A REVISION OF THE GENUS *AMBLYOMIA* STÅL (HETEROPTERA: COREIDAE: COREINAE: LEPTOSCELINI)

HARRY BRAILOVSKY
Instituto de Biología, UNAM, Departamento de Zoología, Apdo Postal 70153 México 04510 D.F. México

ABSTRACT

The genus *Amblyomia* Stål is revised and two new species, *A. foreroi* and *A. promeceops* from Colombia, are described. New host plant and distributional records of *A. bifasciata* Stål are given; habitus illustrations and drawings of male and female genitalia are included as well as a key to the known species. The group feeds on bromeliads.

Key Words: Insecta, Heteroptera, Coreidae, Leptoscelini, *Amblyomia*, Bromeliaceae

RESUMEN

El género *Amblyomia* Stål es revisado y dos nuevas especies, *A. foreroi* y *A. promeceops,* recolectadas en Colombia, son descritas. Plantas hospederas y nuevas localidades para *A. bifasciata* Stål son incluidas; se ofrece una clave para la separación de las especies conocidas, las cuales son ilustradas incluyendo los genitales de ambos sexos. Las preferencias tróficas del grupo están orientadas hacia bromelias.

Palabras clave: Insecta, Heteroptera, Coreidae, Leptoscelini, *Amblyomia*, Bromeliaceae

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The neotropical genus *Amblyomia* Stål was previously known from a single Mexican species, *A. bifasciata* Stål 1870. In the present paper the genus is redefined to include two new species collected in Colombia. This genus apparently is restricted to feeding on members of the Bromeliaceae, and specimens were collected on the heart of *Ananas comosus* and *Aechmea bracteata*.

All measurements are in millimeters.

AMBLYOMIA STÅL

Amblyomia Stål 1870: 171.

Redescription. Head longer than wide, elongate, pentagonal, non-declivent, and produced forward between bases of antennae; tylus blunt, forming rounded elevated ridge, slightly projecting beyond juga; juga unarmed, thickened; mandibular plate unarmed; antenniferous tubercles unarmed, widely separated, space between them slightly more than two times the width of one tubercle; antennal segment I shorter than head, thicker, slightly curving; segments II and III cylindrical, IV fusiform; segment IV longest, segment I shortest, II longer than III; preocellar pit deep, nearly circular; ocelli elevated; eyes hemispherical, prominent; area between eyes convex; postocular tubercle low, almost absent; buccula short, unarmed, not extending beyond antenniferous tubercles; rostrum reaching posterior border of abdominal sternite III;

rostral segment I longest, reaching base of head, segment III shortest, and II longer than IV; neck short. Thorax. Pronotum. Wider than long, trapeziform, gradually declivent; collar wide; anterolateral borders obliquely rounded and entire; frontal and humeral angles rounded, not exposed; posterolateral and posterior borders straight, entire; disc deeply punctate except for smooth callar region. Ventrally smooth, except acetabula, anterior and posterior margin of propleura, posterior margin of mesopleura and metapleura, deeply punctate; prosternum with deep excavation; mesosternum shallowly sulcate; metasternum flat; anterior lobe of metathoracic peritreme elevated, reniform, posterior lobe small, acute. Legs. Femora ventrally armed with two rows of spines, dorsally with scattered spines or low tubercles; hind femora incrassate, moderately in females, strongly in males; hind tibiae shorter than hind femora, sulcate, triquetrous in cross section, and armed on distal third with short spines on low tubercles, conspicuous in males, hard to see in females. Scutellum. Triangular, flat, wider than long; apex subacute; disc punctate. Hemelytra. Macropterous, extending far beyond apex of last abdominal segment; costal margin emarginate, apical margin weakly sinuate; clavus and corium deeply punctate. Abdomen. Connexival segments higher than margin of hemelytron at rest; posterior angles of connexival segment complete, not extending on a short spine; abdominal spiracle submarginal, close to middle third; sternum smooth, without punctures. Male genitalia. Genital capsule simple; posteroventral margin with a shallow median notch (Fig.1). Parameres. Shaft robust; anterior lobe convex, posterior lobe elongate, slender, and nearly perpendicular to shaft (Fig.2). Female genitalia. Spermatheca. Distal bulb oval; sclerotized duct leading from bulb moderately coiled; proximal duct slightly widened near distal flange; distal duct membranous, narrowed (Fig.3).

This genus is related to *Coribergia* Casini (1984) and *Dalmatomammurius* Brailovsky (1982), but differs in a number of characters: posttylar sulcus absent, antennal segment I much shorter than head, humeral pronotal angles rounded, and hind tibiae slightly expanded. In the other two genera the posttylar sulcus is present, antennal segment I longer, humeral pronotal angles acute to subacute, and hind tibiae cylindrical and sulcate. In *Dalmatomammurius* the antenniferous tubercles are externally lobulate; they are truncated and unarmed in *Amblyomia* Stål and *Coribergia*.

The suprageneric position of these three genera is complex. "Packauskas (personal communication) believes that the genus *Amblyomia* deserves its own tribe. He also believes, based on aedeagal characters and lack of a posttylar sulcus, that its affinities may be closer to members of the tribe Nematopodini or even the subfamily Meropachydinae.

KEY TO SPECIES

1. Buccula and rostral segment I bright orange . . . . . *promeceops* new species

1´. Buccula black to reddish brown; rostral segment I black 2

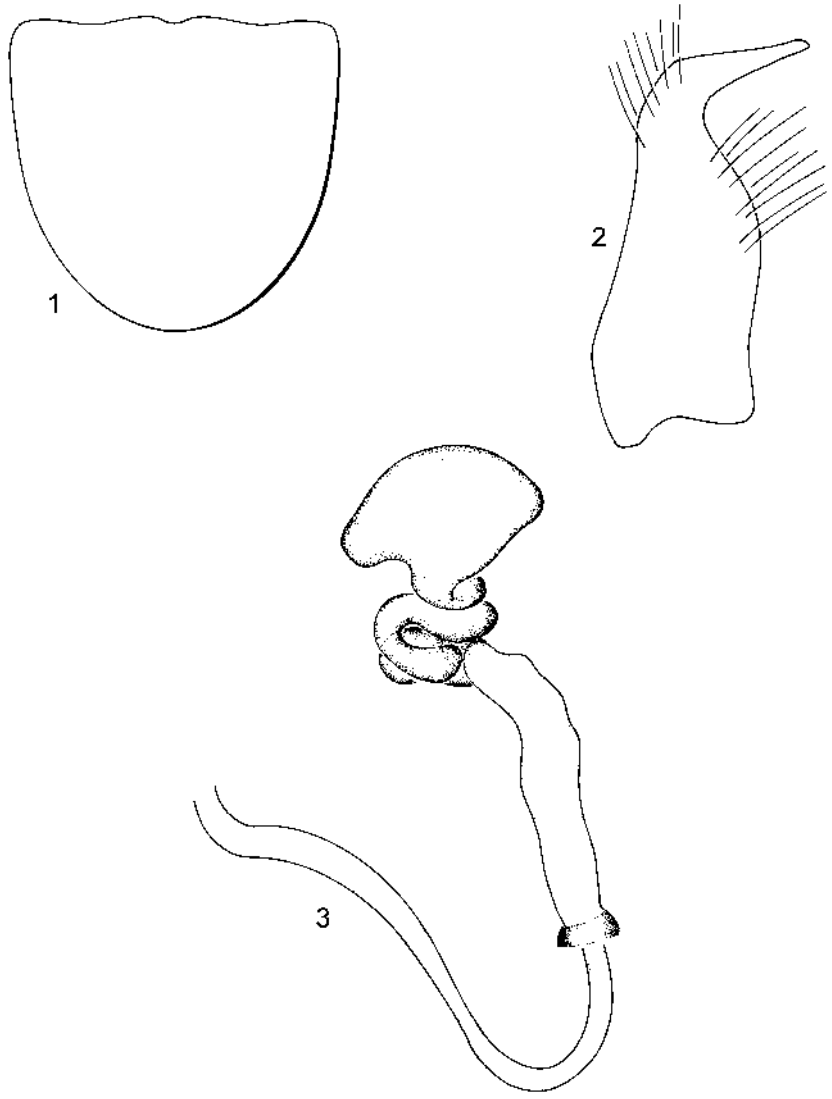
2. (1´).Acetabula black; pronotal disc with a wide orange transverse fascia; posterior margin of pronotum black; pronotal collar white to yellow; corium with wide yellow to orange transverse fascia (Fig. 4) . . . . . *bifasciata* Stål

2´. Acetabula orange; pronotal disc, including posterior margin yellow; pronotal collar black; corium without orange or yellow transverse fascia . . . . . *foreroi* new species

Amblyomia bifasciata Stål
Figs. 1-4

Amblyomia bifasciata Stål 1870: 172

Redescription. Body including antennal segments (apex of IV pale brown to orange), rostral segments, hemelytral membrane, connexival segments, abdominal seg-



Figs. 1-3. *Amblyomia bifasciata* Stål. 1, Caudal view of male genital capsule. 2, Paramere. 3, Spermatheca.

ments, and legs black; head with short orange stripe below eye and external to ocelli; pronotum with white to yellow collar, and posterior portion of pronotal disc with a wide orange transverse fascia; corium with yellow transverse fascia almost straight; posterior margin of abdominal sterna IV to VI without or with orange irregular spots lateral to middle third.

Measurements. ♂ first, then ♀. Head length 1.76, 2.04; width across eyes 1.52, 1.80; interocular space 0.84, 1.00; interocellar space 0.52, 0.48; preocellar distance 0.98, 1.14; length antennal segments: I, 1.00, 1.16; II, 1.80, 2.04; III, 1.24, 1.48; IV, 2.76, 3.04. Pronotum. Total length 1.84, 2.44; width across frontal angles 1.20, 1.40; width across humeral angles 3.16, 3.88. Scutellar length 1.28, 1.56; width 1.44, 1.64. Total body length 10.68, 12.97.

Biology. Nothing has been known of its biology. Numerous adults and late-instar nymphs were taken on the heart of pineapple (*Ananas comosus*) (Bromeliaceae) in the State of Chiapas (Municipio de Ocosingo, Santo Domingo, México), and a few adults on the heart of *Aechmea bracteata* (Bromeliaceae) in the State of Veracruz (Los Tuxtlas, México).

Distribution. This species was originally described from México, without data.

New Records. México: 2 ♀, Veracruz, Los Tuxtlas, 22-VII-1968 (Carlos Beutelspacher); 1 ♂, 2 ♀, same locality, 22-V-1969 (Carlos Beutelspacher). 1 ♀, Tabasco, Cardenas, 4-VIII-1970 (R. Arias); 1 ♀, Chiapas, Bonampak, 20-V-1980 (Harry Brailovsky); 1 ♂, 1 ♀, Chiapas, Simojovel, 25-XII-1968 (Carlos Beutelspacher); 2 ♂, 2 ♀, Chiapas, Rio Santo Domingo, 16-I-1983 (Ernesto Barrera); 10 ♂, 10 ♀, Chiapas, Municipio de Ocosingo, Santo Domingo, 24-26- VIII-1893 (Vicente Hernandez); 1♀, Chiapas, Palenque, 25-XII-1990 (M.J. and C.A. Tauber).

It may be distinguished from the new species by possession of a white to yellow collar, the orange transverse fascia crossing the posterior lobe of the pronotum, and by the yellow transverse fascia of the corium.

Amblyomia foreroi Brailovsky, **New Species**
Fig. 5

Description. Head black with short orange stripe below eye and external to ocelli; antennal segments I to III bright red brown, IV black with apex pale orange yellow; pronotum yellow with collar, calli, and anterolateral margins (except humeral angles) black; scutellum, clavus, corium, and hemelytral membrane black, with following areas dull orange: apex of scutellum, irregular stripe on clavus, and two small spots near middle third of corium; connexival segments III to VI dark brown, with posterior margin orange; segment VII dark brown; abdominal segments III to VI orange, VII dark brown. Ventral coloration. Head black, with middle third dark hazel; rostral segments I to IV red brown; thorax black to red brown, with acetabula and posterior margin yellow; anterior and posterior lobe of metathoracic peritreme dark hazel; evaporative area dull black; legs dark hazel; abdominal sterna orange hazel, with posterior margin of sterna IV to VI yellow; genital capsule dark brown.

Measurements. Head length 1.88, width across eyes 1.62, interocular space 0.96, interocellar space 0.47, preocular distance 1.12; length of antennal segments: I, 1.36, II, 2.20, III, 1.64, IV, 3.08. Pronotum: Total length 2.32, width across frontal angles 1.40, width across humeral angles 3.64. Scutellar length 1.44, width 1.52. Total body length 12.28.

Holotype: ♂ Colombia: Municipio Risaralda Pueblo Rico, Santa Cecilia, II-1992 (F. Fernandez). In Universidad Nacional de Colombia, Santa Fe de Bogota (Instituto de Investigaciones de Recursos Biologicos Alexander von Humboldt).

Etymology: Named for Dimitri Forero.

Amblyomia foreroi is readily distinguishable because it is the only known species in the genus with the pronotum yellow, except the collar, calli, and anterolateral mar-

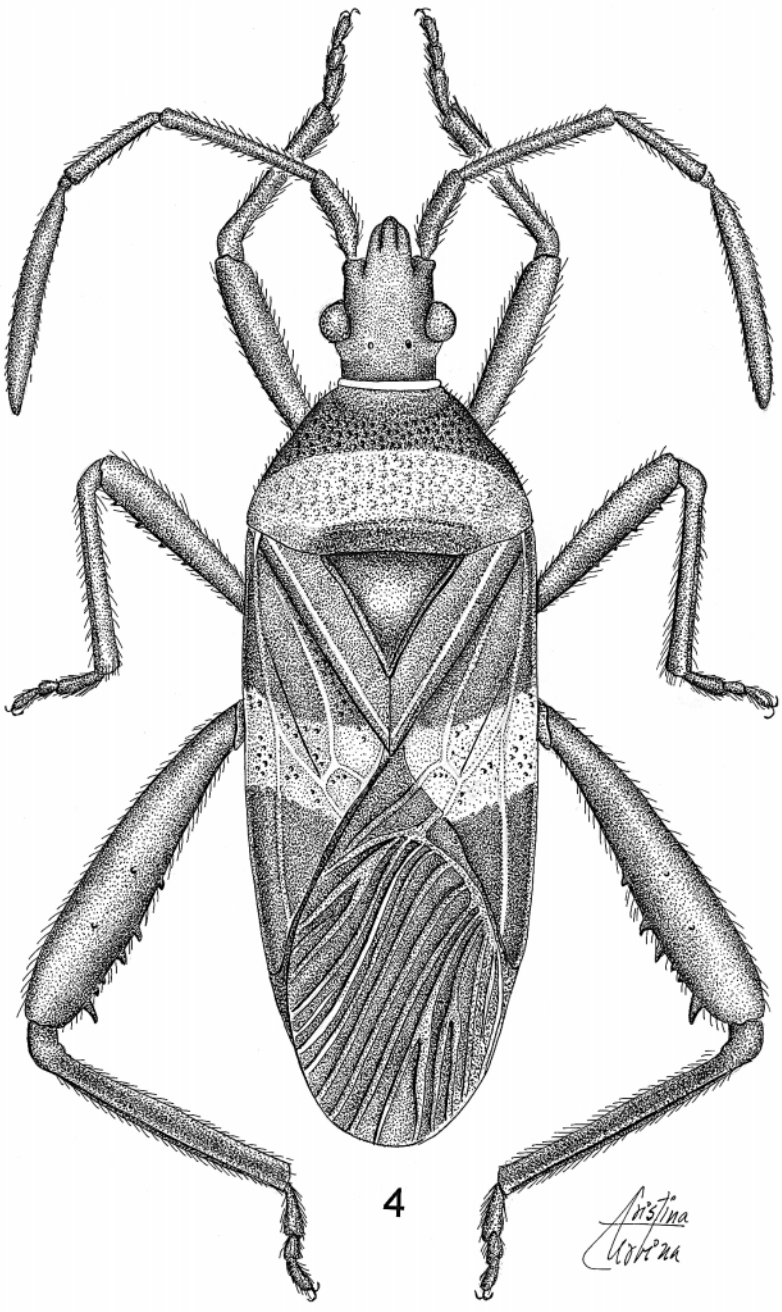


Fig 4. Amblyomia bifasciata Stål, dorsal view.

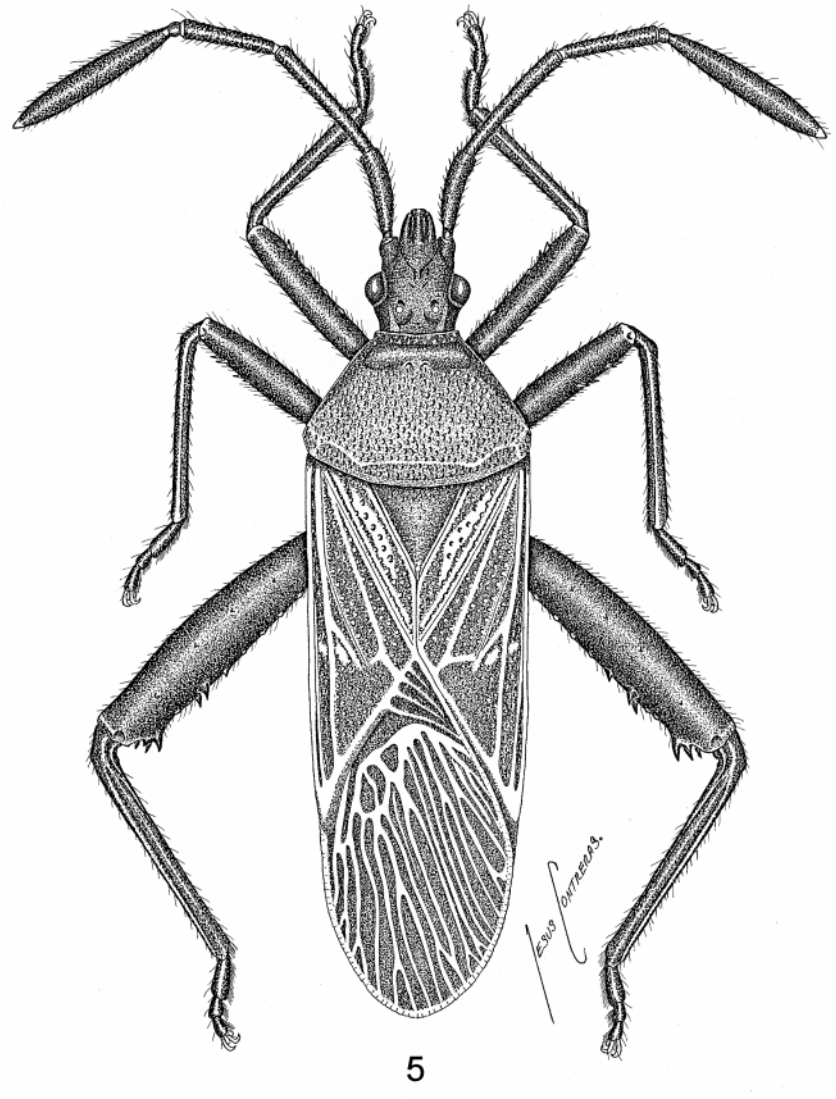


Fig. 5. *Amblyomia foreroi* Brailovsky, New Species, dorsal view.

gins (except humeral angles) black, and corium lacking yellow or orange transverse fascia. *Amblyomia bifasciata* Stål, the most closely related species, has the pronotum black, with the collar white to yellow, and a wide orange transverse fascia over the pronotal disc, and the corium is black with the yellow transverse fascia almost straight.

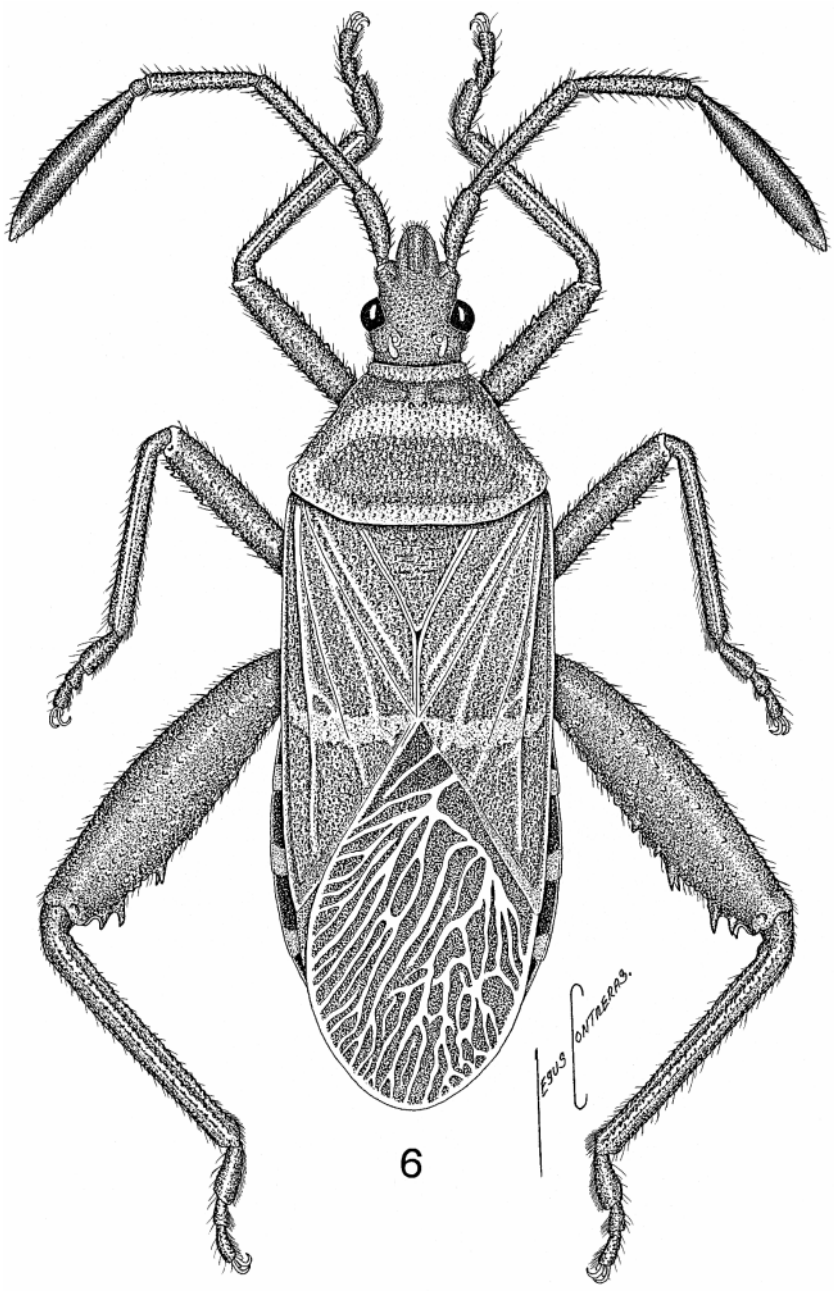


Fig. 6. *Amblyomia promeceops* Brailovsky, New Species, dorsal view.

Amblyomia promeceops Brailovsky, **New Species**
FIG. 6

Description. Dorsal coloration. Head black with short orange stripe below eye and external to ocelli; antennal segments I to IV black (apex of IV pale orange); pronotum black with following areas orange: collar, humeral angles, posterior margin and narrow arcuate transverse fascia over pronotal disc; scutellum, clavus, and hemelytral membrane black; corium black with narrow orange transverse fascia near middle third; connexival segments III to VI black with posterior margin orange; segment VII almost entirely black; abdominal segments III to VI pale brown, VII black. Ventral coloration. Head black with buccula orange; rostral segment I bright orange, II hazel with basal joint orange, III hazel with posterior half orange, and IV hazel; thorax including anterior and posterior lobe of metathoracic peritreme bright to dull black with following areas orange: collar, acetabula, and posterior margin of propleura, mesopleura, and metapleura; legs red brown; coxae pale hazel; abdominal sterna and genital capsule black, with posterior margin of sterna III to VII orange.

Measurements. Head length 1.88, width across eyes 1.60, interocular space 0.88, interocellar space 0.46, preocular distance 1.04; length of antennal segments: I, 1.16, II, 2.04, III, 1.52, IV, 2.80. Pronotum: Total length 2.20, width across frontal angles 1.48, width across humeral angles 3.52. Scutellar length 1.44, width 1.52. Total body length 12.15.

Holotype: ♂ Colombia: Rio Negro, Cundina-Maria, 1000m, 10-I-1965 (W. Schmidt). In Forschungsinstitut und Naturmuseum Senckenberg, Frankfurth am Main, Germany.

Etymology: From the Greek, *promeces*, elongate, and *ops*, face.

Amblyomia promeceops differs from all other members of the genus in having the buccula and rostral segment I orange, the corium black with a narrow orange transverse fascia, and the pronotum black with following areas orange: collar, humeral angles, posterior margin, and narrow arcuate transverse fascia over pronotal disc. The other two species differ in having the buccula and rostral segment I black, and the pronotum and corium with other color pattern.

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REFERENCES CITED

BRAILOVSKY, H. 1982. Hemiptera-Heteroptera de México XXIV. Nuevos registros de la tribu Mictini y descripción de un nuevo género y dos nuevas especies (Coreidae). An. Inst. Biol. Univ. Nal. Autón. México, Ser. Zool. 52 (1981): 277-288.

CASINI, C. 1984. Descripción de un nuevo género de la Familia Coreidae y algunas consideraciones sobre el género *Mamurius* (Hemiptera). Bol. Soc. Zool. del Uruguay (2da. época) 2: 5-11.

STÅL, C. 1870. Enumeratio Hemipterorum I. K. Svenska Vetensk-Akad. Handl. 9 (1): 171-172.

A NEW SPECIES OF *CHLOROCORIS* (HETEROPTERA: PENTATOMIDAE) FROM JAMAICA

DONALD B. THOMAS
USDA-ARS Subtropical Agricultural Research Center
2301 S. International Blvd., Weslaco TX 78596

ABSTRACT

A new subgenus and new species of *Chlorocoris* is described from the Caribbean island of Jamaica. This is the first species of this genus reported from the Antilles. The species is remarkable for an unusually enlarged metatarsus in males and the angulate apex of the femora.

Key Words: Pentatomidae, stink bug, taxonomy, Jamaica

RESUMEN

Un subgénero nuevo, y una especie nueva, del género *Chlorocoris* es descrito con origen en la isla Jamaica del mar Caribe. La misma es el primer registro de este género para las Islas Antillas. La especie nueva es notable porque tiene el metatarso alargado en los machos y el ápice de la superficie superior del fémur está angulada.

When the genus *Chlorocoris* Spinola was last revised (Thomas 1985) no material was available, nor had any species been reported, from the West Indies. In recent years four specimens have come to light representing a new species from the island of Jamaica. Based on the triangular form of the head (Fig. 1), the new species would be assignable to the nominate subgenus. However, a characteristic feature of the nominate subgenus is the presence of a pair of elongated processes on the male proctiger. This character is lacking in the new species. Another remarkable feature of the Jamaican species is a sexual dimorphism. The metatarsus of the male has an unusually enlarged basal segment (Figs. 2-3). In addition, the dorsal apex of each femur is angulate. In all other species the apex is rounded. In the keys to genera including *Chlorocoris* and its relatives the presence or absence of a stout spine at the apex of the femur is a diagnostic character (Eger 1978, Rolston & McDonald 1984). The new species will key to *Chlorocoris* if the angulation is not confused with a true spine. Because of the above stated differences, I am assigning the new Jamaican species to a new subgenus of *Chlorocoris*, described below.

MATERIALS AND METHODS

The material available for study consisted of four specimens, two males and two females, from two localities in Jamaica. The Jamaican specimens were compared to material in my reference collection, including paratypes from my earlier revision of *Chlorocoris*. Type depositions are indicated by acronyms: United States National Museum [USNM], Florida State Collection of Arthropods [FSCA], and Donald B. Thomas collection [DBTC]. The habitus drawing of the new species was prepared by Daniel Schmidt of Schuyler, Nebraska, based on one paratype specimen. All other illustrations were tracings from camera lucida with a Wild M-5 dissecting microscope at magnification 25x and 50x. All measurements are from the male holotype unless

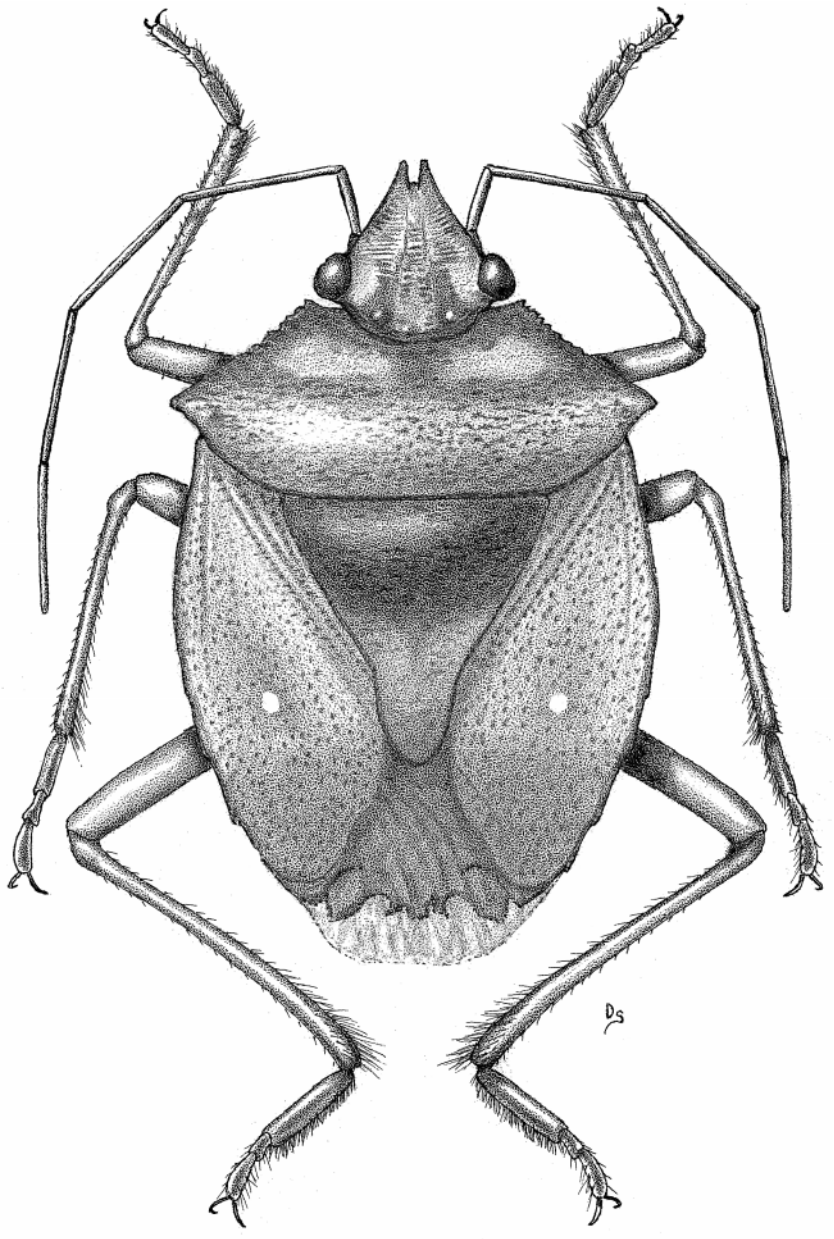


Fig. 1. *Chlorocoris tarsalis*, new species.

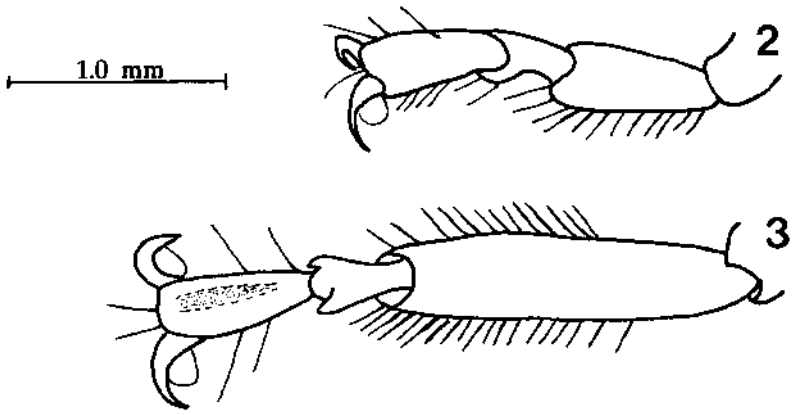


Fig. 2-3. Sexual dimorphism in metatarsi of *C. tarsalis*. 2. female, 3. male.

otherwise indicated. Measurements were made with a Zeiss SV8 dissecting microscope with a 10x graduated ocular. Anatomical nomenclature follows Nichols (1989).

Arawacoris, **New Subgenus**

Type species: *Chlorocoris tarsalis*, **New Species**.

Description. Head triangular, lateral margins of juga straight, or nearly so, to beyond apex of tylus. Rostrum long, apex reaching third visible abdominal sternite in repose. Superior apices of femora angulate. Dorsum of male proctiger inornate, lacking processes. Inferior margin of posterior rim of pygophore with pair of articulated appendages, one each side of midline, each about same size as a paramere. Other characters as in *Chlorocoris* (see Thomas 1985).

Etymology: a latinized combination of "Arawak," the indigenous people of Jamaica, and greek "koris," meaning "bug."

Chlorocoris tarsalis **New Species**
Figs. 1-6

Description. Dorsal color green fading to yellow. Form oval, depressed dorsoventrally, with angular head and humeri. Length 13.3 mm (female 16.1 mm), width across pronotum 8.5 mm (female 9.6 mm).

Head. Dorsum flat, surface strigose, devoid of black punctations except at apices of juga. Lateral margins of juga straight to apices. Apex of each jugum subacuminate, exceeding apex of tylus and forming sinus before tylus, sinus twice as long as wide. Cranial length (tip of jugum to imaginary line connecting ocelli) 3.0 mm, width (across anteocular angles) 2.2 mm. Antennal segment I immaculate, its apex attaining apex of jugum. Segments II-IV subequal (exact proportions vary among individual specimens), each about twice length of I; V slightly shorter than IV. Posterior termination of each buccula evanescent in profile. First rostral segment slightly longer than buccula. Rostrum in repose attaining third visible abdominal sternite.

Thorax. Anterolateral pronotal margins rectilinear in dorsal view, serrate. Humeri angular, prominent, acute. Dorsum of pronotum devoid of black punctations except in

area immediately adjacent to humeral angles. Length of pronotum at midline 3.1 mm. Scutellum and hemelytral coria devoid of markings except for pale pustule on disc of latter. Length of scutellum 4.9 mm. Posterior margin of corium sinuate. Hemelytral membrane clear, transparent, with scattered green flecks. Auricle of metathoracic scent gland orifice short, reaching about one-fifth distance to metapleural margin. Femora and tibiae immaculate. Superior surface of apex of each femur angulate but not spinous. Basal tarsal segment of metathoracic legs of male fusiform, thickened, and notably elongate (Fig. 3) compared to mesotarsi, or metatarsi of female (Fig. 2).

Abdomen. Midline of first three abdominal sternites sulcate for reception of rostrum. Spiracular margins and apices of sternites concolorous with disc of sternum. Connexivum without spots or stripes. Greatest width of abdomen, 8.3 mm.

Genitalia. Male pygophore (Fig. 4) broadly open posteriorly and dorsally with ventroposterior rim deflexed. Surface of pygophore at lateral angles dense with short bristles and with a smaller patch of dense bristles on either side of midline just ectal to inferior margin. Inferior margin bearing, on either side of midline, an articulated, sclerotized L-shaped "pseudoclasper" (hypandrium?), projecting into lumen of proctiger. Basal portion of each pseudoclasper bearing a porrect, angular tooth. Erect arm of pseudoclasper subfoliate. Proctiger inornate. Parameres large with broad compressed base expanding into two angular projections: a dorsal, flat, rectangular projection, and a ventral, flat, acutely angled, rhomboidal projection (Fig. 5).

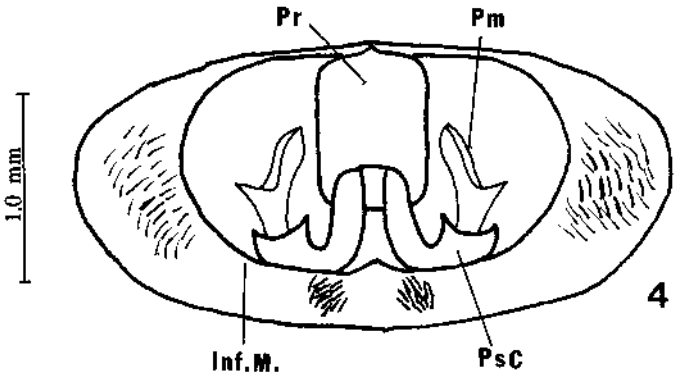
First gonocoxites of female thickened, posterior margin strongly sinuate (Fig. 6). Ninth paratergites elongate, apices acuminate, exceeding posterior margin of eighth paratergite. Apex of eighth paratergite subspinose with spiracles present but displaced to notch of basal angle.

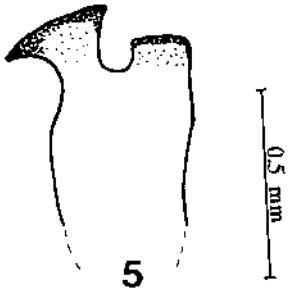
Holotype. Male. verbatim label data: JAMAICA: Green Hills. 13-20-XI-66. A. B. Gurney [USNM]. Allotype: Female, with same label data as holotype [USNM]. Paratypes: Female, with same label data as holotype [DBTC]. Male labeled: (a) JAMAICA: Parish of St. Andrew, 4,000 ft. Holywell Forest Camp. Blacklight. (b) R. E. Woodruff, 16-VI-75, Blacklight Trap [FSCA].

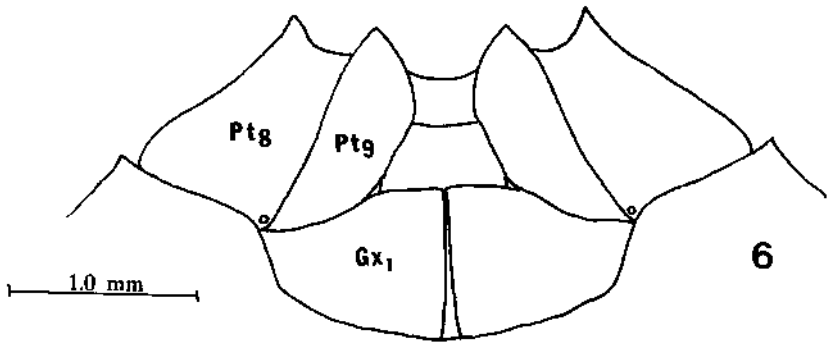
DISCUSSION

The genus *Chlorocoris* is superficially similar and probably closely related to a group of genera that includes *Chloropepla* Stål, *Loxa* Amyot & Serville, *Fecelia* Stål, and *Mayrinia* Horvath. All of these genera, except *Chlorocoris*, have the superior apex of the femora terminating in a minute spine. All known species of *Chlorocoris*, with the exception of the new Jamaican species, have the apex of the femora rounded. The new species has an intermediate condition with the apex of the femora angulate. Inasmuch as the condition of the femoral apex is important in distinguishing genera, and considering the unusual sexual dimorphism of the metatarsus, one might plausibly erect a new genus for this species. I, therefore, reviewed the characteristics of each genus and searched for trends that might support this position.

Within this generic complex, the most striking morphological variation is found in the male genital apparatus. In fact, the terminalia are elaborate to a degree that would almost seem to impede rather than enhance coition. One structure in particular, referred to in the description of the new species as a "pseudoclasper," is especially enigmatic. It is an appendage situated at the middle of the posterior rim of the pygophore. Its function is unknown. In most species of *Chlorocoris*, and its ally *Mayrinia*, the appendage is a singular, erect structure, fused to the rim of the pygophore, the size and conformation of which vary greatly among the individual species. It is referred to as the "hypandrium" in the revision of *Mayrinia* by Grazia-Vieira (1972). In *Chlo-*







Figs. 4-6. Genitalia of *C. tarsalis*. 4. pygophore, posterior view. 5. left paramere, ental view. 6. Female genitalia. Gx_1 = first gonocoxite, Inf.M. = inferior margin, Pm = paramere, Pr = proctiger, PsC = pseudoclasper, Pt_8 = eighth paratergite, Pt_9 = ninth paratergite.

ropepla, *Fecelia*, and *Loxa*, there is a pair of articulated structures in the same position, variously called "hypandria" (Grazia 1968, 1976) or "pygophoral appendages" (McDonald 1966). I can only presume, as did Grazia, that the paired, articulated structures found in some genera are homologous to one another and to the erect structure fused to the rim of the pygophore in others. Within the genus *Chloropepla*, *C. vigens* Stål has a pair of appendages, while *C. aurea* Grazia has none. Likewise, among the species of *Loxa* they may be present or absent (Eger 1978). In most species of *Chlorocoris*, as in *Mayrinia*, the appendage is completely fused to the rim of the pygophore. But in *C. rufispinus* Dallas and *C. rufopictus* Walker, the appendage is articulated, or at least, there is a perceptible line of attachment that is not evident in the other species. The subgenus *Arawacoris* has a pair of articulated appendages and thus is exceptional in this regard from all other *Chlorocoris*. Yet, it seems hazardous to attach much significance to the difference. The degree of homoplasy in the structure of the pygophore is mirrored in the ornamentation of the proctiger. The nominate subgenus of *Chlorocoris* has a pair of elongate processes that overlie, and are parallel to, the dorsum of the proctiger. In the subgenus *Monochrocerus* the processes are short and are oriented horizontal to the length of the proctiger. But some species, including *C. tarsalis*, have no processes at all. Similarly, in the genus *Loxa*, some species have paired spines on the proctiger and others none.

The pattern of divergence and convergence in the form of the genitalia in this complex presents a challenge to those who are firm in the belief that phylogeny can be objectively derived from the cladistic nesting of character-states. Having failed in my own feeble efforts to find clear evolutionary trends among the recognized genera with respect to these genital characters, I am reluctant to erect a new genus. Rather, I opine that this somewhat aberrant Jamaican species is best classified as an isolated subgenus of *Chlorocoris*.

