration ( $y = -0.016x + 1.505$ ), I determined that Surround deposition equivalent to 71% of the recommended concentration is required to obtain a 50% reduction in leaf consumption. Inhibition of oviposition by the particle film was stronger compared with inhibition of leaf consumption (Fig. 1). Weevils caged with untreated citrus leaves produced 5.6 times as many eggs as weevils in cages with leaves treated at the recommended concentration (Table 1). By regressing percent reduction in oviposition on concentration ( $y = -0.011x + 0.962$ ), I determined that 50% reduction of oviposition would occur at Surround deposition equivalent to 41% of the recommended concentration. The roughly proportional decline in leaf consumption and oviposition with increasing particle film coverage suggests that significant repellency to root weevils during the rainy season in Florida would require multiple applications during the rainy season to maintain repellency.

The effect of Surround on oviposition may be attributed to a combination of two factors. First, oviposition may have been directly affected by a deterrent effect of the particle film on ovipositional behavioral (Lapointe 2000). Second, reduced feeding probably contributed to reduced fecundity, thereby decreasing the oviposition potential of females fed treated leaves, particularly after day 10 (Fig. 1). Oviposition was observed even at the highest concentration in the greenhouse under no-choice conditions, as has been reported previously (Lapointe 2000).

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#### SUMMARY

The deterrence of Surround® WP particle film to feeding and oviposition by the *Diaprepes* root weevil was proportional to the concentration of application to citrus leaves in a greenhouse trial. Reduced oviposition appeared to be due to the combined effect of reduced feeding and behavioral deterrence. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

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## ESTABLISHMENT OF THE NEOTROPICAL ORCHID BEE *EUGLOSSA VIRIDISSIMA* (HYMENOPTERA: APIDAE) IN FLORIDA

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Neotropical orchid bees (Hymenoptera: Apidae: Euglossini) have been reported only twice from the United States of America; once near Brownsville, Texas (23.III.1908) and more recently (4.IV.1994) near Silverbell, Arizona (32°22'N, 111°26'W). In each case, a single male *Eulaema polychroma* Mocsáry 1899 had strayed north of the border from breeding populations in northern Mexico (Minckley & Reyes 1996).

During the summer of 2003, however, several male *Euglossa viridissima* Friese 1899 were trapped around Fort Lauderdale (26°08'N, 80°08'W), Florida, by USDA employees in the fruit fly monitoring program and sent to the Florida State Collection of Arthropods for identification (Wiley 2004). To date, more than 50 males and several females have been reported (Table 1). Neither the exact location of the introduction nor the current distribution in Florida is known. However, observations point to an accidental introduction around Butterfly World, Coconut Creek, Broward County—likely as a nest inside a wooden object (shipping pallet, bamboo furniture etc.)—followed by a southward spread to Dade County in 2004.

*Euglossa viridissima* is native to Mexico and most of Central America and recorded from near sea level up to 1,900 m.a.s.l. (Ramírez et al. 2002). The natural distribution mainly follows the range of the tropical dry forest on the Pacific Coast, from NW Costa Rica (ca. 10°N) to the northernmost population near 27°N in the State of Sonora, Mexico (Janzen et al. 1982; Bürquez, 1997), although the species has adapted to habitats ranging from lowland tropical rain forest over arid, scrubby secondary forest to oak-pine forest at 1,300-1,800 m.a.s.l. (pers. obs., Fierros-Lopez 1998). Adult males are active when ambient temperature exceeds 20°C, and the “typical” habitat will be 25-28°C with 70-80% relative humidity (pers. obs.).

*Euglossa viridissima* is a robust, medium-sized bee (3-4 mm wide, 11-13 mm long), bright metallic green, and with a long tongue (10 mm) neatly folded underneath the body. The male has a characteristic cushion of blond hairs on the second sternum (Roubik & Hanson 2004). *E. viridissima* nests in natural as well as man-made cavities (Friese 1922; Aquino Vázquez & Cuadriello Aguilar 1990). The females gather resin to seal off any cracks in the cavity, leaving only a small entrance hole, and to construct barrel-shaped cells for the mass-provisioned offspring. Each cell is

about 11-12 mm tall, 5-8 mm across, and “glued” together with neighboring cells in a roughly hexagonal pattern in one plane (Friese 1922). A nest holds 4-20 cells, which can stand on the floor, or be glued to the walls, giving a “flying carpet” impression (Aquino Vázquez & Cuadriello Aguilar 1990). Sometimes two groups of cells can be found in the same cavity, probably founded independently. There is evidence of cell reuse, and seven females co-existed in a nest with only two open cells, indicating some level of sociality (Aquino Vázquez & Cuadriello Aguilar 1990). In Chiapas, Mexico, the species is multivoltine and generations can overlap. The life expectancy for an individual *E. viridissima* hardly exceeds a few months (60-90 days). During this time, the female can lay a minimum of 6-8 eggs (Friese 1922). The development from egg to adult in the nest studied by Aquino Vázquez & Cuadriello Aguilar (1990) was at least 53 days, but developmental time is expected to vary due to a negative correlation with temperature (Roubik & Hanson 2004).

Male orchid bees leave the natal nest upon eclosion and never return; they do not aid in construction, maintenance, provisioning, or defense of the nest. Instead they devote a considerable amount of time and energy collecting volatile compounds produced in fungus-infested wood, rotting vegetation, and specialized “perfume” flowers in Orchidaceae, Araceae, and a few other families (reviewed in Roubik & Hanson 2004). The fragrances are kept in hind tibiae that are uniquely modified for their storage, and are, most likely, used in species-specific recognition and/or as evidence of male fitness (Eltz et al. 1999). Male *Euglossa viridissima* are known to collect fragrances from at least eleven genera of orchids (Ramírez et al. 2002). They also can be attracted to pure compounds in field bioassays. Eugenol (clove oil) is especially attractive (Cameron & Fenster 1984), followed by cineole, methyl salicylate, *trans*-methyl cinnamate, and benzyl acetate. Males also collect terpinen-4-ol, veratrole, phenylethanol, *p*-cresyl acetate, and geraniol (pers. obs.).

A number of plant families provide resources for *Euglossa viridissima*. Flowers of *Dalechampia* spp. (Euphorbiaceae) that excrete a pliable resin (Armbruster 1988) are the only documented sources of resin for *E. viridissima*. However, Friese (1922) suggested cashew (*Anacardium occidentale*, Anacardiaceae) and conifers (Coni-

TABLE 1. DOCUMENTED OBSERVATIONS OF *Euglossa viridissima* (HYMENOPTERA: APIDAE: EUGLOSSINI) IN FLORIDA, 2003-2004, COMPILED WITH HELP FROM DR. M. MEDINA HAY-ROE AND A. CHIN LEE (MCGUIRE CENTER FOR LEPIDOPTERA AND ENVIRONMENTAL RESEARCH), DR. J. M. GONZÁLEZ (UNIVERSITY OF GEORGIA), DR. D. W. ROUBIK (SMITHSONIAN TROPICAL RESEARCH INSTITUTE), AND E. BERNIER (EJB PHOTOGRAPHY). THE FORT LAUDERDALE LOCATION REFERS TO FERN FOREST NATURE PARK. THE JACKSON TRAPS ARE EMPLOYED TO LURE FRUIT FLIES WITH SYNTHETIC FOOD ATTRACTANTS SUCH AS ME (METHYL EUGENOL) OR ML (MULTI LURE: PUTRESCINE (DIAMINOBUTANE), AMMONIUM ACETATE, AND TRIMETHYLAMINE). THE SCENT BAITS CONSIST OF BLOTTER PAPER SATURATED WITH SYNTHETIC AROMAS: C (CINEOLE = EUCALYPTOL), E (EUGENOL = CLOVE OIL), OR MS (METHYL SALICYLATE = OIL OF WINTERGREEN). AN ASTERISK IDENTIFIES SPECIMENS OR PHOTOGRAPHS EXAMINED BY THE AUTHORS. MOST SPECIMENS WERE RELATIVELY YOUNG AS EVIDENCED BY INTACT WING MARGINS (WING WEAR IS AN AGE CORRELATE).

