A SURVEY OF GROUND-DWELLING ANTS (HYMENOPTERA: FORMICIDAE) IN GEORGIA

REID M. IPSER, MARK A. BRINKMAN, WAYNE A. GARDNER AND HAROLD B. PEELER
Department of Entomology, University of Georgia, College of Agricultural and Environmental Sciences
Griffin Campus, 1109 Experiment Street, Griffin, GA 30223-1797, USA

ABSTRACT

Ground-dwelling ants (Hymenoptera: Formicidae) were sampled at 29 sites in 26 counties in Georgia with pitfall traps, leaf litter extraction, visual searching, and bait stations. We found 96 ant taxa including nine species not previously reported from Georgia: *Myrmica americana* Weber, *M. pinetorum* Wheeler, *M. punctiventris* Roger, *M. spatulata* Smith, *Pyramica wrayi* (Brown), *Stenamma brevicorne* (Mayr), *S. diecki* Emery, *S. impar* Forel, and *S. schmitti* Wheeler, as well as three apparently undescribed species (*Myrmica* sp. and two *Stenamma* spp.). Combined with previous published records and museum records, we increased the total number of ground-dwelling ants known from Georgia to 144 taxa.

Key Words: ground-dwelling ants, Formicidae, survey, Georgia, species.

RESUMEN

Hormigas que habitan en el suelo (Hymenoptera: Formicidae) fueron recolectadas en 29 sitios en 26 condados del estado de Georgia con trampas de suelo, extración de hojarasca, busqueda visual, y trampas de cebo. Nosotros encontramos 96 taxa de hormigas incluyendo nueve especies no informadas anteriormente en Georgia: *Myrmica americana* Weber, *M. pinetorum* Wheeler, *M. punctiventris* Roger, *M. spatulata* Smith, *Pyramica wrayi* (Brown), *Stenamma brevicorne* (Mayr), *S. diecki* Emery, *S. impar* Forel, y *S. schmitti* Wheeler, además de tres especies aparentemente no descritas (*Myrmica* sp. y dos *Stenamma* spp.). Al juntar estos datos con las publicaciones y registros de museos, nosotros aumentamos el número de hormigas conocidas que habitan el suelo en Georgia a un total de 144 taxa.

The state of Georgia in the southeastern United States is characterized by a relatively wide range of soil, topographic and climatic conditions. The eight Major Land Resource Areas (MLRAs) identified in the state are (1) Atlantic Coast Flatwoods, (2) Southern Coastal Plains, (3) Carolina and Georgia Sand Hills, (4) Black Lands, (5) Southern Piedmont, (6) Southern Appalachian Ridges and Valleys, (7) Sand Mountains, and (8) Blue Ridge (USDA–SCS 1981). Each MLRA is characterized by a unique combination or pattern of soils, climate, water resources, and land use. These factors, in turn, affect the biotic communities and habitats as well as the floral and faunal characteristics of each.

The diversity and abundance of ants (Hymenoptera: Formicidae) in Georgia are relatively unknown. Wheeler (1913) published a list of 72 ant species collected in Georgia by J. C. Bradley and W. T. Davis; taxonomic revisions have since decreased this list to 62 species. Since that publication, museum records and collections have been the primary sources of occurrence and distribution of ant species in the state; these data are limited in scope. With the exception of Florida (Johnson 1986; Deyrup 2003) and South Carolina (Smith 1934), surveys for ant species are also limited from areas bordering Georgia.

The objective of the study reported herein was to collect, identify, and catalog ground-dwelling ant species from representative MLRAs in Georgia. Undisturbed habitats were purposely sampled to avoid high population levels of two invasive ant species—*Solenopsis invicta* Buren and *Linepithema humile* (Mayr)—that occur throughout the state and reportedly compete with and displace other ant species (Porter & Savignano 1990; Holway 1999).

MATERIALS AND METHODS

Sample Methods and Sites

Twenty-nine sites were sampled 1 to 4 times between June 2000 and September 2002 for ground-dwelling ants (Fig. 1). Most sites were located in state parks; others were on state-owned properties. The sites represented six of the eight MLRAs identified in Georgia. Information and characteristics of each collection site are listed in Table 1.