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REFERENCES CITED

EGER, J. E. 1978. Revision of the genus *Loxa* (Hemiptera: Pentatomidae). J. New York Entomol. Soc. 86: 224-259.

GRAZIA, J. 1968. Sôbre o gênero "*Chloropepla*" Stål, 1867, com a descricâo de uma nova espécie (Hemiptera, Pentatomidae, Pentatominae). Rev. Bras. Biol. 28: 193-206.

GRAZIA, J. 1976. Revisâo do gênero *Fecelia* Stål, 1872 (Heteroptera, Pentatomidae, Pentatomini). Rev. Bras. Biol. 36: 229-237.

GRAZIA-VIEIRA, J. 1972. O genero *Mayrinia* Horvath, 1925 (Heteroptera, Pentatomidae, Pentatomini). Rev. Peruana Entomol. 15: 117-124.

MCDONALD, F. J. D. 1966. The genitalia of North American Pentatomoidea (Hemiptera: Heteroptera). Quaest. Entomol. 2: 7-150.

NICHOLS, S. W. (compiler). 1989. The Torre-Bueno Glossary of Entomology. New York Entomological Society, New York, NY.

ROLSTON, L. H., AND F. J. D. MCDONALD. 1984. A conspectus of Pentatomini of the Western Hemisphere, Part 3 (Hemiptera: Pentatomidae). J. New York Entomol. Soc. 92: 69-96.

THOMAS, D. B. 1985. Revision of the genus *Chlorocoris* Spinola (Hemiptera: Pentatomidae). Ann. Entomol. Soc. Am. 78: 674-690.

POLLINATORS OF *CHAPMANNIA FLORIDANA* (FABACEAE) AND THEIR FORAGING PREFERENCES

MARGARET M. MAYFIELD
Reed College 3203 SE Woodstock, Portland, OR 97202-8199

ABSTRACT

The visitation rates of two major insect visitors, *Bombus impatiens* Cresson and *Augochloropsis* sp., of the Florida endemic plant species *Chapmannia floridana* Torrey & A. Gray, (Fabaceae); are determined for different microhabitats at the Archbold Biological Station in the summer of 1995. Significant differences in the total number of visits to each site were observed. Each pollinator species was found to visit different vegetation densities and sites with different frequencies. Relationships were also found between visitation rates and temperature and flower size. Variation in visitation rates did not significantly affect seed set in *C. floridana*. Disturbance did not seem to play a major role in determining visitation rates of either of the pollinators. Vegetation composition and flower density appear to be the best indicators of visitation rates to populations of this plant species.

Key Words: *Bombus impatiens*; *Augochloropsis*; Florida scrub; disturbance; pollination; foraging patterns; *Chapmannia floridana*

RESUMEN

Las tasas de visitación de los dos insectos visitantes principales, *Bombus impatiens* Cresson y *Augochloropsis* sp., de una planta endémica de Florida, *Chapmannia floridana* Torrey y & A. Gray (Fabaceae); fueron determinadas para los micro-habitats diferentes en la Estación Biológica Archbold en el verano de 1995. Se observaron diferencias apreciables entre los números de visitaciones totales en cada terreno. También había relaciones entre las tasas de visitación y la temperatura, y el tamaño de la flor. La variación en las tasas de visitación no mostró efecto apreciable en el número de semillas de *Chapmannia floridana*. El disturbio no fue importante para determinar las tasas de visitación de ningún polinizador. La composición de la vegetación y la densidad de las flores parecen ser los mejores indicadores de las tasas de visitación a poblaciones de esta especie de planta.

Many studies have shown that disturbance, both natural and human induced, can cause significant changes in the structure and dynamics of ecosystems. Some of the most obvious changes caused by habitat destruction or damage are changes in biodiversity, niche occupation, and animal feeding behavior (Armesto & Pickett 1985, Glitzenstein et al. 1986, Coffin & Lauenroth 1988, Foster & Zebryk 1993, Aizen & Feinsinger 1994). When disturbance is natural, occurring because of fire, storm damage, or landslides, biodiversity in an area tends to increase and niche occupations and animal feeding preferences remain unchanged (Armesto & Pickett 1985, Feinsinger et al. 1987). However, when areas are damaged by human activities such as grazing, agricultural development, and logging, drastic changes in species composition and foraging behavior are often observed (Brian 1959, Halpern 1988).

Despite the growing interest in the effects of human disturbance on ecosystems and on specific rare plants and animals, there are still many gaps in our understand-

ing of how human disturbance affects the life history of these organisms and their interactions with each other. In this study, I examine the apparent success of the plant species *Chapmannia floridana* Torrey & A. Gray; (Fabaceae) in relation to the visitation rates of its major pollinators. I selected *C. floridana* for this study because of the differences in its population structure in disturbed and undisturbed areas. *C. floridana* is a very unusual Florida endemic scrub plant because of its success in disturbed areas. Most endemic plant species in central Florida are disappearing as central Florida scrub disappears. Undoubtedly, there are many factors that contribute to the success of *C. floridana* in disturbed areas. I was interested in determining if the differences in disturbed and undisturbed habitat and in population structures affect pollination services to this plant, possibly contributing to its success.

In this study, variation in pollinator composition and visitation rates to *C. floridana* were determined for six different sites varying in vegetation composition and disturbance. The effects of flower size and temperature on the pollinator composition visiting this plant were also determined (Cruzan et al. 1988). Information on the foraging behavior of this plant's pollinators can provide valuable information about how human and natural disturbance affects the relationship between *C floridana* and its pollinators.

Materials and Methods

Species description of *Chapmannia floridana:*

Chapmannia floridana is an endemic Florida herb ranging from Clay to Collier Counties in central Florida (Gunn, 1980). It can be found growing in open scrub, sandhills, and disturbed areas such as pastures and road sides. Little attention has been paid to *C. floridana* since its original description by Torrey and Gray in 1838. The information available about this species is minimal and often inconsistent (Gunn 1980). My greenhouse studies on this species suggested that selfing does not occur, but results were inconclusive and more study is needed. *C. floridana* is not thought to be a nitrogen fixer.

C. floridana blooms between mid-April to early September. It is commonly found in large numbers in highly disturbed roadsides and pastures and in smaller numbers in undisturbed or burned habitat. Flowering individuals range in height from 20-101 cm and usually consist of 1 to 10 flowering stalks. Flowers are yellow-orange and range in size from 2.0-4.0 cm from top to the bottom and 0.9-4.0 cm horizontally across the petals. Flowers open at about 8:00 am early in the season and about 6:00 am later in the season. Flowers remain open from three to five hours for a single day, depending on air temperature and light conditions. I never observed flowers open after 10:30 am. Each flower produces one to four seeds in a legume covered with small viscid hairs (Gunn 1980).

Site description

I chose six sites for this study at the Archbold Biological Station in Highlands County, Florida. All sites were located in different micro-habitats (Table 1). Sites A, B, D, and E were 288 m^2 (12 m × 24 m) and sites C and F were 240 m^2 (12 m × 20 m). Each site was defined by its disturbance level and the density of *C. floridana* (Table 1). In this study, human disturbance was any area that had been grazed, plowed, bulldozed, or driven over extensively within the past 15 years. Burned sites were not considered disturbed since fire is a natural part of *C. floridana*'s environment. I was not able to find any high density-undisturbed sites or low density-disturbed sites.

TABLE 1. SITE DESCRIPTIONS USED FOR ANALYSIS OF FIELD EXPERIMENTS. MEAN NUMBER OF FLOWERS ARE BASED ON FLOWER COUNTS MADE ON DAYS WHEN OBSERVATIONS WERE MADE AT EACH SITE. DENSITY RATING REFERS TO THE DENSITY OF FLOWERING C. FLORIDANA.

Site	Mean # Flowers	Density Rating	Disturbance	Habitat Type	Major Vegetation
A	10	Medium	Disturbed	Damaged Scrub	Scrub oak, small herbaceous species
B	43	High	Disturbed	Former pasture	Legumes, weedy herbs, oaks, palmettos, grasses
C	175	High	Disturbed	Road side near a citrus orchard	Surrounded by oaks, weedy herbs, grass
D	3	Low	Undisturbed	Scrubby Flatwoods	Oaks, palmettos, and other Scrubby flatwoods
E	5	Low	Undisturbed	Southern Ridge Sandhill	Turkey oak, hickory, and pine, scrub oaks, palmettos, native herbs
F	13	Medium	Undisturbed	Rosemary Bald	Scrub rosemary, oaks, palmettos, and native herbs

Field Experiments

I observed pollination events in each of the six sites five days a week for all weeks between June 26 and July 28, 1995. Each morning I observed one of the six sites for 2 h. The exact time observations began and ended depended on weather conditions and when flowers began to close. This 2 h period encompassed the majority of the time these flowers were open and the sun was up.

Within a selected site, individual flowering *C. floridana* were observed for 10 minute intervals. In each observation period the number, type, and the time spent by insect visitors at flowers were recorded. I watched between 1 and 8 flowers at a time, depending on the orientation and distance of the plants from my observation point (~ 1 m from the nearest plant). All flowers on a selected plant were observed together. In low density sites, all flowering *C. floridana* were observed at the same time and in high density sites plants were selected arbitrarily. Different plants at high and medium density sites were selected throughout the summer of 1995.

Prior to each ten minute observation period, I recorded the air temperature. After observations were completed each day, the flower dimensions of all observed flowers were measured (Kearns & Inouye 1993), the number of flowers blooming during the observation period were counted, and the approximate vegetation cover in a 1 m radius around each *C. floridana* plant was determined. For vegetation cover I used five arbitrary catagories: 0%-15% (mostly open sand), 15%-30%, 30%-60%, 60%-80% and 80%-100% (the most dense vegetation). Seed set was determined several weeks after visitation observations.

Insect identifications were based on the insect collection at Archbold Biological Station. One time visitors were not always identified.

Statistical Analysis

Splus (MathSoft Inc., Seattle, WA), SAS (SAS Institute Inc., Cary, NC), and Statview (Abacus Concepts Inc., Berkley, CA) were used to analyze the data from this study. Because of the low number of visits of both species, visits by each species were considered independent of one another for all statistical tests. The low number of visits also made it necessary to use a Poisson regression (A Poisson regression is a regression based on a Poisson rather than normal distribution), an analysis of deviance based on a Poisson distribution and analysis of variance (ANOVA) were used to look at differences in pollinator visitation frequency (visitation rate of each species per 10 minute interval) (Snedecor & Cochran 1989). Visits by minor visitors were analyzed with a one-group t-test, and the average time spent on a flower by a visitor was analyzed with ANOVA.

RESULTS

Pollinators

In this study, *C. floridana* flowers were commonly visited only by two types of bees: bumble bees, *Bombus impatiens* (Hymenoptera: Apidae; bumble bees), and several species of metallic green solitary bees in the genus *Augochloropsis* (Hymenoptera: Halictidae). These bees were usually observed ripping holes in the sides of the keel petals of the flowers and vibrating their wings to get pollen out of the floral tube. In a past study on *C. floridana* pollination, *Dialictus pilosus* (Smith) (Hymenoptera: Halictidae), a small solitary bee, was the only major insect visitor to this plant (Gunn et al. 1980). In my entire study, a single *Dialictus nymphalis* (Smith) was observed visiting a *C. floridana* flower and no *D. pilosus* were seen. The observed *Dialictus* sp. did not vibrate its wings to get pollen from the flower and it was too small to touch the stigma of these flowers.

Several minor insect visitors were observed foraging *C. floridana* flowers within my plots, including: *D. nymphalis*, *Geron vitripennis* Loew (Diptera: Bombyliidae) and *Copestylum barei* Loew (Diptera: Syrphidae), and several other unidentified bees and flies. In total, 15 of the 262 observed insect visits were by insects other than *B. impatiens* and *Augochloropsis* spp. Ten of these visits occurred at Site D, four at Site B, and one was observed visiting a single flower at Site F. No ants or beetles were observed visiting flowers, although several beetles were seen eating petals in the afternoon after the flowers were closed. Honey bees were seen foraging at other flowering species in all sites, but were never observed visiting *C. floridana*.

Results of Statistical Analysis

An analysis of deviance test (Snedecor & Cochran 1989) on the total number of insect visits to *C. floridana* flowers showed that the total number of insect visits was significantly different between site, bee type, temperature, percentage vegetation cover, and flower size as well as all interactions with bee type (all p-values ≤ 0.001 except for flower size which had a p-value of 0.0153). Specific effects were determined using a Poisson regression (Table 2).

A single group t-test indicated that there was no significant difference between the number of minor visitors visiting in each site or between disturbed and undisturbed sites. No significant differences (based on an ANOVA test) in the time spent by visitors foraging in 10 minute intervals were found for any variable. There were also no significant differences in seed set for any of the variables listed above.

TABLE 2. POISSON REGRESSION OF THE TOTAL NUMBER OF BEES OF BOTH SPECIES (B. IMPATIENS AND AUGOCHLOROPSIS SP.) VISITING C.FLORIDANA IN 10 MINUTE INTERVALS. NS INDICATES THAT THE EFFECT ON THE TOTAL NUMBER OF BEES VISITING WAS NOT SIGNIFICANT.

Variable	Coefficient	Standard Error	Z-value	P-value
Site A (intercept)	4.65	0.856	5.44	< 0.01
Site B	-0.667	0.287	-2.33	< 0.05
Site C	0.658	0.308	2.13	< 0.05
Site D	-1.43	0.473	-3.02	< 0.01
Site E	-0.561	0.359	-1.56	NS
Site F	-1.56	0.329	-4.75	< 0.01
Bee Type	-6.17	1.57	-3.94	< 0.01
Temperature	-0.237	0.0314	-7.53	< 0.01
60% Cover	-0.895	0.210	-4.27	< 0.01
30% Cover	0.963	0.216	4.45	< 0.01
15% Cover	0.0126	0.393	0.0321	NS
0% Cover	0.477	0.281	1.70	NS
Flower Area (cm^2)	0.614	0.0255	2.41	< 0.05
Site B vs. Bee Type	-1.12	0.724	-1.55	NS
Site C vs. Bee Type	-2.061	0.582	-3.54	< 0.01
Site D vs. Bee Type	1.103	0.767	1.44	NS
Site E vs. Bee Type	0.0650	0.551	0.118	NS
Site F vs. Bee Type	0.573	0.494	1.16	NS
Temp vs. Bee Type	0.242	0.0588	4.12	< 0.01
60% Cover vs. Bee Type	0.483	0.478	1.010	NS
30% Cover vs. Bee Type	-1.71	0.437	-3.920	< 0.01
15% Cover vs. Bee Type	-0.0444	0.894	-0.0496	NS
0% Cover vs. Bee Type	-0.264	0.445	-0.594	NS

[1]Null Deviance: 1304.3 on 1289 degrees of freedom
[2]Residual Deviance: 894.9 on 1267 degrees of freedom

For the Poisson regression shown in Table 2, the dependent variable is the number of bees visiting during a 10 min observation period. All main effects and interaction effects are compared to the values for Site A at 0°C (the assumed intercept for this model), 80-100% vegetation cover, and with a flower area of 1 cm^2. For all of the main effects, negative Z-values indicate that there were fewer total visits than expected. The significant negative Z-value for bee type indicates that there were significantly fewer *Augochloropsis* than expected. The negative Z-value for the temperature indicates that the number of total insect visits decreased as the temperature increased. The significant positive Z-value for flower area indicates that flowers with larger flower areas had significantly more insect visits than smaller flowers. For interaction effects, negative values indicate fewer *Augochloropsis* than expected. Positive values indicate fewer *B. impatiens* than expected. The significant negative interaction effect

of bee type and temperature shows that as the temperature increased *B. impatiens* visitation decreased significantly, while the number of *Augochloropsis* visitation did not differ significantly according to temperature. The significance of the main temperature effect is due mainly to the change in the number of *B. impatiens* visiting flowers at higher temperatures.

The results of the Poisson regression also indicate that sites B, D, and F had significantly fewer total pollinator visits than expected. Site C had significantly more total visits than Site A but significantly fewer *Augochloropsis* visitors than expected (Table 2). The fitted means in Table 3 show differences between the number of visits to each site by *Augochloropsis* and *B. impatiens*. The mean number of *Augochloropsis* visits are lower than the mean number of *B. impatiens* visits at all sites except site D, a low density undisturbed site. The mean number of *B. impatiens* visiting is highest in the three disturbed sites A, B, and C.

Both bee types showed no vegetation preferences, although each type of bee was observed visiting significantly more frequently in certain vegetation covers. The Poisson analysis indicates a significantly lower number of visits by both species to areas of 60-80% vegetation cover and significantly more total visits to 30-60% vegetation cover but significantly fewer *Augochloropsis* visits indicating that the total increase in visitation in 30-60% vegetation cover was due to high visitation rates by *B. impatiens* (Table 4). An analysis of variance, type three sums of squares, indicates that there is a significant interaction effect of site and percentage vegetation on the number of total bee visits (p-value = 0.0056) and with the number of *B. impatiens* visits (p-value = 0.0001). The frequencies of visits by each bee type within each vegetation coverage are shown in Table 5.

Discussion

Both *B. impatiens* and *Augochloropsis* sp. exhibited complex foraging behavior while visiting *C. floridana* flowers. Statistical results indicate that both pollinators preferred larger flowers. This preference could indicate that the pollinators are using flower size to identify flowers with larger pollen or nectar rewards. The results also indicate that the number of bees visiting decreased significantly as temperature in-

Table 3. Mean Number Of Visitors Per Flower Based On All Observed Visits. Mean Flower Number Is The Mean Number Of C. Floridana Blooming In Each Site Each Day. The Visitation Means Are Low Due To The High Number Of Time Intervals Observed With No Visits.

		Bombus Impatiens			*Augochloropsis*		
Site	Mean Flower #	Mean	Std Dev	H	Mean	Std Dev	H
A	10	0.70	1.14	33	0.47	1.14	22
B	43	0.53	0.90	37	0.06	0.24	8
C	175	0.56	0.96	62	0.06	0.26	11
D	3	0.16	0.62	7	0.21	0.47	9
E	5	1.40	0.70	20	0.18	0.44	9
F	13	0.19	0.49	13	0.15	0.47	16

TABLE 4. MEAN NUMBER OF VISITS BY EACH MAJOR BEE SPECIES OBSERVED IN EACH PERCENTAGE VEGETATION COVERAGE FOR ALL SITES COMBINED.

	Bombus Impatiens			*Augochloropsis*			*Total*		
Veg Cover	Mean	Std Dev	N	Mean	Std Dev	N	Mean	Std Dev	N
0-15%	0.49	0.95	15	0.20	0.41	20	0.69	0.99	35
15-30%	0.25	0.50	6	0.07	0.26	11	0.69	1.05	17
30-60%	0.66	1.04	84	0.09	0.33	13	0.31	0.55	97
60-80%	0.20	0.38	15	0.07	0.49	5	0.75	1.12	20
80-100%	0.57	0.10	43	1.22	2.26	26	0.87	1.40	69

creased. It is likely that the bees' visitation rates decreased as flowers began to close later in the morning when temperatures were warmer and it is possible that floral rewards were depleted later in the morning.

Although the total number of insects visiting each site varied significantly for all but one site, only site C had a significantly different number of *Augochloropsis* visiting. Site C had the greatest number of flowering *C. floridana* and was a highly disturbed site on the edge of a citrus orchard. The high number of *B. impatiens* visiting this site could have been related to the larger number of flowers at site C (Table 3), proximity to other flower sources, or the presence of near by nests. The low number of *Augochloropsis* forging at this site could have been caused by overwhelming competition from the abundant *B. impatiens* at this site.

The amount of vegetation cover most often visited by each type of pollinator did not follow a pattern except when considered in connection with the sites. Vegetation preferences of *Augochloropsis* sp. were not related to sites dominated by a particular vegetation coverage while *B. impatiens* visits were (Tables 4 and 5).

There was no clear indication from this study that the foraging patterns of *C. floridana*'s pollinators were directly affected by the level of human disturbance in the area. Seed set levels did not differ significantly between sites, indicating that despite the significant difference in pollinator visitation between sites, all populations observed in this study were receiving similar pollination services. *Bombus impatiens* were observed visiting sites A, B, and C more frequently than in the undisturbed sites, but this was not a significant difference. *Augochloropsis* was found visiting most frequently in disturbed site A and least frequently at the other two disturbed sites B and

TABLE 5. THE FREQUENCY OF CHAPMANNIA FLORIDANA OBSERVED IN EACH SITE IN EACH VEGETATION COVER.

Vegetation Cover	Site A	Site B	Site C	Site D	Site E	Site F
0-15%	9	4	30	59	12	0
15-30%	1	0	170	30	0	0
30-60%	0	39	49	42	26	39
60-80%	0	0	0	0	0	76
80-100%	33	0	0	21	12	20

C (Table 3). Sites B and C had the highest flowering densities and high visitation rates by *B. impatiens* indicating that competition may play a role in the visitation rates of *Augochloropsis* to this plant. Site A was bordered on three sides by undisturbed habitat which may have contributed to the visitation rates of *Augochloropsis*.

The visitation rates of these pollinators were undoubtedly affected by factors not determined in this study. From the data collected, the factors that appear to play the greatest role in determining visitation rates to *C. floridana* are temperature, flower size, and flowering density. Although disturbance does not seem to strongly affect the pollinator visitation rates or resulting seed set in this plant, the higher numbers of flowers in disturbed areas clearly attract more pollinators than the low density undisturbed populations do. This relationship between flowering density and visitation rate may be important to the reproductive success of this plant species in the long term or at least in years with fewer pollinators.

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REFERENCES CITED

AIZEN, M. A., AND P. FEINSINGER. 1994. Forest fragmentation, pollination, plant reproduction in a chaco dry forest, Argentina. Ecol. 75: 330-351

ARMESTO, J. J., AND S. T. A. PICKETT. 1985. Experiments on disturbance in old-field plant communities: impacts on species richness and abundance. Ecol. 66: 230-240.

BRIAN, A. D. 1959. Differences in the flowers visited by four species of bumble-bees and their causes. J. Animal Ecol. 26: 71-98.

COFFIN, D. P., AND W. K. LAUENROTH. 1988. The effects of disturbance size and frequency on a shortgrass plant community. Ecol. 69: 1609-1617.

CRUZAN, M. B., P. R. NEAL, AND M. F. WILSON. 1988. Floral display in phyla-incisa consequences for male and female reproductive success. Evolution 42: 505-515.

FEINSINGER, P., J. H. BEACH, Y. B. LINHART, W. H. BUSBY, AND G. MURRAY. 1987. Disturbance, pollinator predictability, and pollination success among Costa Rican cloud forest plants. Ecol. 68: 1297-1305.

FOSTER, D. R., AND T. M. ZEBRYK. 1993. Long-term vegetation dynamics and disturbance history of a Tsuga-dominated forest in New England. Ecol. 74: 982-998.

GLITZENSTEIN, J. S., P. A. HARCOMBE, AND D. R. STRENG. 1986. Disturbance, succession, and maintenance of species diversity in an east Texas forest. Ecological Monographs 56: 243-258.

GUNN, C. R., E. M. NORMAN, AND J. S. LASSETTER. 1980. *Chapmannia floridana* Torrey and Gray (Fabaceae). Brittonia 32: 178-185.

HALPERN, C. B. 1988. Early successional pathways and the resistance of forest communities. Ecology, 69: 1703-1715.

KEARNS, C. A., AND D. W. INOUYE. 1993. Techniques for pollination biology. The University Press of Colorado. Niwot, CO.

SNEDECOR, G. W., AND W. G. COCHRAN. 1989. Statisitcal Methods: Eighth edition. Iowa State University Press, Ames.

VOLATILES ATTRACTIVE TO THE MEXICAN FRUIT FLY (DIPTERA: TEPHRITIDAE) FROM ELEVEN BACTERIA TAXA

DAVID C. ROBACKER[1], ADELAIDO J. MARTINEZ[2], JOSE A. GARCIA[1], AND ROBERT J. BARTELT[3]

[1]Crop Quality and Fruit Insects Research, USDA, Agricultural Research Service 2301 South International Blvd., Weslaco, TX 78596

[2]Mission Biological Control Center, USDA, Animal and Plant Health Inspection Service, Box 2140, Mission, TX 78573

[3]National Center for Agricultural Utilization Research, Bioactive Agents Research, USDA, Agricultural Research Service, 1815 N. University Street, Peoria, IL 61604

ABSTRACT

Filtrates of 11 bacteria representing 4 higher taxonomic categories were attractive to Mexican fruit flies, *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in laboratory bioassays. All bacterial filtrates were more attractive at pH 9 than at pH 5 although filtrates at pH 5 were more attractive than water controls. The effects of pH on attractiveness of filtrates were consistent with an hypothesis that attractive principals of bacterial filtrates were various nitrogen-containing compounds and carboxylic acids that became more volatile at specific pH's resulting in increased attractiveness. Volatiles produced by the bacteria were sampled by solid-phase microextraction and identified by GC and GC-MS. Attractive principals identified were ammonia, aliphatic amines, pyrazines, imines, and acetic acid. Relative amounts of most of the chemicals were not closely tied to bacteria taxonomy.

Key Words: *Anastrepha ludens*, attractants, bacteria, amines, acetic acid, solid phase microextraction (SPME)

RESUMEN

Los filtrados de 11 bacterias que representan a 4 categorías altas taxonómicas atrayeron a las mosca de la fruta mexicana, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), en bioensayos de laboratorio. Todos los filtrados bacteriales fueron más atrayentes al pH 9 que al pH 5, aunque los filtrados de pH 5 fueron más atrayentes que el testigo. Los efectos del pH sobre la atracción de los filtrados fueron consistentes con la hipótesis de que los químicos atrayentes de los filtrados bacteriales eran varios compuestos de nitrógeno y ácido carboxílico que se hacen más volátiles a pH específicos resultando en un aumento en su atracción. Volátiles producidos por las bacterias fueron colectados usando micro-extracción de fase sólida y fueron identificados por cromatografía de gas y espectrómetro de masa. Los químicos atrayentes fueron identificados como amoníaco, aminos alifáticos, pirazines, imines y ácido acético. Las concentraciones de varios químicos no estuvieron muy cercanamente relacionadas a la taxonomía de las bacterias.

Volatile chemicals from bacterial fermentations attractive to fruit flies have come under increased scrutiny during the last 10 years as possible attractants. Several papers have reported identification of ammonia from bacteria cultures (Gow 1954, Drew

& Fay 1988, Robacker & Flath 1995). Ammonia has long been known as a powerful attractant for fruit flies (Jarvis 1931). Other studies have resulted in identification of additional volatile chemicals from cultures of bacteria (Hayward et al. 1977, Lee et al. 1995, Robacker & Flath 1995, DeMilo et al. 1996, Robacker & Bartelt 1997).

Attractiveness of chemicals (other than ammonia) identified from bacterial odors has been demonstrated in only a few studies. Drew (1987) demonstrated that the bacteria-produced chemicals 2-butanone and 1-butanol were attractive to *Bactrocera tryoni* (Froggatt), purportedly because of their structural similarity to the parapheromone cuelure. Robacker & Flath (1995) and Robacker & Bartelt (1997) identified and demonstrated attractiveness for ammonia, several amines, imines, pyrazines and acetic acid from three species of bacteria.

In this research, principals attractive to the Mexican fruit fly, *Anastrepha ludens* (Loew) were identified from 11 strains of bacteria that had not been investigated before. A 3-step procedure was used. First, attractiveness of each bacterium was verified. Second, the effect of fermentation pH on attractiveness was determined to characterize the classes of chemicals involved in the attraction response. Third, chemicals that fit the attractive-principal profile as determined in the pH tests were identified and quantified.

The purposes of the work were to determine if similar bacteria produce similar attractive chemicals and conversely if dissimilar bacteria produce different, perhaps novel, chemicals. Novel chemicals, along with knowledge gained in this work of general patterns of volatiles produced by attractive bacteria, could be used in development of new lures for fruit flies.

Materials and Methods

Insects and Test Conditions

Flies used to test attractiveness of bacterial preparations were from a culture that originated from yellow chapote, *Sargentia greggii* Coult., (Rutaceae), fruit, a native host of the fly, collected in Nuevo Léon, Mexico, in 1987. Fly handling and laboratory maintenance were as described in Robacker & Flath (1995). Flies were sugar-fed and protein-starved (since eclosion) because previous work indicated this physiological state maximizes attraction to bacterial odor (Robacker & Garcia 1993). Flies were used when 6-10 days old.

Bacterial Preparations

Bacteria species used in this work were: *Enterobacter cloacae* (Jordan); *Alcaligenes faecalis faecalis* Castellani & Chalmers; *Micrococcus luteus* Schroeter; *Bacillus sphaericus* Meyer & Neide; *B. subtilis* Ehrenberg; *B. megaterium* de Bary; *B. popilliae* Dutky; and *B. thuringiensis* Berliner subspecies *shandongiensis*, *coreanensis*, *konkukian*, and *darmstadiensis*. Strains obtained from the American Type Culture Collection (ATCC) (Rockville, MD) were: *E. cloacae* (ATCC strain 961); *A. f. faecalis* (ATCC strain 8750); *M. luteus* (ATCC strain 23259); *B. sphaericus* (ATCC strain 4525); *B. subtilis* (ATCC strain 6051); *B. megaterium* (ATCC strain 14581); and *B. popilliae* (ATCC strain 14706). Strains obtained from the Institut Pasteur (Paris, France) were: *B. t. shandongiensis* (strain 22001); *B. t. coreanensis* (strain 25001); and *B. t. konkukian* (strain 34001). *B. t. darmstadiensis* (strain GUAT1) was obtained from a soil sample from Guatemala (Martinez et al. 1997).

These taxa were chosen to survey volatile chemicals attractive to the Mexican fruit fly produced by bacteria over both broad and narrow levels of classification. The four

genera represent four distinct higher taxonomic categories: *Enterobacter*, facultatively anaerobic, gram-negative rods; *Alcaligenes*, aerobic gram-negative rods and cocci; *Micrococcus*, gram-positive cocci; and *Bacillus*, endospore-forming, gram-positive rods (Holt 1984). The five species of *Bacillus* and the 4 subspecies of *B. thuringiensis* allow an analysis of volatiles produced by more closely related strains.

All ATCC strains were fermented in trypticase soy broth (BBL, Baltimore, MD) in a shaker for 5 days at 30°C. *B. t. shandongiensis*, *B. t. konkukian*, and *B. t. darmstadiensis* were fermented in Bacto nutrient broth (DIFCO Laboratories, Detroit, MI) in a shaker for 3, 6, and 3 days, respectively, at 30°C. *B. t. coreanensis* was fermented in a shaker in a growth medium (medium B) developed by Dulmage et al. (1970) for 5 days at 30°C. Fermentation times and media were based on preliminary bioassays showing maximum attractiveness for these times and media. Bacterial cultures were centrifuged and the resulting supernatants were filtered to remove bacterial cells as described previously (Robacker & Flath 1995). Martinez et al. (1994) demonstrated that filtered and unfiltered cultures of several bacteria species were equally attractive indicating that the attractants were dissolved in the filtrate. Because attractive chemicals were retained in filtrate, there was no concern that bacterial cultures may have been too old to contain actively growing cells at the time they were harvested. Five fermentations of each bacterium were conducted.

Evaluation of Attractiveness of Bacterial Filtrates

Attractiveness of each bacterial filtrate was evaluated using cage-top bioassays as described in Robacker & Flath (1995) with water as the control. The three culturing media were also tested against water. Briefly, the bioassay was conducted by placing two filter paper triangles containing 10 μl of bacterial or growth-medium filtrate and two papers containing 10 μl of water on the top of an insect cage. The filter papers were raised 5 mm above the cage top using plastic rings. Each bioassay cage contained 180-200 flies. The number of flies beneath each filter paper was counted once each minute for 10 min following application of the test materials to the papers. The 11 bacterial filtrates and the three growth-medium filtrates were tested in random order. Two bioassay replications were conducted for each fermentation.

To analyze bioassay results, total flies counted at water control papers were subtracted from total flies counted at treatment papers for each cage-top bioassay to obtain a bioassay count difference. The two bioassay count differences per fermentation were averaged. The resulting fermentation-level means were then used as data points in one-way analysis of variance (ANOVA) using SuperANOVA (Abacus Concepts 1989) to compare attractiveness of the various bacteria. Means separations were conducted by Fisher's protected least significant difference method (LSD).

Effects of pH on Attractiveness of Bacterial Filtrates

For one of the five fermentations, the pH of bacterial filtrates and growth-medium filtrates was adjusted to 5, 7, and 9 with 85% phosphoric acid (Fisher Scientific, Fair Lawn, NJ) or saturated sodium hydroxide (Fisher). Attractiveness of each pH treatment was tested against water controls using cage-top bioassays. The purpose was to determine if attractive principals of the bacterial filtrates were nonionizing chemicals that would not be affected by pH or chemicals that ionize into relatively nonvolatile forms and therefore contribute little to attractiveness at certain pH's. Thus, carboxylic acids (pKa's 4-5) would contribute little to attractiveness at pH 9; ammonia (pKa 9.2) and amines (aliphatic amines, pKa's 10-11) would contribute little to attractiveness at pH 5;

imines (1-pyrroline, pKa 6.7) would be most attractive at pH > 7; and pyrazines (pyrazine, pKa 0.6) and nonionizing compounds would be volatile and attractive throughout the pH range tested (pKa's from March 1968, Amoore et al. 1975, Weast 1976).

Each replication of the experiment consisted of one cage-top bioassay for each of the three pH treatments of all filtrates, tested in random order. Ten replications of the experiment were conducted. Each bacterial filtrate or growth medium filtrate was analyzed separately by one-way ANOVA to compare pH effects. Bioassay count differences (described above) were used as data and pH means were separated by Fisher's protected LSD.

Volatiles Sampling

Chemicals were sampled in the headspace above filtrates of bacteria and uninoculated growth media by solid phase microextraction (SPME) with a 100 μm polydimethylsiloxane-coated fiber (Supelco, Inc., Bellefonte, PA). The fiber was inserted through a septum into the headspace above 1 ml of filtrate in a 4 ml vial for 30 min at 21-23°C before analysis by GC or at 25-27°C before analysis by GC-MS.

Chemical Identifications

Two methods were used to identify chemicals. For bacteria that had volatiles profiles similar to those of three species of bacteria studied previously (Robacker & Flath 1995, Robacker & Bartelt 1997), chemicals were identified by matching GC retention times and detector response ratios with those of standards. The gas chromatograph was a Shimadzu GC-17A (Shimadzu Scientific Instruments, Inc., Columbia, MD) with flame ionization (FID) and flame thermionic (FTD) (Model FTD-17) detectors. A DB-1 capillary column (J & W Scientific, Folsom, CA) with a 5 μm film was used. FTD/FID response ratios were obtained to establish the presence of C-N bonds. A detailed description of the GC method can be found in Robacker & Bartelt (1997).

For bacteria that had nitrogen-containing peaks not observed in previous studies, chemicals were identified by GC-MS. GC-MS data were acquired using a Hewlett Packard 5890 GC with a HP 5970 mass selective detector (electron energy = 70 eV). GC-MS identifications were based on computer matching of unknown spectra with those in the Wiley 138K Mass Spectral Database (John Wiley and Sons, New York). Identifications were authenticated by comparing spectra with those of standards for most chemicals. A DB-1 column with a 5 μm film also was used. A detailed description of the GC-MS method can be found in Robacker & Bartelt (1997). Chemicals were sampled from headspace above unaltered filtrates, and filtrates to which sodium hydroxide was added to enhance volatilization of basic compounds.

GC Analysis of Headspace Volatiles

Relative amounts of eight attractive chemicals in the headspace of bacterial filtrates (at unaltered pH of filtrates) and the 3 uninoculated growth media filtrates were measured. This analysis was conducted to compare amounts of the various chemicals produced by different bacteria taxa.

Analyses of seven nitrogen-containing chemicals were conducted using the Shimadzu GC-17A with FTD as described above. GC peak heights were measured using Millennium 2010 Chromatography Manager software (Waters Corporation, Milford, MA). Two headspace analyses were conducted for each of five fermentations of the 11 bacteria strains and the three growth media. One headspace analysis per fermentation was also done using FID for acetic acid.

Peak heights from the 2 GC-FTD analyses were averaged to give a fermentation-level mean. A one-way ANOVA was conducted for each chemical to assess amounts in headspace above the various filtrates, using the fermentation-level means as data points. ANOVA was also conducted to analyze individual GC-FID peak heights (not means) of acetic acid in the various filtrates. Means were separated by Fisher's protected LSD.

GC Standards

Ammonium carbonate, 2-methylpropanamine, 2-methylbutanamine, 3-methylbutanamine, 2-phenylethanamine, pyrazine, methylpyrazine, 2,3-dimethylpyrazine, 2,5-dimethylpyrazine and trimethylpyrazine were obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI). Methylamine HCl, trimethylamine HCl, and cyclohexylamine were obtained from Sigma Chemical Company (St. Louis, MO). Acetic acid was obtained from Fisher Scientific (Pittsburgh, PA) and 2-methylpropanoic acid and 3-methylbutanoic acid were obtained from Eastman Chemical Products, Inc. (Kingsport, TN).

Five imines were synthesized. 1-pyrroline was synthesized by acid hydrolysis of 4-aminobutyraldehyde diethyl acetal (Aldrich) according to methods of Schopf & Oechler (1936). 2, 3, 4, 5-tetrahydropyridine was synthesized by reaction of N-chlorosuccinimide (Aldrich) with piperidine (Matheson, Coleman & Bell, Norwood, OH, 98%) to form N-chloropiperidine, followed by elimination of HCl from N-chloropiperidine with KOH (Fisher) (Quick & Oterson 1976). Other imines were prepared by the general method of addition of aldehydes to primary amines (March 1968). N-isopentylidene-3-methylbutanamine was prepared by addition of 3-methylbutanal (Aldrich) to 3-methylbutanamine in methylene chloride (Fisher) at room temperature. Anhydrous sodium sulfate (EM Science, Cherry Hill, NJ) was then added to clear turbidity due to water formed as a reaction byproduct. Likewise, N-phenylmethylene-2-methylpropanamine and N-phenylmethylene-3-methylbutanamine were prepared by addition of benzaldehyde (Aldrich) to 2-methylpropanamine and 3-methylbutanamine, respectively.

Results and Discussion

Attractiveness of Bacterial Filtrates

All bacterial filtrates were significantly more attractive than uninoculated media ($F = 7.8$; df = 13,56; $P < 0.0001$) (Fig. 1). No major differences in attractiveness occurred among the bacteria strains except that the *B. thuringiensis* group was generally less attractive than the others. In this work and in previous studies (Robacker & Flath 1995, Robacker & Bartelt 1997), cultures of many species, genera, and higher taxa of bacteria have been demonstrated attractive to Mexican fruit flies. We conclude that Mexican fruit fly attraction to bacteria cultured in aqueous laboratory media is a general phenomenon.

Effects of pH on Attractiveness of Bacterial Filtrates

The pH's of bacterial filtrates, before manipulation with phosphoric acid or sodium hydroxide, generally were between 7.8 and 9.2. These solutions contained more equivalents of bases than acids. Exceptions were *M. luteus* and *B. t. coreanensis* that had pH's of 7.1 and 5.3, respectively. The *B. t. coreanensis* filtrate contained more acids than bases. Uninoculated growth media had pH's between 6.7 and 7.0.

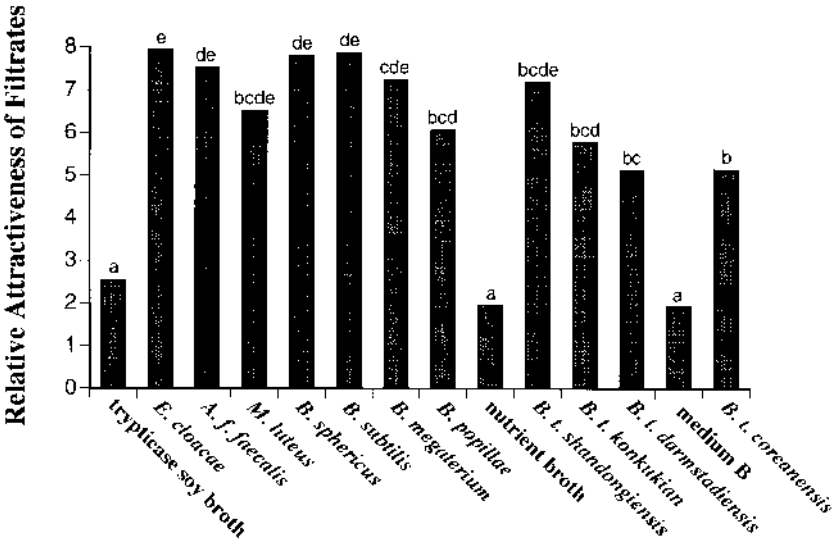

Fig. 1. Attractiveness of bacterial filtrates to *A. ludens* in cage-top bioassays. Relative attractiveness = mean counts at papers containing filtrates, divided by mean counts at papers containing water. Bars with the same letter are not significantly different from each other by Fisher's protected LSD ($P < 0.05$).

Attractiveness of all filtrates was greatly affected by changing filtrate pH (smallest $F = 9.2$; df = 2,27; $P < 0.001$ for nutrient broth) (Fig. 2). Most filtrates at pH 7 and all at pH 9 were more attractive than filtrates at pH 5. This is a critical result because it indicated that the most important attractive principals are compounds containing protonizable nitrogen with pKa's of 7 or above because these chemical classes would be largely ionized and nonvolatile at pH 5.

All filtrates at pH 5 except *E. cloacae* and *A. f. faecalis* were significantly more attractive than water controls, although attractiveness of most was not high compared with attractiveness at pH 7-9. However, *B. popillae* and *B. t. coreanensis* filtrates at pH 5 were much more attractive than water controls (paired *t*-test for *B. popillae*, $t = 7.5$, df = 9, $P < 0.001$; for *B. t. coreanensis*, $t = 6.6$, df = 9, $P < 0.001$). Chemicals that could account for the attractiveness of filtrates at pH 5 include carboxylic acids, pyrazines, and various nonionizing chemicals such as hydrocarbons, alcohols, aldehydes, ketones, esters, etc., that would exist largely in nonionized, volatile forms at pH 5.