Date	Locality	Collector's notes	Specimens	Collector
17.VI.2003	Broward Co.: Coconut Creek	ML trap in mango ( <i>Mangifera indica</i> )	1 male*	Paul Moeser USDA
13.VIII.2003	Broward Co.: Pompano Beach	Jackson trap in <i>Mimusops</i>	1 male*	Julie Palm USDA
8.X.2003	Broward Co.: Margate	Photographed while visiting pale lavender pentas <i>Pentas lanceolatas</i> (Rubiaceae) for nectar	1 (sex unknown)	Beth Bernier EJB Photography
IX.2003	Broward Co.: Wilton Manors	Flying around in foliage outside window on 2 <sup>nd</sup> floor	1 female*	Melissa Hope Univ. of Florida
17.IX.2003	Broward Co.: Pompano Beach	ME trap in <i>Calophyllum</i>	2 males	David T. Benner USDA
18.XI.2003	Broward Co.: Plantation	Jackson trap	1 male	John Pieper USDA
XI.2003	Broward Co.: Coconut Creek	Photographed on blue porterweed, <i>Stachytarpheta jamaicensis</i> (Verbenaceae)	1 male*	Alan Chin Lee Butterfly World
31.III.2004	Broward Co.: Coconut Creek	ME trap	3 males*	Rick McKay USDA
14.IV.2004	Broward Co.: Sunrise	ML trap	1 male	Brian Cairns USDA
16.V.2004	Broward Co.: Ft. Lauderdale	Scent bait (C)	16 males	David Roubik STRI
28.V.2004	Dade Co.: Miami	Jackson trap ( <i>Mangifera indica</i> )	2 males* (1 examined)	Javier C. del Hierro USDA
17.VIII.2004	Broward Co.: Margate	Photographed while visiting a purple flower	1 (sex unknown)	Beth Bernier EJB Photography
1.X.2004	Broward Co.: Fort Lauderdale	Scent baits (C, MS)	Ca. 20 males	David Roubik STRI
6.X.2004	Broward Co.: Davie	<i>Persea americana</i> (avocado)	1 male*	Miryam Briceno USDA
20.X.2004	Broward Co.: Ft. Lauderdale	Photographed while visiting blue porterweed, <i>Stachytarpheta jamaicensis</i> (Verbenaceae) for nectar	3 males and 2 females observed, photographs verified both sexes	Beth Bernier EJB Photography
4.XI.2004	Broward Co.: Margate	Photographed while visiting morning glory, <i>Ipomoea</i> sp. (Convolvulaceae) for nectar	1 male	Beth Bernier EJB Photography
9.XII.2004	Broward Co.: Margate	Scent baits (artificial vanilla, crushed cloves)	Min. 7 males	Beth Bernier EJB Photography
12.II.2005	Broward Co.: Margate	Scent baits (C, E)	2 males*	Charlotte Skov Univ. of Florida
13.II.2005	Broward Co.: Margate	Feeding on lavender <i>Lantana</i> (Verbenaceae)	1 female	Beth Bernier EJB Photography
14.II.2005	Broward Co.: Davie	Scent baits (C, E)	23 males*	Charlotte Skov Univ. of Florida

ferae) as potential resin sources in Costa Rica. In contrast, the bee is quite opportunistic in choice of food plants. Ramírez et al. (2002) list five families,

including Apocynaceae and Bignoniaceae, as sources of nectar and/or pollen. The species also has been observed in flowers of Fabaceae (Fierros-

Lopez 1998) and Passifloraceae (Aquino Vázquez & Cuadriello Aguilar 1990), and females collect pollen from Caesalpinaeae, Commelinaceae, and Nyctaginaceae (pers. obs.). In Mexico, males and females have readily adapted to forage for nectar from introduced plants such as the spice cardamom (*Elettaria cardamomum*, Zingiberaceae) from India (Aquino Vázquez & Cuadriello Aguilar 1990) and the ornamental allamanda (*Allamanda cathartica*, Apocynaceae) from northern Brazil and the Guayanas (pers. obs.). In Florida, *E. viridissima* visits blue and violet flowers of pickerel weed (*Pontederia cordata*, Pontederiaceae), pentas (*Pentas lanceolatas*, Rubiaceae), porterweed (*Stachytarpheta jamaicensis*, Verbenaceae), and morning glories (*Ipomoea* spp., Convolvulaceae) (Table 1).

In conclusion, Southern Florida represents an almost ideal habitat for *Euglossa viridissima*. The temperature and precipitation ranges south of Lake Okeechobee (McPherson & Halley 1996) match those of its native habitats; there are ample floral resources year-round for a long-tongued generalist bee to forage from; pine plantations that could provide resin for nests in urban and natural settings; and there are ornamental peace lilies (*Spathiphyllum* spp., Araceae) and tropical orchids in private gardens and nurseries from which males could collect fragrant compounds. The data presented in Table 1 point to a successful establishment of *E. viridissima* near Fort Lauderdale and we predict a further spread of the species.

### SUMMARY

A description of the orchid bee *Euglossa viridissima* and its nesting biology is given as well as notes on climate requirements and plant resources in its native habitats in Costa Rica and Mexico. An assessment is made for the establishment and spread in Florida.

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## TWO NEW NATIVE HOST PLANT RECORDS FOR *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) IN ARGENTINA

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*Anastrepha fraterculus* (Wiedemann) (South American fruit fly) is a polyphagous cryptic species complex (Steck 1991) distributed throughout continental America from USA (it has occasionally been trapped in extreme south Texas but does not seem to be established) and Mexico to Argentina (Aluja 1999; Norrbom et al. 1999b). It and *Ceratitis capitata* (Wiedemann) (Mediterranean fruit fly) are the only two economically important fruit fly species found in Argentina (Aruani et al. 1996). There are some genetic differences between *A. fraterculus* collected from *Psidium guajava* L. in the Buenos Aires (central-eastern region) and Tucumán (northwestern region) Provinces (Sonvico et al. 1996), but Alberti et al. (2002) concluded that Argentine populations of the complex are conspecific. Within Argentina, *A. fraterculus* is mainly restricted to the northern region between 22° and 31°S latitude where it breeds in native and wild exotic plant species (Ovruski et al. 2003), whereas *C. capitata* occurs from the northern region to as far south as 40°S latitude in Patagonia (southern region), mainly in the Río Negro Valley, commonly infesting commercial exotic fruits (Sanchez et al. 2001).

Of 29 fruit species recorded as hosts of *A. fraterculus* in Argentina, only seven are from plants known to be indigenous to the country (Rust 1918; Ogloblin 1937; Hayward 1960; Blanchard 1961; Putruele 1996; Nasca et al. 1996; Ovruski et al. 2003). Unfortunately, most of these host records did not include data on field infestation level, fruiting phenology, part of the fruit being used by larvae, nor taxonomists performing the plant and fly identifications (Ovruski et al. 2003). Many records excluded specimen or voucher data. All this information is needed to unequivocally consider a plant species as a natural host (Norrbom & Kim 1988; Aluja 1999). This work provides new host plant records for *A. fraterculus* and a more complete picture of the native host range of this economically important tephritid species in Argentina.