Each site was 600 m^2 and was located in wooded areas and at least 60 m from any paths, roads, or right-of-ways. Sampling methods employed were pitfall trapping, extraction from leaf litter collections, visual searching, and baiting as described by Agosti & Alonso (2000) and Bestle-

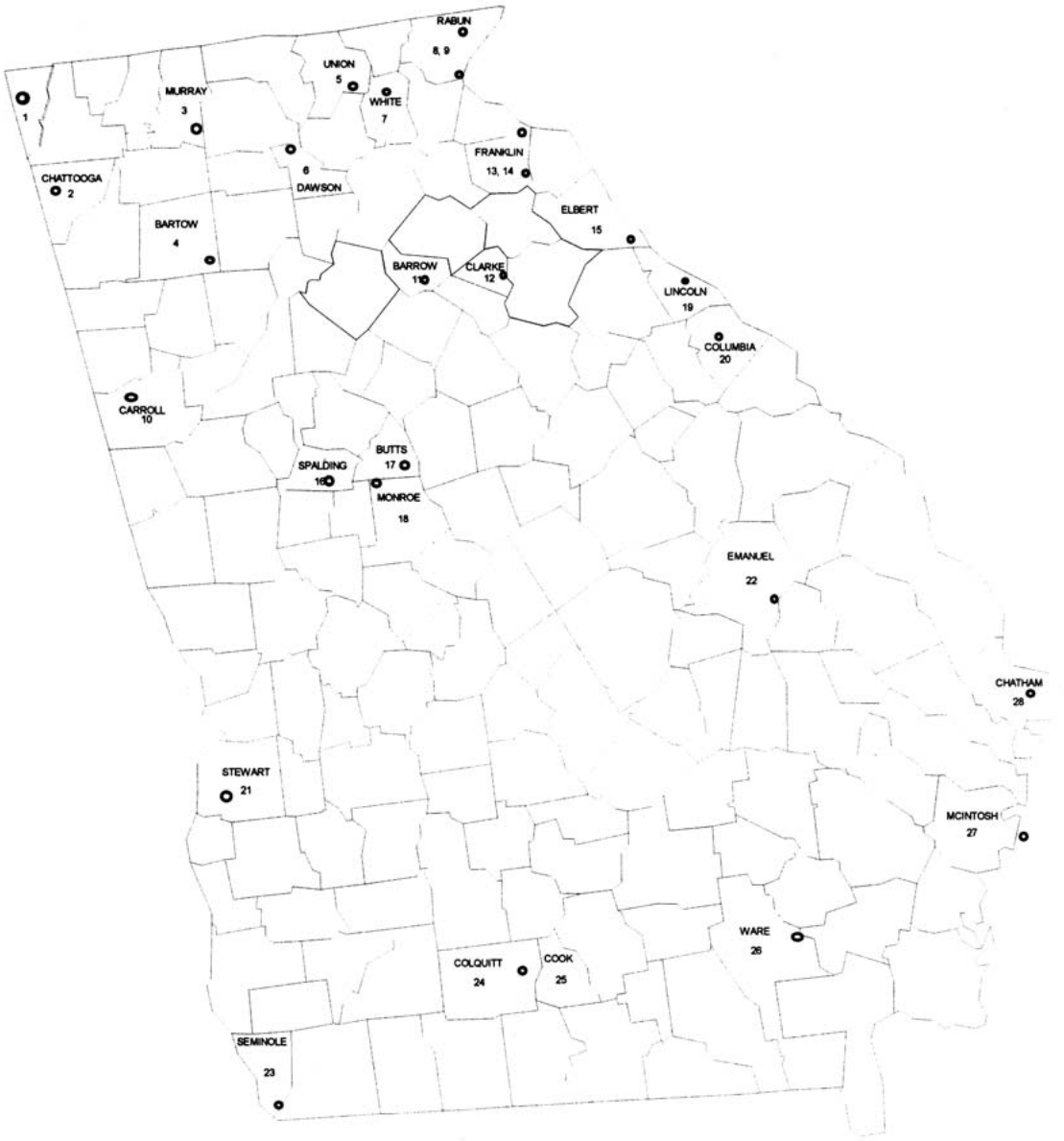

Fig. 1. Georgia sites sampled for ground-dwelling ants, 2000-2002.