Chemical Identifications

Because all filtrates were most attractive at pH 9, chemical identifications were focused on chemicals containing protonizable nitrogen. The relatively low attractiveness of most filtrates at pH 5 indicated that nonionizing chemicals probably did not play major roles in attractiveness and were not identified in this work. However, the moderate attractiveness of the naturally acidic *B. t. coreanensis* filtrate also led us to identify carboxylic acids from the bacterial volatiles.

Filtrates of *A. f. faecalis*, *B. popillae*, and *B. t. coreanensis* were analyzed by GC-MS. Nitrogen-containing chemicals and carboxylic acids that were identified are

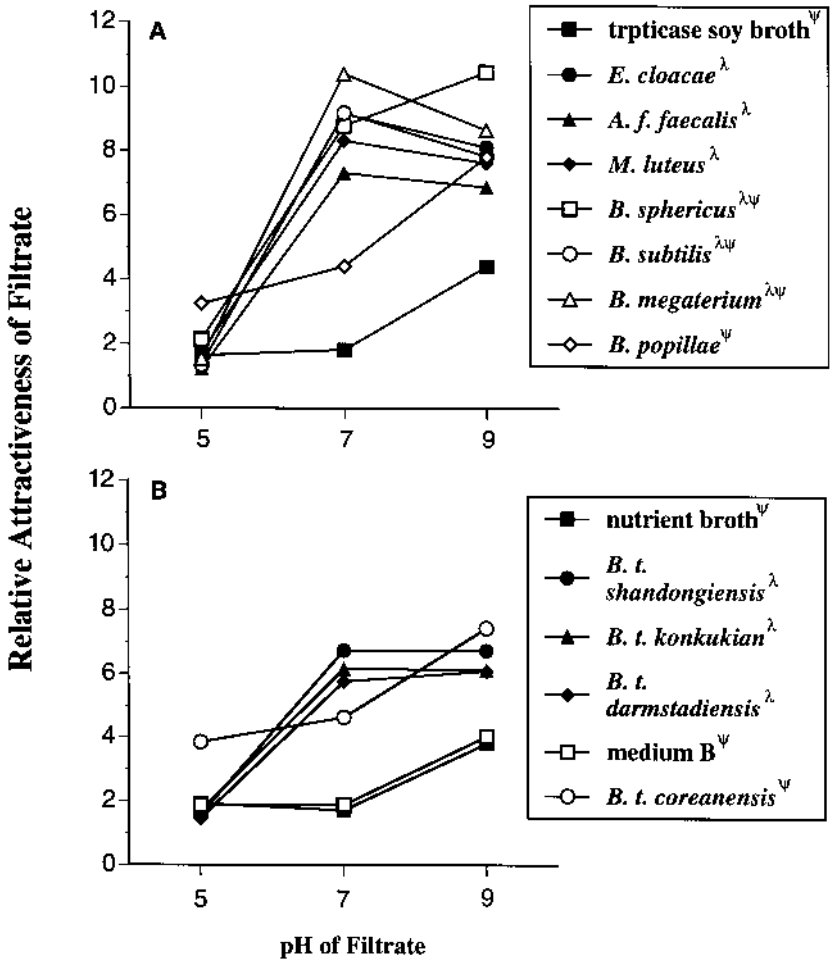

Fig. 2. Attractiveness of pH altered bacterial filtrates to *A. ludens* in cage-top bioassays. Relative attractiveness = mean counts at papers containing filtrates, divided by mean counts at papers containing water. λ indicates significant difference in attractiveness between pH 5 and 7; ψ indicates significant difference in attractiveness between pH 7 and 9; $P < 0.05$ by Fisher's protected LSD.

shown in Table 1. Of these chemicals, trimethylamine, 2-methylpropanamine, 3-methylbutanamine, 2-methylbutanamine, methylpyrazine, 2,5-dimethylpyrazine and trimethylpyrazine had been reported from one or more of the bacteria *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Citrobacter freundii* (Robacker & Flath 1995, Robacker & Bartelt 1997). Lee et al. (1995) and DeMilo et al. (1996) identified numerous pyrazines from headspace of *K. pneumoniae* and *C. freundii* including most of those in Table 1, and others. Acetic acid had been reported from headspace of *S. aureus* (Robacker & Flath 1995) and 2-methylpropanoic acid and 3-methylbutanoic acid from

TABLE 1. NITROGEN-CONTAINING CHEMICALS AND CARBOXYLIC ACIDS IDENTIFIED BY GC-MS IN HEADSPACE ABOVE UNALTERED BACTERIAL FILTRATES AND FILTRATES WITH ADDED NAOH[1].

	Aff	Aff + NaOH	Bp	Bp + NaOH	Btc	Btc + NaOH
trimethylamine	-	+	-	-	-	+
2-methylpropanamine	+	+	++	+++	+	+
3-methylbutanamine	+	++	++	+++	-	+
2-methylbutanamine	-	-	-	+++	-	-
cyclohexylamine	-	+	-	+	-	+
2-phenylethanamine[2]	-	-	+	+	-	-
N-isopentylidene-3-methylbutanamine	-	-	+	++	-	-
N-phenylmethylene-2-methylpropanamine	-	-	-	++	-	-
N-phenylmethylene-3-methylbutanamine	-	-	-	+++	-	-
2,4,5-trimethyl-3-oxazoline[2]	-	-	-	+	-	++
methylpyrazine	+	+	+	+	-	+
2,5-dimethylpyrazine	++	++	++	++	++	++
2,3-dimethylpyrazine	-	-	-	-	+	+
trimethylpyrazine	+	+	+	+	++	++
tetramethylpyrazine[2]	-	-	+	+	+	+
ethyldimethylpyrazine isomer[2]	-	-	+	+	+	+
diethylmethylpyrazine isomer[2]	-	-	-	-	+	+
acetic acid	-	-	-	-	++	-
2-methylpropanoic acid	-	-	-	-	+	-
3-methylbutanoic acid	-	-	-	-	++	-

[1]Aff = *A. f. faecalis*, Bp = *B. popillae*, Btc = *B. t. coreanensis*, - = not detected above baseline, + = trace (< 500 area counts), ++ = minor (500 - 5000 area counts), +++ = major (> 5000 area counts).
[2]Good library match, but not verified with standard.

headspace of *K. pneumoniae* (Lee et al. 1995, Robacker & Bartelt, 1997). Cyclohexylamine, 2-phenylethanamine, N-isopentylidene-3-methylbutanamine, N-phenylmethylene-2-methylpropanamine, N-phenylmethylene-3-methylbutanamine, and 2,4,5-trimethyl-3-oxazoline had not been reported from any bacteria, to our knowledge. N-isopentylidene-3-methylbutanamine has been found in volatiles of NuLure, a protein bait for fruit flies (Flath et al. 1989).

Additional chemicals were identified by GC-FID and GC-FTD. These were ammonia, pyrazine, 1-pyrroline and 2,3,4,5-tetrahydropyridine. The latter 3 chemicals were identified by the highly sensitive GC-FTD technique. They had been identified previously by GC-MS in headspace of other bacteria (Robacker & Flath 1995, Robacker & Bartelt 1997). Ammonia was also identified from these other bacteria by GC-FID and GC-FTD. It was not identified by GC-MS because of its low molecular weight. All four chemicals were verified by GC analyses of standards.

Attractiveness of Chemicals Identified by GC-MS/GC Analyses

Many of the chemicals have been evaluated for attractiveness to Mexican fruit flies (Robacker & Warfield 1993, Robacker & Flath 1995, Robacker et al. 1996, Robacker & Bartelt 1997, Robacker et al. 1997). Ammonia, 2-methylpropanamine, 3-methylbutanamine, 2-methylbutanamine and acetic acid were 2-3 times more attractive than water controls. Trimethylamine, 1-pyrroline, 2,3,4,5-tetrahydropyridine, pyrazine, 2,5-dimethylpyrazine, and trimethylpyrazine were 1.1 to 1.5 times more attractive than water. Methylpyrazine, 2-phenylethananime, and 3-methylbutanoic acid were not attractive. N-isopentylidene-3-methylbutanamine was not attractive to four species of fruit flies that did not include the Mexican fruit fly in olfactometer tests (Flath et al. 1989). The other chemicals in Table 1 have not been tested for attractiveness to fruit flies.

Comparison of Attractants Produced by Bacteria Taxa

Results of the GC-FTD analyses of 8 attractive components of bacterial volatiles are shown in Tables 2 and 3. Ammonia was produced in about the same amounts by all of the bacteria. Emission of most other chemicals varied greatly from strain to strain. Some generalizations can be observed in the tables regarding production of some chemicals by closely related taxa. For example, the only two bacteria that produced large amounts of 2-methylpropanamine and 3-methylbutanamine were in the genus *Bacillus*. However, the other three species of *Bacillus* produced very little of these two chemicals. Thus, chemicals were not produced in similar amounts by related taxa in many cases. In other cases, chemicals were produced by distantly related taxa but not by closely related ones. An example of this is trimethylpyrazine that was produced in relatively high amounts by *E. cloacae* and *B. t. coreanensis* but in lower amounts by other *Bacillus* and even other strains of *B. thuringiensis*.

Some of the differences in volatiles production may be attributable to differences in culturing media, but in other cases, bacteria grown on different media produced the same chemicals. For example, highest amounts of 2, 5-dimethylpyrazine were produced by bacteria cultured on trypticase soy broth and highest amounts of trimethylamine were produced by the *B. thuringiensis* strains cultured on nutrient broth. On the other hand, the trimethylpyrazine example discussed above is a case in which two bacteria grown on different media produced about the same amount of a chemical.

The discussion of similarities and differences in volatiles profiles can be expanded by including results of previous analyses of bacteria volatiles. Profiles of *K. pneumoniae* and *C. freundii* (Robacker & Bartelt 1997), members of the family Enterobacteriaceae along with *E. cloacae*, differed from the profile of *E.òacae* (Tables 2 and 3) in amounts of 3-methylbutanamine, 2, 5-dimethylpyrazine and trimethylpyrazine but were similar with regard to several other chemicals. Also, amounts of trimethylamine, 3-methylbutanamine and acetic acid produced by *S. aureus* (Robacker & Flath 1995), a member of the Micrococcaceae along with *M. luteus*, differed dramatically from amounts produced by *M. luteus* (Tables 2 and 3). Conversely, acetic acid production by *S. aureus* was high as in *B. t. coreanensis*, a species that is not in the Micrococcaceae. These examples suggest a great diversity of metabolic pathways in bacteria that do not tie closely to currently held views of taxonomic relatedness.

Note that peak sizes do not reflect absolute amounts of different chemicals. For example, the small ammonia peaks indicate filtrate concentrations in the 100 μg/ml to 1 mg/ml range while the large 2-methylpropanamine peaks indicate concentrations only in the 1 to 10 μg/ml range (Robacker & Bartelt, 1997).

TABLE 2. PEAK HEIGHTS (MV) OF CHEMICALS IN HEADSPACE ABOVE FILTRATES OF UNINOCULATED GROWTH MEDIA AND 11 BACTERIA STRAINS GROWN ON THOSE MEDIA[1].

	ammonia	trimethyl-amine	2-methyl-propanamine	3-methyl-butanamine
trypticase soy broth	0.14 abc	0.1 a	0.2 a	0.1 a
E. cloacae	0.30 cde	0.6 a	0.4 a	0.2 a
A. f. faecalis	0.30 cde	0.4 a	0.9 a	0.6 a
M. luteus	0.29 cde	0.6 a	1.0 a	0.7 a
B. sphericus	0.32 de	0.5 a	126.1 b	61.7 b
B. subtilis	0.35 de	0.8 a	3.2 a	1.4 a
B. megaterium	0.28 cde	0.7 a	0.8 a	0.4 a
B. popilliae	0.25 bcd	0.6 a	107.4 b	53.6 b
nutrient broth	0.05 a	1.7 a	0.4 a	0.2 a
B. t. shandongiensis	0.25 bcd	14.6 b	1.5 a	0.8 a
B. t. konkukian	0.39 de	29.7 c	0.4 a	0.3 a
B. t. darmstadiensis	0.44 e	34.2 c	0.5 a	0.3 a
medium B	0.09 ab	0.4 a	0.3 a	0.2 a
B. t. coreanensis	0.34 de	2.0 a	0.4 a	0.2 a

[1]Flame thermionic detection. For a given chemical, mean peak heights followed by the same letter were not significantly different from each other by Fisher's protected LSD ($P < 0.05$, n = 5 fermentations).

Attractive Principals vs. pH of Filtrates

Experiments with filtrate pH indicated the importance of chemicals containing protonizable nitrogen to the attractiveness of the bacterial filtrates (Fig. 2). Ammonia, amines, imines, and pyrazines, all chemicals previously demonstrated attractive to Mexican fruit flies, were then identified from the filtrates (Tables 1-3). Also, these chemicals had been identified previously as the attractive principals of three other bacteria (Robacker & Flath 1995, Robacker & Bartelt 1997) and nonionizing chemicals identified from odor of two of those bacteria were not attractive to flies primed for response to bacterial odor. We conclude that the attractive principals of the naturally basic bacterial filtrates tested in this work (pH 7.8 to 9.2), as well as all filtrates adjusted to pH 9, were ammonia, amines, imines and pyrazines.

Two bacteria, *B. t. coreanensis* and *B. popillae*, were also moderately attractive at pH 5 (Fig. 2). The chemical most responsible for the moderate attractiveness of these two filtrates at pH 5 probably was acetic acid. These two filtrates had the largest peak heights for acetic acid (Table 3). As discussed above, acetic acid is very attractive to Mexican fruit flies. Because the *B. t. coreanensis* filtrate was pH 5.3 before manipulation with phosphoric acid, we conclude that acetic acid played a major role in attractiveness of this filtrate at its natural pH. Pyrazines may also contribute to attractiveness of these and all filtrates at pH 5 because of their low pKa's. Possible minor roles of nonionizing chemicals at all pH levels have not been determined.

TABLE 3. PEAK HEIGHTS (MV) OF CHEMICALS IN HEADSPACE ABOVE FILTRATES OF UNINOCULATED GROWTH MEDIA AND 11 BACTERIA STRAINS GROWN ON THOSE MEDIA[1].

	pyrazine	2,5-dimethyl-pyrazine	trimethyl-pyrazine	acetic acid
trypticase soy broth	3.4 b	69.9 bcd	3.0 a	0.1 a
E. cloacae	5.1 bc	152.5 f	20.9 b	0.0 a
A. f. faecalis	4.0 b	112.1 def	4.4 a	0.1 a
M. luteus	5.4 bc	91.8 cde	6.2 a	0.0 a
B. sphericus	4.8 bc	160.2 f	6.3 a	0.2 a
B. subtilis	6.4 cd	161.5 f	7.1 a	0.2 a
B. megaterium	5.4 bc	128.4 ef	5.7 a	0.0 a
B. popilliae	6.2 cd	127.4 ef	7.7 a	0.6 a
nutrient broth	0.0 a	4.1 a	0.5 a	0.0 a
B. t. shandongiensis	0.9 a	48.2 abc	7.7 a	0.2 a
B. t. konkukian	3.9 b	37.5 ab	3.2 a	0.1 a
B. t. darmstadiensis	1.2 a	44.4 abc	3.4 a	0.1 a
medium B	8.1 de	7.9 a	0.7 a	0.0 a
B. t. coreanensis	8.6 e	67.7 bcd	24.5 b	3.0 b

[1]Pyrazines determined by flame thermionic detection; acetic acid determined by flame ionization detection. For a given chemical, mean peak heights followed by the same letter were not significantly different from each other by Fisher's protected LSD ($P < 0.05$, n = 5 fermentations).

Novel Attractants from Bacterial Odors

Several novel chemicals fitting the attractive-principal profile were identified from the four major bacteria taxa that were investigated. However, the principal differences among the bacteria were quantitative rather than qualitative in that they produced mostly the same chemicals but in widely different amounts, at least when grown on laboratory media. Thus, an exhaustive investigation of bacteria species for potential new attractants would likely result in relatively few candidate compounds.

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REFERENCES CITED

ABACUS CONCEPTS. 1989. SuperANOVA. Abacus Concepts, Inc., Berkeley, CA.

AMOORE, J. E., L, J. FORRESTER, AND R. G. BUTTERY. 1975. Specific anosmia to 1-pyrroline: The spermous primary odor. J. Chem. Ecol. 1: 299-310.

DEMILO, A. B., C. -J. LEE, D. S. MORENO, AND A. J. MARTINEZ. 1996. Identification of volatiles derived from *Citrobacter freundii* fermentation of a trypticase soy broth. J. Agric. Food Chem. 44: 607-612.

DREW, R. A. I. 1987. Behavioural strategies of fruit flies of the genus *Dacus* (Diptera: Tephritidae) significant in mating and host-plant relationships. Bull. Entomol. Res. 77: 73-81.

DREW, R. A. I., AND H. A. C. FAY. 1988. Comparison of the roles of ammonia and bacteria in the attraction of *Dacus tryoni* (Froggatt) (Queensland fruit fly) to proteinaceous suspensions. J. Plant Prot. Tropics. 5: 127-130.

DULMAGE, H. T., J. A. CORREA, AND A. J. MARTINEZ. 1970. Coprecipitation with lactose as a means of recovering the spore-crystal complex of *Bacillus thuringiensis*. J. Invertebr. Pathol. 15: 15-20.

FLATH, R. A., K. E. MATSUMOTO, R. G. BINDER, R. T. CUNNINGHAM, AND T. R. MON. 1989. Effect of pH on the volatiles of hydrolyzed protein insect baits. J. Agric. Food Chem. 37: 814-819.

GOW, P. L. 1954. Proteinaceous bait for the oriental fruit fly. J. Econ. Entomol. 47: 153-160.

HAYWARD, N. J., T. H. JEAVONS, A. J. C. NICHOLSON, AWND A. G. THORNTON. 1977. Methyl mercaptan and dimethyl disulfide production from methionine by *Proteus* species detected by head-space gas-liquid chromatography. J. Clin. Microbiol. 6: 187-194.

HOLT, J. G. 1984. Bergey's Manual of Systematic Bacteriology, Vol. I & II, 1599 pp., Williams & Wilkins, Baltimore, MD.

JARVIS, H. 1931. Experiments with a new fruit fly lure. Queensland Agric. J. 36: 485-491.

LEE, C. -J., A. B. DEMILO, D. S. MORENO, AND A. J. MARTINEZ. 1995. Analysis of the volatile components of a bacterial fermentation that is attractive to the Mexican fruit fly, *Anastrepha ludens*. J. Agric. Food Chem. 43: 1348-1351.

MARCH, J. 1968. Advanced organic chemistry: reactions, mechanisms, and structure, 1098 pp., McGraw-Hill, New York.

MARTINEZ, A. J., D. C. ROBACKER, AND J. A. GARCIA. 1997. Toxicity of an isolate of *Bacillus thuringiensis* subspecies *darmstadiensis* to adults of the Mexican fruit fly (Diptera: Tephritidae) in the laboratory. J. Econ. Entomol. 90: 130-134.

MARTINEZ, A. J., D. C. ROBACKER, J. A. GARCIA, AND K. L. ESAU. 1994. Laboratory and field olfactory attraction of the Mexican fruit fly (Diptera: Tephritidae) to metabolites of bacterial species. Florida Entomol. 77: 117-126.

QUICK, J., AND R. OTERSON. 1976. A convenient synthesis of pelletierine (2-piperidylpropanone). Synthesis 1976: 745-746.

ROBACKER, D. C., AND R. J. BARTELT. 1997. Chemicals attractive to the Mexican fruit fly from *Klebsiella pneumoniae* and *Citrobacter freundii* cultures sampled by solid-phase microextraction. J. Chem. Ecol. 23: 2897-2915.

ROBACKER, D. C., AND R. A. FLATH. 1995. Attractants from *Staphylococcus aureus* cultures for the Mexican fruit fly, *Anastrepha ludens*. J. Chem. Ecol. 21: 1861-1874.

ROBACKER, D. C., AND J. A. GARCIA. 1993. Effects of age, time of day, feeding history, and gamma irradiation on attraction of Mexican fruit flies (Diptera: Tephritidae), to bacterial odor in laboratory experiments. Environ. Entomol. 22: 1367-1374.

ROBACKER, D. C., A. B. DEMILO, AND D. J. VOADEN. 1997. Mexican fruit fly attractants: Effects of 1-pyrroline and other amines on attractiveness of a mixture of ammonia, methylamine, and putrescine. J. Chem. Ecol. 23: 1263-1280.

ROBACKER, D. C., D. S. MORENO, AND A. B. DEMILO. 1996. Attractiveness to Mexican fruit flies of combinations of acetic acid with ammonium/amino attractants with emphasis on effects of hunger. J. Chem. Ecol. 22: 499-511.

ROBACKER, D. C., W. C. WARFIELD, AND R. F. ALBACH. 1993. Partial characterization and HPLC isolation of bacteria-produced attractants for the Mexican fruit fly, *Anastrepha ludens*. J. Chem. Ecol. 19: 543-557.

SCHOPF, C., AND F. OECHLER. 1936. Die Synthese des desoxyvasicins unter physiologischen bedingungen. Annalen der Chemie. 523: 1-29.

WEAST, R. C. 1976. Handbook of chemistry and physics, 57th ed., 2390 pp., CRC Press, Cleveland, OH.

VOLTINISM IN *MERRAGATA BRUNNEA* (HETEROPTERA: GERROMORPHA: HEBRIDAE) IN SOUTHERN ILLINOIS

STEVEN J. TAYLOR[1] AND J. E. MCPHERSON[2]
[1]Center for Biodiversity, Illinois Natural History Survey, 607 East Peabody Drive
Champaign, Illinois 61820

[2]Department of Zoology, Southern Illinois University at Carbondale
Carbondale, Illinois 62901

ABSTRACT

Voltinism in *Merragata brunnea* Drake was studied in southern Illinois during 1989 and 1990. This species overwintered as adults, which became active in early March. First instars were found from mid-May through mid-September, second instars from mid-May through mid-October, third instars from early June through mid-October, fourth instars from late May through early November, fifth instars from late May through late October, and adults from early March through mid-November. The sequences of peaks of nymphal instars and adults indicate that this species is bi- or trivoltine in southern Illinois.

Key Words: *Merragata brunnea*, voltinism, southern Illinois, life history

RESUMEN

Se estudió el voltinismo en *Merragata brunnea* Drake en el sur de Illinois durante 1989 y 1990. *Merragata brunnea* invernaron como adultos, los que se activaron a principios de marzo. Se encontraron instares del primer estadío desde mediados de mayo a mediados de septiembre, del segundo estadío de mediados de mayo a mediados de octubre, del tercer estadío de principios de junio a mediados de octubre, del cuarto estadío de finales de mayo a principios de noviembre, del quinto estadío de finales de mayo a finales de octubre, y adultos de principios de marzo a mediados de noviembre. La secuencia de crestas de instares ninfales y de adultos indica que esta especie es bi- o trivoltina en el sur de Illinois.

The velvet water bug *Merragata brunnea* Drake, based on scattered records, occurs from New Jersey south to Florida, and west to Minnesota, Nebraska, and Texas; it also occurs in "S. Canada" (Polhemus and Polhemus 1988). In Illinois, it has been found only in the southernmost counties (i.e., Alexander, Jackson, Johnson, Massac, and Union) (Taylor 1996).

Little has been reported on this insect's life history. It occurs in a variety of habitats (e.g., lakes, ponds, swamps, roadside ditches, and rivers) and often is associated with floating vegetation (Taylor 1996). It has been collected in various months from April to November in New Jersey (Chapman 1959), Minnesota (Bennett and Cook 1981), Illinois (Taylor 1996), Missouri (Froeschner 1949), and Mississippi (Wilson 1958); and during most of the year from January to December in Florida (Chapman 1958). Porter (1950) noted that of 127 adults he examined from across much of the range of the species, 5 were collected in April, 4 in May, 84 in July, and 34 in August.

Wilson (1958) reported that of 65 specimens he examined from Mississippi, 1 had been collected in May, 2 in July - September, 60 in September, and 2 in October.

Both brachypterous and macropterous adults have been reported. Adults primarily are brachypterous in Florida (Chapman 1958), Minnesota (97.5% of 81 adults; Bennett and Cook 1981), and Wisconsin (97% of 301 adults; Hilsenhoff 1986).

Porter (1950) reared this species in the laboratory and briefly described the immature stages. He reported the incubation period and stadia for the egg and first through fifth instars as 8-12, 3-6, 3-4, 3-4, 5-6, and 5-6 days, respectively.

During 1989 and 1990, we studied voltinism in a population of this species at President's Pond on the campus of Southern Illinois University at Carbondale, Jackson County, Illinois (see Taylor [1996] for detailed description of pond). President's Pond is a roughly triangular 0.29 hectare (0.71 acre) pond. It is connected at the northern end to the adjacent Lake on the Campus by a narrow channel (approximately 2-5 m wide, 2 m deep). Along the eastern shore (where the present study was conducted), water depth increased sharply between 1 and 2 m from shore and commonly exceeded 2 m at 2.5 m from shore.

Floating, emergent, and shoreline vegetation associated with the pond was diverse (Taylor 1996). The western margin was bordered by a dense, but narrow, band of cattails (*Typha angustifolia* L.). The southern border was comprised of a riprap dam covered with soil and crossed by a paved road. The eastern margin was bordered by overhanging trees and other vegetation. During the summer, the pond filled with a dense growth of aquatic vascular plants and filamentous algae. Near the shoreline, and wherever the aquatic plants reached the water surface, duckweeds built up into dense mats. Air currents tended to move the duckweeds (i.e., *Lemna minor* L., *Spirodela polyrhiza* (L.) Scheiden, and *Wolffia papulifera* Thompson) around the pond unless the plants were partially anchored in the underlying aquatic vegetation.

This paper presents information on voltinism in *M. brunnea*, including times of occurrence of the adults and nymphal instars.

Materials and Methods

Samples were collected weekly from 18 March to 25 November 1989, and biweekly from 11 February to 2 December 1990, along the eastern shore. Sampling was confined to this area because (1) the cattails along the western shoreline prevented use of the quadrat sampler (see below); (2) the riprap shoreline of the southern border was unnatural and, often, disturbed by fishermen; and (3) the water surface along the eastern shore, which was a mosaic of open water, duckweeds, and emergent stems, supported a diverse gerromorphan fauna.

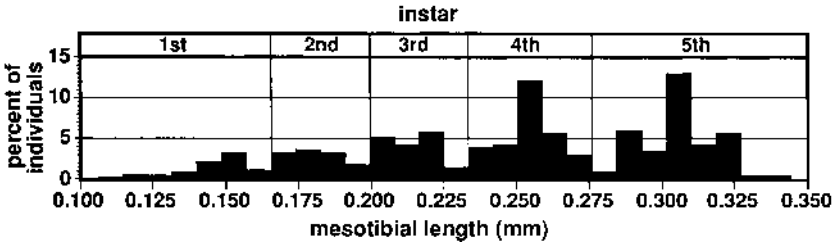

Fig.1. Approximate instars of *M. brunnea* (n = 340), as delineated by mesotibial length. Specimens collected in 1989 and 1990 from President's Pond, Southern Illinois University at Carbondale campus, Jackson County.

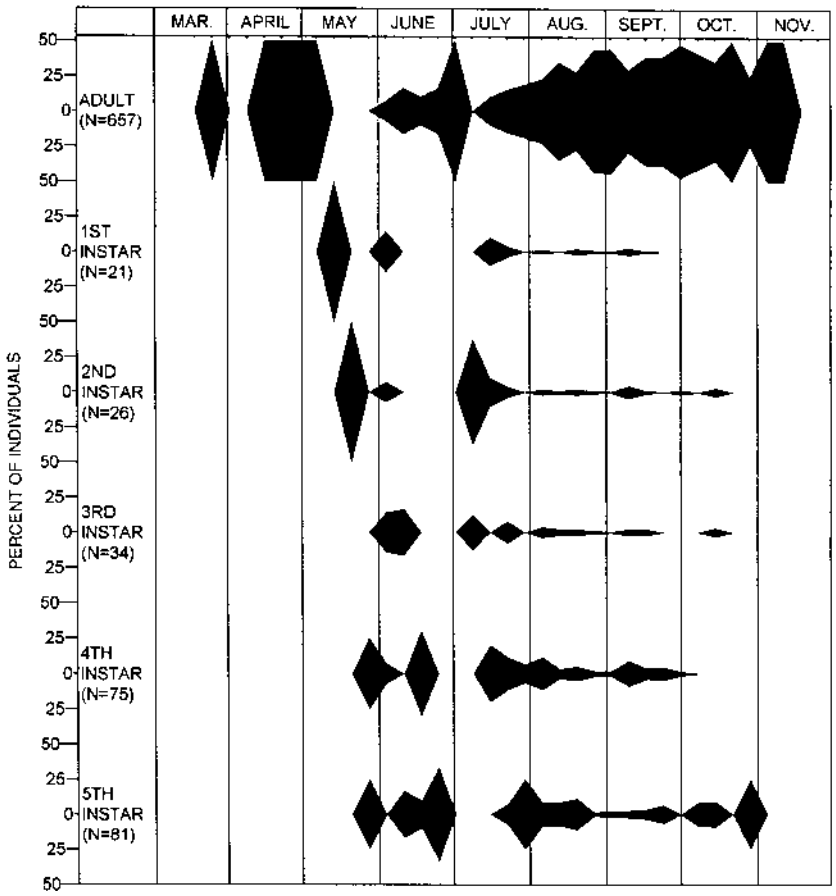

Fig. 2. Percent of individuals in each stage per sample of *M. brunnea* collected at President's Pond, Southern Illinois University at Carbondale campus, Jackson County, during 1989. Beginning and end points of each shaded area represent sample dates preceding and following collection of specimens, respectively.

Four transects, 60 m in length, were made parallel to a relatively uniform section of the eastern margin at 0, 0.5, 1.0, and 1.5 m from the shoreline. Each sample was collected with a floating quadrat sampler (0.25 x 0.25 x 0.05 m), with four replicates placed randomly along each transect; the resulting 16 quadrat samples, which provided a broad sampling of the habitat, were then pooled. Prior to each sample, the collector (SJT) stood for approximately three minutes to allow the insects to acclimate to the disturbance; then, the sampler was placed on the surface of the water. Specimens were removed with a fine mesh nylon net, preserved in alcohol, and sorted in the laboratory.

Adults could be distinguished from nymphs by their well-developed external genitalia and the presence of wings, even in the brachypterous form. Nymphal instars were difficult to separate because they are small and show little progressive change

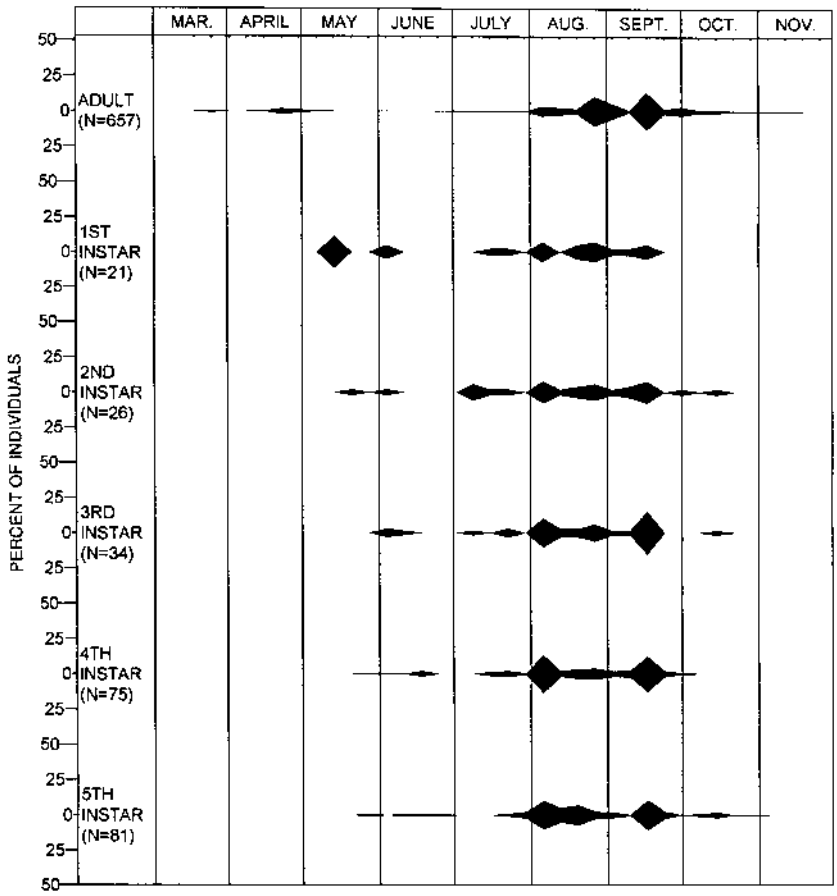

Fig. 3. Percent in each sample of total individuals of same stage of *M. brunnea* collected at President's Pond, Southern Illinois University at Carbondale campus, Jackson County, during 1989. Beginning and end points of each shaded area represent sample dates preceding and following collection of specimens, respectively.

in external characters during development.However, we found that mesotibial length was a useful character for distinguishing instars, although separation between instars was not complete (Fig. 1).

Results and Discussion

In southern Illinois, this species overwintered as adults, which were active from early March through mid-November (Figs. 2-5). Some adults were collected in mid-February in 1990, indicating that the species can be active during warm spells in winter. No eggs were collected. First instars were found from mid-May through mid-September, second instars from mid-May through mid-October, third instars from early

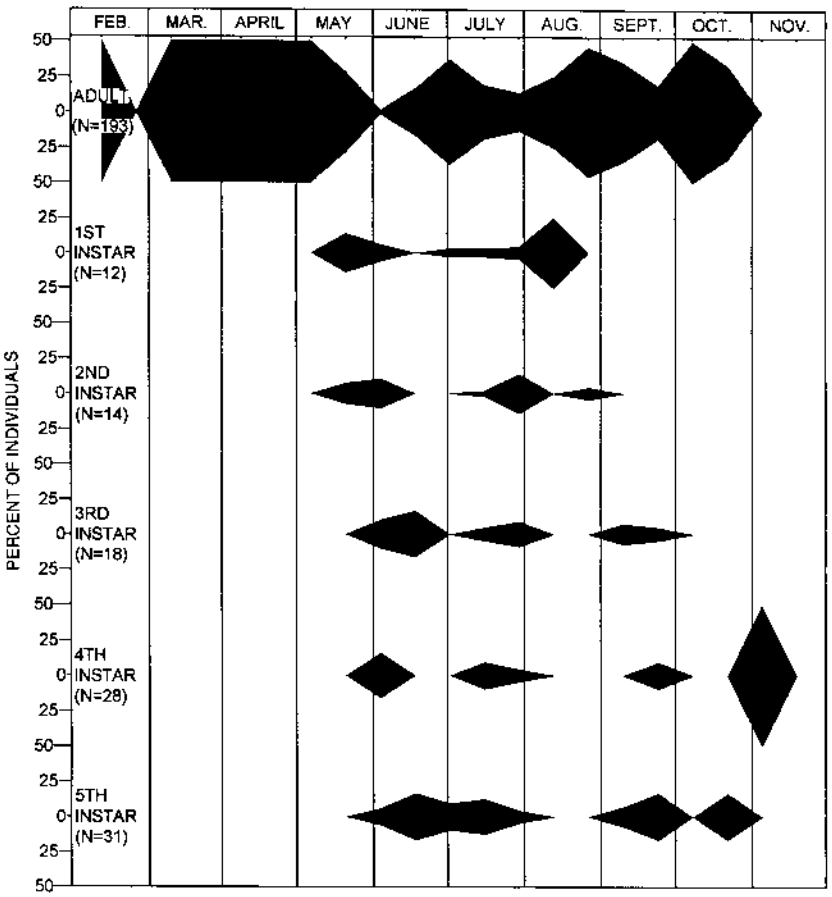

Fig. 4. Percent of individuals in each stage per sample of *M. brunnea* collected at President's Pond, Southern Illinois University at Carbondale campus, Jackson County, during 1990. Beginning and end points of each shaded area represent sample dates preceding and following collection of specimens, respectively.

June through mid-October, fourth instars from late May through early November, and fifth instars from late May through late October.

Merragata brunnea is bi- or trivoltine in southern Illinois. Most fifth instars of the first generation became adults in June, and first instars of the second generation were found in July. The second generation apparently reached adults in late July and August. The second and third generations were not readily distinguishable but a third generation apparently occurred, with fifth instars found in September and October and the resulting adults appearing shortly thereafter. It cannot be determined whether these generations corresponded to the apparent numerical peaks in July and September reported by Porter (1950) and Wilson (1958), respectively.

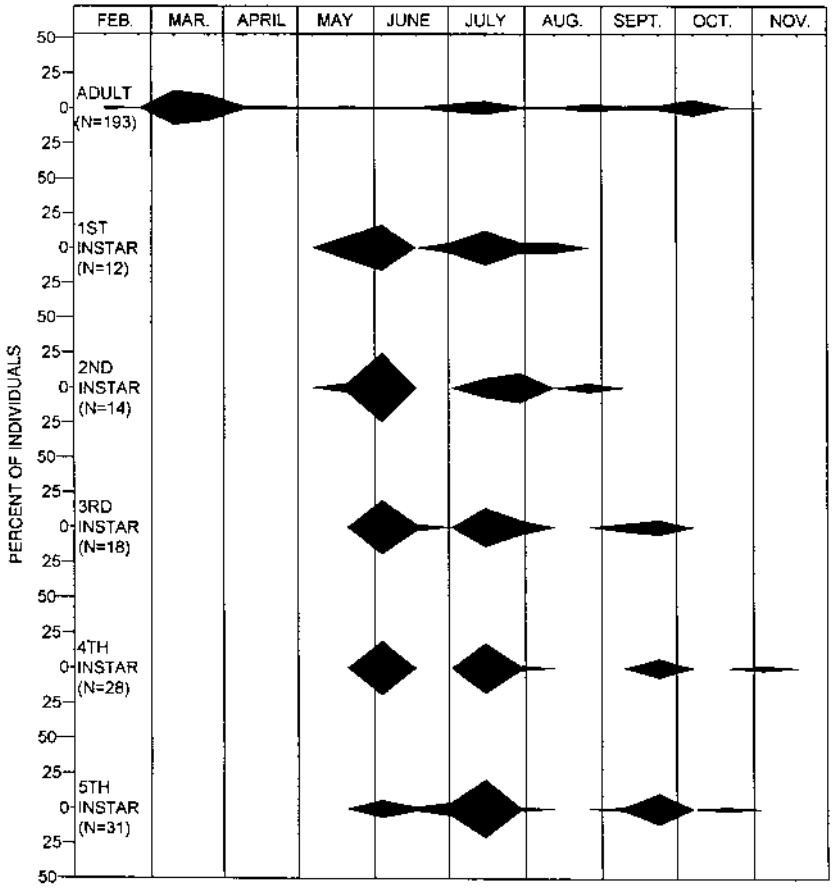

Fig. 5. Percent in each sample of total individuals of same stage of *M. brunnea* collected at President's Pond, Southern Illinois University at Carbondale campus, Jackson County, during 1990. Beginning and end points of each shaded area represent sample dates preceding and following collection of specimens, respectively.

Of the 850 adults collected during this study, 474 were males and 376 were females; of these, 844 were micropterous (♂♂, 99.6%, n=472; ♀♀, 98.9%, n=372), and six were macropterous, thus corroborating the findings of Bennett and Cook (1981), Chapman (1958), and Hilsenhoff (1986). The six macropterous adults were collected in April (1 ♀), July (2 ♀♀), and August (2 ♂♂, 1 ♀).

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LITERATURE CITED

BENNETT, D. V., AND E. F. COOK. 1981. The semiaquatic Hemiptera of Minnesota (Hemiptera: Heteroptera). Minnesota Agric. Exp. Stn. Tech. Bull. 332: 1-59.

CHAPMAN, H. C. 1958. Notes on the identity, habitat and distribution of some semiaquatic Hemiptera of Florida. Florida Entomol. 41: 117-124.

CHAPMAN, H. C. 1959. Distributional and ecological records for some aquatic and semi-aquatic Heteroptera of New Jersey. Bull. Brooklyn Entomol. Soc. 54: 8-12.

FROESCHNER, R. C. 1949. Contributions to a synopsis of the Hemiptera of Missouri, Pt. IV. Hebridae, Mesoveliidae, Cimicidae, Anthocoridae, Cryptostemmatidae, Isometopidae, Meridae (*sic*). Am. Midland Nat. 42: 123- 188.

HILSENHOFF, W. L. 1986. Semiaquatic Hemiptera of Wisconsin. Great Lakes Entomol. 19: 7-19.

POLHEMUS, J. T., AND D. A. POLHEMUS. 1988. Family Hebridae Amyot and Serville, 1843, pp. 152-155. *In* T. J. Henry and R. C. Froeschner [eds.], Catalog of the Heteroptera, or true bugs, of Canada and the continental United States. E. J. Brill, New York. 958 pp.

PORTER, T. W. 1950. Taxonomy of the American Hebridae and the natural history of selected species. Ph.D. Dissertation, University of Kansas, Lawrence. 185 pp. + 12 plates.

TAYLOR, S. J. 1996. Habitat preferences, species assemblages, and resource partitioning by Gerromorpha (Insecta: Heteroptera) in southern Illinois, with a faunal list and keys to species of the state. Ph.D. Dissertation, Southern Illinois University at Carbondale, Carbondale. xviii + 345 pp.

WILSON, C. A. 1958. Aquatic and semiaquatic Hemiptera of Mississippi. Tulane Stud. Zool. 6: 115-170.