Fruit samples consisted of fallen ripe fruit of two native plants, *Chrysophyllum gonocarpum* (Mart. et Eich.) Engler (Sapotaceae) (locally known as "aguay") and *Inga marginata* Willd. (Fabaceae) locally known as "pacay" or "inga del cerro", which were collected in patches of disturbed wild vegetation. The fruit samples of *C. gonocarpum* were collected at El Oculto (Salta Province, NW Argentina) at 23°06'S latitude and 64°29'W longitude, and 530 m above sea level, whereas the fruit samples of *I. marginata* were collected at Horco Molle (Tucumán Province, NW Argentina) at 26°45'S latitude and 65°20'W longitude, and 500 m altitude.

*Chrysophyllum gonocarpum* is a tree that reaches 7-12 m in height with a trunk diameter of 20-50 cm when fully grown (Legname 1982). The fruit is a yellow subglobose berry with five longitudinal grooves,  $2.9 \pm 0.8$  cm (mean  $\pm$  SD) in diameter and  $8.2 \pm 1.3$  g in weight ( $n = 100$ ) when fully ripe. In NW Argentina, it is distributed in the Subtropical Montane Rainforest (locally known as "Yungas" or "tucumano-bolivian" forest), and is found at altitudes of 400-1200 m between the Pre-montane Forest and Montane Forest environmental units of the Yungas (Morales et al. 1995). The fruiting period occurs from October to December (L. Oroño and S. Ovruski, pers. obs.), although according to Legname (1982) it starts in September.

*Inga marginata* is a tree that reaches 4-12 m in height with a trunk diameter of 10-30 cm when fully grown (Legname 1982). The fruit is a yellow-brown indehiscent pod,  $10.8 \pm 2.2$  cm long,  $2.9 \pm 2.3$  cm wide, and  $21.2 \pm 6.3$  g weight ( $n = 100$ ) when fully ripe. In NW Argentina, *I. marginata* is found at altitudes of 300-700 m in the Premontane Forest and Montane Forest of the Yungas (Morales et al. 1995). The fruiting period lasts from January to March (Legname 1982).

Samples ranged from 45 to 130 fruits, depending on fruit availability. These samples were placed in individual cloth bags, and then put in-

side a plastic container ( $45 \times 27 \times 38$  cm) for transport to the laboratory of the Centro de Investigaciones para la Regulación de Poblaciones de Organismos Nocivos (CIRPON), in San Miguel de Tucumán ( $26^{\circ}50'S$ ,  $65^{\circ}13'W$ , 426 m), Tucumán Province. In the laboratory, each fruit was counted, weighed, and placed individually in a plastic tray with damp sand in the bottom as a pupation substrate. All tephritid pupae from each fruit tray were removed every three days, and the *A. fraterculus* and *C. capitata* pupae were separated based on external pupal characters (White & Elson-Harris 1992). The pupae were then isolated in individual plastic container ( $220 \text{ cm}^3$ ) with moistened sterile sand at the bottom. A mesh-covered top was fitted over the glass. All trays containing pupae were kept inside a room at  $25 \pm 1^{\circ}\text{C}$ ,  $75 \pm 5\%$  relative humidity and a photoperiod of 14:10 (L:D) h for two months. Emerged flies and parasitoids were captured alive. Parasitoids and *C. capitata* adults were placed in plastic vials containing 70% ethanol, while *A. fraterculus* adults were placed in transparent Plexiglas cages ( $30 \times 30 \times 30$  cm) and provided with diet (sugar and hydrolysate enzymatic yeast) and distilled water. One week after emergence, *A. fraterculus* adults were killed in 70% ethanol. A. Norrbom and S. M. Ovruski identified the *Anastrepha* specimens, and S. M. Ovruski identified the *C. capitata* and parasitoid specimens. C.B. Martin identified the host plant species. Voucher specimens of the insects are placed in entomological collections of the National Museum of Natural History, Washington, DC, USA, and Fundación Miguel Lillo (FML), in San Miguel de Tucumán, Argentina. Voucher specimens of host plants are placed in the herbarium of FML. Terminology for native host plants follows Morales et al. (1995). Parasitism rates reported here are based on the number of emerged adult flies and parasitoids. Fruit infestation levels are expressed as the mean ( $\pm \text{SD}$ ) number of *A. fraterculus* and *C. capitata* pupae per individual fruit and as the total number of *A. fraterculus* and *C. capitata* pupae per kg of fruit. These indices are given for all fruit samples (uninfested plus infested) and also for infested samples only. Spearman's coefficient of rank correlation was calculated to determine the relationship between individual fruit weight and the number of *A. fraterculus* pupae yielded per fruit.

A total of 168 (1,213.8 g) *C. gonocarpum* fruits were collected from six trees between October 12, 2001 and November 18, 2001, and 407 (8,964.9 g) *I. marginata* fruits were collected from five trees between February 25, 2002 and April 16, 2002. Of the fruit sampled, 50 (29.8%) *C. gonocarpum* and 25 (6.1%) *I. marginata* produced tephritids. *Anastrepha fraterculus* was reared from 47 (27.9%) aguay fruits and from all infested pacay fruits. *Ceratitis capitata* was reared only from 9 (5.4%) aguay fruits. Six (13%) of the *C. gonocarpum* fruit

that produced *A. fraterculus*, also yielded *C. capitata*. *Anastrepha fraterculus* larvae were observed feeding in the pulp of both native plant species. Field infestation data for both host plant species are shown in Table 1.

The infestation level was 3.2 times higher in *C. gonocarpum* than in *I. marginata*, despite the greater number of pacay fruit collected (2.5-fold differences). A positive correlation between fruit size and number of *A. fraterculus* pupae per fruit was observed in both host plant species, but these associations were due to weak correlation coefficients in aguay ( $R_s = 0.17$ ,  $P = 0.03$ ,  $n = 168$ , minimum and maximum individual fruit weight: 4.5–10.5 g) and pacay ( $R_s = 0.19$ ,  $P < 0.001$ ,  $n = 407$ , minimum and maximum individual fruit weight: 8.3–39.4 g). In total, 87 *A. fraterculus* pupae and 9 *C. capitata* pupae were recovered from all infested aguay fruits. From *A. fraterculus* pupae, 32 adult flies (37% emergence rate) and one adult parasitoid [*Aganaspis pelleranoi* (Brèthes) (Hymenoptera: Encyrtidae, Encyrtinae)] were recovered, and from *C. capitata* pupae, 3 adult flies (33% emergence rate) were obtained. Of the 168 *A. fraterculus* pupae recovered from all infested pacay fruits, 64 adult flies (38% emergence rate) and nine adult parasitoids were obtained [8 *Doryctobracon brasiliensis* (Szépligeti) (Hymenoptera: Braconidae, Opiinae), and 1 *A. pelleranoi*]. Parasitism rates were 12.3% and 3% in *I. marginata* and *C. gonocarpum*, respectively.