meyer et al. (2000). For each sampling event, 20 pitfall traps were placed individually at 1-m intervals along a transect. Traps were 40-ml plastic vials filled to 60% of container volume with propylene glycol. The vials were placed in the ground with the upper opening level with the soil surface. The traps remained in the ground for 7 d when they were removed, capped, and transported to the laboratory for processing. Leaf litter was gathered by hand from several locations within the 600 m^2 site. These were combined and placed in a 50-L plastic bag, stored on ice, and transported to the laboratory. In the laboratory, litter samples were divided and placed in Berlese funnels (Agosti & Alonso 2000) for 24 h to separate ants. Bait stations used were those described by Brinkman et al. (2001). Tuna packaged in oil, was placed in a thin layer over the surface of a 2.5-cm diam filter paper disk (Whatman no. 1) in a plastic Petri dish (10 × 35 mm). Ten stations were placed individually at 2-m intervals along a transect. The stations remained uncovered on the ground for 2 h. They were then covered, placed on ice, and transported to the laboratory for processing. The ground, tree trunks, fallen trees, and other surfaces were visually searched for ants at

TABLE 1. LOCATIONS AND CHARACTERISTICS OF SITES SAMPLED FOR GROUND-DWELLING ANTS IN GEORGIA, 2000-2002. ALL STUDY SITES WERE IN STATE-OWNED PROPERTY (STATE PARKS OR UNIVERSITY OF GEORGIA).

Survey Site	Sites	County	N;W	Major Land Resource Areas	Elevation
1	Cloudland Canyon	Dade	34°50.4; 085°28.9	Sand Mountain	602 m
2	Sloppy Floyd	Chattooga	34°26.4; 085°20.2	Sand Mountain/Southern Appalachian	303 m
3	Fort Mountain	Murray	34°46.6; 084°42.5	Southern Appalachian/Blue Ridge	906 m
4	Red Top Mountain	Bartow	34°08.6; 084°42.2	Southern Appalachian/Blue Ridge	325 m
5	Vogel	Union	34°46.1; 083°54.9	Blue Ridge	236 m
6	Amicalola Falls	Dawson	34°34.2; 084°14.7	Blue Ridge	900 m
7	Unicoi	White	34°43.9; 083°43.6	Blue Ridge	887 m
8	Black Rock Mountain	Rabun	34°54.4; 083°24.3	Blue Ridge	1055 m
9	Tallulah Gorge	Rabun	34°44.4; 083°23.3	Blue Ridge	539 m
10	John Tanner	Carroll	33°36.1; 085°09.9	Southern Piedmont	332 m
11	Fort Yargo	Barrow	33°57.9; 083°43.4	Southern Piedmont	303 m
12	UGA Whitehall Forest	Clarke	33°53.7; 083°21.9	Southern Piedmont	887m
13	Victoria Bryant	Franklin	34°17.7; 083°09.7	Southern Piedmont	236 m
14	Tugaloo	Franklin	24°29.5; 083°04.4	Southern Piedmont	374 m
15	Richard B. Russell	Elbert	34°10.8; 082°45.9	Southern Piedmont	214m
16	Bobby Brown	Elbert	33°58.1; 082°34.6	Southern Piedmont	89 m
17	UGA Griffin Campus	Spalding	33°16.0; 084°17.2	Southern Piedmont	307 m
18	Indian Springs	Butts	33°14.9; 083°55.5	Southern Piedmont	193 m
19	High Falls	Monroe	33°10.3; 084°00.7	Southern Piedmont	178 m
20	Elijah Clark	Lincoln	33°51.3; 082°24.0	Southern Piedmont	154 m
21	Mistletoe	Columbia	33°39.9; 082°22.9	Southern Piedmont	163 m
22	Providence Canyon	Stewart	32°04.0; 084°54.3	Southern Coastal Plain	222 m
23	George L. Smith	Emanuel	32°32.7; 082°07.5	Southern Coastal Plain	123 m
24	Seminole	Seminole	30°48.2; 084°52.7	Southern Coastal Plain	35 m
25	Reed Bingham	Colquitt	31°09.6; 083°32.3	Southern Coastal Plain	78 m
26	Laura S. Walker	Ware	31°08.5; 083°12.9	Atlantic Coast Flatwoods	47 m
27	Sapelo Island Dunes	McIntosh	31°23.4; 081°15.9	Atlantic Coast Flatwoods	0 m
28	North Sapelo Island	McIntosh	31°23.4; 081°15.9	Atlantic Coast Flatwoods	19 m
29	UGA Bamboo Farm	Chatham	31°59.9; 081°16.2	Atlantic Coast Flatwoods	19 m

TABLE 2. LIST OF GROUND-DWELLING ANTS COLLECTED IN GEORGIA 2000-2002 SURVEY WITH COLLECTION SITE (S) NOTED.