RELATIONSHIP OF BROAD MITE (ACARI: TARSONEMIDAE) TO HOST PHENOLOGY AND INJURY LEVELS IN *CAPSICUM ANNUUM*

M. DE COSS-ROMERO[1] AND J. E. PEÑA[2]
[1]Universidad Autonoma de Chiapas, P.O. Box 34, Tapachula, Chiapas, Mexico

[2]University of Florida, Tropical Research & Education Center, 18905 S.W. 280th Street, Homestead, Florida 33031

ABSTRACT

The responses of broad mite, *Polyphagotarsonemus latus* Banks (Acarina: Tarsonemidae), were studied on four phenological stages of pepper plants: vegetative (V), blossoming (B), early fruiting (EF) and late fruiting (LF) stages. All stages of the mite preferred the undersides of the leaves to the uppersides. Plants in V, B, and EF stages had higher numbers of mites per cm^2 of foliage than plants in the late fruiting stage. A damage index scale (0-6) was developed to assess broad mite injury to pepper plants. Eight to nine cumulative mite days/cm^2 were needed to reach a damage index equal to 3 for V, B and EF plant stages. The damage index was also used to relate

broad mite injury to leaf area, height, water content, number of leaves, flowers, buds, fruits and fruit weight of plants infested at four different phenological stages. Plants infested when 14 weeks old (late fruiting stage), had less damage, significantly higher number of fruits and fruit weight than plants infested at earlier plant stages, i.e., vegetative, flowering or early fruiting. The relationship between the damage rating (x) and fruit numbers per plant (y_1) and fruit weight in grams (y_2) was given by $y_1 = 2.83\text{-}0.45x$ and $y_2 = 232.5\text{-}37.234x$, respectively.

Key Words: *Polyphagotarsonemus latus, Capsicum annum,* injury levels, green pepper

RESUMEN

Se estudió la respuesta de cuatro estados fenológicos (vegetativo (V), floración (F), fruta pequeña (EF) y fruta madura(LF)) de pimentón verde a el ataque del ácaro blanco, *Polyphagotarsonemus latus* Banks (Acarina: Tarsonemidae). Los ácaros prefirieron el envés a el haz de las hojas. Aquellas plantas en estado V, F y EF mantuvieron un número más alto de ácaros por cm^2 que aquellas plantas en estado LF. Se estableció una escala de daño del 0 al 6 para evaluar la acción del ácaro blanco en las plantas de pimentón. Un rango de 8 a 9 días cumulativos de ácaros son necesarios para causar un nivel de daño igual a 3 en plantas en estado V, F y EF. Las plantas en estado LF necesitan 6 días-ácaros /cm^2 para alcanzar un nivel de daño igual a 1. La escala de daño fué también utilizada para relacionar el daño causado por los ácaros y el area foliar, altura de la planta, contenido líquido, número de hojas, flores, yemas, frutos, y peso de frutos por plantas en los 4 estados fenológicos. Aquellas plantas infestadas cuando tienen 14 semanas, presentaron menor daño y un número significativamente mayor de frutas y peso de fruta, que plantas en estado V, B y EF. La relación entre la escala de daño (x) y el número de frutas (y_1) y el peso de fruta en gramos (y_2) está descrita por la ecuación: $y_1 = 2.83\text{-}0.45x$ y $y_2 = 232.5\text{-}37.234x$, respectivamente.

Outbreaks of plant feeding tarsonemid mites often occur in vegetative, blossoming or early fruiting stages in the host plant (Jeppson et al. 1975). Because of the tarsonemid's short generation time (approx. 5 days), high fecundity, small size and protected habitat, the injury it produces is often confused with diseases and phytotoxicity Jeppson et al., 1975, Aubert et al. 1981, Cross and Bassett 1982). The impact of the broad mite, *Polyphagotarsonemus latus* (Banks) feeding has been qualitatively described for cotton, cucumber, potatoes, tomatoes, gerberas, beans, papaya, and pepper (Aubert et al. 1981, Bassett, 1981, Beattie & Gellatley 1983, Cross & Basset 1982, Hooper 1957, Laffi 1982, Lo & Chao 1972, Peña & Bullock 1994, Schoonhoven et al. 1978, Jeppson et al. 1975, Ochoa et al. 1994). While these observations suggest a causal relation to host phenology, quantitative assessment of actual impact of feeding by broad mite on growth, leaf area and yield is apparently not well correlated with levels of visible injury and with broad mite densities (Dhoria and Bindra, 1977, Jones & Brown 1983, Peña, 1990). We observed in commercial pepper, *Capsicum annuum* L., that rapid increases of broad mite numbers coincided with early stages of the plant. However, under field conditions it is difficult to determine whether enlarged broad mite populations on early vegetative or reproductive host plant stages resulted from an enhanced mite growth rate compounded over time, or from immigration from outside sources.

Our study was designed to (1) determine under greenhouse conditions the response of mite populations to host phenology in pepper and (2) to measure the impact of mite density on total yield, fruit number, number of leaves and flowers of different developmental stages of pepper plants.

Materials and Methods

Pepper "Early Calwonder"was grown to the stages desired for testing in 3.78 l plastic pots. Plants were fertilized with 20-20-20 NPK, plus micronutrients. Treatments consisted of 15 plants at the vegetative stage(V) (ca. 5 weeks old), 15 plants at the blossoming stage (B) (ca. 7 weeks-old), 15 plants at the early fruiting stage (EF) (ca. 10 weeks old) and 15 plants at the late fruiting stage (LF) (ca. 14 weeks old). Treatments and the untreated controls were replicated 4 times and arranged in a randomized complete block design in a greenhouse maintained at 26 ± 2°C; 75-89% RH. Twelve adult broad mite females from a colony maintained on "Podsquad" garden bean plants, were placed on 2 apical leaves of each treated plant. One leaf was collected 4 days after exposure and thereafter every 4th day until 50 days after exposure from each pepper plant and the number of mites per cm^2 determined under a microscope. Levels of mite populations were measured in cumulative mite-days per cm^2 with 1 mite-d defined as one mite (any motile stage) per leaf for 1 d. Broad mite days were calculated as the sum of the two successive counts (mean number of mites/cm^2) divided by two and multiplied by the number of days between evaluations, and then summed over the evaluation period. It was assumed that the amount of physical injury (removal of cell contents) increased with the number of broad mite days; thus, the term is used interchangeably with injury. Leaf area, fresh weight, dry weight of leaves, were measured weekly during the growing season. Leaf area was determined with a leaf area meter (LI-COR, Lambda Instruments Corporation, Lincoln, NE) and water content was determined by subtracting leaf dry weight from leaf fresh weight. Amount of vegetative growth was determined by dividing the dry leaf weight by the total leaf area.

Damage Index Among Different Plant Ages:

A second experiment consisted of 30 broad mite-infested plants and 30 uninfested plants at four phenological ages (V, B, EF, LF), where the number of leaves, buds, flowers, fruit, fruit weight and mite days and damage index per plant was assessed weekly. To establish a damage index per plant, plants were separated into 6 categories of damage. The damage categories were defined as follows: Category 0.5: apical leaves have begun to curl; the mid vein has become sinuous and the color of the leaves has changed from shiny green to opaque green. Category 1: the mesophyl of the leaf undersides sunken; the basal portion of the apical leaves showed a light green color and the apical leaves curled down. Category 2: a bronze color is present in the apical leaves. If the apical leaves were large, bronzing was a characteristic of the leaf base. If the leaves were small, the leaves were completely bronzed and their tips necrosed. Floral buds were necrosed; leaf area was reduced and damage was observed in axillary leaves. Category 3: petioles of apical leaves have elongated and are thicker than uninfested ones and when stems were necrosed and bronzed, necrosis and/or hypertrophy were observed also in floral buds. Category 4: apical and lateral floral buds have proliferated but are deformed and hypertrophied and have failed to develop. Category 5: apical leaves have become necrotic and necrotic floral buds have aborted. A damage pattern similar to categories 1 to 4 is observed in lateral leaves and floral

buds. Category 6:apical and lateral leaves show lignification, floral buds and flowers are hypertrophied and new leaves are necrotic. New leaves are observed but they show symptoms similar to categories 1 and 4. No fruits are observed or if present, they are deformed. Differences in these categories for infested and uninfested plants were determined by student-t-test (SAS 1987) and analysis of variance (ANOVA) was used to determine differences among plant stages.

Damage Index and Yield Reduction:

Fruit yields were determined by harvesting and weighing all peppers grown from each plant and calculating the total weight of the fruit per plant. Regression analysis was used to determine if there was a relationship between broad mite days (x) and damage index (y). Data were combined over four plant stages. The fruit weight (dependent variable) was also regressed on the damage index (independent variable). To establish injury levels, a linear regression model was used i.e., $y = a + bx$, where y is the percentage of fruit weight reduction per plant, x is the damage index per plant.

Results and Discussion

Mite Dynamics Related to the Abaxial and Adaxial Leaf Surfaces:

Mites were first observed on the underside basal portion of the leaf near the mid and lateral veins. In general, number of broad mites were significantly higher 4 to 14 days after infestation; thereafter, densities remained below 93 broad mites per leaf ($F = 2.78$; $P = 0.0073$; df = 11, 47; N = 59). Oviposition was first observed near the mid and secondary leaf veins but later continued at random on the leaf. During the first 8 days following infestation, higher *P. latus* densities were observed on the leaf underside. Maximum oviposition rate and maximum number of immature and mature stages were observed on the eighth day after infestation (Fig. 1, 2). When the number of eggs was > 200/cm^2 on the leaf underside, the number of females increased on the leaf upperside (Fig. 1A). This population trend might suggest that females search for a new habitat after the carrying capacity of the preferred habitat on the leaf underside has been reached. However, from the 19th through the 73rd day of the evaluation period, the proportion of broad mites on the leaf underside was always higher than on the leaf upperside (Fig 1, 2). This pattern may be due to the propensity of the mites to avoid sunlight or to avoid parts of the plant with low humidity. High light intensity, low humidity and high temperature combinations are unfavorable for *P. latus* (Jeppson et al. 1975, Jones & Brown 1983).

Mite Dynamics Related to Plant Age:

The number of mites feeding varied for different plant stages ($F = 8.54$; $P = 0.0001$; df = 3, 226; N = 230)(Table 1). Even though the same number of females were allocated per plant, higher numbers of eggs deposited per cm^2 were observed on vegetative(V), blossoming (B) and early fruiting (EF) plant stages than on late fruiting stages (LF) (Table 1). The male to female ratio varied from 5: 1 for 5 week-old plants (V) to 3: 1 for the 7-14 week old plants (B, EF, LF). Mite populations on plants in V to EF stages increased significantly more than populations on LF stages of pepper (Fig. 3). These results strongly indicate that *P. latus* responds to some physiological change in the late

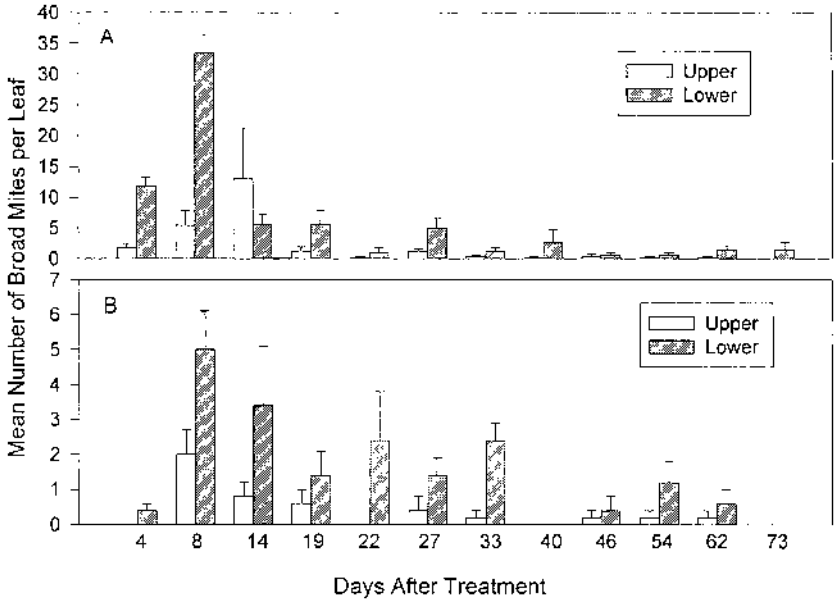

Fig. 1. Mean number of *P. latus* female(A) and male (B) on the upperside and underside of green pepper leaves recorded for 73 days after infestation.

fruiting stage of the plant. Peaks of mite abundance were observed during the first 14, 20, 7 and 20 days following infestation of vegetative, blossoming, early fruiting and late fruiting plants, respectively (Fig. 2).

Early fruiting stage plants had the highest numbers of mites/cm^2 and late fruiting plants had the lowest number of mites/cm^2. There were no significant differences in mites/cm^2 for V and B plant stages (Table 1). Apparently, tarsonemid mouthpart appendages are unsuitable for effective penetration of renitent tissues (Jeppson et al. 1975). Thus *P. latus* may not be able to puncture the more lignified tissues found in 14 week-old plants as opposed to those tissues in 5-10 week old plants. These data may

TABLE 1. COMPARISON OF AVERAGE NUMBER OF DIFFERENT BROAD MITE STAGES PER CM ^{2} ON THE LEAF UPPERSIDE AND LOWERSIDE ON FOUR GROWTH STAGES OF PEPPER.

Plant Age (weeks)	Plant Stage	Mites /cm^2	Eggs	Nymphs	Female	Male
5	V	23.87b	42.68a	10.79b	7.92ab	2.05b
7	B	24.19b	39.84a	11.22b	7.36ab	2.64b
10	EF	45.13a	52.27a	23.38a	13.29a	4.88a
14	LF	9.60c	4.57b	6.49b	1.34c	0.51c

Numbers within the same column followed by the same letter were not significantly different ($P > 0.05$).

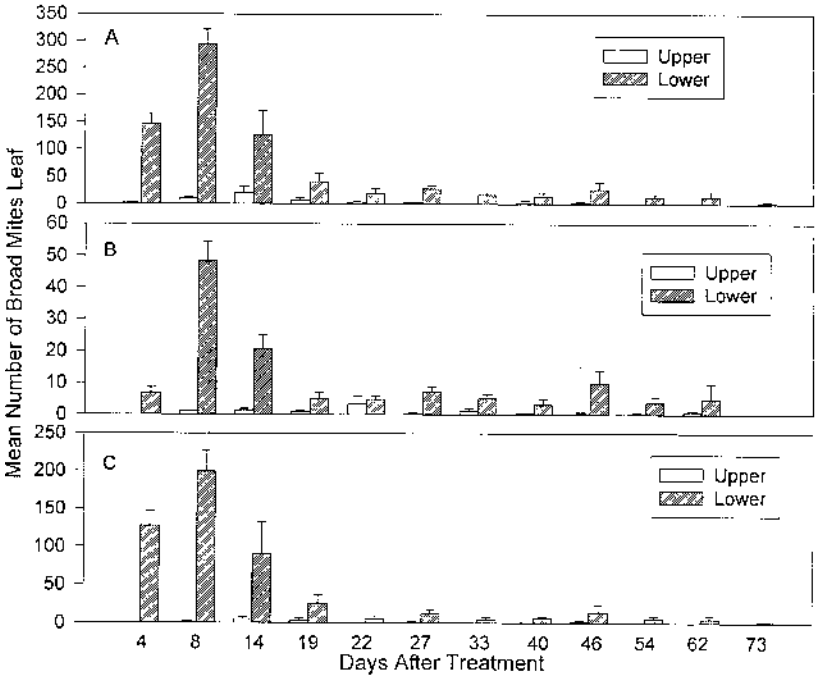

Fig. 2. Mean number of *P. latus* prelarvae (A), nymphs(B) and eggs (C) on the upperside and underside of green pepper leaves recorded for 73 days after infestation.

be of value in programs for evaluating resistance of peppers to *P. latus.* Thus, assessments of plant resistance to *P. latus* made at early growth stages of peppers would be particularly effective for identifying highly resistant plants.

Relationship between damage index and mite-day/cm^2:

The greenhouse experiment indicated that 9.24, 8.24 and 9.24 cumulative mite days/cm^2 are needed for 5, 7 and 10 day-old plants (V, B, EF stages) to reach an average damage index equal to 3.59, 2.56 and 3.02, respectively, whereas 6.31 mite days/cm^2 are necessary to reach a damage index equal to 0.98 for 14 week-old plants (LF stage) (Table 3).

Broad mites significantly reduced the increment in leaf sizes of infested plants compared to the control (Table 4). Fresh leaf weight was reduced for all plant stages, but significant reductions in dry weight were observed only for V and B plant stages. The levels of significance associated with soluble solids were reduced for V, B and EF plant stages. (Table 4). Table 5 shows that the numbers of leaves per plant, plant heights and numbers of fruit per plant were also affected by mite injury during all plant stages. The data suggest that broad mites reduce height in the infested plants, and that they induce lateral shoot growth (Table 5). The number of flowers and buds in V, B and EF plant stages was significantly reduced compared with the uninfested check plants, but corresponding reductions were not observed on LF plants (Table 5).

TABLE 2. CUMULATIVE NUMBER OF BROAD MITE DAYS AND AVERAGE DAMAGE RATING OF PEPPER PLANTS AT FOUR DIFFERENT PLANT STAGES.

Plant Age (weeks)	Plant Stage	Cumulative Number of Mite Days								Average Damage
		2	7	14	20	26	33	46	50 DAI	Rating
5	V	1.40	5.08	7.42	8.12	8.14	8.99	9.13	9.24	3.59
7	B	1.33	3.52	5.17	6.77	7.96	8.21	8.22	8.24	2.56
10	EF	1.36	4.96	8.96	9.08	9.20	9.21	9.21	9.21	3.02
14	LF	1.12	2.24	6.24	6.28	6.29	6.31	6.31	6.31	0.98

DAI = Days after infestation.

TABLE 3. COMPARISON OF MEAN DAMAGE RATING, LEAF AREA, LEAF WATER CONTENT AND GROWTH OF PEPPER PLANTS INFESTED WITH BROAD MITE AT FOUR PLANT STAGES.

Plant Age (weeks)	Plant Stage	Damage Rating		Leaf Area/ leaf (cm^2)		Leaf Fresh weight (g)		Leaf Dry weight (g)		Soluble Solids (g)	
		Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
5	V	3.59a	0.0b	1.76b	5.20a	0.12b	0.16a	0.04b	0.05a	0.08b	0.11a
7	B	2.56a	0.0b	2.12b	5.04a	0.08b	0.09a	0.02b	0.01a	0.07b	0.08a
10	EF	3.02a	0.0b	5.22b	9.94a	0.18b	0.25a	0.03a	0.02a	0.16b	0.23a
14	LF	098a	0.0b	5.10b	7.23a	0.18b	0.20a	0.05a	0.04a	0.14a	0.16a

Means for each parameter within rows for each parameter, followed by the same letter are not significantly differently (t-test; P = 0.05)

TABLE 4. COMPARISON OF MEAN NUMBER OF LEAVES, PLANT HEIGHT, BUDS, FLOWERS, FRUITS AND FRUIT WEIGHT FROM FOUR PEPPER PLANT STAGES INFESTED WITH BROAD MITE.

Plant Age	Plant Stage	Leaves /plant		Plant Height (cms.)		No. Buds /plants		No. Flowers /plant		No. Fruits /plant		Fruit weight/plant (g)	
		Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control	Infested	Control
5	V	17.68b	29.62a	11.97b	20.63a	2.17b	6.79a	0.12b	0.80a	0.08b	1.88a	10.67b	270.51a
7	B	14.44b	19.33a	11.76b	16.11a	1.15b	5.31a	0.01b	0.50a	0.02b	0.37a	3.90b	50.78a
10	EF	18.03b	30.49a	14.50b	22.54a	2.58b	10.83a	0.09b	1.31a	0.34b	0.98a	16.87b	55.62a
14	LF	37.52b	41.39a	30.82b	32.54a	4.41a	5.28a	0.83a	0.93a	2.48b	3.10a	333.17a	346.07a

Means for each parameter within rows followed by the same letter are not significantly different (t-test, P=0.05)

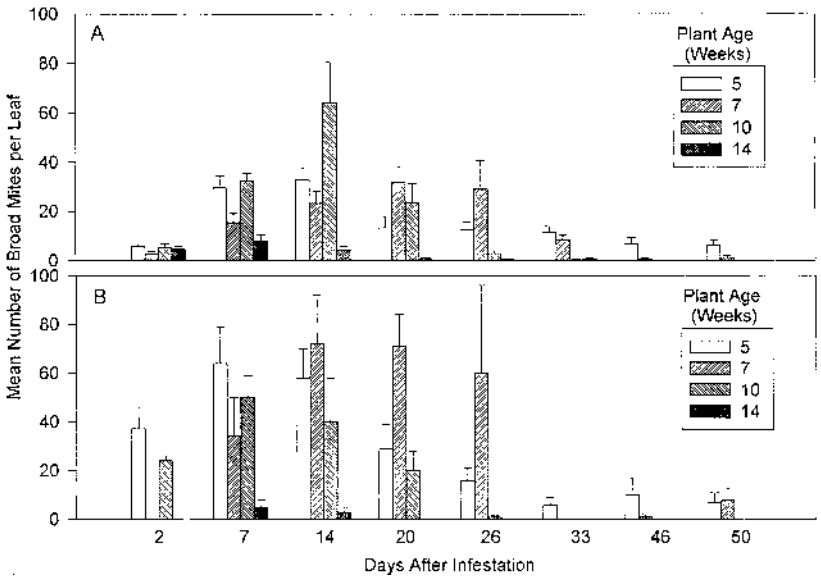

Fig. 3. Densities of *P. latus* motile and prelarva stages (A) and eggs (B) in 5, 7, 10 and 14 week-old pepper plants recorded for 50 days after infestation.

Mean fruit weight on LF plants did not differ significantly between infested and control plants. However, mean fruit weights of V, B, EF plants exposed to broad mite were significantly lower than those of control plants. Fruit weight was consistently lower from V, B and EF broad mite-stressed plants compared to uninfested plants at the same growth stage. Lower fruit numbers were recorded from mite-stressed plants compared to the untreated check.

Relationship between damage index and plant yield:

The damage caused by broad mites appears to be dependent on the stage of development of the pepper plant. Plants infested when 14 weeks-old (LF) had significantly more fruits than plants infested at an earlier plant stage. This experiment indicated an intermediate ($y = 2.83 - 0.45x$; $r^2 = 0.46$; $P = 0.0001$) relationship between damage index (x) and the number of fruits per plant (y). However, the relationship between fruit weight per plant (y) in grams and damage index (x) was less than intermediate ($y = 232.50 - 37.23x$; $r^2 = 0.38$; $P = 0.0001$). Nevertheless, these relationships may be used to predict yield loss for *P. latus* infested pepper plants. For example, using the intercepts of the above equations, and the damage index is 0, the yield of undamaged plants would be 2.83 fruits or 232.50 grams per plant. However, if the damage index (x) is 5, the yield will be reduced by 80%.

High levels of stress induced by *P. latus* feeding resulted in reductions in quantity and quality of fruit, reduction in vegetative growth and flower development responds to some anatomical, physiological or biochemical differences between vegetative and reproductive stage plants. This reductions were due to chronic feeding on plants with younger leaf tissue, which appear to be more susceptible than plants with greater

numbers of mature leaves. This effect has been shown to vary with the phenological development of hedera (Nemestothy et al. 1982). Plants with younger hirsute leaves suffered the strongest damage compared to older plants with leaves with less hairs and where cell differentiation has already occurred. These results are in agreement with the reports of Smith (1935) who stated that the broad mite cannot survive long on the tough, mature leaves of most plants.

Regardless of the causative factors, our results help to explain why outbreak populations of *P. latus* are observed only in vegetative and early reproductive stages of the crop. The response of *P. latus* to pepper phenology appears to be an important component of the broad mites pest potential in the pepper ecosystem. In pepper, flowering and fruit formation induce a significant increase in the growth rate of *P. latus* populations. This rapid increase in mite density, together with the production of new lateral growth, may stimulate mite movement onto new lateral leaves. However, when the leaves are mature (LF), the plants seem to be unable to support broad mite populations. Thus, mites invading plants younger than 14 weeks encounter a potential host suitable for colonization and favorable rapid growth. The sequence of motile mites observed every 8 days, explains why plants at these early ages have the potential for inducing damaging mite outbreaks. This potential is often realized under the exacerbating effects of hot humid weather and certain pesticide programs. The knowledge that the potential of damage arises from mite responses to the phenological stage of the crop can enhance the efficiency and value of broad mite monitoring programs and control strategies by focusing attention on the critical periods prior to flowering and fruiting in pepper. However, yield responses to broad mite damage under field conditions may differ from those observed under controlled conditions in the greenhouse.

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References Cited

AUBERT, B., P. LOSSOIS, J. NARCHAL, J. RABAUD, AND P. DE BOUSVILLIERS. 1981. Mise en evidence de degats causes par *Polyphagotarsonemus latus* (Banks) sur papayer a l'ile de la Réunion. Fruits. 36: 9-24.

BASSETT. P. 1981. Observations on broad mite (*Polyphagotarsonemus latus*) (Acarina: Tarsonemidae) attacking cucumber. Proc. 1981 Br Crop Prot Conf. Pests and Diseases 1: 99-103.

BEATTIE, G. A. C., AND GELLATLEY, J. G. 1983. Mite pests of citrus. Agfacts H2, AE 3, Dept. Agriculture, New South Wales.

CROSS, J. V., AND P. BASSETT. 1982. Damage to tomato and aubergine by broad mite, *Polyphagotarsonemus latus* (Banks). Plant Pathology. 31: 391-393.

DHOORIA, M. S., AND O. S. BINDRA. 1977. *Polyphagotarsonemus latus* (Banks), a mite pest of chili and potato in Punjab. Acarology Newsletter. 4: 7-9.

HOOPER, G. H. S. 1957. The potato broad mite. Queensland Agr. J. 83: 56-58.

JEPSSON, L. R., H. H. KEIFFER, AND E. W. BAKER. 1975. Mites injurious to economic plants Univ. California Press, Los Angeles.

JONES, F. P., AND R. D. BROWN. 1983. Reproductive responses of the broad mite, *Polyphagotarsonemus latus* (Acari: Tarsonemidae), to constant temperature humidity regimes. Ann. Entomol. Soc. Am. 76: 466-469.

LAFFI, F. 1982. Occurrence of *Polyphagotarsonemus latus* (Banks) on capsicum sedbedsin North Italy. Informatore Fitopatologico. 32: 55-57.
LO, P. K., AND S. R. CHAO. 1972. Tea broad mite (*Hemitarsonemus latus* (Banks))(Acarina:Tarsonemidae) injure the string bean plants in greenhouses. J. Taiwan Agric. Research 21: 59-61.
NEMESTHOTY, K., E. VOLCSANSKY, AND N. SIMON. 1982. Influence of damage of the mites *Tarsonemus pallidus* and *Polyphagotarsonemus latus* Banks (Acari: Tarsonemidae) on the morphological properties of fashedera and hedera leaves. Novenyvedelem 10: 437-442
OCHOA, R., H. AGUILAR, AND C. VARGAS. 1994. Phytophagous mites of Central America: An illustrated guide. CATIE, Turrialba 234 p.
PEÑA, J. E. 1990. Relationships of broad mite (Acari: Tarsonemidae) density to lime damage. J. Econ. Entomol. 83: 2008-2015.
PEÑA, J. E., AND R. C. BULLOCK. 1994. Effects of broad mite, *Polyphagotersonemus latus* feeding on vegetative plant growth. Florida Entomol. 77: 180-184.
Schoonhoven, A. V., J. Piedrahita, R. Valderrama, and G. Galvez. 1978. Biologia, daño y control del acaro tropical *Polyphagotarsonemus latus* (Banks) (Acarina: Tarsonemidae) en frijol. Turrialba. 28: 77-80.
SAS INSTITUTE. 1987. SAS user's guide: statistics. SAS Institute, Cary NC.
SMITH, F. F. 1935. Control experiments on certain *Tarsonemus* mites on ornamentals. J. Econ. Entomol. 28: 91-98.

FIELD PRODUCTION OF TWO SPECIES OF PARASITOIDS OF THE DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE)[1]

G. Y. HU[2], E. R. MITCHELL, D. H. SIEGLAFF[2], AND J. S. OKINE[2]

Center for Medical, Agricultural and Veterinary Entomology, U. S. Department of Agriculture, Agricultural Research Service, P.O. Box 14565, Gainesville, FL 32604, USA

[2]Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611- 0620.

ABSTRACT

Two species of parasitoids, *Cotesia plutellae* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae), of diamondback moth, *Plutella xylostella*, (Lepidoptera: Plutellidae), were colonized in cages in cabbage fields west of Bunnell, Florida, from November 1996 to February 1997. Two kinds of cages were used: large-screened cages and screened laundry hampers. Both parasitoids attacked their host during the winter, completed development within the host, and increased in numbers within field cages. Parasitism of diamondback moth larvae by *C. plutellae* was 36-42% in laundry hampers, and 35-65% in large screened cages. The sex ratio of

emerging *C. plutellae* was 1:1-1.2 (♀:♂) in laundry hampers and 1:0.8-1.3 in large screened cages. Parasitism of diamondback moth larvae by *D. insulare* was 55-90%, parasitoid adults emerged from 89% of the cocoons, and the sex ratio was 1:1.4-2.1 (♀:♂) in large screened cages. The results showed that it is possible to rear these parasitoids in field nursery cages to provide parasitoid sources for release to control diamondback moth in cabbage in Florida.

Key Words: *Plutella xylostella*, *Cotesia plutellae*, *Diadegma insulare*, biological control, parasitism, cabbage

RESUMEN

Dos especies de parasitoides, *Cotesia plutellae* (Hymenoptera: Braconidae) y *Diadegma insulare* (Hymenoptera: Ichneumonidae), de la palomilla dorso de diamante, *Plutella xylostella* (Lepidoptera: Plutellidae), fueron colonizadas dentro de jaulas en campos de col al oeste de Bunnell, Florida, de noviembre de 1996 a febrero de 1997. Dos tipos de jaulas fueron usadas: jaulas grandes con tela de malla y canastas para la ropa con tela de malla. Los dos parasitoides atacaron a sus hospederos durante el invierno, completaron su desarrollo dentro de sus hospederos, y aumentaron en sus números dentro de las jaulas en el campo. El nivel de parasitismo de larvas de la palomilla por *C. plutellae* fue de 36-42% en las canastas de ropa y de 35-65% en las jaulas grandes. El coeficiente sexual de los *C. plutellae* que emergieron fue de 1:1-1.2 (♀:♂) en los canastos de ropa y de 1:0.8-1.3 en las jaulas grandes. El nivel de parasitismo de larvas de la palomilla por *D. insulare* fue de 55-90%, el 89% de los parasitoides adultos emergieron de los capullos, y el coeficiente sexual fue de 1:1.4-2.1 (♀:♂) en las jaulas grandes con malla. Los resultados demostraron que es posible criar estos parasitoides dentro de jaulas el en campo para proveer a los parasitoides con recursos para su liberación para controlar la palomilla dorso de diamante en la col en Florida.

The diamondback moth, *Plutella xylostella* (L.), is the most destructive pest of cabbage and other crucifers throughout the world. The annual cost for control of this pest is estimated to be U.S. $1 billion (Talekar & Shelton 1993). In Florida, the annual average production of cabbage is 4,555 hectares with an average total value of $34.4 million, and diamondback moth is one of the major pests of this crop (Leibee 1996). The diamondback moth typically has been controlled using pesticides (Shelton et al. 1993). To prevent damage to cabbage by diamondback moth, Florida growers typically have relied on one or two applications of insecticide per week; however, this has led to problems from insecticide resistance (Leibee 1996).

Integrated pest management (IPM) programs provide the most viable alternative to reliance on pesticides. An IPM approach to control lepidopterous pests in cabbage using multiple tactics is described by Biever et al. (1994). In Florida, an IPM program has been under trial for control of diamondback moth in cabbage fields. This pilot program contains a combination of strategies such as biological control (releases of parasitoids; Mitchell et al. 1997a, 1998), trap crops (Mitchell et al. 1997b), pheromone for disrupting mating (McLaughlin et al. 1994, Mitchell et al. 1997c) and *Bt* pesticides.

Of the parasitoids attacking diamondback moth, *Cotesia plutellae* Kurdjumov and *Diadegma insulare* (Cresson) show the most promise (Ooi & Lim 1989, Ooi 1990, Tabashnik et al. 1990). *Diadegma insulare* is the most important parasitoid of dia-

mondback moth in North America (Latheef & Irwin 1983, Pimentel 1961, Oatman & Platner 1969, Harcourt 1960, 1963, 1986, Losata & Kok 1986, Horn 1987), and has resulted in greater than 90% parasitism in untreated fields (Muckenfuss et al. 1990). It is the most abundant parasitoid of diamondback moth in Florida (Mitchell et al. 1997b). The female wasps primarily attack 2nd and 3rd instars of diamondback moth (Hu, et. al.: unpublished data). After completing larval development, this parasitoid makes its cocoon within the host cocoon. In cabbage fields in northeast Florida, however, *Diadegma insulare* populations typically do not increase until late March (Hu et al. 1997).

An imported diamondback moth parasitoid, *C. plutellae*, has been released in cabbage in Florida. This parasitoid primarily attacks early instars of diamondback moth (Hu et al.: unpublished data). After completing development, the parasitoid larva migrates outside the host larva to form its own cocoon. The results from field releases have shown that *C. plutellae* competes with *D. insulare* for increasing parasitism following inundative releases. Unfortunately, *C. plutellae* has not yet been found to establish in cabbage production areas in Florida (Mitchell et al. 1997a). Moreover, purchasing this parasitoid for release is costly (Mitchell et al. 1998). The objective of this study was to determine the feasibility of colonizing these two parasitoids in field cages to provide local sources for releases in cabbage for control of diamondback moth.

Materials and Methods

Experimental Location

This study was conducted in an area of commercial cabbage production, west of Bunnell, Flagler County, Florida. Approximately 800 hectares of cabbage grow in the winter-spring and fall-winter crop in this area. The fall-winter crop lasts from October to February and the winter-spring season lasts from January to April. Temperature and humidity were recorded in that area while the study was carried out (November 1996 - February 1997). Average daily high temperature was 24°C, ranging from 9.4 to 32.2°C. Average daily low temperature was 9.6°C, ranging from -4.4 to 21.1°C. Relative humidity was 22-100%, with an average of 61.8%.

Cages

Two types of cages were used in this study: screened Sterilite® laundry hampers (Sterilite Corporation, Townsend, MA) and large screened cages. Laundry hampers (Fig. 1, left) were trapezoidal and 61 cm high: the bottom was 38-cm long x 27-cm wide and the top (opening) was 46.4-cm long x 33.7-cm wide. Air vents on the sides were covered using a fine Saran® screen of eight meshes per cm. The large screened cages (Fig. 1, right) were constructed of a wire frame covered with two layers of Saran® screens: the outer layer was fine with 16 meshes per cm and the inner layer was coarse with six meshes per cm. The cage was semicircular in section, 2.5-m long, 2.2-m wide (bottom), and 1.0-m high. Edges of the screens were buried into soil to prevent the insects within the cage from escaping and the insects outside from invading the cage. A fire ant bait, Amdro® (American Cyanamid, Wayne, NJ), was applied onto the ground inside and around the cages once a week to help control *Solenopsis invicta* Buren (Hymenoptera: Formicidae), which attacked immature diamondback moth and its parasitoids in the cages.

Parasitoid Source

Cotesia plutellae used in this study were purchased from Biofac, Inc., Mathis, Texas. Cocoons established on paper towels (about 1,000 each) were placed in plastic

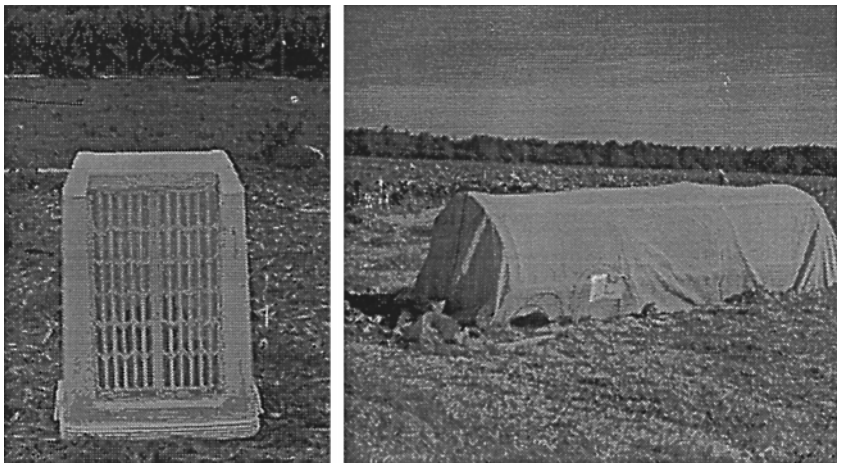

Fig. 1. Screened laundry hampers (left) used for rearing *Cotesia plutellae* and large screened cages (right) used for rearing *C. plutellae* and *Diadegma insulare* in cabbage fields.

bags, wrapped with old newsprint, inserted into styrofoam containers, and shipped to Gainesville, Florida. *Diadegma insulare* (collected from Bunnell, Florida, May 1996) were reared on diamondback moth larvae reared on wheat germ-based artificial diet (Shelton et al. 1991). Both species of parasitoids were fed honey and water, and maintained under a 12:12 h L:D cycle at 25°C and 50% RH.

Parasitoid Rearing in Large-Screened Cages

To initiate rearing, 10 to 20 individually potted collard plants, infested with 100-200 diamondback moth larvae (mixed instars) each, were introduced into each of the two cages. A cumulative total of 75 pairs of *D. insulare* was introduced into one cage from 25 November to 10 December, 1996. On 28 November, 1996, 200 pairs of *C. plutellae* were introduced into the other large cage. No more parasitoids were added to the cages.

Follow-up visits to the cages were made once a week. Each visit included watering the collard plants, removing dead plants and adding new infested plants when needed. Every 2-3 weeks, samples of diamondback moth larvae and parasitoid cocoons were collected from the plants in the cages and brought to our Gainesville laboratory, where the larvae were dissected to determine parasitism. Parasitoid cocoons were allowed to emerge as adults to obtain sex ratio data.

Parasitoid Rearing in Laundry-Hampers

Because *C. plutellae* is an exotic species, no data were available on the possibility for survival in our experimental location. To start tests, two fully-grown potted collard plants were infested with mixed instars of 100-200 diamondback moth larvae, and were covered by an upside down screened laundry hamper. Next, 50 pairs of 3-d old *C. plutellae* were introduced into the hamper. Four pieces of metal wire were used to

anchor each corner of the cage edges to the soil. This test was replicated three times and each replicate included three cages. After the third wk, the collard plants along with the laundry hampers were brought to the Gainesville lab. The diamondback moth larvae collected from the plants were dissected for parasitism, and the cocoons collected from the plants and the laundry hampers were reared to adults to obtain sex ratio data.

Results and Discussion

Cotesia plutellae

A total of 225 diamondback moth larvae (mixed instars) collected from the laundry hampers was dissected to determine parasitism by *C. plutellae*. Parasitism caused by this parasitoid was 36-42% from November 1996 to February 1997. A total of 310 cocoons of *C. plutellae* was collected, from which 256 adults emerged. Emergence success ranged from 80.8 to 85.3%, with an average of 82.8% per trial. Sex ratios of the emerging adults were 1: 1.0-1.2 (♀:♂), with an average of 1: 1.13 (Table 1).

Eighty diamondback moth larvae collected over the four sampling dates from the large screened cage also were dissected for eggs or larvae of *C. plutellae* (Table 1). Parasitism caused by this parasitoid ranged from 35 to 65%, with an average of 55%. Sex ratios of emerging adults were 1: 0.8-1.3, with an average of 1: 1.1 (♀:♂). Adults *C. plutellae* were observed to fly around within the large screened cage during the entire rearing period, but remained on plants when ambient temperature dropped below 5°C.

Diadegma insulare

In the large screened cage, 75 diamondback moth larvae collected from the four sampling dates were dissected to determine parasitism by *D. insulare* (Table 2). Parasitism caused by *D. insulare* ranged from 55 to 90%, with an average of 75%. Sex ratios of emerging adults were 1:1.4-2.1, with an average of 1:1.8 (♀:♂), which is similar to that reported to occur in field populations (Idris & Grafius 1993, Mitchell et al. 1997b) and in our rearing facility. Adult *D. insulare* were observed to fly around within the large screened cage. They were seen to hover a few cm to 20 cm above collard plants or weeds. When ambient temperature dropped below 5°C, however, they stayed on the plants.

The results showed that *C. plutellae* and *D. insulare* attacked diamondback moth larvae, completed their development within the host larvae, and reproduced continuously within the nursery cages during the winter months in east-central Florida. An estimate of three generations completed in cages. At the end of the rearing period (late-February of 1997), the numbers of *D. insulare* and *C. plutellae* increased greatly but we did not attempt to quantify populations of the caged parasitoids.

The unparasitized larvae of diamondback moth used for the rearing developed into cocoons, and adults emerged from the cocoons continuously throughout the rearing period within the cages where *C. plutellae* and *D. insulare* were maintained. The adult parasitoids were observed to stay on collard plants or in weeds, occasionally flying between plants. However, even though diamondback moth adults were continuously present, larvae had to be introduced continuously into the cages with collard plants because larval numbers were very low during the winter and the plant quality decreased over time.

Following lifting the fine (outer layer) screen from the large cage, *C. plutellae* colonized in the large-screened cage were observed to fly out through meshes of the

TABLE 1. SURVIVAL AND HOST-ATTACKING RATES OF *COTESIA PLUTELLAE* UNDER LAUNDRY HAMPERS IN A CABBAGE FIELD DURING WINTER 1996-1997. BUNNELL, FLORIDA. REPLICATES = 3 FOR EACH DATE.

Dates		*Cotesia* Released	Diamondback Moth		Parasitoid Emergence		
Start	End	(Pairs per Cage)	Larvae Checked per Cage	Parasitism (%)	Total No. Cocoons Collected	% Emergence	Sex Ratio (♀:♂)
07/11/96	29/11/96	50	25	41.3 ± 5.0	130	80.8	1:1.2
25/11/96	31/12/96	50	25	42.0 ± 4.9	85	82.4	1:1.2
21/01/97	12/02/97	50	25	36.7 ± 6.7	95	85.3	1:1.0

TABLE 2. PARASITISM OF DIAMONDBACK MOTH LARVAE AND SEX RATIOS OF *DIADEGMA INSULARE* AND *COTESIA PLUTELLAE* REARED IN LARGE NURSERY CAGES IN CABBAGE FIELDS DURING WINTER 1996-1997. BUNNELL, FLORIDA.