*Chrysophyllum gonocarpum* is recorded for the first time from Argentina as a natural host plant for *A. fraterculus*, and it appears to be a good host based on infestation data and number of adult flies reared from fruit samples. As reported previously by Ovruski et al. (2003), high levels of infestation by *A. fraterculus* were also recorded in the Yungas Forest in fruit species weighing between 1 and 60 g, such as the natives *Eugenia uniflora* L., *Myrcianthes pungens* (Berg) Legrand (Myrtaceae), *Juglans australis* Grisebach (Juglandaceae), and the introduced *Prunus armeniaca* L., *P. domestica* L., *P. persica* (L.) Batsch (Rosaceae), and *Psidium guajava* L. (Myrtaceae). Similarly, Ovruski & Schliserman (2003a) found high infestation rates by *A. fraterculus* in the natives *Feijoa sellowiana* (O. Berg) O. Berg (Myrtaceae) and *E. uniflora* from samples collected in a subtropical rainforest in northeastern Argentina. Interestingly, *C. gonocarpum* was previously recorded as a primary host for *A. fraterculus* in southeastern Brazil (Salles 1995; Kovaleski et al. 2000). *Chrysophyllum cainito* L (star apple) was also recorded as a natural host for *A. fraterculus* in Perú (Korytkowski & Ojeda-Peña 1970), but this record was considered questionable (Norrbom & Kim 1988). Among 22 indigenous plant families from which *A. fraterculus* has been reared, the Sapotaceae includes the third highest number of genera and species of native hosts reported (4

TABLE 1. INFESTATION LEVELS FOR ANASTREPHA FRATERCULUS (AF) AND CERATITIS CAPITATA (Cc) IN CHRYSPHYLLUM GONOCARPUM AND INGA MARGINATA IN NORTH-WESTERN ARGENTINA.

Plant species	Total no. of samples	Mean (±SD) no. fruit per sample	Fruit fly pupae no./kg of fruit (infested samples only)		Mean (±SD) no. of fruit fly pupae per fruit (infested samples only)		Fruit fly pupae no./kg of fruit (all samples)		Mean (±SD) no. of fruit fly pupae per fruit (all samples)		
			Af	Cc	Af	Cc	Af	Cc	Af	Cc	Af
<i>Chrysophyllum gonocarpum</i>	3	56.0 ± 32.9	16.7 ± 14.7	86.1	8.9	0.64 ± 1.17	0.07 ± 0.25	71.7	7.4	0.52 ± 1.08	0.05 ± 0.21
<i>Inga marginata</i>	3	135.7 ± 11.9	8.3 ± 7.2	27.0	0	0.61 ± 2.22	0	18.7	0	0.41 ± 1.86	0

genera and 6 species versus 8/26 in Myrtaceae and 4/7 in Rosaceae) (Norrbom et al. 1999a).

*Inga marginata* is a new host plant record for *A. fraterculus*, although it has been previously reported as a host of *A. distincta* Greene in Venezuela (Norrbom & Kim 1988). The low infestation level observed in the native *I. marginata* is similar to values recorded in exotic cultivated fruit growing in northwestern Argentina, such as *Diospyros kaki* L. (Ebenaceae), *Annona cherimola* Mill. (Annonaceae), *Citrus paradisi* Macfadyn (Rutaceae), and *Mangifera indica* L. (Anacardiaceae) (Ovruski et al. 2003). Although the Fabaceae are mainly infested by *A. distincta* (17 species of the genus *Inga* recorded as hosts), five species of this plant family have been reported as hosts for *A. fraterculus* (Norrbom 2004).

As reported previously in northwestern Argentina by Ovruski et al. (2003), we found that *A. fraterculus* is much more abundant in native, wild fruit than *C. capitata*. These authors showed that *A. fraterculus* appears to prefer areas with patches of wild vegetation, whereas *C. capitata* seems to adapt well to highly perturbed environments where exotic plants are more common. A similar situation has been recorded in several regions of Brazil (Malavasi & Morgante 1981; Malavasi et al. 2000).

*Doryctobracon brasiliensis* and *A. pelleranoi*, both native parasitoid species collected in the study areas, were previously recorded from northwestern Argentina in association with *A. fraterculus* in *Prunus armeniaca*, *P. domestica*, *P. persica*, *Psidium guajava*, and in *J. australis* (Ovruski et al. 2004). These two wasp species are solitary, koinobiont endoparasitoids of larvae of the genus *Anastrepha*, belonging to the fruit fly parasitoid guild number "2" defined by Ovruski et al. (2000). In general, the degree of larval parasitism obtained in *I. marginata* and *C. gonocarpum* was similar to values found in other native fruit species such as *F. sellowiana*, *E. uniflora*, *M. pungens* and *J. australis*, and exotic "feral" species such as *P. guajava* and *Prunus* species, which form part of the wild vegetation in perturbed subtropical rainforest of northern Argentina (Ovruski & Schliserman 2003a, b; Ovruski et al. 2004).

*Inga marginata* and *C. gonocarpum* increase to nine the number of native host plant species of *A. fraterculus* recorded for Argentina. Previously, one species of Juglandaceae (*J. australis*) and six species of Myrtaceae (*Eugenia retusa* Berg, *E. uniflora*, *M. pungens*, *Hexachlamys edulis* (Berg.) Krause et Legrand, *Campomanesia crenta* Berg, and *F. sellowiana*) were registered for Argentina (Ovruski et al. 2003; Ovruski & Schliserman, 2003a).

The discovery of these two new native host plants for *A. fraterculus* in northwestern Argentina underscores the importance of conducting fruit surveys in environments with vast areas of native vegetation and over long periods including

several fruiting seasons (Aluja 1996; Aluja et al. 2000). Thus, the information yielded by these types of studies can aid the Argentinean National Fruit Fly Control and Eradication Program to develop management strategies in the fruit-producing regions of northern Argentina, where *A. fraterculus* and *C. capitata* have numerous alternative host plants.

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## SUMMARY

*Chrysophyllum gonocarpum* (Sapotaceae) and *Inga marginata* (Fabaceae) are reported as host plants of *Anastrepha fraterculus* in Argentina for the first time. Infestation rates (number of *A. fraterculus* pupae/kg of fruit) were 86.1 and 27.0 for *C. gonocarpum* and *I. marginata*, respectively. In total, 32 *A. fraterculus*, 3 *Ceratitis capitata*, and 1 *Aganaspis pelleranoi* (parasitoid) adults were recovered from *C. gonocarpum*, while 64 *A. fraterculus*, 8 *Doryctobracon brasiliensis* (parasitoid), and 1 *A. pelleranoi* were obtained from *I. marginata*.

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## BOOK REVIEWS

BASSET, Y., V. NOVOTNY, S. E. MILLER, AND R. L. KITCHING (EDS). 2003. Arthropods of Tropical Forests. Spatio-temporal Dynamics and Resource Use in the Canopy. Cambridge Univ. Press, Cambridge, UK. xvi + 474 pp. ISBN 0-521-82000-6. Hardback. \$110.00.

This book, written by 79 authors contributing to 35 chapters, aims to provide an overview of data collected during recent canopy studies in Australia, Africa, Asia and South America. The idea for it originated at a symposium of the 21st International Congress of Entomology, at Foz do Iguaçu, Brazil in August 2000. The editors invited 'active research groups' to prepare manuscripts containing new research results. Thus, the book does not begin with a history in which it might have been pointed out that a few 19<sup>th</sup> century naturalists (e.g., Bates, Müller, Wallace) opened up the subject. Then, several 20<sup>th</sup> century studies concentrated on mosquitoes, and to a lesser extent on Ceratopogonidae and Psychodidae, with concern not just for their basic biology but with the practical aspect that some of these flies are important vectors of disease. This is mentioned briefly on p. 18, but no research group working on biting flies participated in this book. Also surprisingly, just one chapter (17, pp. 176-185) comes close to dealing with faunal diversity associated with epiphytes or with phytotelmata, when there are several active groups.

Chapters are grouped into five topics. These are (1) Arthropods of tropical canopies: current themes of research, (2) Vertical stratification in tropical forests, (3) Temporal patterns in tropical canopies, (4) Resource use and host specificity in tropical canopies, and (5) Synthesis: spatio-temporal dynamics and resource use in tropical canopies. Chapter 1 reveals controversy in definitions of the terms 'canopy' and 'understorey' (usually spelled 'understory' in U.S. publications). Chapter 2 addresses the now well-known technique of 'canopy fogging' along with use of towers, cranes, canopy walkways, and helium balloons to access the canopy for sampling. This chapter also makes plain the taxonomic difficulties, in which identification of specimens to the species level in the tropics is at best difficult. The ability to collect specimens has exceeded the ability to identify them to the species level by many of the researcher groups who rushed into canopy studies perhaps because the theme has become practicable and is fundable.

Exceptions to the above problem are studies of groups of insects in which the researchers have taxonomic expertise, have identified collections to the species level, and have at least some understanding of the autecology of each species. Chapters 15, 22, and 33 (a powerful data set for saturniid caterpillars on Costa Rican trees by Dan Janzen) take this approach. Such studies can tie

abundance of a species to its way of life. In contrast, studies that claim to have collected e.g., 12,000 specimens of Carabidae and 10,000 of Staphylinidae provide little information other than that tree canopies may be important habitats for who-knows-how-many species of these two families feeding on who-knows-what. They disregard the roles of the species encountered: for example some staphylinids are generalist predators, others are specialists (but on what prey?) while others are fungivores (but on what fungi?), and is not the abundance of food (the resource) an important determinant of presence and abundance?

The chapter I liked best was the conclusion, by all four editors, in a valiant effort to see pattern and generalities in the findings of the diverse chapters. It was a difficult task. Of course we may see that that canopies of some tree species maintain more arthropod species and/or individuals than do canopies of other tree species. Generally we see some seasonal effects. We see that there is vertical stratification of some sort. We also see some weak trends with exceptions. What was hard to draw out was generalities given the heterogeneous nature of the localities, the species studied, and the objectives. Sample size was one of the obstacles. Authors were able to obtain many samples of, e.g., Orthoptera or Formicidae, but when these were subdivided to the level of 'morphospecies', sample size often was not great enough for statistical manipulation. And, when authors lumped together all samples of Orthoptera or Formicidae, this was equivalent to lumping together apples and oranges. My conclusion is that canopy diversity (as diversity everywhere else) occurs at the species level and is best understood from the bottom upward, i.e., from the species level. Few of the included chapters showed that projects had the necessary taxonomic expertise to work at the species level. Apart from obtaining specimens that might at some point be useful to taxonomists (if there really are taxonomists prepared to work on these specimens, and if somehow they are funded to do so), most of the included chapters merely pointed at questions to be asked. It is a shame that so many 'canopy studies' have been funded without evident funding for taxonomists who could identify the species encountered, make sense of the diversity, promote autecological studies, and make some progress.

The book ends in References (pp. 407-467) and an Index of subjects and scientific names combined (pp. 469-474). The 6-page index seems a bit skimpy for such an abundance of data. By error,

in the copy I received from the publisher, pages follow in sequence to p. 416, **then begins a duplicative section** in which pp. 393-416 appear again before pp. 417-467. This duplicative section may form a form a 'signature' (a group of pages stitched together, in this instance 12 leaves or 24 pages) that has been repeated. The entire dupli-

cative section ('signature') may be scissored out and discarded.

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CAPINERA, J. L., R. D. SCOTT, AND T. J. WALKER. 2004. Field Guide to the Grasshoppers, Katydids, and Crickets of the United States. Comstock Publishing Associates (Cornell Univ. Press), Ithaca, NY. vi + 249 pp. ISBN 0-8014-8948-2. Paperback. \$29.95 (also as ISBN-0-8014-4260. Hardback. \$65.00).

I own several books called Field Guides by their various publishers, to various groups of insects and other organisms. For the most part they are very well illustrated and they are about 300 pages long and they now cost about \$20 to \$30. This one is no exception. But it is totally new and does not duplicate a book offered by another publisher. Kudos to Comstock Publishing Associates for producing it.

I own such a book for Orthoptera of the British Isles. The task of its author (Ragge 1965) was much easier than that of the present authors because there are far fewer species in Britain, so all could be included with the addition of stick insects (called walkingsticks in the U.S.A.) and cockroaches (At one time they jointly were considered to form the order Orthoptera). A later book (Ragge & Reynolds 1998) characterizes singing Orthoptera by the sounds they make and covers not just Britain, but western Europe, and comes bundled with two CDs. The book under review (Capinera et al. 2004) does not include a CD of songs, but some of the songs are available cost-free on a website (Walker & Moore 2004). The book under review cannot include all species in America north of Mexico because there are some 1200 species, too many to be included in a book of this size and price. Worse, the taxonomy of the species of America north of Mexico is incomplete: some species have not yet been characterized and cannot yet be included. The authors have selected the species included: about a third of the total occurring in the area, taking care to include the most abundant species as well as representatives of uncommon groups.

I am guessing that most purchasers of such books want and expect to be able to identify any insect seen or captured (of the group in question) to the species level. Field Guides to birds and reptiles do that. For insects in general and for insect orders (or suborders) such as beetles, flies, wasps, moths, and true bugs this will not happen in a North American Field Guide—there are just too many species. Consider the result if this book had included all species of grasshoppers, katydids and crickets. It would have stretched to at least 1000 pages making it bulky, and the multiple of its sale price would have set it at above \$100. Most casual purchasers would have judged this price ‘too high’ and would not have bought it because it would not have been a typical Field Guide. Therefore, the publisher would have to **increase** the price as a specialty book for a small market in order to make a profit. The authors did as well as they could within the constraints of Field Guide format. Unfortunately, the book gives users no way of knowing whether a specimen at hand has been

identified correctly—because so many species lack any mention, and there are no keys to the genus and species level, and no species list.

Is the public ready for Field Guides to family-level or subfamily-level of insects? One on tiger beetles of the U.S.A. and Canada is in press. Would Field Guides to more obscure families and subfamilies sell? Or, for the future, should Field Guides concentrate on regional works? There already are regional Field Guides to tiger beetles. There already has been a ‘Field Guide to butterflies of North America, east of the Great Plains.’ These are pertinent questions for publishers. Then, for serious biologists, there is the question of whether some philanthropist or national funding agency would commission a large set of Field Guides (hundreds of volumes) to cover all of the insects of America north of Mexico, each to be of about 300 pages and each to sell for about \$30, subsidized where necessary. Entomologists now have the expertise to produce many of those volumes, and the rest could be produced as knowledge advances.

I am also guessing that most users of Field Guides expect to encounter vernacular names. In this book quite a few vernacular names are newly coined where none existed. I find it ironic that such coined names come to be called ‘common’ names especially when they have never been used before and in that sense are anything but commonly used. Are readers really averse to using scientific names? Even small children seem to handle scientific names for dinosaurs such as *Tyrannosaurus rex*. Is it necessary to take a perfectly good scientific name such as *Conocephalus strictus* and invent a long-winded second name ‘straight-lanced meadow katydid’ for it?