Dates	No. Larvae Dissected	% Parasitism	Adults Emerging	Sex Ratio (♀:♂)
D. insulare				
Jan.7	20	55	39	1:1.8
Jan. 19	20	90	48	1:1.8
Feb. 4	15	80	34	1:2.1
Feb. 16	20	75	22	1:1.4
Total	75	75	143	1:1.8
C. plutellae				
Jan. 16	20	35	52	1:1.3
Jan. 28	20	65	34	1:0.8
Feb. 14	20	55	27	1:1.1
Feb. 26	20	65	36	1:1.3
Total	80L	55	149	1:1.1

coarse (inner layer) screen and spread into the cabbage in nearby fields. The adults of diamondback moth, however, could not escape through the screen due to their larger size, eliminating the spread of this pest from the nursery cages.

Unfortunately, *Diadegma insulare* could not be released in this way because its size is nearly the size of diamondback moth adults and they could not migrate through the coarse layer screen. Therefore, cocoons of this parasitoid were collected from the cage and placed into adjacent cabbage fields. Unfortunately, released parasitoids could not be evaluated for establishment due to heavy chemical pesticide applications by growers in the fields near the rearing cages.

Diamondback moth larvae sterilized by gamma radiation are just as suitable hosts for the parasitoid (*C. plutellae*) as are unsterilized larvae (Okine et al. 1998) and may be used in the future as the host for rearing *D. insulare* in field cages. The adults of *D. insulare* and those of sterile diamondback moth can then be released simultaneously from the cages into the fields by lifting the cover screens without infesting the field with diamondback moth.

Augmentation of parasitoids to increase their effectiveness involves their direct manipulation, either by mass production and periodic colonization, or by some type of planned genetic improvement, or by employing chemical cues that affect their behavior (Debach & Rosen 1991). Our results showed that *D. insulare* and *C. plutellae* were successfully colonized in field cages. Both parasitoids survived, completed their development within the host, and increased in numbers. Therefore, colonizing these two species of parasitoids in field cages may provide a good source of large numbers of *C. plutellae* and *D. insulare* for control of diamondback moth in cabbage. The parasitoids were ready for release into the cabbage fields either as adults or cocoons. This procedure can be easily adopted by growers of commercial cabbage. Moreover, the rearing facility was not costly and required minimal labor, both important considerations for implementation of biological control.

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ENDNOTES

1. This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.

REFERENCES CITED

BIEVER, K. D., D. L. HOSTETTER, AND J. R. KERN. 1994. Evolution and implementation of a biological control-IPM system for crucifers: a 24-year case study. American Entomol. 40: 103-108.

DEBACH, P., AND D. ROSEN. 1991. Biological control by natural enemies. 2nd ed. Cambridge University, New York.

HARCOURT, D. G. 1960. Biology of the diamondback moth, *Plutella maculipennis* (Curt) (Lepdoptera: Plutellidae) in eastern Ontario III. Natural enemies. Canadian Entomol. 92: 419-428.

HARCOURT, D. G. 1963. Major mortality factors in population dynamics of the diamondback moth, *Plutella maculipennis* (Cur.) (Lepidoptera: Plutellidae). Mem. Canadian Entomol. Soc. 32: 55-56.

HARCOURT, D. G. 1986. Population dynamics of the diamondback moth in southern Ontario, pp. 1-15 *in* N. S. Talekar and T. D. Griggs [eds.], Diamondback moth management. Proc. 1st Inter. Workshop at Asian Veg. Res. and Dev. Center, 11-15 March 1985, Shanhua, Taiwan.

HORN, D. J. 1987. Vegetational background and parasitism of larval diamondback moth on collards. Entomol. Exp. Appl. 43: 300-303.

HU, G. Y., E. R. MITCHELL, AND J. S. OKINE. 1997. Effect of habitat types on population densities and parasitism of the diamondback moth (Lepidoptera: Plutellidae) larvae in cabbage fields. J. Entomol. Sci. 32:56-71.

IDRIS, A. B., AND E. GRAFIUS. 1993. Field studies on the impact of pesticides on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and parasitism by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae). J. Econ. Entomol. 86: 1196-1202.

LASOTA, J. A., AND L. T. KOK. 1986. *Diadegma insulare* (Hymenoptera: Ichneumonidae) parasitism of the diamondback moth (Lepidoptera: Plutellidae) in southwest Virginia. J. Entomol. Sci. 21: 237-242.

LATHEEF, M. A., AND R. D. IRWIN. 1983. Seasonal abundance and parasitism of lepidopterous larvae on *Brassica* greens in Virginia. J. Georgia Entomol. Soc. 18: 164-168.

LEIBEE, G. L. 1996. Insect pest management in crucifers in Florida, pp. 413-422 *in* Rosen D., F.D. Bennett and J. L. Capinera [eds.], Pest Management in the Subtropics: Integrated Pest Management - a Florida Perspective. Intercept Limited, Andover, United Kingdom.

MCLAUGHLIN, J. R., E. R. MITCHELL, AND P. KIRSCH. 1994. Mating disruption of diamondback moth (Lepidoptera: Plutellidae) in cabbage: reduction of mating and suppression of larval populations. J. Econ. Entomol. 87: 1198-1204.

MITCHELL, E. R., F. C. TINGLE, R. C. NAVASERO-WARD, AND M. KEHAT. 1997a. Diamondback moth (Lepidoptera: Plutellidae) parasitism by *Cotesia plutellae* (Hymenoptera: Braconidae) in cabbage. Florida Entomol. 80: 477-484.

MITCHELL, E. R., G. Y. HU, AND J. S. OKINE. 1997b. Diamondback moth (Lepidoptera: Plutellidae) infestation and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae) in collards and adjacent fields. Florida Entomol. 80: 54-62.

MITCHELL, E. R., G. Y. HU, J. S. OKINE, AND J. MCLAUGHLIN. 1997c. Mating disruption of diamondback moth (Lepidoptera: Plutellidae) and cabbage looper (Lepidoptera: Noctuidae) in cabbage using a blend of pheromones emitted from the same dispenser. J. Entomol. Sci. 32: 120-137.

MITCHELL, E. R., G. Y. HU, J. S. OKINE, AND J. E. CARPENTER. 1998. Parasitism of diamondback moth (Lepidoptera: Plutellidae) larvae by *Cotesia plutellae* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae) in cabbage fields after inundative releases of *C. plutellae*. J. Entomol. Sci. 4: (December).

MUCKENFUSS, A. E., B. M. SHEPPARD, AND E. R. FERRER. 1990. Natural mortality of diamondback moth in coastal South Carolina, pp. 27-36 *in* Talekar, N. S. [Ed.], Diamondback Moth and Other Crucifer Pests: Proceedings of the Second International Workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, AVRDC Publication No. 92-368, 603 pp.

OATMAN, E. R., AND G. R. PLATNER. 1969. An ecological study of insect populations on cabbage in southern California. Hilgardia 40: 1-40.

OKINE, J. S., E. R. MITCHELL, J. CARPENTER, AND G. Y. HU. 1998. Oviposition response of *Cotesia plutellae* (Hymenoptera: Braconidae) to sterile and untreated diamondback moth (Lepidoptera: Plutellidae) larvae and spatial dispersion of larvae on collard plants. Environ. Entomol. 27(6): December.

OOI, P. A. C. 1990. Management of diamondback moth in Cameron Highlands. Newlt. Malaysia Plant Prot. Soc. 13, 55-56.

OOI, P. A. C., AND G. S. LIM. 1989. Introduction of exotic parasitoids to control the diamondback moth in Malaysia. J. Plant Prot. Tropics 6: 103-111.

PIMENTEL, D. 1961. Natural control of caterpillar populations in cole crops. J. Econ. Entomol. 54: 889-892.

SHELTON, A. M., R. J. COOLEY, M. K. KROENING, W. T. WILSEY, AND S. D. EIGENBRODE. 1991. Comparative analysis of two rearing procedures for diamondback moth (Lepidoptera: Plutellidae). J. Entomol. Sci. 26: 17-26.

SHELTON, A. M., J. A. WYMAN, N. L. CUSHING, K. APFELBECK, T. J. DENNEHY, S. E. R. MAHR, AND S. D. EIGENBRODE. 1993. Insecticide resistance of diamondback moth (Lepidoptera: Plutellidae) in North America. J. Econ. Entomol. 86: 11-19.

TABASHNIK, B. E., N. L. CUSHING, N. FINSON, AND M. W. JOHNSON. 1990. Field development of resistance to *Bacillus thuringensis* in diamondback moth (Lepidoptera: Plutellidae). J. Econ. Entomol. 83: 1671-1676.

TALEKAR, N. S., AND A. M. SHELTON. 1993. Biology, ecology, and management of the diamondback moth (Lepidoptera: Plutellidae). Annu. Rev. Entomol. 38: 275-301.

ADIPOKINETIC HORMONES IN FIFTH INSTAR *ROMALEA GUTTATA* (ORTHOPTERA: ACRIDIDAE): ACTIVATION OF GLYCOGEN PHOSPHORYLASE DOES NOT PRODUCE HYPERTREHALOSEMIA

JOHN D. HATLE AND JEFFREY H. SPRING
University of Southwestern Louisiana, Department of Biology, PO Box 42451
Lafayette, LA 70504

ABSTRACT

Romalea guttata Houttuyn (= *R. microptera* Beavois) is flightless, lethargic, aposematic, and chemically defended. *R. guttata* stores large quantities of two adipokinetic hormone (AKH) family peptides in its corpora cardiaca. In adults, these peptides (Rom-CC-I and Grb-AKH) activate fat body glycogen phosphorylase but are not hypertrehalosemic. Because juvenile *R. guttata* contain sufficient peptide to be bioactive, we sought to determine whether these peptides are hypertrehalosemic, phosphorylase activating, or hyperlipemic in juveniles. Late fifth (= last) instar and adult *R. guttata* activated phosphorylase in response to Rom-CC-I injections. These same individuals showed no hypertrehalosemia in response to Rom-CC-I. We hypothesize that the glycogenolysis pathway is not started by activation of glycogen phosphorylase in response to Rom-CC-I. From fourth instar through third week adult, *R. guttata* showed a slight, statistically insignificant hypolipemia, but clearly no hyperlipemia. *R. guttata* differs from *Locusta migratoria* in that it appears to show neither hypertrehalosemia nor hyperlipemia at any developmental stage.

Key Words: lubber grasshopper; chemical defense; glycogenolysis; adipokinetic hormone

RESUMEN

Romalea guttata Houttuyn (= *R. microptera* Beavois) es una especie que no vuela, es letárgica, es aposemática, y se defiende por medios químicos. *R. guttata* guarda grandes cantidades de dos péptidos de la familia de las hormonas adipokinéticas (AKH) en la glándula corpora cardiaca. En los adultos, estos péptidos (Rom-CC- I and Grb-AKH) activan fosforilasas de glicógeno del cuerpo graso pero no son hipertrehalosémicos. Como los *R. guttata* juveniles contienen suficientes péptidos para que sean bioactivos, hemos tratado de determinar si estos péptidos son hipertrehalosémicos, activadores de fosforilasas, o hiperlipémicos en los juveniles. El quinto (último) instar y el adulto de *R. guttata* activaron la fosforilasa como respuesta a injecciones de Rom-CC-I. Estos mismos individuos no mostraron ninguna hipertrehalosemia como respuesta a Rom-CC-I. Nosotros suponemos que el proceso de glicogenolisis no es iniciado con la activación de la fosforilasa del glicógeno como respuesta a Rom-CC-I. Del cuarto estadío al adulto de tres semanas, los *R. guttata* mostraron una hipolipemia estadísticamente insignificante, pero claramente ninguna hyperlipemia. *R. guttata* difiere de *Locusta migratoria* en que parece no mostrar ni hipertrehalosemia ni hyperlipemia en ninguna fase de su desarrollo.

The Eastern Lubber Grasshopper, *Romalea guttata* Houttuyn (= *R. microptera* Beavois), is seasonally common in the Southeastern US, flightless, lethargic, aposematic, and chemically defended (Whitman et al. 1990). Adult *R. guttata* store large

quantities of two small peptides in their corpus cardiacum (CC; Gäde and Spring 1986). These peptides (Rom-CC-I and Grb-AKH) are members of the adipokinetic / red-pigment concentrating hormone (AKH/RPCH) family by both bioactivity in appropriate test species (Gäde and Spring 1986) and their primary structures (Gäde et al. 1988). Further, the peptides are released under *in vitro* physiological conditions (Spring and Gäde 1991). In adults, the endogenous functions of both Rom-CC-I and Grb-AKH appear to be control of fat body glycogen phosphorylase (GP), converting inactive GP to the active form. In adult *R. guttata*, separate injections of Rom-CC-I and Grb-AKH activated GP in a dose dependent manner (Gäde and Spring 1989). As is true with the well-studied migratory locust (*Locusta migratoria* L.), the activation of GP was not concurrent with hypertrehalosemia (Goldsworthy 1994). Also, the peptides elicited no hyperlipemic response in adult *R. guttata* (Spring and Gäde 1987). In sum, injections of endogenous AKHs into adult *R. guttata* do not appear to affect hemolymph metabolite levels.

Juvenile *R. guttata* contain sufficient Rom-CC-I and Grb-AKH to be biologically active (Spring and Gäde 1991). For example, early fourth instar *R. guttata* contain about 230 pmol Rom-CC-I and about 25 pmol Grb-AKH in their CC (Spring and Gäde 1991). These quantities are much greater than the minimum dosage (≈1 pmol) needed for maximal activation of GP in adults. We hypothesized that because Rom-CC-I and Grb-AKH are not known to play a biologically important role in adult *R. guttata*, they may function to control hemolymph metabolites in juveniles. Alternatively, endogenous AKHs in *R. guttata* may not serve any metabolite control functions that endogenous AKHs serve in other grasshoppers, such as *L. migratoria*.

We asked four primary questions. First, are juvenile *R. guttata* significantly hypertrehalosemic in response to synthetic Rom-CC-I (sRom-CC-I)? Second, do these same grasshoppers activate GP in response to sRom-CC-I? Third, are decapitated *R. guttata* significantly hypertrehalosemic in response to sRom-CC-I? Fourth, are juvenile *R. guttata* hyperlipemic in response to injections of CC homogenates?

Materials And Methods

Experiment 1—Responses of Hemolymph Carbohydrates to sRom-CC-I Injections through Development.

Experimental animals. In April, 1996, we collected first instar *R. guttata* near Lydia, LA, USA. We brought the insects to the laboratory and raised them as described by Whitman (1986). Briefly, we kept *R. guttata* at 30 ± 2°C on a 14L:10D photoperiod. *R. guttata* were fed oatmeal and Purina Cricket Chow® *ad libitum,* Romaine lettuce daily, and green beans, green onions, carrot tops, and apple occasionally.

*Determination of hemolymph carbohydrates***.** We measured changes in hemolymph carbohydrates in response to sRom-CC-I injections at six stages of development: instar 4 day 4 (= L4-d4); L5-d2; L5-d6; L5-d10; adult days 3 and 4 (= Ad-d3); and Ad-d9. To determine the hemolymph carbohydrate concentrations, we collected 2 µl hemolymph samples and measured total carbohydrates as anthrone positive material with trehalose standards (Spik and Montreuil 1964). We then injected each grasshopper with either 5 µl deionized H_2O or 20 pmol sRom-CC-I (Peninsula Laboratories Inc.; San Carlos, CA) in 5 µl deionized H_2O and measured hemolymph carbohydrates again 90 min later.

*Statistics***.** We tested the changes in carbohydrates for statistical differences between treatments with ANOVA and Tukey's post-tests.

Experiment 2—Responses of Active GP to sRom-CC-I Injections through Development**.**

*Experimental animals***.** Following the determination of carbohydrates, we offered each *R. guttata* Romaine lettuce and kept them isolated at 25 ± 2°C overnight until the GP experiments the next day. Half of the grasshoppers injected with deionized H_2O for carbohydrate determination were also injected with deionized H_2O for active GP determination. The remainder of the grasshoppers injected with deionized H_2O for carbohydrate determination were injected with sRom-CC-I for active GP determination. This treatment control influenced our data only once, for L5-d6 grasshoppers. At this developmental stage, insects injected with sRom-CC-I the previous day did not activate GP, but insects injected with deionized H_2O the previous day did activate GP. Because this was the sole influence of our treatment control, we combined the data for clarity of presentation.

Determination of active GP. We assayed active GP by following glycogen breakdown according to the methods of Ziegler et al. (1979) as modified by Gäde and Spring (1989).

*Statistics***.** We tested the changes in percent active GP for statistical differences between treatments by ANOVA with Tukey's post-tests.

Experiment 3—Responses of Hemolymph Carbohydrates to sRom-CC-I Injections in Decapitated Adults**.**

*Experimental animals***.** In August, 1996 we collected adult *R. guttata* from the same collection site used in Experiment 1. These *R. guttata* were kept as described above for three to six days before experimentation.

*Determination of hemolymph carbohydrates***.** The night before an experiment, we decapitated the grasshoppers and sealed the exposed orifices with liquid wax. We measured changes in hemolymph carbohydrates in response to either deionized H_2O injection or sRom-CC-I injection identically to Experiment 1, except that we took hemolymph samples from the coxal membrane.

*Statistics***.** We compared the data using student's t-tests.

Experiment 4—Determination of Hemolymph Lipids in Response to CC Homogenate Injections through Development**.**

Experimental animals. We collected *R. guttata* from April to August, 1994 from the same collection site used in Experiment 1. We fed these grasshoppers lettuce daily and Purina Cricket Chow® *ad libitum*. Grasshoppers were held in the laboratory at least 48 h prior to use. In all other respects, we maintained these grasshoppers identically to the grasshoppers used in Experiment 1.

CC homogenates. We prepared CC homogenates by the method of Gäde and Spring (1989). Our CC homogenates contained both Rom-CC-I and Grb-AKH, the predominant *R. guttata* CC peptides (Spring and Gäde, 1987).

Lipid assays. We measured changes in hemolymph lipids in response to CC preparation injections at six stages of development: instar 3 (= L3); L4; L5; week 1 adults (= Ad-w1); Ad-w2; Ad-w3. We used the method of Spring and Gäde (1987) to determine if *R. guttata*'s competence to CC homogenates with respect to hyperlipemia changes through development. We first collected a hemolymph sample from each grasshopper, and then we injected 5 μl aliquots of test solution (= 0.1 CC-equivalents) intra-abdominally. Second samples were taken 90 min post-injection. We measured total lipids as vanillin-positive material using vegetable oil standards following the method of Zöllner and Kirsch (1962).

Statistics. We tested the changes in lipid concentrations for statistical differences among developmental stages by ANOVA.

RESULTS

Experiment 1—Responses of Hemolymph Carbohydrates to sRom-CC-I Injections through Development.

Changes in hemolymph carbohydrate concentrations after test solution injections varied widely among the six developmental stages examined (Fig. 1). Except for L5-d2 ($P < 0.05$; Tukey's test), injection of sRom-CC-I did not statistically change hemolymph carbohydrate concentrations in comparison to water injection within any developmental stage.

Experiment 2—Responses of Active GP to sRom-CC-I Injections through Development.

Synthetic Rom-CC-I injection activates GP during the developmental period from L4-d4 to Ad-d9; an ANOVA revealed a statistically significant effect of sRom-CC-I injection ($P = 0.009$), but no significant effects for developmental stage ($P = 0.651$) or the interaction of developmental stage and sRom-CC-I injection (0.417; Fig. 2). In general, activation of GP was stronger in the older *R. guttata,* with competence to sRom-CC-I appearing to develop by L5-d10.

Experiment 3—Responses of Hemolymph Carbohydrates to sRom-CC-I Injections in Decapitated Adults

Changes in hemolymph carbohydrate concentrations in decapitated adults that were injected with water ($\bar{x} = 0.0151$ mg/ml; SE = 0.0054; n = 9) did not differ signif-

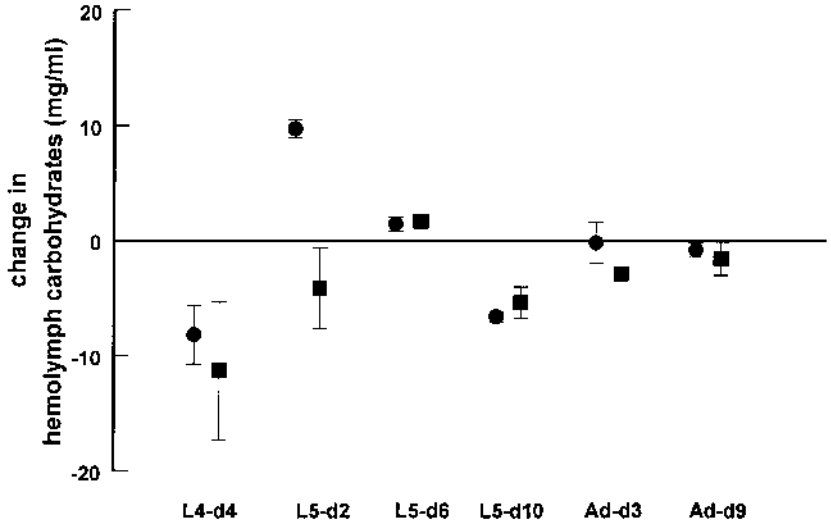

Fig. 1. Changes in hemolymph carbohydrate concentrations in response to injections at six developmental stages of *R. guttata*. Dots represent deionized water injected groups (n = 13-20), and squares represent sRom-CC-I injected groups (n = 7-10). For abbreviations, see text.

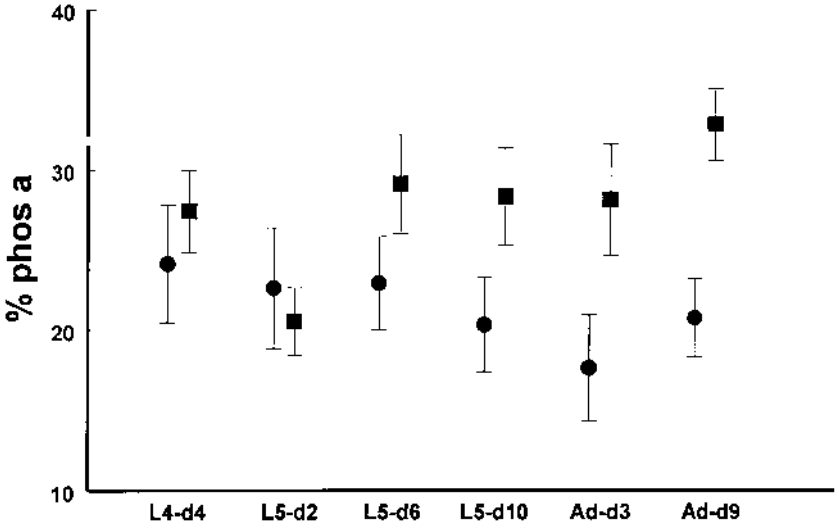

Fig. 2. Percent active fat body glycogen phosphorylase in response to injections in six developmental stages of *R. guttata*. Dots represent deionized water injected groups (n = 7-8), and squares represent sRom-CC-I injected groups (n = 13-17). For abbreviations, see text.

icantly from concentration changes in decapitated adults that were injected with sRom-CC-I ($\bar{x}$ = 0.0091 mg/ml; SE = 0.0038; n = 12).

Experiment 4—Changes in Hemolymph Lipids in Response to CC Homogenate Injections through Development.

Injections of CC extracts produced a hypolipemic affect in all developmental stages from L3 to Ad-w3 (Fig. 3). ANOVA revealed no significant differences in the responses among any of the developmental stages (P = 0.127). In general, adults showed the strongest hypolipemic responses, and larvae showed the weakest hypolipemic responses.

Discussion

Activation of GP but Absence of Hypertrehalosemia

R. guttata significantly activate GP in response to sRom-CC-I injections (Fig. 2). The interaction of developmental stage and sRom-CC-I injection was insignificant; nonetheless, it appears from our data that the competence to AKHs in *R. guttata* develops in the late fifth instar. Regardless of the developmental moment of the onset of competence, it is clear that the older *R. guttata* in our study activated GP in response to sRom-CC-I injections.

R. guttata has no competence to sRom-CC-I with respect to hypertrehalosemia at any developmental stage from L4-d4 through Ad-d9 (Fig. 1). For the well-studied *L. migratoria*, there have been at least two explanations postulated in the literature for this lack of hypertrehalosemia concurrent with activation of GP: 1) not enough glyco-

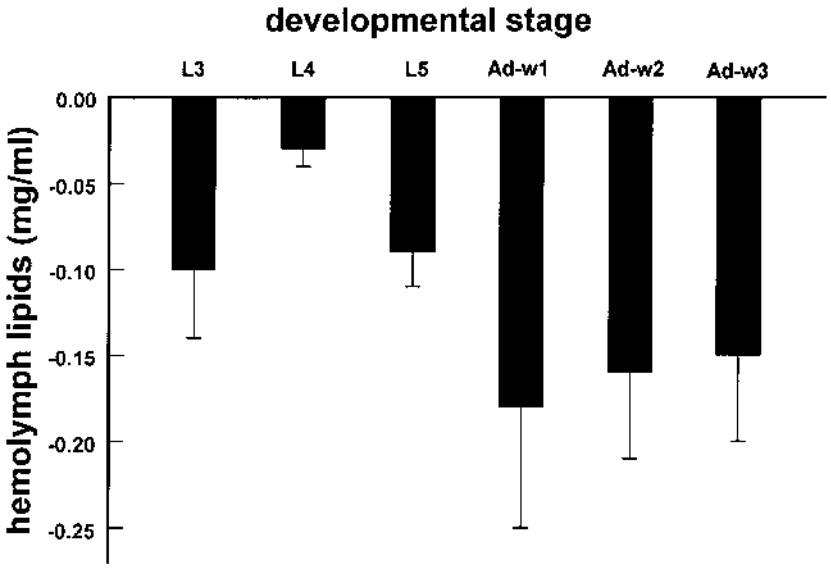

Fig. 3. Changes in hemolymph lipid concentrations in response to injections of corpus cardiaca preparations at six developmental stages (n = 7-10) of *R. guttata*. For abbreviations, see text.

gen in the fat body (Goldsworthy 1994), and 2) inhibition of hyperglycemia by an unspecified "head factor" (Loughton and Orchard 1981). Adult *R. guttata*, especially those fed daily in the laboratory (as ours were), have sufficient glycogen in the fat body to produce a hypertrehalosemic effect (Spring and Gäde 1987). Second, the lack of hypertrehalosemia in decapitated adults (see Experiment 3) suggests that, in *R. guttata*, a head factor is not necessary for the prevention of hypertrehalosemia.

Alternatively, it may be that the additional quantity of GP activated in our experiments (≈ 10%) was not sufficient to induce hypertrehalosemia. We do not believe this to be true for two reasons. First, Gäde and Spring (1989) showed stronger activations of GP (≈ 30%) but still no hypertrehalosemia. Second, the catalytic nature of enzyme function requires only a small change in enzyme activation to produce a large change in metabolite concentrations.

Fifth instar *L. migratoria* are moderately hypertrehalosemic in response to the endogenous Lom-AKH-I (Van Marrewijk et al. 1984), whereas fifth instar *R. guttata* are clearly not hypertrehalosemic in response to sRom-CC-I. In fact, our data suggest that L5-d2 *R. guttata* may be hypotrehalosemic in response to sRom-CC-I. We therefore hypothesize that the glycogenolysis pathway is not started by activation of GP in response to sRom-CC-I in *R. guttata*. *R. guttata* may activate GP for some function other than the mobilization of sugars, but this seems highly unlikely. Barring this explanation, the competence to sRom-CC-I in *R. guttata* appears to be an evolutionary remnant of the development of flight physiology in last instar Acrididae. Importantly, our data suggest that the physiology of *R. guttata*, as well its behavior, is different from other grasshoppers, and that this difference reflects its flightless, lethargic, chemically defended life style.

Hypolipemic Response to CC Homogenates?

R. guttata are slightly hypolipemic in response to CC homogenate injections at developmental stages from L3 through Ad-w3. Rom-CC-I and Grb-AKH are the predominant peptides in *R. guttata* CC homogenates. Hence, at the very least, *R. guttata* are not hyperlipemic in response to injections of endogenous AKHs. In contrast, *L. migratoria* develop competence with respect to hyperlipemia to the synthetic endogenous AKH as L5 (Van Marrewijk et al. 1984). The lack of hyperlipemia in L5 and adult *R. guttata* is further evidence that the physiology of these grasshoppers is different from the physiology of grasshoppers that fly.

Divergent Physiology?

We have shown three ways that *R. guttata* differs physiologically from *L. migratoria*. All three of these differences make sense in light of *R. guttata*'s flightless and lethargic behavior: 1) absence of hypertrehalosemia in late L5 grasshoppers in concert with activation of GP; 2) absence of hypertrehalosemia in decapitated adults of *R. guttata*; 3) absence of hyperlipemia in L5 and adult *R. guttata.* Taken together, these data suggest that *R. guttata* may have diverged physiologically from grasshoppers that can fly, and that *R. guttata's* responses mirror its flightless, lethargic, chemically defended life-style.

References Cited

GÄDE, G., AND J. H. SPRING. 1986. Presence and preliminary characterization of factors regulating carbohydrate and lipid metabolism isolated from the corpus cardiacum of the Eastern lubber grasshopper. pp. 191-194 *in* Borkovec A. B. and D. B. Gelman [eds]. Insect Neurochemistry and Neurophysiology. Humana Press, Clifton, NJ.

GÄDE, G., AND J. H. SPRING. 1989. Activation of fat body glycogen phosphorylase in the eastern lubber grasshopper (*Romalea microptera*) by the endogenous neuropeptides Ro I and Ro II. J. Exp. Zool. 250: 140-149.

GÄDE, G., C. HILBICH, K. BEYREUTHER, AND K. L. RINEHART. 1988. Sequence analyses of two neuropeptides of the AKH/RPCH-family from the lubber grasshopper, *Romalea microptera.* Peptides 9: 681-688.

GOLDSWORTHY, G. 1994. Adipokinetic hormones of insects: are they the insect glucagons? pp. 486-492 *in* Davey, K. G., R. E. Peter, and S. S. Tobe. Perspectives in Endocrinology. National Research Council Of Canada, Ottawa.

LOUGHTON, B. G., AND ORCHARD, I. 1981. The nature of the hyperglycemic factor from the glandular lobe of the corpus cardiacum of *Locusta migratoria*. J. Insect. Physiol. 27: 383-385.

SPIK, G., AND J. MONTREUIL. 1964. Deux causes d'erreur dans les dosages colorimetriques des oses neutres totaux. Bull. Soc. Chim. Biol. 46: 739-749.

SPRING, J. H., AND G. GÄDE. 1987. Factors regulating carbohydrate and lipid metabolism isolated from the corpus cardiacum of the Eastern lubber grasshopper, *Romalea microptera*. J. Exp. Zool. 241: 41-50.

SPRING, J. H., AND G. GÄDE. 1991. Storage and release of neuropeptides from the corpus cardiacum of the Eastern lubber grasshopper, *Romalea microptera*. J. Exp. Zool. 258: 60-68.

WHITMAN, D. W. 1986. Laboratory biology of *Taeniopoda eques* (Orthoptera: Acrididae). J. Entomol. Sci. 21: 87-93.

WHITMAN, D. W., M. S. BLUM, AND D. W. ALSOP. 1990. Allomones: chemicals for defense. pp. 289-351 *in* Evans, D. L., and J. O. Schmidt [eds]. Insect Defenses. State University of New York Press, Albany

VAN MARREWIJK, V. J. A., A. TH. M. VAN DEN BROEK, D. J. VAN DER HORST, AND A. M. TH. BEENAKKERS. 1984. Hypertrehalosaemic and hyperlipaemic responses to adipokinetic hormone in fifth larval instar locusts, *Locusta migratoria*. Insect. Biochem. 14: 151-157.

ZIEGLER, R., M. ASHIDA, A. M. FALLON, L. T. WIMER, S. S. WYATT, AND G. R. WYATT. 1979. Regulation of glycogen phosphorylase in fat body of *Ceropia* silkmoth pupae. J. Comp. Physiol. B131: 321- 332.

ZÖLLNER, N., AND K. KIRSCH. 1962. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophospho-vanillin Reaktion. Z. Ges. Exp. Med. 135: 545-561.

INSECTS, COFFEE AND OCHRATOXIN A

FERNANDO E. VEGA AND GUY MERCADIER
European Biological Control Laboratory, United States Department of Agriculture Agricultural Research Service, Parc Scientifique Agropolis II,34397 Montpellier Cedex 5, FRANCE

A problem with coffee consumption is the possible presence of ochratoxin A, a potent toxin produced by *Aspergillus ochraceus* Wilh. and *Penicillium viridicatum* Westl. Several studies have demonstrated the presence of this toxin in green coffee beans, roasted coffee, and coffee brews, including instant coffee (Levi et al. 1974, Tsubochi et al., 1984, Micco et al. 1989, Studer-Rohr et al. 1994, Patel et al. 1997). In the UK, out of 100 retail coffee samples tested for ochratoxin A, 81 tested positive (Patel et al. 1997). Coffee exported from Brazil to Greece and Lebanon must be tested for ochratoxin A and levels must be below 20 mg/kg (Milanez et al. 1995). After collecting coffee beans infected with the coffee berry borer (*Hypothenemus hampei* (Ferrari), Coleoptera: Scolytidae) in Uganda and Benin, as part of a project aimed at finding new biological control agents against this insect pest, we isolated *A. ochraceus* from adult insects emerging from the beans. In Uganda, of 636 insects emerging from coffee beans collected in 26 sites, 34 (5.3%) were infected with *A. ochraceus*. In Benin, out of 564 insects originating in one site, 98 (17.4%) were infected with *A. ochraceus*. *H. hampei* females lay eggs inside the coffee bean where both larval development and mating occur. If the mother is infected with *A. ochraceus* while entering the bean, it is likely that the adult progeny leaving the bean will also be infected, thereby disseminating the fungus. This insect, endemic to Africa, has now spread to most coffee growing regions in the world; therefore, its potential to serve as a vector for this cosmopolitan fungus is high. Other insects are known to serve as vectors for toxicogenic fungi, including *Aspergillus flavus* Link: Fr. to corn (Dowd 1998). Our finding indicates that *H. hampei*, in addition to being a direct pest of coffee, could also serve as a vector for *A. ochraceus*. Plans aimed at reducing ochratoxin contamination in coffee beans should take into consideration the presence of the insect in the field.

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SUMMARY

A search for natural enemies of the coffee berry borer *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae) in Uganda and Benin revealed that the insect serves as a carrier for *Aspergillus ochraceus* K. Wilh., a fungus known to produce ochratoxin A. Contamination with this toxin is a serious problem for the coffee industry. Plans aimed at reducing this problem should take into consideration the presence of this insect in the field.

REFERENCES CITED

DOWD, P. F. 1998. The involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions. *in*: K. K. Sinha and D. Bhatnagar [eds.] Mycotoxins in Agriculture and Food Safety. Marcel Dekker, New York, pp. 307-350.

LEVI, C. P., H. L. TRENK, AND H. K. MOHR. 1974. Study of the occurrence of ochratoxin A in green coffee beans. J. Assoc Off. Anal. Chem. 57: 866-870.

MICCO, C., M. GROSSI, M. MIRAGLIA, AND C. BRERA. 1989. A study of the contamination of ochratoxin A of green and roasted coffee beans. Food Add. Contam. 6: 333-339.

MILANEZ, T. V., M. SABINO, AND L. C. A. LAMARDO. 1995. Comparison of two methods for the determination of ochratoxin A in green coffee beans. Rev. Microbiol. 26: 79-82.

PATEL, S., C. M. HAZEL, A. G. M. WINTERTON, AND A. E. GLEADLE. 1997. Survey of ochratoxin A in UK retail coffees. Food Add. Contam. 3: 217-222.

STUDER-ROHR, I., D. R. DIETRICH, J. SCHLATTER, AND C. SCHLATTER. 1994. The occurrence of ochratoxin A in coffee. Food Chem. Toxicol. 33: 341-355.

TSUBOCHI, H., K. YAMAMOTO, K. HISADA, AND Y. SAKABE. 1984. A survey of occurrence of mycotoxins and toxicogenic fungi in imported green coffee beans. Proc. Jpn. Assoc. Mycotoxicol. 19: 14-21.

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REDISCOVERY OF A SPRINGTAIL AND A GRASSHOPPER IN FLORIDA

Krista E. M. Galley[1] and R. Wills Flowers[2]

[1]Longleaf Pine Restoration Project, The Nature Conservancy, P.O. Box 875, Niceville, FL 32588

[2]Agricultural Research Programs, Florida A&M University, Tallahassee, FL 32307

Ecosystem management programs of the U.S. Forest Service (Hermann et al., in press) and a cooperative agreement between The Nature Conservancy and the Department of Defense have promoted study of the increasingly rare longleaf pine (*Pinus palustris*) ecosystem (Biondo 1997) and the arthropods inhabiting it. In the Florida Panhandle, two research projects on longleaf pine restoration ecology have led to the rediscovery in Florida of the springtail *Sminthurus floridanus* MacGillivray (Collembola: Sminthuridae) and the grasshopper *Gymnoscirtetes morsei* Hebard (Orthoptera: Acrididae). Both species have been searched for in Florida in recent years without success.

S. floridanus was described from one specimen collected in "Florida" (MacGillivray 1893). This species was known from that single specimen until Snider (1982) redescribed *S. floridanus* from several series collected at the Savannah River Plant, Aiken, South Carolina. These series were swept from roadside grass beneath tall loblolly pines.

In 1995-1997, *S. floridanus* was collected on Eglin Air Force Base (EAFB) in northwest Florida. All specimens were taken in an area subject to frequent fires, characterized by a nearly pure stand of longleaf pine, a sparse hardwood midstory, and a dense groundcover of grasses and forbs, including bluestems (*Andropogon* spp. and *Schizachyrium* spp.), low panic grasses (*Dichanthelium* spp.), pineywoods dropseed (*Sporobolus junceus*) and wiregrass (*Aristida beyrichiana*). Collection data are: Florida, Okaloosa Co., Eglin Air Force Base, T1S-R25W-sec. 30, 31-V-1995, 21-IX-1995, 01-VI-1996, 16-VI-1997, D-Vac and sweep net, 70% EtOH, Longleaf Pine Restoration Project, Site 2C-W.

A second series of *S. floridanus* specimens was collected by D-Vac in the Apalachicola National Forest (ANF), Florida. Specimens were also taken from longleaf pine-wiregrass habitats that experience regular fires. Collection data are: Florida, Liberty Co., Apalachicola Nat. For., Hwy 379 NW Sumatra, Compartment 95, 11-VI-1997, 30-X-97; Compartment 100, 30-X-1997; Hwy 85 N of Wilma, Compartment 11, 14-VIII-1997, 30-X-1997.

S. floridanus is a distinctive sminthurid, due to the sharp contrast between the dark blue dorsum and the yellowish venter, plus the acuminate dorsal protuberance anteriad of the anal papilla (habitus in Snider [1982: 223] and Borror et al. [1989: 167]). All EAFB and ANF specimens exhibit this dorsal protuberance. Voucher specimens from EAFB and ANF are deposited in the Entomology Museum, Michigan State University (East Lansing) and Florida State Collection of Arthropods (Gainesville).

Gymnoscirtetes morsei Hebard is one of two species in this genus. Both species are found in the southeastern United States and can be recognized by their small size and lack of any trace of wings or wingpads in the adult. *G. morsei* was described from DeFuniak Springs, Florida (Hebard 1918) and has since been found only in the Florida Panhandle between the ANF and Mobile, Alabama, and in some adjacent Alabama counties. Collection records in the Florida State Collection of Arthropods and other institutions, notably the University of Michigan (T. J. Cohn, Museum of Zoology, University of Michigan, personal communication) all date from before the early 1950s.

In 1996, four specimens were collected in June and August on Hurlburt Field, located on 6,634 acres in south Okaloosa County west of Mary Esther and Ft. Walton Beach, Florida. Hurlburt Field contains a variety of habitats, including wet longleaf pine savannah, where the specimens of *G. morsei* were collected. This collection locality was burned in early 1997. In April, 1997, large numbers of *G. morsei* nymphs were observed in the burned area. The grasshopper was very common early in the growing season and became somewhat less abundant as the summer progressed. Voucher adults were first collected during a visit in June, the last adults of the year were seen in late October, and nymphs were collected again in April, 1998. *G. morsei* appears to favor a moist microclimate: grasshoppers jumped up from near the ground to the tops of grasses and low bushes when approached. One female was observed feeding on leaves of gallberry (*Ilex glabra*). Several other adults were confined in plastic bags with various common plants from their groundcover habitat; only gallberry leaves showed any evidence of feeding after several days. Collection records for Hurlburt Field are: Florida, Okaloosa Co., Ft. Walton Beach, Hurlburt Field, 9-VIII-1996; same locality, S of EOD [Explosive Ordinance Disposal], 27-VI-1997, 11-VII-1997, 17-IV-1998.

Another population of *G. morsei* was found in 1997 on Whitmier Island, a wet prairie on the northern border of EAFB. The groundcover habitat where the *G. morsei* were taken was very similar to the Hurlburt Field groundcover layer. Collection records from this locality are: Florida, Santa Rosa Co., Whitmier Island, T1N-R26W-sec. 19, 23-VIII-1997, Eglin AF Base, W side of RR 717, *ex* wet prairie. Voucher specimens from Hurlburt Field and EAFB are deposited in the Florida State Collection of Arthropods (Gainesville).

Gymnoscirtetes morsei is distinguishable from the more widespread *G. pusilla* Scudder by the external male genitalia (Blatchley 1920); however, descriptions of the aedeagi of the two species have not been published. There is some evidence that *G. morsei* may be conspecific with *G. pusilla* (G. Folkerts, Dept. Zoology and Wildlife, Auburn University, personal communication). Both morphospecies are found most commonly in the herbaceous groundcover under open pine canopy. Moist flatwoods, where pitcher plants grow and where gallberry is present, seem particularly favored by *Gymnoscirtetes.*

Until recently, there has been little study of arthropod communities in fire-maintained longleaf pine habitats (Folkerts et al. 1993). This could be due to an erroneous perception that burned areas have less zoological richness; even entomologists sometimes misunderstood the importance of fire in maintaining the rich biodiversity of longleaf pine ecosystems (cf. Klots 1951: 33). As arthropod faunas of the longleaf pine landscape become better known, discoveries of new species and rediscoveries of rare and uncommonly collected species will become increasingly frequent.

We thank R. J. Snider for identifying *S. floridanus* and for bringing the status of this species to KEMG's attention. T. J. Cohn, D. R. Gordon, B. J. Herring, and L. Provencher provided useful comments on the manuscript.