On pages 219-221 the authors explain the pronunciation of scientific names. They say there are two systems of pronunciation in English-speaking countries. One is the system of pronunciation explained in Latin textbooks published during the last 50 years; it is taught in Latin classes in the U.S.A. and internationally. The second is Latin as it had come to be pronounced in English-speaking countries by the 19th century [corrupted because it had come, over the centuries, to be pronounced more or less like English]. Its use was abandoned by Latin teachers by the mid-twentieth century. Instead of being allowed to die, it is perpetuated in an entomology textbook (Borror et al. 1989). The authors of this book explain how U.S. (and English-speaking Canadian) orthopterists tend to pronounce the names, and the explanation closely mirrors the Borror et al. (1989) explanation. I am waiting for a future edition of Borror et al. to drop its explanation of

corrupted Latin pronunciation, and instead adopt Latin pronunciation as it is now taught—perhaps then will we (entomologists in the USA and English-speaking Canada) be able to communicate scientific names to entomologists in non-English-speaking countries.

The two paragraphs above do nothing to belittle the accomplishment of these authors. For the first time in the U.S.A. we have an attractive, up-to-date, and beautifully illustrated guide to some of the Orthoptera of the United States (and Canada). As centerfolds, it even has 48 plates of colored drawings of (mainly) lateral views of adult Orthoptera, of high quality, that must have taken months of work. Each species diagnosis includes a distributional map. Species diagnoses, where necessary, include drawings of diagnostic characters. There is an introduction (pp. 5-42). Pictorial keys to subfamily are included (pp. 43-51). Waveforms and spectrograms are included for songs of some species. There is a 3-page bibliography of further reading, a glossary of terms, and an index.

I would have added a classificatory list of all known species, whether diagnosed in the book or not, with names of describers (authors), a fairly easy task for the authors. Perhaps this will be included in a later edition. I would have tried to in-

clude keys to all known genera and species, a much more difficult task, which would lengthen the book. But, in all humility, "I would have . . ." does not indicate that I could have written this book.

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- RAGGE, D. R., AND W. J. REYNOLDS. 1998. The songs of the grasshoppers and crickets of western Europe. Harley Books; Great Horsham, England. xii + 591 pp. With a boxed set of two CD-ROM discs titled "A sound guide to the grasshoppers and crickets of western Europe." [Reviewed in *Florida Entomol.* 83: 115-116]
- WALKER, T. J., AND T. E. MOORE. 2004. Singing insects of North America. (<http://Buzz.ifas.ufl.edu>).

SAMWAYS, M. J. 2005. Insect Diversity Conservation. Cambridge Univ. Press, Cambridge, U.K. viii + 342 pp. Paperback. ISBN 0-521-78947-8. \$55.00 (Hardback, ISBN 0-521-78338-0, \$110.00).

"Wilderness is the ultimate natural value" (p. 206). Given that so much of the world is today altered by humans, how can we conserve that natural value? By setting aside parks and preserves, we attempt to do so, but for various reasons we fall short of perfection. Parks and preserves may be illegally encroached upon by humans. They may be too small or not in all the right places for their purpose. They may be invaded by immigrant species (including those whose presence was inadvertently aided by humans) and introduced species. They may be damaged by human-caused pollution or altered by global warming. Then again, why do we bother to try to conserve insect diversity?

The author (who lives in South Africa) of this book addresses these questions and more from a global viewpoint. He begins with a chapter on ethical foundations, and includes Insect Utility, Ethical Philosophy, Insect Rights, and Spiritual (religious) Conceptions. And yes, this is the appropriate place to begin because the entire framework depends upon human values. According to the author, representatives of all five major religions (Buddhist, Christian, Hindu, Jewish, and Muslim) at least pay lip service to wildlife conservation, although none expressly mentions insects. In the U.S.A., federal and state laws on wildlife implicitly or explicitly include insects, and insects are the vast majority of animal species. However, 'wildlife specialists' in the U.S.A. typically emphasize vertebrate animals, and through lack of knowledge or interest or time, pay little attention to insects. Somewhere in here there is confusion between the right of the individual animal (insect) to live out its life and the right of the species to exist. The author states "best we let individuals live" (p. 11), and he does not mean that an individual of an endangered species has any special rights over an individual of a widespread species. When he gets to rights of species, he wonders how to take into account that 99% of all species that have existed on earth are now extinct.

This made me examine my own position. I have no compunction about killing small or large numbers of individuals of those species that I consider to be pests, but it has to be my definition of pest, not that of the average Florida resident as exemplified by Dave Barry's statement "insects, if they get anywhere near you . . . whomp them with a hard-cover work of fiction at least the size of Moby Dick" (Barry 1990). The only difference is that I have a narrower definition of pests and perhaps a greater tolerance of small numbers of them. I also kill individual insects that I want to study or as necessary for some experiment. Other than that, I am reluctant to kill insects, and occasionally I even rescue them. At the species level, I believe in the idea of conservation of endangered and threat-

ened insect species. However, I do not extend this belief to species of all phyla of organisms: I would have no remorse if all malarial parasites (*Plasmodium* spp.) of humans were to be eradicated worldwide. Rationally, perhaps all species of all phyla (including *Plasmodium* spp.) could be assigned equal 'rights' to existence. Does anyone truly believe in that level of rationality from the standpoint of ethics? So I have my own values and am not being rational. But, back to killing insects: May we generalize that we all kill insects (either directly or by proxy) according to perceived need, but that perception of need differs between people? Does that explain why some people perceive the need to use electrocuting insect traps that kill any insect unlucky enough to be caught? Or use broad-spectrum chemical pesticides on their lawns? Or enthuse about insectivorous bats simply because they consume lots of insects?

The author then presents the following chapters, and this is where the science comes in: (2) The special case of insects in conservation biology, (3) Insects and the conservation of ecosystem processes, (4) Insects and the changing world, (5) Responses by insects to the changing land mosaic, (6) Threats from invasive aliens, biological control, and genetic engineering, (7) Global climate change and synergistic impacts, (8) Conserving and managing insect diversity, (9) Mapping, inventorying and monitoring, (10) Managing for insect diversity, (11) Restoration of insect diversity, and (12) Conventions and social issues in insect diversity conservation. All of these chapters are well written and well documented and this book is an up-to-date compendium. But the author wrote the first chapter appropriately because conservation is rooted in ethics. Ethics is not science but a grab-bag of human perceptions.

A statement: "Broadly, the doing of conservation has two components. The first is research, or the finding out. The second is the practical implementation . . ." (p. 156) makes me wonder just how we will ever succeed in documenting the abundance of all the  $\approx$  5 million insect species in the world, or even the  $\approx$  12,500 in Florida. Certainly abundance of 'charismatic' native species (butterflies and dragonflies) is being documented in Florida, but for the vast majority we have little idea of the species that are rare, threatened and endangered, despite the good intentions of Mark Deyrup and Richard Franz (Deyrup & Franz 1994). This is because (a) current funding agencies have insufficient funds to support such studies by students or professionals (perhaps because the general public is disinterested—the 'Dave Barry syndrome' wishes most insects dead), and so concentrate their funding effort on 'charismatic' species, and (b) because, unlike in Europe

and perhaps Japan, there is only a tiny core of dedicated amateurs making such studies, although the Research Associates program of the Florida State Collection of Arthropods is trying to build one. All of the insect species in Florida are not yet described, and for many thousands of them—most of them—we have but trivial information about population densities and threats to existence. We hardly know which ones are threatened and endangered.

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DEYRUP, M., AND R. FRANZ. 1994. Rare and endangered biota of Florida. Volume IV. Invertebrates. University Press of Florida, Gainesville. xxx + 798 pp.

KOUL, O. 2005. Insect Antifeedants. CRC Press, Boca Raton, FL. xii + 1005 pp. ISBN 0-415-33400-4. Hardback. \$189.95.

There has been much interest in recent years in manipulating the behavior of pest insects to protect crop plants (Foster & Harris 1997). Pheromones have been most widely studied for this purpose and have enjoyed considerable commercial success. Another fruitful avenue, and relatively unexploited from a commercial standpoint (except for neem-based products), is the use of antifeedants to change the feeding behavior of insects. Plants have evolved a tremendous array of allelochemicals that act as feeding deterrents to herbivores. It is these allelochemicals that have been extensively studied as plant protectants during the past two decades. This book brings together much of the literature on the discovery, identification, and chemistry of insect antifeedants of plant origin, information that is widely scattered in entomological and chemical journals.

The format of the book is a compendium with six introductory chapters and 900 pages of bioefficacy and chemical data pertaining to individual compounds with insect antifeedant activity. There are references after each of the introductory chapters although many of the references are relatively old (many pre-1990). References for the bioefficacy data in the monograph section are placed on the page where each chemical is described. There is an excellent index at the end of the book that includes the scientific names of plants, greatly facilitating the task of locating chemically characterized antifeedants in a plant of interest.

The first chapter begins with a brief overview of semiochemical terminology and definition of the term antifeedant as "a peripherally mediated behavior-modifying substance (i.e., acting directly on the chemosensilla in general and the deterrent receptors in particular) resulting in feeding deterrence" (Isman 1994). This definition purposefully excludes compounds that reduce feeding after ingestion, either through sub-lethal effects or direct action on the central nervous system. The author continues with a discussion of coevolution and the myriad classes of defensive compounds (alleloones) that plants have evolved, ending with a short history of the study of plant-produced antifeedants with activity against insects.

The second chapter begins with a brief description of the chemosensory system, the system upon which antifeedants act. Although little is known about the mechanisms of action of antifeedants on insect sensory neurons, some information about neuronal specificity and sensitivity has been gleaned through electrophysiological studies. The author goes on to describe several specific cases where the mechanism of action of antifeedants on the chemosensory system is known.

The third chapter describes the many types of bioassays that are performed to test the efficacy

against insects of suspected antifeedants. This chapter is quite complete in content but tends to overdo the methodological details of some of the bioassays.

In the fourth chapter the author attempts to summarize the structure-activity relationships of the major classes of antifeedants (limonoids, quassinoids, mono-, sesqui- and diterpenes, coumarins, isoflavonoids, alkaloids, maytansinoids, ellagitannins, and aristolochic acids). Slight changes in functional group and stereochemistry can cause significant changes in bioactivity so the author does not attempt to generalize structure-activity relationships beyond what is necessary to help guide systematic search for and modification of bioactive compounds.

In the fifth and sixth chapters, the author describes the current state of commercialization of antifeedants and the many practical reasons for their lack of commercialization, with the exception of neem-based products. The major roadblocks to commercialization are the lack of technology to produce large quantities of commercial-grade antifeedants and consequently the costly and labor-intensive nature of their production. Few studies of antifeedants have moved beyond the laboratory to the field, but it is expected that problems such as insect desensitization (habituation) to the deterrent and rapid environmental degradation will be encountered. The author ends on the hopeful note that antifeedants will find a place in integrated pest management programs targeted against specific combinations of crop plants and pest insects (Isman 2002).

The last chapter contains bioefficacy monographs on more than 800 antifeedant compounds. Each compound, listed alphabetically, has chemical data such as molecular formula, weight, and structure and occasionally other useful data such as melting and boiling points, optical rotation, and mammalian LD<sub>50</sub>. The source of the compound is described by scientific name of the plant, including author, common name, if any, and family. Common name and plant family cannot be searched in the index but scientific name can. The final section of each monograph is the activity profile where the author lists all the insects against which this particular compound has been tested and found effective as a feeding deterrent. Details in this section include the testing method, compound concentration or dosage, and efficacy, which the author has standardized to EC<sub>50</sub> (effective concentration that deters feeding in 50% of the population), if the data allowed it.

This book would be a welcome addition to the library of any scientist or student interested in the chemistry of plant-insect interactions, or the potential use of antifeedants for pest manage-

ment, either conventional or organic. Its best feature is the monograph section because many biological and chemical details on antifeedant compounds are collected from disparate literature sources. There is no other source that I know of that provides this information in one location. The first six chapters describe nicely much of the basic information amassed over a quarter century of research on insect antifeedants and are a bonus in a compendium such as this. The English usage in the introductory chapters is a little cumbersome but the advantage of having general background information and chemical details on antifeedants in one book outweighs any negative aspects.

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## FLORIDA PIONEER WILMON NEWELL: THE PAST, PRESENT AND FUTURE OF INSECT PEST CONTROL

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Dr. Wilmon Newell was a pioneer whose roots extended far deeper than the primordial Florida Entomological Society. Imagine the field of entomology in 1878 when he was born, and the great body of work he accomplished as Florida established agriculture and associated pest management capabilities. For perspective, he was almost 20 years old when the battleship Maine exploded in Havana Harbor signaling the beginning of the Spanish American War. It was not until the turn of the century that the U.S. Army Yellow Fever Commission, headed by Walter Reed, proved that yellow fever was a viral disease transmitted by *Aedes aegypti*. Soon thereafter, on January 5, 1916, the Florida Entomological Society was established with J. R. Watson as president and Wilmon Newell the secretary-treasurer. Newell had recently been appointed Plant Commissioner of the Florida State Plant Board, created in 1915 initially to eradicate citrus canker. He directed the first Mediterranean fruit fly eradication campaign in 1929-30, and was instrumental in successfully eliminating citrus canker from Florida in the early 1930s. This Florida pioneer entomologist cooperatively accomplished these extraordinary pest management feats before the era of synthetic chemical pesticides. To provide an historical perspective for Dr. Newell's distinguished career, John T. Creighton wrote his obituary for the Journal of Economic Entomology in 1943, himself honored as a Florida Entomological Society Pioneer in 2000.

Wilmon Newell was born at Hull, Iowa on March 4, 1878, receiving his B.S., M.S., and honorary Sc.D. degrees from Iowa State College in 1897, 1898, and 1920, respectively. He served as Assistant in Entomology at the Iowa Agricultural Experiment Station from 1897 to 1899, held the same position at the Ohio Agricultural Experiment Station from 1899 to 1902, became Associate Entomologist and Apiarist at the Texas Agricultural Experiment Station for a year, moved again to be the State Entomologist of Georgia from 1903 to 1904, transferred in 1904 to the position of Entomologist for the State Crop Pest Committee of Louisiana, and returned to Texas in 1910 as Entomologist of the Experiment Station and State Entomologist, a position he held until 1915 when he came to Florida as Plant Commissioner of the newly established State Plant Board. In addition to being Plant Commissioner, in 1921 he became Dean of the College of Agriculture and Director of the Florida Agricultural Experiment Station and the Agricultural Extension Division. In 1938, he was appointed Provost of the



Dr. Wilmon Newell

College of Agriculture at the University of Florida. Dr. Newell's tremendous zeal for work and executive ability were responsible for the number of positions he held and in great part accounted for the prominence of the Florida Agricultural Experiment Station in the 1920s through the 1940s.