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Summary

Research on Eglin Air Force Base, Hurlburt Field, and the Apalachicola National Forest has led to rediscovery in Florida of the springtail *Sminthurus floridanus* (Collembola: Sminthuridae) and the grasshopper *Gymnoscirtetes morsei* (Orthoptera: Acrididae). *S. floridanus* was collected in fire-maintained longleaf pine/wiregrass stands. *G. morsei* was found in wet areas, including longleaf pine savannahs, flatwoods, and prairies.

References Cited

Biondo, B. 1997. In defense of the longleaf pine. Nature Conservancy 47(4): 10-17.

Blatchley, W. S. 1920. Orthoptera of northeastern America. The Nature Publ. Co., Indianapolis, IN. 784 pp.

Borror, D. J., C. A. Triplehorn, and N. F. Johnson. 1989. An introduction to the study of insects, 6th ed. Saunders College Publ., Philadelphia, PA. 875 pp.

Folkerts, G. W., M. A. Deyrup, and D. C. Sisson. 1993. Arthropods associated with xeric longleaf pine habitats in the southeastern United States: a brief overview, pp. 159-192 *in* S. M. Hermann [ed.], Proceedings of the Tall Timbers Fire Ecology Conference No. 18, Tall Timbers Research Station, Tallahassee, FL.

Hebard, M. 1918. New genera and species of Melanopli found within the United States. Transactions of the American Entomological Society 44: 141-169.

Hermann, S. M., T. Van Hook, R. W. Flowers, L. A. Brennan, J. S. Glitzenstein, D. R. Streng, J. L. Walker, and R. L. Myers. 1998. Fire and biodiversity: studies of vegetation and arthropods pp. 384-401 *in* G. W. Kelly [ed.], Trans. 63rd No. Amer. Wildl. and Natur. Resour. Conf. Vol. 63.

Klots, A. B. 1951. A field guide to the butterflies. Peterson Field Guide Series (1958 ed.). Houghton Mifflin Co., Boston, 349+xvi pp.

MacGillivray, A. D. 1893. North American Thysanura. Canadian Entomol. 25: 127-128.

Snider, R. J. 1982. Redescription of *Sminthurus floridanus* MacGillivray, 1893 (Collembola: Sminthuridae). Florida Entomol. 65: 221-227.

COMPARATIVE TOXICITY OF SPINOSAD TO *FRANKLINIELLA* SPP. (THYSANOPTERA: THRIPIDAE), WITH NOTES ON A BIOASSAY TECHNIQUE

J. E. EGER, JR.[1] J. STAVISKY[2], AND J. E. FUNDERBURK[2]
[1]Dow AgroSciences, 2608 S. Dundee Blvd., Tampa, FL 33629

[2]North Florida Research and Education Center, University of Florida, Route 3 Box 4370, Quincy, FL 32351-9500

The western flower thrips *Frankliniella occidentalis* (Pergande) is a very serious worldwide pest of ornamental, vegetable, and fruit crops in the field and greenhouse (Tommasini and Maini 1995). It is an efficient vector for tomato spotted wilt virus, a serious disease of a wide variety of plants, including vegetable, flower, and ornamental crops (Allen et al. 1990). Western flower thrips are difficult to control effectively with insecticides (Brodsgaard 1994), and resistance has developed to organophosphate, carbamate, pyrethroid, and macrocyclic lactone insecticides after repeated exposure (Immaraju et al. 1992).

Spinosad (DowElanco, Indianapolis, IN), a new natural macrocyclic lactone insect control product with a unique mode of action, was highly efficacious against *F. occidentalis* in field experiments with pepper conducted in North Florida during 1996 and 1997 (J. E. F, J. S., and S. M. Olson, unpublished data). Another abundant flower thrips species, *F. tritici* (Fitch), was not significantly suppressed by spinosad in these experiments and spinosad did not have detrimental effects on populations of the minute pirate bug, *Orius insidious* (Say), a key predator of *F. occidentalis.* Thus, spinosad has the potential to be an important new tool for managing *F. occidentalis.* For this reason and because *Frankliniella* species differ in their ability to transmit tomato spotted wilt virus (Sakimura 1962, 1963), an understanding of the comparative toxicity of spinosad to various species of *Frankliniella* is needed. Knowledge of spinosad toxicity and the development of an effective bioassay will facilitate resistance monitoring for these pests.

Previous resistance or efficacy bioassays for *F. occidentalis* have employed either topical application (Robb 1989), detached leaves as a substrate for the insecticide (e.g., Immaraju et al. 1992), or a residue-on-glass technique (Brodsgaard 1994). Our objectives were to develop an insecticide bioassay procedure suitable for three common species of flower thrips in Florida, F. *occidentalis, F. tritici, and F. bispinosa* (Morgan), and to determine the toxicity of spinosad to these flower thrips species as a possible explanation for control differences in field plots.

Flower thrips were collected from wild radish, *Raphanus raphanistrum* L., growing 50-300 m from pepper fields at the North Florida Research and Education Center of the University of Florida in Quincy. Collection dates were May 27-29, 1997. Adults were aspirated into glass tubes (6 mm diam.) and then emptied into individual plastic diet cups (35 ml) with uncoated paper caps. The cups were provided with sections (20 mm) of snap bean pods sealed at either end with a thin layer of paraffin. After sealing, bean pod sections were submerged for 30 sec in 10 different concentrations of a 0.24 kg ai/l suspension concentrate of spinosad (SpinTor® 2SC, Dow AgroSciences, Indianapolis, IN) in distilled water, and allowed to air dry for 1 hr. Individual cups were placed into a sealed plastic rearing container (5.7 liter), the bottom of which was covered with moist paper toweling. These containers were held in a controlled-environment chamber maintained at 23° ± 2°C, 60% RH and a photoperiod of 14: 10 (L: D). The trial was replicated four times with three diet cups per replicate.

Because of the fragile nature of thrips and the difficulty in separating living individuals into the three species, no attempt was made to standardize the numbers of each species or the total number of individuals placed in each diet cup. We attempted to place a minimum of 20 individuals into each cup. Table 1 lists the range and mean numbers of each species used in this trial. These numbers are representative of the relative species abundance on wild radish on the collection dates. If less than 5 of any one species was present in any replicate, that replicate was repeated the following day using newly prepared solutions.

Although bioassay development will not be dealt with in detail here, a few observations are relevant. We initially evaluated glass snap-cap vials in addition to plastic diet cups as bioassay containers. The vials had a small opening which made them more difficult to use and plastic caps which promoted the formation of excess moisture, thus diet cups were chosen for further evaluation. We evaluated thrips survival in empty diet cups, in cups with bean sections only, with a small piece of moistened paper toweling only, and with both bean sections and toweling. Thrips survival at 24 hrs was minimal in empty cups. The paper toweling resulted in excess moisture which trapped and drowned some thrips. Cups with snap bean sections only resulted in the highest (virtually 100%) survival. Uncoated paper caps were chosen over wax coated caps because the latter resulted in excessive moisture in the cups. The sealed plastic rearing containers with moist paper toweling did not promote excess moisture in the cups, but moisture from the paper toweling did result in a slight expansion of the paper caps to provide a better seal and less desiccation of bean sections. The ends of bean sections were coated with paraffin to reduce desiccation and to serve as a barrier to prevent thrips from crawling inside the bean section. Finally, we chose to treat only the bean sections and not the cup itself because preliminary trial observations suggested that thrips spent most of their time on the bean sections.

Mortality was evaluated at 24 ± 1 hrs. Thrips were considered dead if they were unable to stand upright and/or move forward when probed. Individuals were segregated into living and dead, placed in alcohol and the respective numbers of each species determined under a dissecting microscope. No mortality was observed in untreated controls for *F. occidentalis* and *F. tritici*. For *F. bispinosa*, mortality (3%) was observed in only one replicate. Mortality for the various doses were corrected for control mortality (Abbott 1925). Data were analyzed with analysis of regression using a log-probit model. The analytical software used was Statgraphics Plus® (Manugistics, Inc., Rockville, MD).

The most common species in our samples was *F. bispinosa*, while *F. occidentalis* was the least common (Table 1). Numbers of *F. bispinosa* used in the bioassay were >3X those of *F. occidentalis* and numbers of *F. tritici* were >2X those of *F. occidentalis*. In contrast, Salguero Navas et al. (1991), also working in North Florida, found that *F. oc-*

TABLE 1. RANGE AND MEAN NUMBERS OF EACH *FRANKLINIELLA* SPECIES USED IN THE BIOASSAY.

Species	Mean/ Replicate	Range/ Replicate	Total # Tested	Sex Ratio F:M
F. occidentalis	8.8	5-21	379	2.14:1.0
F. tritici	18.3	7-42	803	2.00:1.0
F. bispinosa	30.7	14-62	1352	2.21:1.0
All species combined	57.6	34-87	2534	2.13:1.0

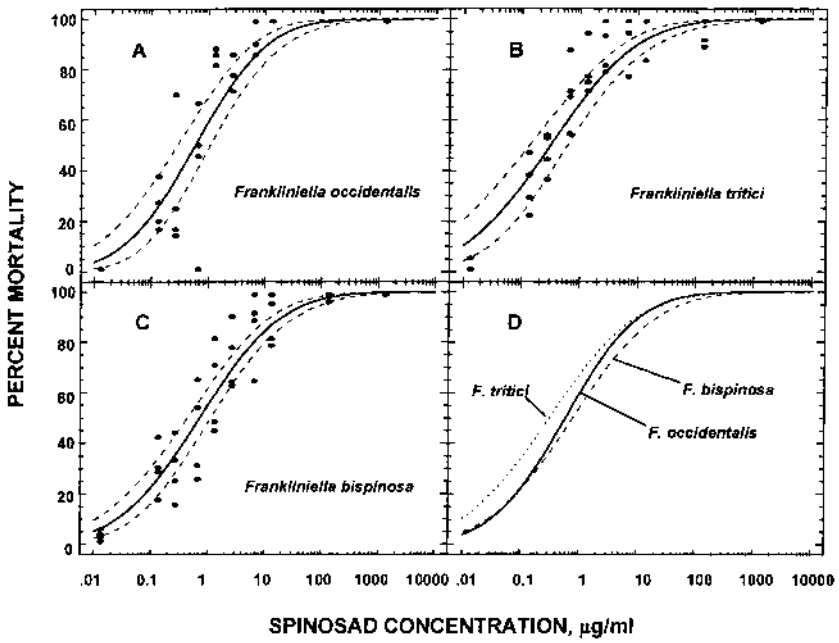

Fig. 1. Mortality of *Frankliniella* spp. in response to spinosad; responses of *F. occidentalis* (A), *F. tritici* (B), and *F. bispinosa* (C), and all three species combined (D). Solid lines are the predicted dose response curves (log-probit model) and dashed lines represent corresponding 95% confidence intervals. Dose response curves for all three species of *Frankliniella* are compared in Figure D.

cidentalis was generally the most abundant species of *Frankliniella* in tomato flowers in the spring and *F. bispinosa* was relatively uncommon in their study. Our results may represent a host preference of *F. bispinosa* for wild radish. An unusually warm winter in 1996-97 may have also contributed to the differences. Sex ratios of *Frankliniella* spp. tested are also given in Table 1. There was roughly a 2 to 1 ratio of females to males with only minor differences between species. Dose responses of males and females were not significantly different for any species, so data for the two sexes were combined.

Dose-response curves were similar for all three *Frankliniella* species (Figure 1). Regressions of dose/mortality data were highly significant for all three species (R^2 = 75-85%, $P < 0.00001$) (Table 2). The regression slope for the *F. occidentalis* data was significantly higher than that for the other two species based on non-overlapping standard error values. Standard error values around the regression slopes for *F. tritici* and *F. bispinosa* data did overlap, indicating that slopes for these species were not significantly different. Although *F. tritici* was numerically more susceptible than *F. bispinosa* as indicated by the lower LC_{10}, LC_{50}, LC_{90} and LC_{99} values, the 95% confidence intervals around these values for the three species overlapped. Thus, there were no significant differences among the three species.

Data presented here suggest that spinosad is equally toxic to the three species of *Frankliniella* tested and that differential toxicity of spinosad to *Frankliniella* spp. is probably not responsible for differences in relative species abundance between non-

TABLE 2. PARAMETERS OF REGRESSIONS (LOG-PROBIT MODEL) AND PREDICTED VALUES DESCRIBING SPINOSAD TOXICITY TO *FRANKLINIELLA* SPP.

	F. occidentalis	*F. tritici*	*F. bispinosa*
R-Squared (%)	76.35	74.96	85.50
Probability Level	< 0.00001	< 0.00001	< 0.00001
Slope (SE)	0.437 (0.040)	0.368 (0.035)	0.383 (0.026)
Intercept (SE)	0.227 (0.132)	0.427 (0.115)	0.124 (0.085)
LC_{10} (µg/ml)	0.032	0.0096	0.025
95% Confidence Limits	0.0097 - 0.075	0.0022 - 0.028	0.011 - 0.051
LC_{50} (µg/ml)	0.594	0.31	0.72
95% Confidence Limits	0.293 - 1.09	0.14 - 0.61	0.44 - 1.13
LC_{90} (µg/ml)	11.19	10.18	20.59
95% Confidence Limits	6.07 - 23.03	5.47 - 20.95	12.73 - 35.93
LC_{99} (µg/ml)	122.50	173.98	315.96
95% Confidence Limits	53.80 - 368.86	72.72 - 568.82	156.89 - 760.15

treated and spinosad-treated field plots (J. E. F., J. S., and S. M. Olson, unpublished data). Field rates of spinosad that have demonstrated activity against *Frankliniella* spp. (75-100 g ai/ha) will result in concentrations of 50-200 ppm of spinosad in normal application volumes. Although laboratory results may not translate directly to field activity, these concentrations exceed concentrations needed to provide greater than 90% mortality of all three species based on our bioassay. Differences in species abundance in spinosad-treated field plots may thus be due to factors other than differential toxicity (e. g., migration or competition).

Populations of *F. occidentalis* have been shown to have multiple resistance mechanisms and have developed cross-resistance to insecticides within the same chemical group and to those in other classes (Zhao et al. 1995). Consequently, alternating insecticides from different classes with different modes of action as a sole resistance management tactic poses risks. Insecticide selection pressure can be minimized by using noninsecticidal methods in conjunction with carefully selected insecticides used only when needed. The efficacy of spinosad demonstrated in our research reported here, combined with its compatibility with the key natural enemy of flower thrips, make it a potentially important tool for integrated pest management programs. Further, the baseline knowledge of spinosad toxicity reported here will help to develop a resistance monitoring program to determine the effectiveness of integrated pest management programs in minimizing resistance development.

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SUMMARY

A bioassay technique was developed and used to determine the toxicity of spinosad to three species of *Frankliniella*: *F. bispinosa*, *F. occidentalis*, and *F. tritici*. Dose response curves for the three species were similar and regressions of dose/mortality data were highly significant (R^2 = 75-85%, P < 0.00001 for all species). 95% confidence intervals around LC_{10}, LC_{50}, LC_{90} and LC_{99} values for the three species overlapped, suggesting that there were no significant differences among the three species tested.

REFERENCES CITED

ABBOTT, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

ALLEN, W. R, J. A. MATTEONI, AND A. B. BROADBENT. 1990. Susceptibility of cultivars of florist's chrysanthemum to tomato spotted wilt virus. Can. J. Plant Pathol. 12: 417-423.

BRODSGAARD, H. F. 1994. Insecticide resistance in European and African strains of western flower thrips (Thysanoptera: Thripidae) tested in a new residue-on-glass test. J. Econ. Entomol. 87: 1141-1146.

IMMARAJU, J. A., T. D. PAINE, J. A. BETHKE, K. L. ROBB, AND J. P. NEWMAN. 1992. Western flower thrips (Thysanoptera: Thripidae) resistance to insecticides in coastal California greenhouses. J. Econ. Entomol. 85: 9-14.

ROBB, K. L. 1989. Analysis of *Frankliniella occidentalis* (Pergande) as a pest of floricultural crops in California greenhouses. Ph.D. Dissertation, University of California, Riverside.

SAKIMURA, K. 1962. *Frankliniella occidentalis* (Thysanoptera: Thripidae), a vector of the tomato spotted wilt virus, with special reference to the color forms. Ann. Entomol. Soc. Am. 55: 387-389.

SAKIMURA, K. 1963. *Frankliniella fusca,* an additional vector of the tomato spotted wilt virus, with notes on *Thrips tabaci,* another vector. Phytopathology 53: 412-415.

SALGUERO NAVAS, V. E., J. E. FUNDERBURK, R. J. BESHEAR, S. M. OLSON, AND T. P. MACK. 1991. Seasonal patterns of *Frankliniella* spp. (Thysanoptera: Thripidae) in tomato flowers. J. Econ. Entomol. 84: 1818-1822.

TOMMASINI, M. G., AND S. MAINI. 1995. *Frankliniella occidentalis* and other thrips harmful to vegetable and ornamental crops in Europe, p. 1- 42. *in*: A. J. M. Loomans, J. C. van Lenteren, M. G. Thomasini, S. Maini, and J. Riudavets (eds.). Biological Control of Thrips Pests. Wageningen Agric. Univ. Papers 95-1, Wageningen, The Netherlands.

ZHAO, G., W. LIU, J. M. BROWN, AND C. O. KNOWLES. 1995. Insecticide resistance in field and laboratory strains of western flower thrips (Thysanoptera: Thripidae). J. Econ. Entomol. 88: 1164-1170.

INTRASPECIFIC DUELING IN PALM APHIDS, *CERATAPHIS BRASILIENSIS* (HOMOPTERA: HORMAPHIDIDAE)

F. W. HOWARD[1], SUSAN HALBERT[2], AND ROBIN GIBLIN-DAVIS[1]
[1] University of Florida, Fort Lauderdale Research & Education Center, 3205 College Avenue, Fort Lauderdale, Florida 33314

[2] Florida Department of Agriculture and Consumer Services, Division of Plant Industry, P. O. Box 147100, Gainesville, Florida 32614

Two subfamilies of aphids (Aphididae), Pemphiginae and Hormaphidinae, exhibit intra- and interspecific aggression. For example, *Pemphigus betae* Doane (Pemphigidae) fights duels for feeding sites on *Populus angustifolia* James, in which two aphids may kick and shove each other for up to two days (Whitham 1979). Foster (1996) recently described duels for feeding sites among colony mates of *Astegopteryx minuta* van der Goot (Hormaphidinae). Interspecific aggression in these subfamilies is displayed in the soldier caste of some species (Aoki 1977; Foster 1990).

In its native range in Southeast Asia, the palm aphid, *Cerataphis brasiliensis* (Hempel) (Hormaphidinae), alternates between a dicotyledonous tree, *Styrax benzoin* Dryand, where colonies form galls, and palms where they live externally on green tissue. The gall-inhabiting colony has a soldier caste that attempts to protect the colony from predators (Stern et al. 1995).

Cerataphis spp. have been introduced into many tropical areas and survive exclusively on palms where *Styrax* or other suitable alternate hosts are not present. They are pests of palms in some countries (Enobakhare 1994; Reinert and Woodiel 1974). Flat, circular and aleyrodid-like (Fig. 1), all stages bear a pair of minute spikes, or 'horns', on the front of the head. Some species additionally have several pairs of minute dagger-like setae on the ventral side of the head. The function of these structures has been presumed to be of an offensive or defensive nature but this presumption has not been confirmed until now.

Palm aphids are common on coconut palm, *Cocos nucifera* L., and several other palm species in southern Florida. They usually occur on the unopened frond and the youngest two or three fronds and sometimes on young fruits. They normally remain motionless, apparently feeding for long periods. They are associated with ants, and show the typical mutualistic ant-aphid relationship involving protection by the ants in exchange for honeydew.

One of our students, Claudia Vanderbilt, called our attention for the first time to two palm aphids involved in an altercation. Since then, we have observed dueling palm aphids about 15 times, have videotaped three dueling pairs (running time approximately 60 minutes), and report the behavior in this note.

The altercations that we observed were on excised palm frond tissue under the microscope in a laboratory at about 23°C. When manipulated slightly with a probe made of a human hair, the aphids sometimes secrete a small drop of honeydew, as aphids in general do when ants manipulate them. With insistent probing, they retract their stylets from the palm tissue and begin to crawl.

Disturbed aphids wander slowly and randomly. Occasionally one aphid encounters another aphid that is motionless and apparently feeding. The moving aphid sometimes explores the feeding aphid with its antennae and then moves on; at other times the moving aphid butts the outer margins of the feeding aphid with its horns. After several butts, the feeding aphid typically rotates a few degrees and appears to withdraw from feeding. Moving slowly, the aphid that was feeding turns to face the in-

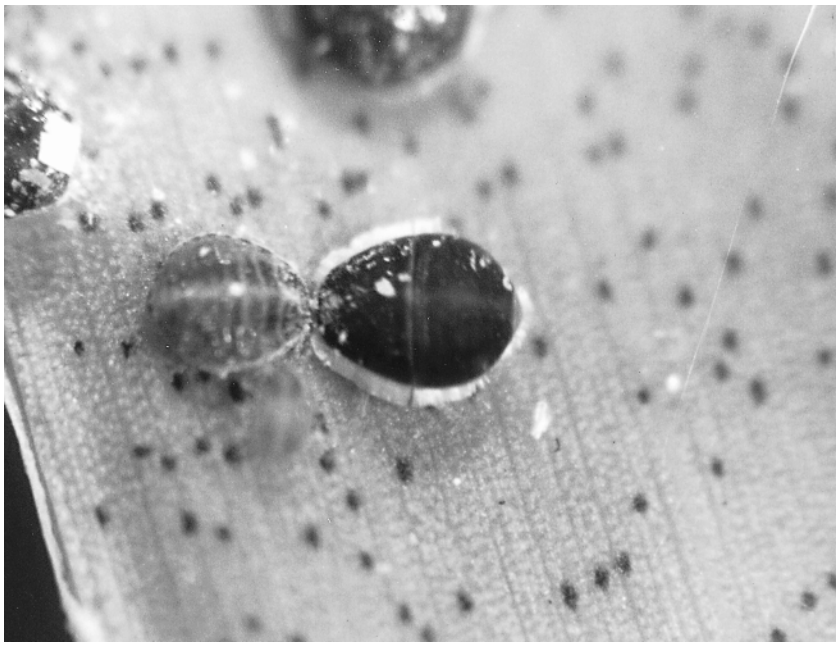

Fig. 1. Dueling palm aphids.

truder. Shortly, the aphids engage in a butting duel. To butt another aphid, an aphid lowers its head, places its horns beneath the head of the other aphid, then snaps its head upward while simultaneously thrusting forward with the legs. The motion often lifts the other aphid at its margin. Each of the dueling pair responds to being butted within a few seconds by butting its opponent. The altercation may last up to 19 minutes, the aphids often exchanging blows about 40 times per minute and alternately resting for intervals of several minutes. We were unable to observe any role of the minute dagger-like setae mentioned above.

The dueling aphids usually seemed well matched, even when younger nymphs challenge larger adults. Neither one seemed to be injured by the other, and one gained ground over the other only after prolonged butting. It was not clear to us what factors brought about the end of these duels. Often, in what would appear to be the middle of a well matched duel, the opponents appeared to pause slightly, after which one of them would climb upon the other, rotate clockwise, remain for a few minutes, then climb down and walk off; some aphids would then encounter another aphid and begin a new duel. Some altercations between three aphids simultaneously were observed, in which case one aphid would be butted from both sides or from front and back. Only one aphid that displaced a feeding aphid appeared to occupy the loser's feeding site, as observed by Foster (1996) for *A. minuta*.

The aphids readily engaged each other upon contact, but ignored other small arthropods placed with them, including nymphs and adults of brown citrus aphids (*Toxoptera citricida* Kirkaldy) and nymphs of psyllids *(Ceropsylla sideroxyli* Riley), the latter which were of similar size and shape as the palm aphids. The palm aphids did

not attempt to defend themselves against a small larva of a coccinellid beetle that attacked and consumed several of them.

Dueling among palm aphids may usually be for feeding sites, since this is known in their close relatives (Foster 1996; Whitham 1979). Aphids generally have highly specific requirements not only in their host plants, but in the sites on the plant in which they feed (Dixon 1985). Also, palm tissue is notoriously tough and fibrous. Perhaps an aphid expends less energy in displacing a feeding aphid than in finding a suitable new site and penetrating it.

However, the objective of the dueling was not apparent in our observations. More extensive investigation of the palm-inhabiting phase of palm aphids in nature may further elucidate this behavior.

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Summary

Duels between palm aphids, *Cerataphis brasiliensis,* infesting palm tissue were observed and videotaped in the laboratory. The objective of this aggression was not clear. In nature they presumably duel for feeding sites, as do related species. This is the first report of intraspecific aggression in the palm-infesting phase of palm aphids.

References Cited

AOKI, S. 1977. *Colophina clematis* (Homoptera: Pemphigidae), an aphid species with "soldiers". Kontyû, Tokyo 45: 276-282.

DIXON, A. F. G. 1985. Aphid Ecology. Glasgow and London: Blackie. 157 p.

Enobakhare, D. A. 1994. Occurence and distribution of *Cerataphis palmae* (Ghesquierei) (Homoptera: Pemphigidae) on *Raphia* palms in southern Nigeria. Insect Science and its Applications 15(1): 101-104.

FOSTER W. A. 1990. Experimental evidence for effective and altruistic colony defence against natural predators by soldiers of the gall-forming aphid *Pemphigus spyrothecae* (Hemiptera: Pemphigidae). Behavioral Ecology and Sociobiology 27: 421-430.

FOSTER, W. A. 1996. Aphids: Duelling aphids: intraspecific fighting in *Astegopteryx minuta* (Homoptera: Hormaphididiae). Animal Behavior 51: 645-655.

REINERT, J. A., AND N. L. WOODIEL 1974. Palm aphid control on 'Malayan dwarf' coconut palms. Florida Entomologist 57(4): 411-414.

STERN, D. L., S. AOKI, AND D. U. KUROSU 1995. The life cycle and natural history of the tropical aphid *Cerataphis fransseni* (Homoptera: Aphididae: Hormaphidinae), with reference to the evolution of host alternation in aphids. Journal of Natural History 29: 231-242.

WHITHAM, T. G. 1979. Territorial behaviour of *Pemphigus* gall aphids. Nature 279(5711): 324-325.

LARRA BICOLOR (HYMENOPTERA: SPHECIDAE: LARRINAE) COLLECTED IN PHEROMONE- AND PHENYLACETALDEHYDE-BAITED TRAPS

R. L. MEAGHER, JR.[1] AND J. H. FRANK[2]

[1] Center for Medical, Agricultural and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32604

[2] Department of Entomology and Nematology, University of Florida Gainesville, FL 32611-0630

Several efforts have been made to import natural enemies of *Scapteriscus* mole crickets since their arrival in the southeastern USA about 1900. One of these natural enemies was the sphecid wasp *Larra bicolor* F. Populations of *L. bicolor* from Bolivia were released between October 1988 and June 1989, and became established in Alachua County, Florida (Frank et al. 1995). Since there is no demonstrated method to sample for *L. bicolor*, the nectar-bearing plant *Spermacoce verticillata* L. was established near several release sites so that wasp visits could be observed. Wasps were not observed until the fall of 1993, and wasps continued to be seen through September 1994. By 1995, it was concluded that *L. bicolor* had dispersed at least a distance of 4 km from release sites (Frank et al. 1995). Our note documents the collection of *L. bicolor* adults in agricultural fields in northwestern Alachua County that are at least 22 km from the original release sites.

From 18 June to 10 October 1997, white plastic funnel traps ("bucket" or Universal Moth Traps, International Pheromone Systems, Wirral, Merseyside, England) were placed in an area planted to over 470 ha. of cotton, *Gossypium hirsutum* L., to attract beet armyworm, *Spodoptera exigua* (Hübner) and fall armyworm, *S. frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae). Traps were baited with either commercially-produced sex pheromones, phenylacetaldehyde ($C_6H_5CH_2CHO$, a floral attractant obtained from Aldrich Chemical Co., Milwaukee, WI) in plastic caps (20 mm diameter, 13 mm height; 0.2 or 0.5 ml phenylacetaldehyde per cap), or a combination of pheromone and phenylacetaldehyde. Pheromone lures were attached to the bottom of a cork that was placed in a hole in the canopy of the bucket trap. The phenylacetaldehyde cap was hot-gun glued (Arrow Fastener Co., Saddle Brook, NJ) to the bottom of the cork, which was placed in the trap canopy. The combination lure was composed of a cork with attached cap and the pheromone lure attached to the outside of the cork. Three tests were conducted in separate cotton fields. The first used Hercon® (Hercon Environmental Corp., Emigsville, PA) pheromone lures for *S. exigua*, the second used Scentry® (Ecogen, Inc., Langhorne, PA) lures for *S. exigua*, and the third used Trécé® (Trécé, Inc., Salinas, CA) lures for *S. frugiperda*. Four replications of the three treatments were placed within each field along pivot roads or along the field edges. Traps were observed three times weekly and pheromone and phenylacetaldehyde lures were replaced every two weeks.

Larra bicolor was collected in 2 of the 3 fields over seven different dates. The first wasp was collected in the Hercon field 18 June, with subsequent collections in the Trécé field 23 June (1 collected) and 11 July (2 collected). Higher numbers of wasps were collected in the fall, as 53 wasps were found in late September--early October. Peak capture was 29 September when 48 wasps were collected over a 5 day period in 6 separate traps. Of the total 57 *L. bicolor* collected, 32 were found in traps baited with the pheromone-phenylacetaldehyde combination, 23 were found in the phenylacetaldehyde-baited traps, and only 2 were found in the pheromone-baited traps.

Nontarget Hymenoptera have been collected in bucket traps placed in field crops which were baited for several different noctuid species (Adams et al. 1989, Mitchell et al. 1989, Gauthier et al. 1991), however, this is the first report for collection of *L. bicolor*.

SUMMARY

Larra bicolor was collected as a nontarget species in white bucket traps baited with sex pheromones and the floral attractant phenylacetaldehyde in an agricultural area in northwestern Alachua County, Florida. The first wasp was collected in mid-June, but larger numbers of wasps were collected in late September and early October. More wasps were collected in traps that had phenylacetaldehyde as a lure. This collection method may aid researchers in determining the dispersal and effectiveness of this natural enemy of *Scapteriscus* mole crickets.

REFERENCES CITED

ADAMS, R. G., K. D. MURRAY, AND L. M. LOS. 1989. Effectiveness and selectivity of sex pheromone lures and traps for monitoring fall armyworm (Lepidoptera: Noctuidae) adults in Connecticut sweet corn. J. Econ. Entomol. 82: 285-290.

FRANK, J. H., J. P. PARKMAN, AND F. D. BENNETT. 1995. *Larra bicolor* (Hymenoptera: Sphecidae), a biological control agent of *Scapteriscus* mole crickets (Orthoptera: Gryllotalpidae), established in northern Florida. Fla. Entomol. 78: 619-623.

GAUTHIER, N. L., P. A. LOGAN, L. A. TEWKSBURY, C. F. HOLLINGSWORTH, D. C. WEBER, AND R. G. ADAMS. 1991. Field bioassay of pheromone lures and trap designs for monitoring adult corn earworm (Lepidoptera: Noctuidae) in sweet corn in southern New England. J. Econ. Entomol. 84: 1833-1836.

MITCHELL, E. R., H. R. AGEE, AND R. R. HEATH. 1989. Influence of pheromone trap color and design on capture of male velvetbean caterpillar and fall armyworm moths (Lepidoptera: Noctuidae). J. Chem. Ecol. 15: 1775-1784.

PHENYLACETALDEHYDE ENHANCES UPWIND FLIGHT OF MALE FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) TO ITS SEX PHEROMONE

R. L. MEAGHER, JR. AND E. R. MITCHELL
Center for Medical, Agricultural and Veterinary Entomology, Agricultural Research Service, U.S. Department of Agriculture, Gainesville, FL 32604

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is a migratory polyphagous pest that attacks several important crops (Luginbill 1928). Currently, adult male populations are monitored using a synthetic blend of sex pheromone components as a lure (Tumlinson et al. 1986, Mitchell et al. 1989). Chemicals other than sex pheromones have been assayed as moth attractants. For instance, floral compounds that attract noctuid moths have been isolated and identified. Baits of phenylacetaldehyde,

first isolated from a flower (*Araujia sericofera* Brothero) (Asclepiadaceae), captured hundreds of noctuid moths in field traps, including *S. frugiperda* (Cantelo & Jacobson 1979). Phenylacetaldehyde, benzaldehyde, 2-phenylethanol, and benzyl alcohol were identified from flowers of the shrub *Abelia grandiflora* (André) (Caprifoliaceae) (Haynes et al. 1991), a plant that elicits flight responses from cabbage looper, *Trichoplusia ni* (Hübner) (Grant 1971). Benzaldehyde, benzyl acetate, and phenylacetaldehyde were collected from night-blooming jessamine *Cestrum nocturnum* (L.) (Solanaceae), a plant known to attract looper moths (Noctuidae: Plusiinae) (Heath et al. 1992).

Phenylacetaldehyde in combination with sex pheromones or blacklights increased moth trap capture (Smith et al. 1943, Creighton et al. 1973, Cantelo & Jacobson 1979). Both male and female *T. ni* were attracted to phenylacetaldehyde in flight tunnel, greenhouse, and screen cage bioassays (Haynes et al. 1991, Landolt et al. 1991, Heath et al. 1992). Our objective was to determine if phenylacetaldehyde enhances the attractiveness of sex pheromones to fall armyworm in a flight tunnel bioassay.

Fall armyworms used in the bioassays were reared in the laboratory on a pinto bean-based artificial diet according to the procedures of Guy et al. (1985). Pupae were sexed and placed in 163 ml (5.5 oz.) paper cups (Sweetheart, Chicago, IL) that were placed in 24 × 24 cm screen cages for eclosion. Pupae were maintained under reversed photoperiod (14: 10, light:dark) in an environmental chamber held at 26°C and 70% RH. Adults had access to cotton balls saturated with distilled water and a honey-sugar solution. Pupae were transferred daily so that cages contained adult males of a known age.

The tunnel used was a Plexiglas rectangular box (2.0 by 0.6 by 0.6 m). The floor had alternating black and white panels (ea. panel 10 cm long). Air was pulled past an activated charcoal filter and through the tunnel at the rate of 0.22 m/sec by a blower motor. A cylindrical moth release cage (9 cm by 5.1 cm diameter) and a two-compartment source cage (6 cm long by 2 cm diameter), both made from metal screen, were hung in the middle at the downwind and upwind portions of the tunnel, respectively. Distance between the cages was 1.4 m. Room conditions during testing were ≈ 26°C and 65–80% RH, and observations were aided by overhead red lights.

Adults were placed in the tunnel room at least one hour before testing and tests were conducted 1-4 h post-scotophase. The commercial pheromone lures used were purchased from Scentry® (Ecogen, Inc., Langhorne, PA) and from Trécé® (Trécé, Inc., Salinas, CA). A standard lure containing 2 mg of the pheromone blend (*Z*)-9-tetradecen-1-ol acetate (*Z*9-14: AC) (80.3%), (*Z*)-11-hexadecen-1-ol acetate (*Z*11-16: AC) (19.2%), and (*Z*)-7-dodecen-1-ol acetate (*Z*7-12: AC) (0.5%), loaded in a solvent refined rubber septum, was prepared by J. H. Tumlinson (USDA-ARS CMAVE). A hexane solution of 10 mg/ml of phenylacetaldehyde (Aldrich Chemical Co., Milwaukee, WI) was prepared, and 100 μl of this solution was pipetted onto filter paper. The bioassay protocol was to place an individual moth in the release cage, hold it in the plume for 10 seconds, and then release it down the tunnel. Each moth was observed for 2 min and upwind flight and contact with the source screen cage was scored. Each replicate contained between 5 and 20 moths, with totals of from 70–150 moths tested per treatment. The experiment was designed as a randomized complete block, and percentage responses were transformed into arcsine-square roots before analysis of variance (PROC GLM, SAS Institute 1995). Comparisons between each pheromone lure and lure plus phenylacetaldehyde combination were tested using the contrast statement in PROC GLM.

The addition of phenylacetaldehyde in the source cage increased upwind flight and contact with the lure in all combinations tested. Phenylacetaldehyde in combination

with the standard lure increased upwind flight from 68.6% ± 5.6 (SE) to 87.4% ± 7.3 (df = 1, 28; F = 12.5; P = 0.0014), and increased contact with the source cage from 51.9% ± 7.6 to 76.6% ± 6.1 (df = 1, 28; F = 8.0; P = 0.0085). The combination of phenylacetaldehyde with a Scentry lure increased upwind flight from 61.1% ± 6.3 to 80.0% ± 9.1 (df = 1, 28; F = 4.3; P = 0.0468, and increased contact from 30.8% ± 6.3 to 56.3% ± 9.0 (df = 1, 28; F = 3.9; P = 0.0584). Similar results were obtained with a Trécé lure, upwind flight increased from 66.5% ± 6.8 to 88.3% ± 3.3 (df = 1, 28; F = 9.8; P = 0.0041) and contact increased from 48.7% ± 8.0 to 75.0% ± 8.7 (df = 1, 28; F = 10.9; P = 0.0026) when phenylacetaldehyde was added. No differences were found in upwind flight or contact among lures lacking phenylacetaldehyde ($P > 0.05$), and fall armyworm males did not respond to phenylacetaldehyde alone (n = 50).

Phenylacetaldehyde generally has enhanced trap catch of moths in pheromone traps or with blacklights (Creighton et al 1973, Cantelo & Jacobson 1979). Our study is the first to show that a floral compound such as phenylacetaldehyde can increase attraction of *S. frugiperda* males to a pheromone source. Pheromone-baited traps for *S. frugiperda* have been used to detect seasonal population trends (Tingle & Mitchell 1977, Waddill et al. 1982), document migration patterns over large areas (Mitchell et al. 1991), and predict larval populations and plant infestation (Silvain & Ti-A-Hing 1985, Silvain 1986, Linduska & Harrison 1986). Phenylacetaldehyde as an enhancement may increase trap capture in the field, thereby improving current uses of pheromones and potentially creating new lure and toxicant systems for management of this pest (Landolt et al. 1991).

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Summary

More male fall armyworms, *Spodoptera frugiperda* (J. E. Smith), flew upwind to combinations of pheromone-treated septa and phenylacetaldehyde than to pheromone-treated septa alone in flight tunnel bioassays. No moths flew upwind to phenylacetaldehyde alone at the dose tested. This compound may increase pheromone-baited trap captures in the field, thereby improving current uses of pheromones and potentially creating new lure and toxicant systems for management of this pest.

References Cited

CANTELO, W. W., AND M. JACOBSON. 1979. Phenylacetaldehyde attracts moths to bladder flower and to blacklight traps. Environ. Entomol. 8: 444-447.

CREIGHTON, C. S., T. L. MCFADDEN, AND E. R. CUTHBERT. 1973. Supplementary data on phenylacetaldehyde: an attractant for Lepidoptera. J. Econ. Entomol. 66: 114-115.

GRANT, G. G. 1971. Feeding activity of adult cabbage loopers on flowers with strong olfactory stimuli. J. Econ. Entomol. 64: 315-316.

GUY, R. H., N. C. LEPPLA, J. R. RYE, C. W. GREEN, S. L. BARETTE, AND K. A. HOLLIEN. 1985. *Trichoplusia ni*, pp. 487-494. *In* P. Singh & R. F. Moore [eds.], Handbook of insect rearing, vol. 2. Elsevier, Amsterdam.

HAYNES, K. F., J. Z. ZHAO, AND A. LATIF. 1991. Identification of floral compounds from *Abelia grandiflora* that stimulate upwind flight in cabbage looper moths. J. Chem. Ecol. 17: 637-646.

HEATH, R. R., P. J. LANDOLT, B. DUEBEN, AND B. LENCZEWSKI. 1992. Identification of floral compounds of night-blooming jessamine attractive to cabbage looper moths. Environ. Entomol. 21: 854-859.

LANDOLT, P. J., B. LENCZEWSKI, AND R. R. HEATH. 1991. Lure and toxicant system for the cabbage looper (Lepidoptera: Noctuidae). J. Econ. Entomol. 84: 1344-1347.
LINDUSKA, J. J., AND F. P. HARRISON. 1986. Adult sampling as a means of predicting damage levels of fall armyworm (Lepidoptera: Noctuidae) in grain corn. Florida Entomol. 69: 487-491.
LUGINBILL, P. 1928. The fall armyworm. USDA Tech. Bull. 34. 92 p.
MITCHELL, E. R., H. R. AGEE, AND R. R. HEATH. 1989. Influence of pheromone trap color and design on capture of male velvetbean caterpillar and fall armyworm moths (Lepidoptera: Noctuidae). J. Chem. Ecol. 15: 1775-1784.
MITCHELL, E. R., J. N. MCNEIL, J. K. WESTBROOK, J. F. SILVAIN, B. LALANNE-CASSOU, R. B. CHALFANT, S. D. PAIR, V. H. WADDILL, A. SOTOMAYOR-RIOS, AND F. I. PROSHOLD. 1991. Seasonal periodicity of fall armyworm, (Lepidoptera: Noctuidae) in the Caribbean basin and northward to Canada. J. Entomol. Sci. 26: 39-50.
SAS INSTITUTE. 1995. SAS/STAT guide for personal computers, version 6.11 ed. SAS Institute, Cary, NC.
SILVAIN, J. F. 1986. Use of pheromone traps as a warning system against attacks of *Spodoptera frugiperda* larvae in French Guiana. Florida Entomol. 69: 139-147.
SILVAIN, J. F., AND J. TI-A-HING. 1985. Prediction of larval infestation in pasture grasses by *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from estimates of adult abundance. Florida Entomol. 68: 686-691.
SMITH, C. E., N. ALLEN, AND O. A. NELSON. 1943. Some chemotropic studies with *Autographa* spp. J. Econ. Entomol. 36: 619-621.
TINGLE, F. C., AND E. R. MITCHELL. 1977. Seasonal populations of armyworms and loopers at Hastings, Florida. Florida Entomol. 60: 115-122.
TUMLINSON, J. H., E. R. MITCHELL, P.E.A. TEAL, R. R. HEATH, AND L. J. MENGELKOCH. 1986. Sex pheromone of fall armyworm, *Spodoptera frugiperda* (J. E. Smith): identification of components critical to attraction in the field. J. Chem. Ecol. 12: 1909-1926.
WADDILL, V. H., E. R. MITCHELL, W. H. DENTON, S. L. POE, AND D. J. SCHUSTER. 1982. Seasonal abundance of the fall armyworm and velvetbean caterpillar (Lepidoptera: Noctuidae) at four locations in Florida. Florida Entomol. 65: 350-354.