Some of Dr. Newell's finest research was on control methods for the cotton boll weevil, Argentine ant, and American foul brood in honeybees. During his long career, he published technical papers on cotton and scale insects, apiculture, quarantine programs and procedures, and insect eradication. Dr. Newell had a particular interest in ant taxonomy, but also conducted pioneering research on boll weevil control in Louisiana and maintained a deep interest in apiary work in Texas and other states. However, he was best known for his activities in control and eradication of plant pests. He directed eradication from Florida of the Mediterranean fruit fly, citrus canker, and citrus blackfly. He also surveyed extensively for the Argentine ant along the Gulf Coast, particularly in Louisiana. His ant collection is in the Florida State Collection of Arthropods, Division of Plant Industry, in Gainesville. The State Plant Board became the Division of Plant Industry in 1960, one of 10 divisions in the Florida Department of Agriculture and Consumer Services.

Dr. Newell attained many honors, including member of the advisory council of the Southern Forestry Experiment Station of the Southern Forestry Service, member and president in 1920 of the American Association of Economic Entomologists, charter member of the Cotton States Branch of the Association, member and president in 1929 of the Association of Southern Agricultural Workers, University of Florida representative of the Institute for Research in Tropical America, member of the Soil Science Society, administrator of Florida State Soil Conservation, chairman of the Florida Land-Use Planning Committee, and chairman of the Advisory committee on Agriculture of the Florida Defense Council. He was also a Mason and a Shriner, a member of Kappa Sigma social fraternity, and member of the honorary fraternities of Alpha Zeta, Phi Kappa Phi, and Gamma Sigma Delta. The students in the Department of Entomology in the College of Agriculture held him in such high esteem that they named their professional society the Newell Entomological Society in his honor. Subsequent to Dr. Newell's death, the Florida State Board of Control and The State Board of Education, at the direction of Governor Spessard L. Holland, dedicated the rebuilt Agricultural Experiment Station Building on the University of Florida campus as Newell Hall, a memorial in his honor.

Harold Denmark, a past president of the Florida Entomological Society and Pioneer Lecturer, knew Wilmon Newell and shared some memories of this eminent pioneer and leader of Southern agriculture. Dr. Newell was a very personable man who helped many colleagues and students become professional entomologists. He was intensely concerned about invasive insects and diseases that affected agriculture in Florida and used the tools at hand to control or eradicate the most damaging pests of his time. His "scorched earth" approach to eradicating the Mediterranean fruit fly was conducted without regard to the environment and at extreme economic losses to growers. He was high-handed because he considered eradication to be critical. Perhaps he was justified based on the numerous awards he received for meritorious service. It is easy to criticize these somewhat imperious methods—but they were successful. Dr. Newell literally gave his life to serve Florida agriculture, sleeping only four hours a night. One of his last comments to John T. Creighton was the wish that he could live longer to solve more pest problems in Florida and the world. Dr. Newell was passionate about his life's mission and worked relentlessly to protect Florida's agriculture.

Wilmon Newell's remarkable successes in pest control during the first half of the last century were taken as the starting point for this Pioneer Lecture. His successes are now considered in light of current control efforts directed at thrips pests—these being the target organisms of much of my

research, as well as that of Joe Funderburk, 1998 president of the Florida Entomological Society. There can be no doubt that Newell's dynamic approach to such problems would be socially unacceptable today. Newell lived in a time when actions intended for the greater good of society held precedence over personal rights. These days, disrupting the lives of citizens by blanket pest control methods would quickly lead to litigation, and entomologists must work within this very different social milieu. Only in parts of the world where citizens have far more limited civil rights than in Florida can the "Newell Approach" still be practiced.

A second problem associated with Newell's eradication methods is that his very success contributed to one of the greatest problems that face us in pest control—the view that it is actually possible to eradicate one type of organism without affecting the lives of other organisms. Such a view runs deep through the human psyche. Consider the response of a U.S. funding agency to a research proposal by an eminent entomologist who wished to examine the epidemiology of thrips-born tospoviruses that cause serious crop losses. The formal rejection notice from the funding agency stated that in the view of the committee all that was needed was for "the thrip be killed". This chilling response, the Curse of Cain, is all too human and all too frequent, but as scientists we represent the sons of Abel and must try to do things differently.

The magic initials IPM represent a banner around which biologists can gather and organize new methods of crop production—methods that recognize that our children will need clean water and clean air as well as biological diversity in the landscapes within which they live. But, at least in pest thrips control, we find that these initials too often represent *Ignorance, Prejudice and Mismanagement*. Amongst a range of growers visited in recent years, there have been those who could not see insects as small as thrips, some insisting that their tospovirus problems are due to aphids. There are those who blame their neighbors for the heavy thrips and virus infestations that are destroying their lettuce crops, while rejecting the fact that their beautiful beds of French marigolds are the source of their problems. And there are those who, at considerable expense, cull all infested plants but then thoughtlessly dump these in a heap just up-wind of their crop, so that the viruliferous thrips fly straight back into the crop. In contrast, other growers keep their crops clear of thrips and tospoviruses—and it is instructive and heartening to talk to such growers and recognize their deep appreciation of insect behavior and insect/plant relationships. Thus, we can do better—but it requires that effort that is so difficult for all of us, thinking.

Good IPM demands that we learn all that we can about the biology of our target pests and their relationships to our crops, and that we also have a

sound knowledge of the other organisms that are in and around those crops. It recognizes that these different organisms are interdependent, and that disrupting one will have effects on others. In the 1998 Pioneer Lecture, I drew an analogy between pest control and human warfare, remembering that Clausewitz emphasized that all items of information about an enemy,—economic, social, and behavioral—are useful in leading to victory. Similarly, Sherman's lateral thinking in marching from Atlanta to the sea was responsible for shortening America's bloody civil conflict—not through killing, but through disrupting and demoralizing his opponents. What can we as biologists learn from these proponents of "total warfare"? Are we still in the paradigm of Sherman's colleagues who advocated massive frontal attacks?

The important lesson is that we can never know too much about our opponents—how they live, how they feed, their behavioral prejudices and preferences. In contrast, the approach to thrips pests around the world still places great emphasis on killing, rather than on studying. Our understanding of thrips biology has improved considerably in recent years, but our knowledge of the biology of pest species is often remarkably weak (Mound 2004). Even simple questions are too often ignored, such as where populations of thrips in a crop come from—from the soil in which the crop is growing or through immigration from surrounding plants; or where do thrips pupate—on the crop, in leaf duff, or in soil? Thrips workers in Florida are among the few worldwide who recognize the problems inherent in this lack of information about thrips biology, and who are finding alterna-

tive ways of reducing crop losses to these pests. The University of Florida web site gives some details of their success <[http://ipm.ifas.ufl.edu/success-stories/tomato/vanquishing\\_virus.pdf](http://ipm.ifas.ufl.edu/success-stories/tomato/vanquishing_virus.pdf)>, and their thrips control program has resulted in the team receiving a prestigious USDA Honor Award for Excellence (Funderburk et al., 2005). The focus in Florida on the ecology of thrips pests contrasts with the Rambo-esque approach of grabbing a spray gun that so many in society find emotionally reassuring, even when economically ineffective. Wilmon Newell was an important Pioneer in his day, but these thrips workers represent a new generation of pioneers. We should not aspire to emulate our predecessors but build on their successes and, through expanding our knowledge base concerning pests, search for new ways in which we can optimize crop production and pest management for future generations.

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