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ATTRACTION OF THE LOVEBUG, *PLECIA NEARCTICA* (DIPTERA: BIBIONIDAE) TO ANETHOLE

RON CHERRY
Everglades Research and Education Center, P.O. Box 8003, Belle Glade, FL 33430

Plecia nearctica Hardy is the lovebug that motorists frequently encounter as a serious nuisance when traveling in southern states. The insects are smashed against windshields obscuring the vision of motorists. Cars may overheat when radiators become clogged and the smashed insects damage car paint if the body fluids are not removed soon after contact (Callahan & Denmark 1973). The insect was first described by Hardy (1940) from Galveston, Texas, who reported it to be widely spread, but more common in Texas and Louisiana than other Gulf Coast states. It has now progressed

to all states bordering on the Gulf of Mexico, as well as Georgia, South Carolina, and parts of Central America. It was first collected in Florida in 1949 and today is found throughout Florida (Denmark & Mead 1992).

Several studies have been conducted on adult attractants and adult sampling for lovebugs. Callahan & Denmark (1973) observed large numbers of lovebug adults congregating at intersections, traffic lights, and filling stations. Their data showed that lovebugs were attracted to automobile exhaust fumes irradiated with 3600 Å UV light. Whitesell (1974) observed adults flying to heat sources such as recently parked, warm cars and engines. His data showed that greater numbers of lovebug adults were caught on a heated box than on sound, exhaust, or control boxes. A mobile trap mounted on top of a car has been used to measure population density of adult lovebugs on highways (Sharpe 1974). In field tests, visual observations were used by Leppla et al. (1974) to measure rhythmic activity of adult lovebugs. Thornhill (1976) marked adult lovebugs with an ultraviolet dust and used large, white sticky traps to recapture the adults in order to measure the dispersal of the adults. Callahan et al. (1985) postulated that lovebugs are attracted to highways by automobile exhaust fumes. They tested irradiated automobile exhaust fumes and their components as attractants for adult lovebugs. Of the five different aldehydes tested, formaldehyde and heptaldehyde were the most attractive. In this report, I provide data on the attraction of adult *P. nearctica* to anethole and the use of anethole baited sticky traps to sample adult populations of *P. nearctica*.

Cherry et al. (1996) reported on the attraction of adult beetles of *Anomala marginata* (Robinson) to anethole in Japanese beetle traps. During the course of that study, I observed adult lovebugs hovering in large numbers around Japanese beetle traps baited with anethole. However, since Japanese beetle traps are designed to capture heavy-bodied insects such as beetles, I decided to see if lovebugs would be attracted to anethole in sticky traps which are more suitable for catching smaller insects. Yellow sticky traps (Pherocon AM, no bait) made by Trece, Inc. Salinas, California were used in these tests. Ten pairs of traps (anethole versus control) were set-up at ten different locations on the Everglades Research and Education Center at Belle Glade, Florida. Traps at each location were 10 m apart and hung one m above the ground on metal rods. A sponge (3 by 3 by 3 cm) was wedged into each trap. Control traps were unbaited and each anethole trap had 10 ml of anethole poured into the sponge. The anethole was obtained from Acros Organics, Fairlawn, New Jersey and was greater than or equal to 99 percent. Tests were conducted when large numbers of adults were observed flying at the research center. Six tests were conducted during April-May, 1996 and 1997 (see Table 1). Traps were exposed for 24 h in each test and then covered with clear cellophane and taken to a laboratory. Lovebug adults on each trap were counted under microscopic examination. The sex ratio of adults on the traps was determined by scraping 100 adults from different control traps and 100 adults from the anethole baited traps. These adults were placed in gasoline to dissolve the adhesive from the trap and then sexed using characters described by Denmark & Mead (1992). Statistical differences in adult numbers of control versus anethole baited traps in each of the six tests were determined using paired t-tests (SAS 1996). A two by two contingency table using Chi-square analysis (Dixon & Massey 1969) was used to determine if the adult sex ratio was significantly different in control traps versus anethole baited traps.

Data in Table 1 show that significantly more adult lovebugs were caught on sticky traps baited with anethole than unbaited control traps in all six tests. By far, the most lovebugs caught on any date occurred in both control and anethole traps on May 1, 1997. Reasons for the large catches during that test are not known for sure. However,

TABLE 1. ADULT *P. NEARCTICA* CAUGHT ON YELLOW STICKY TRAPS BAITED WITH ANETHOLE.

	Control[b]			Anethole[b]		
Date[a]	Mean	SD	Range	Mean[a]	SD	Range
May 16, 1996	3.0	3.5	0-11	186.5	99.0	48-291
May 20, 1996	2.2	2.7	0-7	75.3	50.5	7-153
May 29, 1996	7.5	4.6	0-14	88.7	46.1	14-141
April 15, 1997	11.4	7.5	2-22	113.4	55.6	24-225
April 17, 1997	5.6	2.3	3-11	101.4	34.5	51-151
May 1, 1997	258.8	85.5	123-368	887.8	199.2	648-1240

[a]Date of start of test. Traps exposed for 24 h.
[b]Adults per trap. Paired t-test (SAS 1996) showed significantly ($P<0.01$) more adults were caught on anethole baited traps than controls during all six testing dates.

field observation indicated large numbers of adults were flying that day probably due to large populations and calm winds which did not hinder flight. The sex ratio was 49:51 (M:F) in the control traps and 46:54 (M:F:) in the anethole baited traps. Chi-square analysis showed that there was no significant difference (Chi-square = 0.3, 1 d.f., $P > 0.05$) in the sex ratio of adults in control versus baited traps. Anethole is an essential oil found in plants (Morrison & Boyd 1973) and adult lovebugs are known to feed on different plants (Hetrick 1970). Previous studies have shown anethole to be attractive to diverse insects such as bees (Ladd & Tew 1983), scarabs (Cherry et al. 1996), and wireworms (Lehman 1932).

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SUMMARY

Significantly more adult lovebugs were caught on sticky traps baited with anethole than unbaited control traps in six tests. These data show that sticky traps baited with anethole can be used as a simple and efficient sampling tool for adult P. nearctica.

REFERENCES CITED

CALLAHAN, P. S., AND H. A. DENMARK. 1973. Attraction of the "lovebug" *Plecia nearctica* (Diptera: Bibionidae) to UV irradiated exhaust fumes. Florida Entomol. 56: 113-119.

CALLAHAN, P. S., T. C. CARLYSE, AND H. A. DENMARK. 1985. Mechanism of attraction of the lovebug, *Plecia nearctica* to southern highways: further evidence for the IR-dielectic waveguide theory of insect olfaction. Applied Optics 24: 1088-1093.

CHERRY, R. H., M. G. KLEIN, AND W. S. LEAL. 1996. Attraction of adult *Anomala marginata* (Coleoptera: Scarabaeidae) to anethole. J. Agric. Entomol. 13: 359-364.

DENMARK, H. A., AND F. W. MEAD. 1992. Lovebug, *Plecia nearctica* Hardy (Diptera: Bibionidae). Fla. Dept. Agric. Consumer Serv. Entomol. Circ. No. 350.

DIXON, W. J., AND F. J. MASSEY. 1969. Introduction to statistical analysis. McGraw-Hill, New York.

HARDY, D. E. 1940. Studies in New World *Plecia* (Bibionidae: Diptera). Part 1. Kansas Entomol. Soc. 13 (1): 15-27.

HETRICK, L. A. 1970. Biology of the "love-bug", *Plecia nearctica* (Diptera: Bibionidae) Florida Entomol. 53: 23-26.

LADD, T. L., AND J. E. TEW. 1983. Attraction of honey bees (Hymenoptera: Apidae) to traps baited with lures for Japanese beetles (Coleoptera: Scarabaeidae) J. Econ. Entomol. 76: 769-770.

LEHMAN, R.S. 1932. Experiments to determine the attractiveness of various aromatic compounds to adults of the wireworms. J. Econ. Entomol. 25: 949-958.

LEPPLA, N.C., J. L SHARP. W. K. TURNER, E. W. HAMILTON, AND D. R. BENNETT. 1974. Rhythmic activity of *Plecia nearctica*. Environ. Entomol. 3: 323-326.

MORRISON, R. T., AND R. N. BOYD. 1973. Organic chemistry, Allyn and Bacon Inc. Boston.

SAS INSTITUTE. 1996. SAS Systems for Windows. Version 6.12 SAS Institute, Cary, N.C.

SHARPE, H. 1974. Love bugs are drawn to heat. Sunshine State Agric. Res. Rep. Vol. 19, No. 3-4. Pages 3-4.

THORNHILL, R. 1976. Dispersal of *Plecia nearctica* (Diptera: Bibionidae). Florida Entomol. 59: 45-53.

WHITESELL, J. J. 1974. Heat, sound, and engine exhaust as "lovebug" attractants (Diptera: Bibionidae: *Plecia nearctica*) Environ. Entomol. 3: 1038-1039.

PREDATORY BEHAVIOR OF A PIT-MAKING ANTLION, *MYRMELEON MOBILIS* (NEUROPTERA: MYRMELEONTIDAE)

JOSEPH F. NAPOLITANO[1]
Department of Entomology, Clemson University, Clemson, SC 29634

[1]Current address: Bureau of Water, South Carolina Dept. of Health and Environmental Control, 2600 Bull St., Columbia, SC 29201

The larvae of antlions (Neuroptera: Myrmeleontidae) are renowned for their predatory tactic: the construction of funnel-shaped pitfall traps in sandy substrate, beneath which they wait for prey. Pit-building behavior, however, is limited to the tribe Myrmeleontini (New 1986) and is characteristic of the genus *Myrmeleon* (Lucas & Stange 1981). The lie-in-wait predation strategy suggests that various prey will be encountered by the antlion larva. Plasticity of predatory behavior should increase the efficiency by which an opportunistic predator subdues and processes different types of prey. Therefore, I asked the question: does the behavioral response of a pit-building antlion, *Myrmeleon mobilis* Hagen, differ among prey types? In this study I characterize the predatory behaviors of *M. mobilis* and compare the sequence and frequency of these behaviors in response to three prey types.

Thirty late first- and second-instar *M. mobilis* larvae were collected from sheltered, sandy areas in Clemson, Pickens County, South Carolina, on 8 October, 1995. Larvae were placed individually in containers with 3 cm of sterilized sand, and held at 25 ± 1°C, 65 ± 5%RH, and a photoperiod of 12:12 (L:D). Each larva was allowed to construct a pit and then fed a maintenance diet of earwigs, *Euborellia annulipes* Lu-

cas (Carcinophoridae); rearing continued for 12 days, until all individuals had reached late second instar. The three experimental prey species (length ± SD/max. width ± SD) were the termite *Reticulitermes flavipes* Kollar (Rhinotermitidae) [5.7 ± 0.85 × 1.2 ± 0.08 mm], the ant *Prenolepis imparis* Say (Formicidae) [4.22 ± 0.20 × 1.5 ± 0.17 mm], and the beetle *Alphitobius diaperinus* Panzer (Tenebrionidae) [6.13 ± 0.82 × 2.72 ± 0.13 mm].

Behavioral trials were conducted at 23-25°C. Each *M. mobilis* larva was presented with one individual of a randomly selected prey species. Prey was dropped into the center of the pit, to standardize introduction (Griffiths 1980), and the resulting interaction was videotaped at a distance of ca. 7 cm. Recording began with prey introduction and ended when the prey either escaped, or was consumed, and the larva returned to the pre-introduction 'ready position' (jaw set). Ten trials of each prey species were recorded and no larva was used in more than one trial. Descriptions of predatory behaviors were based on videotaped trials and direct observation. Each trial was reviewed, and sequence and frequency of behaviors noted. Significant behavioral transitions ($p = 0.05$) were identified using a first order, preceding-following, behavioral transition matrix (after Willey et al. 1992). Flow diagrams of significant transitions were constructed.

The following 12 discrete predatory behaviors were identified in the behavioral catalog of *Myrmeleon mobilis*:

1. Attack.

The head is moved rapidly forward while closing the mandibles, and is often flicked rapidly back, expelling sand from the pit.

2. Holding.

The prey is gripped securely in the mandibles.

3. Submergence.

Holding prey, the larva moves down and back into the substrate until the entire larva and at least part of the prey are not visible.

4. Emergence.

Holding prey, the larva moves up and forward until the entire prey and at least part of the larva's head/mandibles is visible.

5. Prey Beating.

Holding prey, the larva rapidly flicks its head up and down (4-5 beats per bout) (Fig. 1.), often drumming the prey on the substrate.

6. Feeding.

While at least one mandible tip is inserted, fluids are extracted from the prey, often alternating with mandibular probing and manipulation of the prey.

7. Pit Clearing.

The head is moved laterally, accumulating sediment on the dorsal surface, then flicked rapidly back, expelling sediment.

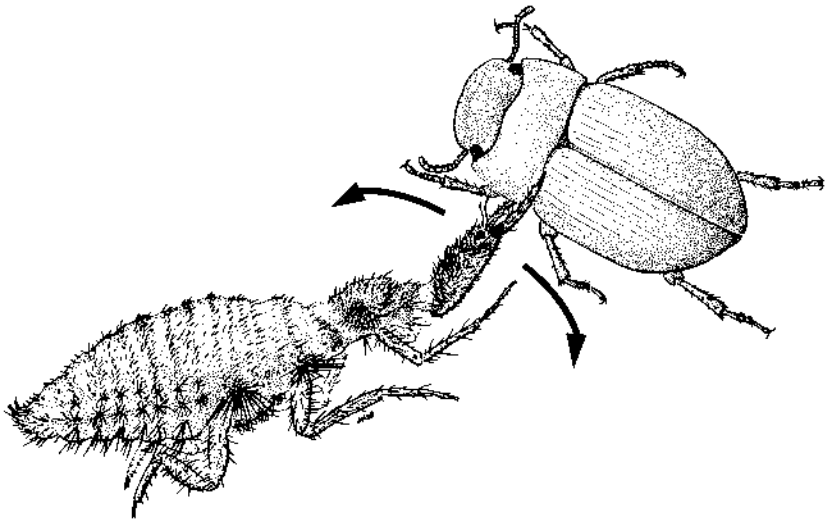

Fig. 1. Prey-beating behavior exhibited by *Myrmeleon mobilis* with beetle prey, *Alphitobius diaperinus*.

8. Head Roll.

The head is raised and swept in a circular motion along the pit wall, accumulating sediment in the pit center.

9. Prey Clearing.

The mandibles are used to position prey on the dorsal head surface, then the head is flicked rapidly back, expelling prey.

10. Grooming.

The tip of one mandible is moved along the groove on the inside edge of the opposing mandible.

11. Quiescence.

Larva remains motionless, without prey, for 7+ seconds.

12. Jaw Set.

The larva pulls beneath the sand, while fully opening the mandibles. The eyes, antennae and mandible tips remain visible.

Sequences for all prey types typically followed a core pattern of behaviors (Fig. 2), starting with attack and holding, followed by submergence, emergence, and feeding. After feeding ended, maintenance behavior generally occurred (prey clearing, pit clearing, head roll, and grooming) and, finally, jaw set. The major difference in behavioral sequence was prey beating behavior: 90% of the beetle prey-trials resulted in

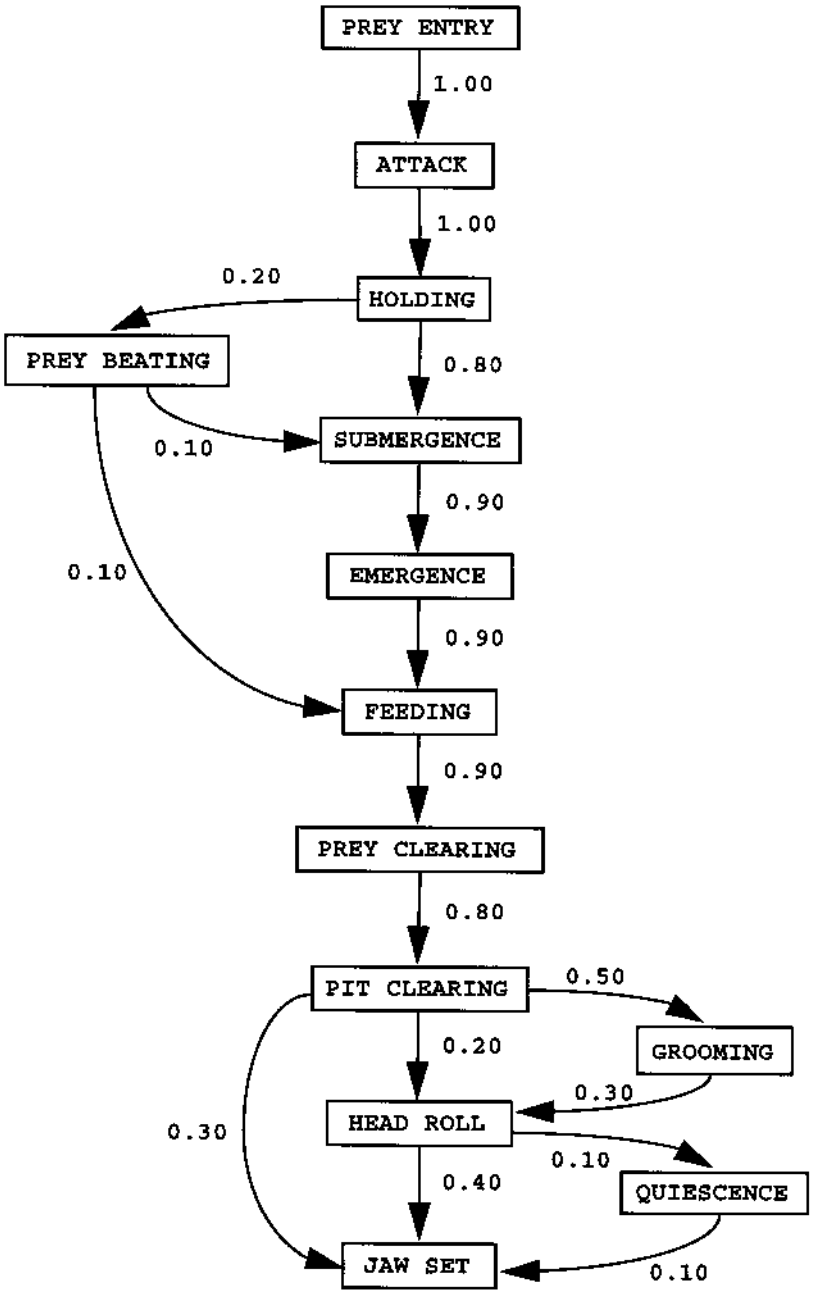

Fig. 2. Flow diagram of predatory behavior for *M. mobilis*, showing sequence of significant behavioral transitions (p = 0.05) and transition frequency (n = 10 trials) when presented with ant prey, *Prenolepis imparis*.

prey beating, compared to 20% of the ant trials, and 10% of the termite trials. The mean frequency of prey-beating bouts for the beetle (42.40 ± 12.59SE) was significantly different ($p \leq 0.005$) from that for both the termite (2.00 ± 2.00SE) and the ant (8.90 ± 7.57SE); the latter two were not significantly different (Tukey's, $p > 0.05$).

My field observation in areas of *M. mobilis* habitation revealed that taxa including Hymenoptera, Coleoptera, Orthoptera, and non-insect arthropods are consumed by antlion larvae. In the laboratory, *M. mobilis* larvae ate both soft and hard-bodied prey. However, trials with highly sclerotized prey (beetles) differed significantly from those with softer prey (ants and termites) in sequence and frequency of prey-beating behavior, demonstrating that the predatory response of *M. mobilis* varies with prey type. Prey-beating behavior may be an adaptation to facilitate mandibular penetration (in beetles, this usually occurred in a coxal joint or between tagma), or to disorient and subdue vigorously struggling prey. Griffiths (1980) described behavior similar to prey beating for 'difficult' prey in the feeding biology of *Morter obscurus* Rambur, and noted that treatment of hard and soft-bodied ant prey varied with respect to mandibular insertion. In addition, previous research suggests that phylogeny may be reflected by behavior (Mansell 1988, Matsura & Murao 1994). An interspecies comparison of predatory behavior in Myrmeleontidae may prove worthwhile in relating behavioral differences to phylogeny.

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Summary

The predatory behavior of a pit-making antlion, *Myrmeleon mobilis*, is characterized. Behavioral sequences among three prey types were similar, when compared via flow diagrams. A significant difference in behavioral frequency existed between hard-bodied and soft-bodied prey types.

References Cited

GRIFFITHS, D. 1980. The feeding biology of ant-lion larvae: prey capture, handling and utilization. J. Anim. Ecol. 49: 99-125.

LUCAS, J. R., AND L. A. STANGE. 1981. Key and descriptions to the *Myrmeleon* larvae of Florida (Neuroptera: Myrmeleontidae). Florida Ent. 64: 207-216.

MANSELL, M. W. 1988. The pitfall trap of the Australian ant-lion *Callistoleon illustris* (Gerstaecker) (Neuroptera: Myrmeleontidae): an evolutionary advance. Australian J. Zool. 36: 351-356.

MATSURA, T. A., AND T. MURAO. 1994. Comparative study on the behavioral response to starvation in three species of antlion larvae (Neuroptera: Myrmeleontidae). J. Insect Behav. 7: 873-884.

NEW, T. R. 1986. A review of the biology of Neuroptera Planipennia. Neuroptera International, Suppl. 1: 1-57.

WILLEY, M. B., JOHNSON, M. A., AND P. H. ADLER. 1992. Predatory behavior of the basilica spider, *Mecynogea lemniscata* (Araneae, Araneidae). Psyche 99: 153-167.

DEVELOPMENT OF *COPTERA HAYWARDI* (HYMENOPTERA: DIAPRIIDAE) IN IRRADIATED AND UNIRRADIATED PUPAE OF THE CARIBBEAN FRUIT FLY AND THE MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

ELLEN MENEZES, JOHN SIVINSKI, TIM HOLLER[1], MARTIN ALUJA[2], FELIPE JERONIMO[3], AND EDWIN RAMIREZ[4]
USDA-ARS, CMAVE, P. O. Box 14565, Gainesville, FL, 32604

[1]USDA-APHIS, Gainesville Plant Protection Station, P. O. Box 147100, Gainesville, FL 32614-7100

[2]Instituto de Ecologia, A. C. 91000 Xalapa, Veracruz, Mexico

[3]USDA-APHIS, Methods Development, 12 Calle 6-96 Zona 10, Guatemala City, Guatemala

[4]MOSCAMED, Guatemala City, Guatemala

Augmented releases of tephritid parasitoids have suppressed populations of both the Mediterranean fruit fly (*Ceratitis capitata* [Weidemann]) and the Caribbean Fruit fly, (*Anastrepha suspensa* [Loew]) (Wong et al. 1991, Sivinski et al. 1996). Typically, braconid parasitoids of larvae, such as *Diachasmimorpha longicaudata* (Ashmead), are employed. However, parasitoids that attack fruit fly pupae might be useful additions to such programs since they are able to attack flies that might otherwise escape parasitism. Flies developing within large fruits are less likely to be parasitized by braconids; parasitoids are less able to reach them with their ovipositors (e.g., Sivinski 1991, Sivinski et al. 1997). Since tephritid larvae typically leave fruits to pupate in the soil, fruit size is less important to pupal parasitoids foraging for hosts.

The diapriid *Coptera haywardi* (Ogloblin) is a widespread native of Latin America, where it has been collected from the pupae of several *Anastrepha* species (Loiacono 1981). It appears to attack only species of Tephritidae (Sivinski et al. 1998). Unlike many common, ectoparasitic, pteromalid pupal parasitoids of cyclorrhaphous Diptera, *C. haywardi* develops as an endoparasitoid (Sivinski et al. 1998). This more intimate relationship with its host may result in greater specialization and a narrower host range (see Godfray 1994). Specialized parasitoids are particularly valuable in augmentative releases since they are less likely to harm beneficial insects and more likely to focus their foraging on declining numbers of target pests.

There is a possibility of adding pupal parasitoids to existing braconid mass-rearing programs. For example, fruit fly larvae could be exposed to a braconid parasitoid, and after host pupation the pupae could then be placed with pupal parasitoids. Unparasitized pupae would typically be available for the second parasitoid since only a few braconid rearing operations consistently reach parasitism levels of 50%, and some, such as early efforts with raising *Diachasmimorpha tryoni* (Cameron), average as low as 20% (pers. observ. of the authors). This scheme would be most effective with a pupal parasitoid that, 1) would not hyperparasitize the primary braconid parasitoid, and 2) was able to develop in flies whose maturation was disrupted by radiation. Irradiation of larvae prior to parasitization is used in mass-rearing programs in Florida, Mexico, and Guatemala to prevent mixed lots of parasitoids and fertile flies (Siv-

inski and Smittle 1990; Sivinski personal observation). Previous studies have found no indication of *C. haywardi* hyperparasitism of *D. longicaudata* developing in *A. suspensa* (Sivinski et al. 1998).

In order to determine if *C. haywardi* would develop in pupae that had been formed by irradiated larvae, we provided the diapriid with irradiated and unirradiated pupae of *A. suspensa* in the following manner. Mixed lots of unparasitized pupae and pupae parasitized by *D. longicaudata* were obtained from the Florida Division of Plant Industry, Gainesville, Florida (see Sivinski et al. 1996). At the start of the experiment, *A. suspensa* had been in colony for ~ 9 years (150 generations). *D. longicaudata* had been in colony ~ 6 years and *C. haywardi* had been colonized on *A. suspensa* for ~ 1 year. These lots had been previously derived from late 3rd instar larvae that had been either irradiated in a Cesium 137 source (Nordion International Inc., Model M; Kanata, Ontario, Canada) at 6 kR or left unirradiated. Depending on availability, either 10 ml (~400 pupae) or 3 ml (~120 pupae) of 1-day old pupae were placed with 15 unsexed individuals of *C. haywardi* in 250 ml cardboard cups containing moist vermiculite, honey, and water. The cups were covered with a fine-mesh cloth and left at 26 (± 1)°C and ambient humidity for 1 week. At the end of this period pupae and adult parasitoids were separated and the pupae held at 29°C and 70% humidity for 1 month. At this point the adult insects that had emerged were identified and counted. Unemerged pupae were dissected to determine their contents. There were 8 replicates of 3 ml and 7 replicates of 10 ml cups of pupae. Each replicate consisted of 5 cups of irradiated and 5 cups of unirradiated pupae, so that a total of 470 ml of irradiated and 470 ml of unirradiated pupae were exposed to a total of 1125 diapriids each. Since the results of the experiment were consistent and unambiguous, the data from cups containing different amounts of pupae were pooled.

There was not a single successful development of *C. haywardi* in pupae formed by irradiated *A. suspensa* larvae. In the unirradiated lots, a total of 9772 *A. suspensa* eclosed. In the irradiated lots, 1 *A. suspensa* and 0 *C. haywardi* eclosed. Parasitism by *D. longicaudata* was 31% (SE = 7%) in unirradiated lots and 29% (SE = 7%) in irradiated lots. Parasitism by *C. haywardi* of unirradiated pupae averaged 12% (SE = 0.3%).

A similar experiment, examining the development of *C. haywardi* in irradiated pupae of *C. capitata* was conducted in the "Aurora" USDA-APHIS/MOSCAMED facility in Guatemala City, Guatemala. At the time of the experiment, *C. haywardi* had been reared for ~5 generations on *C. capitata* pupae. *Coptera haywardi* was presented with pupae of *C. capitata* formed from larvae either irradiated at 14.5 kR with a Cobalt 60 source at the MOSCAMED rearing facility at El Pino, Guatemala or left unirradiated. Lots of 1180 pupae, either irradiated or unirradiated, were exposed for a period of three days to ~ 800 unsexed individuals of *C. haywardi* housed in a 1 m by 1 m plexiglass cage. Pupae then were removed and held at 26°C and 60-70% humidity for one month. There were six replicates, so that a total of 7080 irradiated and 7080 unirradiated pupae were exposed to parasitism.

As in the case of irradiated *A. suspensa*, there was no emergence of adult *C. haywardi* from irradiated *C. capitata.* Unirradiated pupae yielded 231 *C. haywardi* (~ 4% parasitism).

Thus, *C. haywardi* lacks the useful attribute of being able to exploit irradiated fruit flies in those mass-rearing programs which expose larvae to high levels of radiation. This does not preclude its use in other types of mass-rearing programs. For example, in a parasitoid mass-rearing system previously used in Hawaii there was a sufficient difference in the developmental periods of the braconid *Diachasmimorpha tryoni* (Cameron) and its host *C. capitata* to allow separation of the adults of the two species without the use of radiation. (Wong and Ramadan 1992). In this instance, the

adult fruit flies emerged first and were allowed to die off before the braconid was "harvested". Assuming that *C. haywardi* would not hyperparasitize *D. tryoni,* this lack of irradiation permits the exposure of pupae to *C. haywardi.* Genetic "sexing-strains" of host flies sometimes generate sexually dimorphic pupal colors that allow the mechanical separation of male and female pupae in mass-rearing programs (Willhoeft et al. 1996). Since female pupae are considered detrimental in sterile releases, they are discarded after sorting. These might be more profitably employed to provide hosts for pupal parasitoids (Jorge Hendrichs, pers. comm.). In the Aurora Facility in Guatemala, *C. haywardi* has been successfully reared on the female pupae of *C. capitata* generated by the "Temperature Sensitive Lethal" sorting strain (see Franz et al. 1996).

Ed Burns, Avi Eitam, and Pat Greany made many improvements to an earlier draft. Gina Posey assisted in the laboratory, and Valerie Malcolm swiftly prepared the manuscript.

Summary

Coptera haywardi, a diapriid parasitoid of tephritid pupae, failed to develop in pupae formed by *Anastrepha suspensa* larvae that had been previously irradiated at 6 kR. Irradiation is typically used in the mass-rearing of braconid larval parasitoids such as *Diachasmimorpha longicaudata*. Neither was *C. haywardi* able to develop in *Ceratitis capitata* pupae formed by larvae irradiated at 14.5 kR. A scheme that sequentially exposes irradiated fruit fly hosts to first a braconid, such as *D. longicaudata,* and then to *C. haywardi* is impracticable. However, mass rearing programs of other pest fruit flies and natural enemies that do not require irradiation to separate braconid parasitoids from adult hosts, such as the production of *Diachasmimorpha tryoni* on *C. capitata,* may allow for the integration of *C. haywardi*.

References Cited

Franz, G., P. Kerremans, P. Rendon, and J. Hendrichs. 1996. Development and application of genetic sexing systems for the Mediterranean fruit fly based on a temperature sensitive lethal, pp. 185-191 *in* B. McPheron and G. Steck [eds.] Fruit Fly Pests. St. Lucie Press, Delray Beach, FL.

Godfray, H. 1994. Parasitoids. Princeton Univ. Press. Princeton, NJ.

Loiacono, M. S. 1981. Notas sobre Diapriinae neotropicales (Hymenoptera, Diapriidae). Rev. Soc. Entomol. Argentina. 40: 237-241.

Sivinski, J. 1991. The influence of host fruit on parasitism rates in the Caribbean fruit fly (*Anastrepha suspensa* [Loew]). Entomophaga 36: 447-455.

Sivinski, J., C. Calkins, R. Baranowski, D. Harris, J. Brambila, J. Diaz, R. Burns, T. Holler, and G. Dodson. 1996. Suppression of a Caribbean fruit fly (*Anastrepha suspensa* [Loew]) population through releases of the parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Cont. 6: 177-185.

Sivinski, J., M. Aluja, and M. Lopez. 1997. Spatial and temporal distributions of braconid parasitoids of Mexican *Anastrepha* spp. in the canopies of host fruit trees. Ann. Entomol. Soc. America 90: 604-618.

Sivinski, J., K. Vulinec, E. Menezes, and M. Aluja. 1998. The bionomics of *Coptera lopezi* Masner (Diapriidae: Hymenoptera) and other pupal parasitoids of tephritid fruit flies (Diptera). Biol. Cont. 11: 193-202.

Sivinski, J., and B. Smittle. 1990. Effects of gamma radiation on the development of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) and the subsequent development of its parasite *Diachasmimorpha longicaudata* (Ashmead). Entomol. Exp. et. Appl. 55: 295- 297.

WILLHOEFT, U., G. FRANZ, AND D. MCINNIS. 1996. Toward the application of genetic sexing in tephritid fruit fly SIT programs, pp. 179-184 *in* B. McPheron and G. Steck [eds.] Fruit Fly Pests. St. Lucie Press. Delray Beach, FL.

WONG, T., AND M. RAMADAN. 1992. Mass rearing biology of larval parasitoids (Hymenoptera: Braconidae) of tephritid fruit flies in Hawaii, pp. 405-476 *in* T. Anderson and N. Leppla [eds.] Advances in Insect Rearing for Research and Pest Management. Westview Press, Boulder, CO.

WONG, T., M. RAMADAN, D. MCINNIS, N. MOCHIZULI, J. NISHIMOTO, AND J. HERR. 1991. Augmentative releases of *Diachasmimorpha tryoni* (Hymenoptera: Braconidae) to suppress a Mediterranean fruit fly (Diptera: Tephritidae) population in Kula, Hawaii. Biol. Cont. 1: 2-7.

PARASITES ASSOCIATED WITH THE PONERINE ANT *ECTATOMMA TUBERCULATUM* (HYMENOPTERA: FORMICIDAE): FIRST HOST RECORD FOR THE GENUS *DILOCANTHA* (HYMENOPTERA: EUCHARITIDAE)

JEAN-PAUL LACHAUD[1,2], GABRIELA PÉREZ-LACHAUD[2] AND JOHN M. HERATY[3]
[1]Laboratoire d'Ethologie et Psychologie Animale, CNRS-UMR 5550, Université Paul-Sabatier, 118 route de Narbonne, 31062 Toulouse Cedex (France)

[2]El Colegio de la Frontera Sur, Apdo. Postal 36, 30700 Tapachula, Chiapas (Mexico)

[3]Department of Entomology, University of California, Riverside, CA 92521 (USA)

The ant subfamilies Ecitoninae, Myrmicinae, Formicinae and Dolichoderinae frequently harbor a variety of commensals exhibiting myrmecophilous, scavenger and/or parasitic habits (Wheeler 1910, Rettenmeyer 1963, Wilson 1971, Lachaud 1981, Hölldobler & Wilson 1990). However, among the more primitive ant subfamilies, such intruders are poorly known, and most reports deal essentially with the dipteran or hymenopteran parasites affecting the host brood. Among these, various genera of Eucharitidae (*Austeucharis*, *Chalcura*, *Neolosbanus*, *Propsilogaster*, *Schizaspidia* and *Tricoryna*) from the subfamily Eucharitinae (*sensu* Heraty 1994), are known to parasitize species of *Myrmecia* (Myrmeciinae), *Odontomachus*, *Rhytidoponera*, *Gnamptogenys*, and *Hypoponera* (Ponerinae) (Wheeler & Wheeler 1937, Bouček 1988, Heraty 1994). In the New World, only two genera of Eucharitinae have been reared from the cocoons of ponerine ants: four species of *Kapala* from *Odontomachus* and *Pachycondyla* (Wheeler 1907, Myers 1931, Wheeler & Wheeler 1937, Clausen 1941, Heraty 1994) and *Isomerala coronata* (Westwood) from *Ectatomma tuberculatum* (Cook 1905, Wheeler 1907, Wheeler & Wheeler 1937). *Kapala* and *Isomerala* belong to a distinct clade of New World Eucharitini that includes the genera *Dilocantha*, *Dicoelothorax*, *Galearia*, *Lasiokapala*, *Lirata*, *Liratella*, *Parakapala* and *Thoracantha* (J. M. H., un-

published data), all of which are probably parasitoids of large Ponerinae. Wheeler and Wheeler (1937) reported *Pogonomyrmex badius* Latr. (Myrmicinae) as the host of *Kapala floridana* (Ashmead), but this record was based only on the opinion of W. H. Ashmead and cited in Wheeler (1907). Also, a single adult female of *Galearia bruchi* (Gemignani) was found in the scrap pile of a nest of *Pogonomyrmex cunicularius* Mayr, but the association was indirect (not reared) and the condition of the adult (alive or dead) was not recorded (Gemignani 1933). *Pogonomyrmex* are not known to be host to any Eucharitidae. With accurate rearing information, *Kapala* and related genera have only been associated with large Ponerinae.

During a survey of the seasonal population variation in colonies of the neotropical ponerine ant *Ectatomma tuberculatum* (Olivier), performed between January 1995 and February 1996 (J. P. L., unpublished data), a total of 10 colonies (of which 7 were queenright) were collected in the Soconusco region of Chiapas (Mexico), in a coffee plantation (*Coffea arabica*) with open vegetation. The site was located at "Finca Santa Elena", on one side of the road to Nueva Alemania, Tapachula municipality.

On January 29, 1995, two adults (one male: 3.6 mm in length and one female: 4.4 mm in length) of *Dilocantha lachaudii* Heraty emerged from one colony collected 2 days previously in Finca Santa Elena and temporarily stored in a plastic box (30 × 20 × 8 cm). The close examination of the nest material brought back to the laboratory allowed us to separate the remains (thoraces) of four additional adults (sex undetermined). During the first hours the parasites were generally ignored, but even when persistent antennal contact occurred, aggression was never exhibited by the ant workers, apart from some openings of the mandibles. In such a situation, the wasp tended to immobilize and, on some occasions, adopted a pupal position, which triggered a typical transport behavior from workers of *E. tuberculatum*. The carried wasp was held by the thorax, with its legs and antennae folded in and its body curved over the carrier's back, and was transported within the box for a few centimeters before being released by the ant. The immobilization of the wasp after contact with an ant was in clear contrast to its numerous jumps, when moving freely (in a clear attempt to escape the box), and the buzzing displayed when experimentally held with forceps. After repeated contacts with the workers of *E. tuberculatum*, the wasps appeared to be handled more roughly by their host, and the seizure by the mandibles for transport appeared to be more vigorous. After three days, both eucharitids were found dead and dismembered, their remains abandoned on a refuse pile in a corner of the box.

Another colony of *E. tuberculatum*, collected from the same site on February 8, 1995, provided two additional adult females of *D. lachaudii* (one already dead, the other attempting to escape the nest), and two more colonies, also collected from Finca Santa Elena but on February 20, 1996, contained six females, three males and two thoraces (sex undetermined) in one colony, and two females in the other.

An additional collection was made on July 7, 1997, to examine pupae and larvae for parasitism by juvenile stages of *Dilocantha*. Of six *E. tuberculatum* colonies, three were parasitized but at a very low rate (Table 1): an unfed first-instar larva (planidium) parasitizing an ant larva in one colony, a fed first-instar larva on an ant worker prepupa within the host cocoon in the second one, and finally two unfed planidia on two ant larvae in the third colony.

The parasitism of *E. tuberculatum* by *D. lachaudii* is the first host record for this genus and adds support to the hypothesis that *Kapala* and related genera within the New World are parasitic on large Ponerinae attributed to the Ponerini, Odontomachini and Ectatommini tribes.

Dilocantha are unique within Eucharitidae for having a patch of specialized hook-shaped setae filling a deep depression in the scutellum at the scutoscutellar sulcus

TABLE 1. COLONIES OF *ECTATOMMA TUBERCULATUM* (ALL COLLECTED AT FINCA SANTA ELENA, TAPACHULA MUNICIPALITY, CHIAPAS, MEXICO), WITHIN WHICH WERE ENCOUNTERED ADULT OR JUVENILE STAGES OF *DILOCANTHA LACHAUDII*.

Date	Colony population[3]	Eucharitids[4]
27/01/95[1]	1 Q + 0 Qa + 8 M + 491 W + 35 P + > 100 L	6 eucharitid adults (1M + 1F + 4?)
08/02/95[1]	1 Q + 0 Qa + 4 M + 407 W + 3 P + > 100 L	2 eucharitid adults (2 F)
20/02/96[1]	1 Q + 0 Qa + 0 M + 281 W + 46 P + ≈ 180 L	11 eucharitid adults (3 M + 6 F + 2?)
	1 Q + 0 Qa + 0 M + 428 W + 30 P + ≈ 70 L	2 eucharitid adults (2 F)
07/07/97[2]	1 Q + 0 Qa + 1 M + 491 W + 17 P + ≈ 130 L	1 planidium on ant larva
	Q + 0 Qa + 0 M + 191 W + 2 P + 21 L	nothing
	0 Q + 0 Qa + 0 M + 109 W + 69 P + 55 L	nothing
	1 Q + 0 Qa + 0 M + 299 W + 123 P + ≈ 250 L	1 fed first-instar larva on worker prepupa
	4 Q + 0 Qa + 1 M + 120 W + 10 P + ≈ 65 L	nothing
	0 Q + 6 Qa + 0 M + 261 W + 37 P + 20 L	2 planidia on ant larva

[1]Brood not examined for parasitism. [2] Brood examined for parasitism. [3]F: female, L: larvae, M: male, P: pupae, Q: queen, Qa: alate queen, W: workers. [4]?: sex undetermined.

and having this associated with external secretions (Heraty 1998). The similarity of the patch and secretion to that of myrmecophilous Staphylinidae suggested that the patch could act as an ant appeasement structure (Heraty 1998). The absence of aggression from *E. tuberculatum* workers during the first hours following the emergence of eucharitid adults could, in part, support this hypothesis. However, no licking behavior by the ants was observed, and if an appeasement really occurred it was only temporary, since, in the inability to escape the artificial nest, the wasps were killed in fewer than three days. Such an observation would instead support the acquisition of a cuticular hydrocarbon profile similar to the host, as demonstrated in *Orasema xanthopus* Cameron (Oraseminae) parasitic on *Solenopsis invicta* Buren (Vander Meer et al. 1989), followed by a progressive loss of this chemical camouflage. Similar amicable treatment, followed by aggressive behavior by the ant host after several days, was observed for *Orasema viridis* parasitic on *Pheidole tepicana* Pergande (Wheeler 1907) and a species of *Orasema* parasitic on *Pheidole dentata* Mayr (J. M. H., unpublished data). Within Eucharitinae, workers of the host species of *Formica* attempted to drag freshly emerged adults of *Eucharis* back into the nest (Clausen 1941). Chemical camouflage is probably widespread, if not universal in Eucharitidae, but the specialized hair patch and associated secretion is unique to adults of *Dilocantha*. Only more detailed behavioral observations of adults recently emerged and a fine microscopic analysis of the cellular structures associated with the scutoscutellar patch of setae would provide a confident answer of the patch's function.

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Summary

Adults of the eucharitid wasp *Dilocantha lachaudii* Heraty were reared from the ponerine ant *Ectatomma tuberculatum* (Olivier). This is the first host record for this genus, adding support to the hypothesis that all the species belonging to the distinct clade of New World Eucharitini that includes *Kapala*, *Dilocantha* and related genera, are specifically parasitic on large ponerine ants.

References Cited

Bouček, Z. 1988. Australasian Chalcidoidea (Hymenoptera). Wallingford: C. A. B. International. 832 pp.

Clausen, C. P. 1941. The habits of Eucharidae. Psyche 48: 57-69.

Cook, O. F. 1905. The social organization and breeding habits of the cotton-protecting Kelep of Guatemala. U.S.D.A. Technical Series n°10, pp. 1-55.

Gemignani, E. V. 1933. La familia "Eucharidae" (Hymenoptera: Chalcidoidea) en la República Argentina. An. Mus. Nac. Hist. Nat. Buenos Aires 37: 477-493.

Heraty, J. M. 1994. Classification and evolution of the Oraseminae in the Old World, including revisions of two closely related genera of Eucharitinae (Hymenoptera: Eucharitidae). Life Sciences Contributions 157. Royal Ontario Museum. 174 pp.

Heraty, J. M. 1998. The genus *Dilocantha* (Hymenoptera: Eucharitidae). Proc. Entomol. Soc. Washington 100: 72-87.

HÖLLDOBLER, B., AND E. O. WILSON. 1990. The ants. The Belknap Press of Harvard University Press, Cambridge, Massachusetts. 732 pp.

LACHAUD, J. P. 1981. Etude des relations hôte-myrmécophile entre les Diapriidae *Lepidopria pedestris* Kieffer et *Solenopsia imitatrix* Wasmann et la fourmi *Diplorhoptrum fugax* Latreille. Doctoral Thesis, Univ. Paul-Sabatier, Toulouse, 139 pp.

MYERS, J. G. 1931. Descriptions and records of parasitic Hymenoptera from British Guiana and the West Indies. Bull. Entomol. Res. 22: 267-276.

RETTENMEYER, C. W. 1963. Behavioral studies of army ants. Kansas Univ. Sciences Bull. 44: 281-465.

VANDER MEER, R. K., D. P. JOUVENAZ, AND D. P. WOJCIK. 1989. Chemical mimicry in a parasitoid (Hymenoptera: Eucharitidae) of fire ants (Hymenoptera: Formicidae). J. Chem. Ecol. 15: 2247-2261.

WHEELER, G. C., AND J. WHEELER. 1937. New hymenopterous parasites of ants (Chalcidoidea: Eucharidae). Ann. Entomol. Soc. Am. 30: 163-175.

WHEELER, W. M. 1907. The polymorphism of ants, with an account of some singular abnormalities due to parasitism. Bull. Amer. Mus. Nat. Hist. 23: 1-93.

WHEELER, W. M. 1910. Ants. Their Structure, Development and Behavior. Columbia University Press. New York and London. 663 pp.

WILSON, E. O. 1971. The Insect Societies. The Belknap Press of Harvard University Press, Cambridge, Mass. 584 pp.

FIRST REPORT OF *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE) IN THE EASTERN AMAZON, PARÁ, BRAZIL

JANISETE GOMES SILVA[1,2], KEIKO URAMOTO[1], AND ALDO MALAVASI[1]
[1]Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo
Caixa Postal 11461 05422-970 - São Paulo, São Paulo, Brazil

[2]Department of Entomology, Penn State University, 501 A.S.I. Building
University Park, PA 16802

The Brazilian Amazon, which comprises about 45% of the Brazilian territory, contains approximately 180 known native and exotic fruit species (Zucchi et al. 1996). The available data on fruit flies and their hosts are scarce when compared to the high diversity of the available host species that occur in the region (Silva et al. 1996).

Earlier surveys of fruit flies in the Brazilian Amazon reported several *Anastrepha* species reared from collected fruit or captured in traps, but no specimens of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), were found (Malavasi et al. 1980, Silva 1993, Silva et al. 1996, Zucchi et al. 1996).

C. capitata is native to sub-Saharan Africa (White & Elson-Harris 1992) but a global process of colonization has been taking place throughout the last century. In Brazil, where its presence has been reported since the early 1900's (Ihering 1901), *C. capitata* is considered one of the major quarantine pests, preferentially infesting in-

troduced fruit species (Malavasi et al. 1980). Until the 1980's, this species was reported only from the southern and southeastern regions of the country, with the Recôncavo Baiano region, Bahia, as its northernmost limit (Malavasi et al. 1980, Nascimento & Zucchi 1981). However, more recently, it has been reported further north as far as São Luís, Maranhão, infesting tropical almond (*Terminalia catappa* L.) (Morgante 1991).

Until recently, this species had not been reported in the Amazon. According to Silva (1993), the Amazon region imports a large amount of fruit, without any quarantine restrictions, from the State of São Paulo where this pest has long been established. Therefore, he suggested that *C. capitata* may have been introduced into the region but had not become established due to unfavorable local climatic conditions, such as high local temperatures and humidity. Recently, Ronchi-Telles & Silva (1996) reported *C. capitata's* presence for the first time in the southern Amazon, Rondônia, infesting guava (*Psidium guajava* L.).

We collected fruits of nine different plant species from six families in two localities in Pará (Belém, 1°48'S; 48° 30'W, and Quatro Bocas' 2°30'S; 48°18'W) in February, 1997. The fruits were placed in containers with a layer of vermiculite as a pupation medium, and the pupae were held in plastic cups until emergence of adults. Voucher specimens were deposited at the Departamento de Entomologia, Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo, Piracicaba, SP, Brazil.

Table 1 shows the tephritid species and their hosts, as well as parasitoids and other flies reared from our collections.

The presence of medfly is reported for the first time in the eastern Amazon (Belém, Pará) infesting carambola (*Averrhoa carambola* L.) and Barbados cherry (*Malpighia glabra* L.). The presence of this species in Pará may be due to its spread from the neighboring State of Maranhão, where it was detected in the early 1990's, or to introductions from other regions. The role played by human mediated transportation in the dispersal of *C. capitata* should not be overlooked, since Belém is an important port in the Amazon basin.

It seems unlikely that the populations of southern and eastern Amazon (Ouro Preto D'Oeste, 10°42'49" S; 62°14'29" W, and Belém, 1°48'S; 48°30' W, respectively) are contiguous. Further studies, including extensive surveys in the Amazon and the use of genetic markers (Gasparich et al. 1997, Silva 1996) are necessary to resolve questions of colonization and genetic relationships among populations in this region.

Only two *Anastrepha* species were found in this study: *A. obliqua* (Macquart) and *A. striata* Schiner. All of the parasitoids were found associated with *Anastrepha* spp. and belong to the species *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae). The lonchaeid specimens that emerged from the collected fruit belong to the species *Neosilba pendula* (Bezzi). These species have been reported in the region in earlier studies (Malavasi et al. 1980, Silva 1993, Silva et al. 1996, Zucchi et al. 1996).

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SUMMARY

The occurrence of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is reported in the eastern Amazon, State of Pará, for the first time. The specimens were ob-

TABLE 1. TEPHRITOIDS AND ASSOCIATED PARASITOIDS COLLECTED IN BELÉM AND QUATRO BOCAS, PARÁ, BRAZIL.

Host family	Host species	*Anastrepha* species	N	*C. capitata* N	Lonchaeidae N	Parasitoids N
Anacardiaceae	*Mangifera indica* L.	–		–	–	–
	Spondias mombin L.	–		–	–	–
Malpighiaceae	*Malpighia glabra* L.	*obliqua*	45F; 50M	1F	5M	1F
		striata	1F; 1M	–	–	–
Myrtaceae	*Eugenia stipitata* McVaugh	–		–	–	-–
	Psidium acutangulum DC.	*striata*	20F; 18M	–	–	4F
		obliqua	1M	–	–	–
	Psidium guajava L.	*striata*	1F	–	–	–
Oxalidaceae	*Averrhoa carambola* L.	–		20F; 26M	–	–
Passifloraceae	*Passiflora edulis* Sims	–		–	–	–
Sapotaceae	*Manilkara zapota* L.	–		–	–	-–

N = number of adults, M = males, F = females.

tained from infested carambola (*Averrhoa carambola* L.) and Barbados cherry (*Malpighia glabra* L.). Rearing of *Anastrepha* spp., a lonchaeid and a parasitoid is also reported.

References Cited

GASPARICH, G. E., J. G. SILVA, H -Y. HAN, B. A. MCPHERON, G. J. STECK, AND W. S. SHEPPARd. 1997. Population genetic structure of the mediterranean fruit fly (Diptera: Tephritidae) and implications for worldwide colonization patterns. the an. Entomol. Soc. America. 90(6): 790-797.

IHERING, H. VON. 1901. Laranjas bichadas. Rev. Agri., 6: 179.

MALAVASI, A., J. S. MORGANTE, AND R. A. ZUCCHI. 1980. Biologia de "moscas-das-frutas" (Diptera: Tephritidae). I: Lista de hospedeiros e ocorrência. Rev. Brasileira Biol., 40(1): 9-16.

MORGANTE, J. S. 1991. Moscas-das-frutas: (Tephritidae): características biológicas, detecção e controle. Brasília, Minist. Agric. Ref. Agr., SENIR, Bol. Tec., 19 pp.

NASCIMENTO, A. S. DO, AND R. A. ZUCCHI. 1981. Dinâmica populacional das moscas-das-frutas do gênero *Anastrepha* (Dip., Tephritidae) no Recôncavo Baiano. I - Levantamento das espécies. Pesq. Agropec. Brasileira, 16 (6): 763-767.

RONCHI-TELLES, B., AND N. M. DA SILVA. 1996. Primeiro registro de ocorrência da Mosca-do-Mediterrâneo (*Ceratitis capitata*) (Diptera: Tephritidae) na Amazônia Brasileira. An. Soc. Entomol. Brasil. 25(3): 569-570.

SILVA, N. M. DA. 1993. Levantamento e análise faunística de moscas-das-frutas (Diptera: Tephritidae) em quatro locais do Estado do Amazonas. PhD Dissertation. Escola Superior de Agricultura "Luiz de Queiroz" da Universidade de São Paulo, Piracicaba, S.P., Brazil. 152pp.

SILVA, N. M. DA, S. SILVEIRA NETO, AND R. A. ZUCCHI. 1996. The natural host plants of *Anastrepha* in the State of Amazonas, Brazil. pp. 353-357. *In*: B. A. McPheron and G. J. Steck (eds.). Fruit fly pests: a world assessment of their biology and management. St. Lucie Press, Delray Beach, Florida.

SILVA, J. G. 1996. Demografia e estrutura genética de *Ceratitis capitata* (Wiedemann) (Diptera, Tephritidae) e os processos de invasão e colonização no Brasil. PhD Dissertation. Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, S.P., Brazil, 115 pp.

WHITE, I. M., AND M. M. ELSON-HARRIS. 1992. Fruit flies of economic significance: their identification and bionomics. CAB International, Wallingford, UK.

ZUCCHI, R. A., N. M. DA SILVA, AND S. SILVEIRA NETO. 1996. *Anastrepha* species from the Brazilian Amazon: distribution, hosts and lectotype designations. pp. 259-264. *In*: B. A. McPheron and G. J. Steck (eds.). Fruit Fly Pests: a world assessment of their biology and management. St Lucie Press, Delray Beach, Florida.

EVALUATION OF REARING METHODS FOR *DIADEGMA INSULARE* (HYMENOPTERA: ICHNEUMONIDAE), AN ENDOPARASITOID OF THE DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE)

D. H., SIEGLAFF[1], E. R. MITCHELL, AND G. Y. HU[1]
Center for Medical, Agricultural, and Veterinary Entomology, U.S. Department of Agriculture, Agricultural Research Service, P.O. Box 14565
Gainesville, FL 32604, USA

[1]Department of Entomology and Nematology, University of Florida
Gainesville, FL 32611

The diamondback moth, *Plutella xylostella* (L.), is the most important insect pest of cruciferous plants worldwide (Talekar & Shelton 1993). In North America, the diamondback moth typically has been controlled by using synthetic insecticides, but with resistance to commonly used insecticides developing in the mid-1980's (Shelton et al. 1993, Talekar & Shelton 1993), IPM practices for controlling this pest have been adopted (Biever et al. 1994, Leibee 1996). The augmentative release of natural enemies can be an important component to any IPM program, and parasitoids are considered to be essential to any such program for diamondback moth management (Talekar & Shelton 1993). The release of parasitoids for control of diamondback moth has been conducted in many geographic regions (Talekar & Shelton 1993), including North America (Biever et al. 1994, Leibee 1996, Mitchell et al. 1997b). The larval endoparasitoid, *Diadegma insulare* (Cresson), can be a very important natural control of diamondback moth in North America (Lasota & Kok 1986, Idris & Grafius 1993, Muckenfuss et al. 1992) and offers the greatest potential as a biocontrol agent among larval parasitoids of this pest (Idris & Grafius 1993).

Our laboratory has been developing various strategies to combat diamondback moth populations in cabbage growing regions of Florida (Adams 1994), and *D. insulare* could be an important addition to this program. However, *D. insulare* has been exceedingly difficult to maintain in culture. The purpose of the current study was to develop an effective and economically feasible rearing method using the natural insect host coupled with an artificial diet to produce *D. insulare* in sufficient quantities for field release.

Diamondback moth (originally received from Juliet Tang, Cornell University, New York) was reared on a wheat germ-based artificial diet (Shelton et al. 1991), in 0.23 liter paper cups (Sweetheart VS508, Chicago, IL) covered with a Kimwipe (Kimberly-Clark EX-L, Atlanta, GA) and overlaid with a perforated plastic lid (Sweetheart, LS8). Adult diamondback moths were maintained in 30 × 30 × 30 cm mating cages, fed a 10% honey-water solution in small paper cups provisioned with a saturated cotton ball, and provided with several pieces of wrinkled aluminum foil (12.7 × 8.89 cm) treated with collard extract (see description below) as oviposition substrates. The diamondback moth prefers uneven surfaces on which to oviposit. The diamondback moth colony was maintained at 25°C, 50% RH and a photoperiod of 12:12 h (L:D).

D. insulare (collected from Bunnell, Florida, May 1996) were reared on 2 - 3rd instar diamondback moth larvae that had been placed on freshly harvested collard leaves. Following 24 h of exposure to *D. insulare*, host larvae were transferred to an 88 × 30 × 17 cm plastic pan and fed fresh collard leaves ad libitum until pupation. Leaves harboring pupae were placed into emergence cages (30 × 30 × 30 cm), and eclosed *D. insulare* were fed 10% honey-water solution. All *D. insulare* were maintained under a 12:12 h L:D cycle at 25° C and 50% RH.

To prepare collard extract (CE), collard leaves were harvested from a small field plot maintained at our laboratory, blended with water at the rate of 0.071 g/ml using a household blender (Hamilton Beach®, St. Louis, MO.), and filtered through a fine organdy mesh. The CE was used to produce "treated" aluminum foil oviposition substrate, "sting-cups" and amended artificial diet. Aluminum foil was crumpled to create ridges and depressions, straightened, dipped into CE, and allowed to dry overnight to produce "treated" aluminum foil for use as oviposition substrates for diamondback moth and as a possible stimulant source for *D. insulare*. CE also was mixed into cool (< 110° C) wheat germ-based artificial diet to produce 10 and 23% CE diet. "Sting-cups" were produced by impregnating 0.47—liter paper cups (James River 2186, Norwalk, CT) with CE. The cups were filled with 500 ml CE, held for 5 min, emptied-out, and allowed to dry overnight. All substrates were exposed to UV radiation for approximately one hour to sterilize their surfaces.

To determine if the CE would stimulate parasitism by *D. insulare*, five treatments were compared: artificial diet, artificial diet contiguous with "treated" aluminum foil, artificial diet with 10 and 23% CE, and a collard leaf control (as described above for *D. insulare* rearing). Each diet cake (7.6 cm diam and 2.5 cm thickness) was infested with 2nd-3rd instar diamondback moth larvae (200-450) taken from our laboratory colony. The larvae were placed on top surface of the diet cake from where they then spread to all the surfaces. The "treated" aluminum foil was created as previously described except that they were circular (14 cm diam) in shape rather than rectangular. A diet cake without CE was attached over a piece of circular foil to one leg of an A-frame structure (15 × 13 cm) made from five-mm hardware cloth. This arrangement allowed for exposure of all sides of the cake to diamondback larvae and *D. insulare* as the ridges in the foil permitted larvae and parasitoids to crawl between the two surfaces. The A-frames with larvae were placed individually in 5 liter plastic containers (Tristate Plastics 289 NL, Dixon, KY) and covered with a fine organdy mesh. The collard leaf control was also set-up on A-frames with an equivalent number of diamondback moth larvae (this treatment was meant to mimic current rearing procedures). The number of diamondback moth larvae in each treatment was determined, and a diamondback moth larvae: *D. insulare* ratio of 50:1 was used for each treatment (♂:♀ *D. insulare* ratio of 2:1, 24-48 h old). The *D. insulare* were aspirated into each treatment and allowed to oviposit for 24 h. To determine if a longer period of exposure to parasitism would enhance parasitoid production, two additional treatments were tested. Artificial diet cakes without CE were placed atop a U-shaped hardware cloth (5 mm) frame in 0.47 liter paper cups (James River 2186). One set of cups was untreated, i.e., without CE, and the other set was treated by impregnating the interior surface with CE as described for "sting cups." Each cup was infested with larvae as described, and the cups were exposed to *D. insulare* for 48 h. All treatments were maintained at 25° C, 50% RH and 12:12 h (L:D), and each had 11 replications over time.

Measurements were standardized because the number of diamondback moth larvae differed among replicates. The measurements were total *D. insulare* adults produced per *D.* insulare, ♀ *D. insulare* adults produced per *D. insulare*, percentage parasitism (i.e., total emerged *D. insulare* / [total emerged *a D. insulare* + total emerged diamondback moth adults] × 100), and sex ratio. Numbers produced were transformed by square root (n+1) and percentage parasitism was transformed by the square root of the arcsine before analysis. The data were subjected to analysis of variance, and means were separated by Duncan's multiple range test (SAS Institute 1989).

The collard leaf control produced more *D. insulare* adults and had a higher percentage parasitism than any other treatment (Table 1). Among all treatments using artificial diet, the 48 h artificial diet + "sting-cups" and 10% collard extract diet produced

TABLE 1. PRODUCTION OF PARASITOIDS PER DIADEGMA INSULARE _ AND PERCENTAGE PARASITISM OF DIAMONDBACK MOTH LARVAE (= TOTAL % *D. INSULARE* ON VARIOUS DIETS.[1]

Treatment	Total *D. insulare* / *D. insulare* ♀ (SE)	♀ *D. insulare* / *D. insulare*♀ (SE)	Total % *D. insulare* (SE)	Sex Ratio ♂: ♀
Artificial diet	11.6 (3.0) bc	3.1 (1.1) bc	19.6 (4.6) c	3.9:1
Artificial diet + "treated" foil	12.7 (2.5) bc	3.2 (0.7) abc	28.6 (4.6) bc	3.5:1
10% Collard extract diet	15.3 (2.4) b	3.9 (1.2) abc	38.2 (4.8) bc	3.3:1
23% Collard extract diet	6.8 (2.0) c	1.2 (0.6) c	22.9 (4.4) bc	5.4:1
48 hr artificial diet	11.1 (2.2) bc	3.8 (0.9) abc	27.1 (5.0) bc	2.3:1
48 hr artificial diet + "sting-cup"	19.7 (4.3) b	7.7 (2.0) a	46.6 (8.8) b	2.3:1
Collard leaf control	32.0 (3.8) a	5.2 (1.6) ab	77.2 (4.5) a	4.6:1

[1]Column numbers with the same letter are not significantly different ($P < 0.05$, Duncan's multiple range test).

more *D. insulare*, and the 48 h artificial diet + "sting-cups" had a higher percentage parasitism. However, with the exception of 23% CE, the collard leaf control did not produce more ♀*D. insulare* adults than any other treatment. This suggests that a longer exposure period coupled with the use of collard extract may enhance parasitoid production. Therefore, diet treated with collard extract plus a longer exposure of the parasitoid and in larger chambers may be the best combination for mass-production of *D. insulare*, especially when the plant material is not continuously supplied.

The sex ratio of *D. insulare* varied among treatments (i.e., 2.3:1 - 5.4:1 (♂:♀)) (Table 1). The treatments with longer exposure of the parasitoid to the diet had a sex ratio of 2.3:1 which is similar to that usually found in our rearing facility (2:1 (♂:♀)), where parasitoids are allowed to fly freely about in a 3.04 m long × 2.13 m wide × 2.13 m high incubation chamber. The other treatments, however, produced undesirable ratio of ♂:♀ *D. insulare* (3.3:1-5.4:1) which is different from that reported to occur in field populations (Idris & Grafius 1993, Mitchell et al. 1997a) and our rearing facility. Lower parasitism of diamondback moth larvae by *D. insulare* compared with the standard rearing using plant materials may be due to the larvae tunneling into the diet cake preventing attack by the parasitoid. Host quality also may be a factor responsible for reduced reproduction of *D. insulare* females. For example, *D. insulare* responds to sub-optimal nitrogen levels in their host's diet with reduced levels of parasitism and highly male-biased sex ratios (Fox et al. 1990). Sex ratios in other ichneumonids are affected by various factors, such as host size and density (Sandlan, 1979), which also may be important in the biology of *D. insulare.* Further studies of these factors in the *D. insulare* rearing program is currently under way in efforts to improve the rearing of this parasitoid for augmentative release.

Summary

Experiments showed potential for rearing *D. insulare* on diamondback moth-infested artificial diet cakes when a possible stimulant, collard extract, was provided along with allowing adequate time for parasitism to occur. Parasitism increased from 19% in the artificial diet treatment to 46% in the 48 h artificial diet plus collard extract impregnated sting-cup treatment. However, this still was not as productive as using larvae placed on collard leaves (77%). The treatments with longer exposure time (48 h) of the parasitoid to diet produced a sex ratio similar to that found in our rearing facility (about 2:1 ♂:♀). The sex ratio of *D. insulare* produced from the other treatments were heavily biased towards males, a situation that is much different from that which occurs in nature.

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or the recommendation for its use by USDA.

References Cited

ADAMS, S. 1994. Assault on the senses: pheromone overload is first in three-pronged attack on crop pests. Agr. Res. July 1994: 4-7.

BIEVER, K. D., D. L. HOSTETTER, AND J. R. KERN. 1994. Evolution and implementation of a biological control-IPM system for crucifers: a 24-year case study. American Entomol. 40: 103-108.

FOX, L. R., D. K. LETOURNEAU, J. EISENBACH, AND S. VAN NOUHUYS. 1990. Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. Oecologia 83: 414-419.

IDRIS, A. B., AND E. GRAFIUS. 1993. Field studies on the impact of pesticides on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and para-

sitism by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae). J. Econ. Entomol. 86: 1196-1202.

LASOTA, J. A., AND L. T. KOK. 1986. *Diadegma insularis* (Hymenoptera: Ichneumonidae) parasitism of the diamondback moth (Lepidoptera: Plutellidae) in Southwest Virginia. J. Entomol. Sci. 21: 237-242.

LEIBEE, G. L. 1996. Insect pest management in crucifers in Florida, pp. 413-422 *In* D. Rosen, F. D. Bennett and J. L. Capinera [eds.] Pest Management in the subtropics: integrated pest management - a Florida perspective. Intercept Limited, Andover, United Kingdom.

MITCHELL, E. R., G. Y. HU, AND J. S. OKINE. 1997a. Diamondback moth (Lepidoptera: Plutellidae) infestation and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae) in collards and adjacent cabbage fields. Florida Entomol. 80: 54-62.

MITCHELL, E. R., F. C. TINGLE, R. C. NAVASERO-WARD, AND M. KEHAT. 1997b. Diamondback moth (Lepidoptera: Plutellidae): parasitism by *Cotesia plutellae* (Hymenoptera: Braconidae) in cabbage. Florida Entomol. 80: 477-489.

MUCKENFUSS, A. E., B. M. SHEPARD, AND E. R. FERRER. 1992. Natural mortality of diamondback moth in coastal South Carolina, pp. 27-36 In N. S. Talekar [ed.] Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, AVRDC Publication No. 92-368, 603 pp.

SANDLAN, K. 1979. Sex ratio regulation in *Coccygomimus turionella* Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. Ecol. Entomol. 4: 365-378.

SAS INSTITUTE. 1989. SAS/STAT user's guide, version 6.08. SAS Institute, Cary, North Carolina.

SHELTON, A. M., R. J. COOLEY, M. K. KROENING, W. T. WILSEY, AND S. D. EIGENBRODE. 1991. Comparative analysis of two rearing procedures for diamondback moth (Lepidoptera: Plutellidae). J. Entomol. Sci. 26: 17-26.

SHELTON, A. M., J. A. WYMAN, N. L. CUSHING, K. APFELBECK, T. J. DENNEHY, S. E. R. MAHR, AND S. D. EIGENBRODE. 1993. Insecticide resistance of diamondback moth (Lepidoptera: Plutellae) in North America. J. Econ. Entomol. 86: 11-19.

TALEKAR, N. S., AND A. M. SHELTON. 1993. Biology, ecology, and management of the diamondback moth. Annv. Rev. Entomol. 38: 275-301.

FIRST RECORD OF *OCHYROMERA LIGUSTRI* (COLEOPTERA: CURCULIONIDAE) FROM CHINESE PRIVET IN FLORIDA

J.P. CUDA[1] AND M.C. ZELLER[2]
[1]Entomology and Nematology Department, University of Florida, P.O. Box 110620, Gainesville, Florida 32611-0620

[2]Bureau of Aquatic Plant Management, Florida Department of Environmental Protection, 3917 Commonwealth Boulevard, M.S. 710 Tallahassee, Florida 32399-3000

Chinese privet or hedge privet, *Ligustrum sinense* (Lour.) (Oleaceae), an ornamental plant of Asian origin, has become naturalized throughout north Florida, Alabama,

and Georgia (Godfrey 1988) as well as Mississippi (Goddard 1992) and Tennessee (Faulkner et al. 1989). This shrub is frequently used as a hedge or border plant (Whitcomb 1975) and its variegated form added color and design to Florida landscapes (Watkins & Sheehan 1975). Chinese privet is now considered an invasive weed because it has escaped cultivation. Songbirds and bobwhite quail are primarily responsible for spreading the plant by ingesting the fruits and dispersing the seeds (MacRae 1980). In some areas, Chinese privet forms dense thickets which displace more desirable native vegetation (Faulkner et al. 1989). According to Goddard (1992), dense stands of this weedy shrub also may harbor populations of the hard tick *Ixodes scapularis* Say, a suspected vector of Lyme disease in the southern United States (Oliver 1989). The Florida Exotic Pest Plant Council currently lists Chinese privet as a Category I invasive species because it causes severe ecological damage by disrupting native plant communities (EPPC 1997). Since no practical control measures exist for large infestations of Chinese privet in natural areas (Faulkner et al. 1989), this weedy shrub may be a suitable candidate for biological control (Pemberton 1996).

A sample of several hundred seeds was collected (by MCZ) from Chinese privet in Tallahassee, Leon County, Florida, on 2 July 1997 for germination studies. After careful inspection, some seeds were found to contain insect larvae. To determine the identity of the insect, several larvae and a subsample of 120 seeds were transferred to Gainesville for rearing. Sixty seeds were placed individually into 1 oz. (29.6 ml) clear plastic cups and capped with lids with air holes to obtain adults for identification. The remaining seeds were arranged on moist sand (2 cm depth) in a 1 pint (0.55 l) plastic container with holes in the lid for air exchange. The rearing containers were held in a laboratory at 27°C and a 18L:6D photoperiod. No adults or parasitoids emerged from the seeds held individually but six weevils emerged from the pooled seed sample on 11 July 1997. The weevils were identified (by M.C. Thomas, Florida Department of Agriculture and Consumer Services, Division of Plant Industry) as *Ochyromera ligustri* Warner, and the larvae and adults were deposited in the Florida State Collection of Arthropods, Gainesville. On 7 August 1997, the remaining seed stock from Tallahassee was dissected and inspected for the presence of *O. ligustri*. Weevil larvae were found in 89 of the 358 seeds examined, or 24.9%.

Ochyromera ligustri was first discovered in 1959 attacking Japanese privet, *L. japonicum* Thunb., in North Carolina (Wray 1961, Warner 1961). According to Warner (1961), *O. ligustri* was probably brought into the United States from the Orient in nursery stock. Japanese privet is the preferred host plant of the weevil although *O. ligustri* also will attack glossy privet or wax-leaf ligustrum, *L. lucidum* Ait., amur or common privet, *L. amurense* Carr., and possibly lilac, *Syringa* spp. (Warner 1961, Wray 1961). Since its introduction, *O. ligustri* has been reported from Florida, Georgia, South Carolina, North Dakota, South Dakota, and Virginia (O'Brien & Wibmer 1982, Johnson & Lyon 1988). Notes on the biology and life history of *O. ligustri* were first reported by Wray (1961)and are summarized by Johnson and Lyon (1988).

Surveys of the arthropods associated with Chinese privet in Florida during the mid 1970's, although not extensive, suggested this plant was not attacked by *O. ligustri* (Poe et al. 1978). Since the weevil has been collected from Chinese privet in the vicinity of Tallahassee in recent years, this paper represents the first published report of Chinese privet as a host plant. Further studies will be required to determine what role seed predation by *O. ligustri* may play in reducing the invasiveness of Chinese privet in Florida.

We thank J. L. Gillmore for her technical support, M. C. Thomas for identifying the insect, K. C. Burks for confirming the identity of the host plant, and G. R. Bucking-

ham and D. H. Habeck for reviewing the manuscript. Florida Agricultural Experiment Station Journal Series No. R-06189.

SUMMARY

The discovery of *Ochyromera ligustri* on Chinese privet, *Ligustrum sinense*, in Florida is a new host record.

REFERENCES CITED

EXOTIC PEST PLANT COUNCIL. 1997. Florida exotic pest plant council's 1997 list of non-native invasive species. Pamphlet, Ft. Lauderdale.

FAULKNER, J. L., E. E. C. CLEBSCH, AND W. L. SANDERS. 1989. Use of prescribed burning for managing natural and historic resources in Chickamauga and Chattanooga National Military Park, USA. Environ. Manage. 13: 603-612.

GODDARD, J. 1992. Ecological studies of adult *Ixodes scapularis* in central Mississippi: questing activity in relation to time of year, vegetation type, and meteorologic conditions. J. Med. Entomol. 29: 501-506.

GODFREY, R. K. 1988. Trees, shrubs and woody vines of northern Florida and adjacent Georgia and Alabama. University of Georgia Press, Athens, 734 pp.

JOHNSON, W. T., AND H. H. LYON. 1988. Insects that feed on trees and shrubs, 2nd ed. Cornell University Press, Ithaca, 556 pp.

MACRAE, W. A. 1980. Unusual bobwhite foods on abandoned Piedmont farmlands, Georgia, USA. Georgia J. Sci. 38: 49-54.

O'BRIEN, C. W., AND G. J. WIBMER. 1982. Annotated checklist of the weevils (Curculionidae *sensu lato*) of North America, Central America, and West Indies (Coleoptera: Curculionoidea). Mem. Amer. Entomol. Inst. 34: 116.

OLIVER, JR., J. H. 1989. Lyme disease: tick vectors, distribution and reservoir hosts. J. Med. Assoc. Georgia. 78: 3-6.

PEMBERTON, R. W. 1996. The potential of biological control for the suppression of invasive weeds of southern environments. Castanea 61: 313-319.

POE, S. L., S. BURGESS, AND D. LESTER. 1978. Arthropods associated with *Ligustrum sinense* (Lour.). Proc. Florida State Hort. Soc. 91: 253-255.

WARNER, R. E. 1961. The genus *Ochyromera* new to the western hemisphere, with a new species and additions to the Jung-Schenkling Coleopterorum Catalogus (Curculionidae: Prionomerinae, Endaeini). Coleop. Bull. 15: 121-124.

WATKINS, J. V., AND T. J. SHEEHAN. 1975. Florida landscape plants. University Press, Gainesville, 368 pp.

WHITCOMB, C. E. 1975. Know it and grow it. Oklahoma State University Press, Stillwater, 500 pp.

WRAY, D. L. 1961. Biology and life history of the ligustrum weevil (Curculionidae). Coleop. Bull. 15: 119-120.

BOOK REVIEWS

PEARCE, M. J. 1997. Termites. Biology and Pest Management. CAB International; Wallingford, U.K. xii + 172 p. ISBN 0-85199-130-0. Hardback. $65.00.

Surprisingly few books have been written about termites, and those which include information about their control can be counted on one hand – with this book included. In seven brief chapters, Mike Pearce reviews termite systematics, distribution, biology, nest habits, ecology, pest status, and control. Four appendices follow. The first, on collecting, is rather generic. The appendix on culture techniques is more useful and provides some ideas on long-term laboratory maintenance of termites. Those on monitoring and laboratory testing are incomplete and offer little discussion about their purposes and limitations.

On the back cover, the publisher promises that the book "will fill a gap in the market" and states that it is "aimed at advanced students of entomology and pest management, as well as professionals concerned with urban and agricultural pest control". [Also on the back, the publisher advertises six other books on specific pest/insect groups, but the page lengths of these (368-600) suggest more detailed reviews of their subjects. By comparison, a comprehensive book on termite biology and control should be at least 500 pages in length]. My appraisal of the book is more reserved. For those new to termites, Pearce offers a glimpse into the diverse natures of these insects. For those wanting a detailed treatise on the subject, they will find predecessors of this book, listed under general references, more complete, accurate, and rewarding. Pest control professionals will be disappointed by the tentative overview given to both established and new control techniques. Brevity of text is compounded by a lack of photographs or figures that depict basic elements of infestations such as subterranean termite foraging tubes or drywood termite fecal pellets. No mention is made of acoustic emissions, fiber optic, or other detection methods.

Many original figures are inaccurately drawn and mislabeled. For example, the first figure shows the caste composition and life cycle of a generalized member of the family Rhinotermitidae, but is labeled as that of a kalotermitid. Thirteen full-page figures consist of rough free-hand drawings of morphological characters. These should have been detailed line drawings, photographs, or scanning electron micrographs as used elsewhere in the book. In the pictorial keys to termite families and subfamilies, the drawings look like preliminary first-draft sketches that omit or confuse some useful characters. Some figure labels are missing. The 32 color plates are of good quality, but focus mainly on nest structures and unusual damage (e.g., clothing, book, telephone pole, etc. eaten by termites). Only six photographs in the entire book depict adult termites (soldiers or imagos), and three of these are of African *Macrotermes*. Thirteen photographs are of nesting structures. Another general criticism is that the text is only selectively cited so as to obscure the source of much of the information presented.

The introductory chapter and those on biology, nests, and ecology are benign in content and useful reviews for the novice. The eight-page chapter on termite distribution needs revision. Biogeographical information is lacking or outdated as in reference to land bridge dispersal mechanisms instead of tectonic theory. The explanation for the distribution of the drywood genus *Cryptotermes* was incorrectly cited. The distribution maps of the pest groups given are haphazardly inked in and depict errors such as the presence of *Cryptotermes* in the western Nearctic or the exclusion of *Coptotermes* from Texas to Florida and Taiwan. With regard to introduced pests, the exhaustive records in the classic review by Gay (1967, A World Review of Introduced Termite Species. Bull. 286 CSIRO Melbourne 88 p.) and more recent discoveries of intercontinental introductions of species including *Coptotermes*, *Heterotermes*, and *Reticulitermes* are not used.

In light of the book's title and the heavy pace of research on applied termitology over the last decade, I was disappointed that Pearce devoted only 6 pages of text to the control of termites in buildings. An update of the excellent control chapters in Edwards and Mill (1986, Termites in Buildings: Their Biology and Control, Rentokil Ltd. UK, 261 p.) should have been in order in place of an obsolete and incomplete list of chemical and non-chemical controls or a list of socioeconomic considerations. Owing to his experience in termite research in Africa, Pearce is more complete in his discussion of termite management practices in non-paradomestic ecosystems. Likewise, however, it gives the book a bias toward Africa, where advanced control methods are generally not practiced, and target species are in agriculture.

Pearce's experience with control practices outside of rural Africa is tenuous. He confuses the reader by mixing remedial treatments like gallery dusting and fumigation with preventative treatments like wood preservation. He goes so far as to include engine oil as a short-term wood protectant. The author also proposes archaic supplements to soil treatments like filling expansion joints with coal tar. He erroneously refers to a photograph depicting soil treatment as a wood injection or "drill-and-treat" application. When discussing fumigation methods, he confuses gas exclusion polymers with fumigant-retaining tarpaulins, reports incorrect exposure rates, and lists carbonyl sulfide as an alternative fumigant. He does not point out that inert gases can only be used as fumigants in gas-tight chambers, states incorrectly that humidity must be measured in heat treatments, and specifies wrongly that dampness is a requisite for intragallery control of termites using an electrocution device called the Electrogun. With reference to baiting, Pearce puts emphasis on the "attractiveness" of bait systems when, in order to be efficacious, baits need only be palatable after termites find them. He wrongly categorizes the bait toxicants mirex and sulfluramid in the same class with the chitin synthesis inhibitors diflubenzuron and hexaflumuron.

In conclusion, this book would have been more aptly named if the title had been prefaced with the phrase "An Introduction to". The book appears to have been written with some oversight and haste and was not authoritatively reviewed. It can best be recommended for those wanting a brief overview of termite biology and control. Specialists wanting a comprehensive review of this subject will not find it here.

Rudolf H. Scheffrahn, Professor
University of Florida
3205 College Avenue
Fort Lauderdale, Florida 33314

SCHWARTZ, M.D. AND R.G. FOOTFIT 1998. Revision of the Nearctic Species of the genus *Lygus* Hahn, with a Review of the Palearctic Species (Heteroptera: Miridae). Memoirs on Entomology 10. International Associated Publishers. Gainesville, Florida v-vii, 1-428 pp. 497 figs. ISBN 1-566665-066-6. Hardback. $65(plus $5 for post and packing).

It is really impossible in a short review to do justice to this important and exhaustive study. The economically important mirid genus *Lygus* has remained one of the most intractable and perplexing groups of Heteroptera despite the efforts of such outstanding North American plant bug specialists as H. H. Knight and L. A. Kelton. The present study is one of the most comprehensive and thorough monographs to appear

in the heteropterological literature in many years. The limits of the genus as well as the difficulty of species recognition has been a major problem for taxonomists and economic entomologists alike. This has apparently been due not only to the similarity of many species but to the variability within many species both geographically and due to seasonal difference caused by the multivoltine nature of the life cycles.

For the first time the authors have treated both the Nearctic and Palearctic species and have produced a cladogram (with a clear explanation of character polarities) that indicates what are sister taxa throughout the entire genus.

This however is really almost ancillary to the main text of the book which includes a key to all the species (with 79 figures accompanying the key alone and not repeated elsewhere in the text).

For the Nearctic region 29 species are recognized and each is treated exhaustively with one or more full page dorsal view illustrations, scanning micrographs of the head, pronotum, corial surface and of the pretarsus. For most species there is also a dorsal view of the fifth instar nymph, from two to six views of details of the male genitalia, a view of the dorsal wall and the sclerotized rings of the female genitalia and a very detailed map of the distribution. These maps are extremely valuable for they are based upon the examination of an amazing amount of material—over 17,000 specimens of *Lygus lineolaris* (P. B.) and over 15,000 of *Lygus elisus* Van Duzee alone. For each species there is a detailed Diagnosis as well as a formal description, a series of measurements, a discussion of variability, host plant data (much of it new), differences in seasonal generations and an exhaustive list of the locality data.

With a molecular study now in progress it is hard to imagine what the authors could have done to have improved the quality and thoughness of this study.

The Palearctic fauna is treated in a more abbreviated fashion than is the Nearctic, but is especially valuable for comparative purposes.

In addition the limits of *Lygus* are closely defined, and two new genera are established for species previously placed in the genus (one unfortunately with the rather inappropriate name *Nonlygus*). Three new Nearctic species are described and eleven junior synonyms proposed.

For the first time it is now possible to see the genus *Lygus* in an intelligible geographic sense. It is a genus of temperate Holarctic distribution with all of the Oriental, African and Neotropical species removed. In fact the majority of species in the Western Hemisphere have a distinctly northern and western distribution, only one species occurring south well into Mexico and with a number of species occurring only along the Pacific coast and many more that extend from Alaska and or British Columbia eastward only in montane habitats in the Rocky Mountains or the Great Plains. A few species extend across Canada while only two seem to have primarily an eastern distribution in North America. Thus it is surprising that the authors recognize only two species as being Holarctic. This suggests that either more study of the fauna of the eastern Palearctic is needed, or that speciation has been relatively rapid since the breakdown of the Bering land bridge. Even the widespread species may have ranges that have expanded recently as several of these feed on various plants in agricultural areas and suggest range expansions (possibly from the northern Great Plains?) with the spread of agriculture.

With the completion of this study not only can one determine species of this difficult and important genus but it allows study of related taxa in other parts of the world that have previously been placed into what the authors rather inelegantly refer to as "garbage genera".

The careful and complete nature of this admirable study will not only be of value to mirid specialists, but to students interested in relationships of what seem to be strictly Nearctic-Palearctic groups and actually anyone interested in the biogeography of North American insects.

It is a rare pleasure to be able to recommend a book with as much enthusiasm as this reviewer has for this one. It should be on the shelf of all Heteroptera students and those of other taxonomists and many applied entomologists as well.

James A. Slater
Dept. Ecology & Evolutionary Biology
University of Connecticut
Storrs, CT 06269

INDEX TO VOLUME 81

INDEX TO VOLUME 81