Species	Survey sites[1]
Acanthomyops interjectus (Mayr)	5
Amblyopone pallipes (Haldeman)	6,8,21,9
Aphaenogaster ashmeadi (Emery)	10,28
Aphaenogaster fulva Roger	6,10,11,18,19
Aphaenogaster lamellidens Mayr	11,16,18,19
Aphaenogaster miamiana Wheeler	27
Aphaenogaster picea/rudis/texana complex[2]	1,2,3,4,5,6,7,8,9,10,11,12,13,14,16,18,19,20,21,23,29
Aphaenogaster tennesseensis (Mayr)	23
Brachymyrmex depilis Emery	18,19,22,23,24,25,26,29
Brachymyrmex musculus Forel	23,29
Camponotus americanus Mayr	1,2,4,10,11,14,18,19
Camponotus castaneus (Latreille)	10,14,29
Camponotus floridanus (Buckley)	19,24,29
Camponotus nearcticus Emery	10,21,26
Camponotus pennsylvanicus (De Geer)	1,2,4,8,9,10,11,18,19,23
Camponotus subbarbatus Emery	1,19
Crematogaster ashmeadi Mayr	2,4,9,10,11,12,14,16,18,19,20,21,23,26,28,29
Crematogaster cerasi (Fitch)	29
Crematogaster lineolata (Say)	1,2,7,9,11,12,14,18,19,20
Crematogaster minutissima Mayr	10,18,19
Cyphomyrmex rimosus (Spinola)	22,24,25,26
Dorymyrmex bureni Trager	22,25,26,27,29,26
Dorymyrmex insanus (Buckley)	25,29
Forelius analis (Andre)	10,14,18,19,29
Forelius pruinosus (Roger)	25
Formica archboldi Smith	10
Formica exsectoides Forel	1
Formica pallidefulva Latreille	6,10,14,17,19,20,21,23,29
Formica rubicunda Emery	1
Formica schaufussi Mayr	10,19
Formica subintegra Wheeler	10
Formica subsericea Say	1,5,6,7,9,10,11,18,20
Hypoponera opaciceps (Mayr)	5,7,18,20,23,29
Hypoponera opacior (Forel)	10,17,18,19,20,21,23,29
Lasius alienus	5,6,8,18,19,25,29
Lasius neoniger Emery	10
Leptothorax curvispinosus Mayr	2,3,4,9,10,11,29
Leptothorax pergandei Emery	9,16,24,28,29
Leptothorax schaumii Roger	14,19
Leptothorax smithi Baroni Urbani	16
Linepithema humile (Mayr)	10,17,21,25
Monomorium minimum (Buckley)	1,10,18,19,21
Monomorium viride Brown	27
Myrmecina americana Emery	1,3,4,5,8,9,10,11,13,14,16,20,21
Myrmica americana Weber	6,18,23
Myrmica pinetorum Wheeler	19
Myrmica punctiventris Roger	5,7,8,9,10,18,19
Myrmica spatulata Smith	5,9
Myrmica sp. (undescribed)[3]	6
Odontomachus brunneus (Patton)	29

[1]Sites and site information are provided in Table 1.

[2]*Aphaenogaster picea/rudis/texana* complex includes *A. picea* (Wheeler), *A. picea rudis* Enzmann, *A. texana* Wheeler, and *A. texana carolinensis* Wheeler species (S. Cover, personal communication).

[3]Previously undescribed species (S. Cover, personal communication).

[4]*Solenopsis molesta* complex includes *S. carolinensis* Forel, *S. molesta* (Say), *S. pergandei* Forel, *S. texana* Emery, *S. truncorum* Forel species (S. Cover, personal communication).

[5]Two previously undescribed species and first records from Georgia (S. Cover, personal communication).

TABLE 2. (CONTINUED) LIST OF GROUND-DWELLING ANTS COLLECTED IN GEORGIA 2000-2002 SURVEY WITH COLLECTION SITE (S) NOTED.

Species	Survey sites[1]
Pachycondyla chinensis (Emery)	11,14,15,16,18
Paratrechina arenivaga Wheeler	6,10,12,19,22
Paratrechina faisonensis (Forel)	1,7,10,11,14,17,19,21,28,29
Paratrechina parvula (Mayr)	19
Paratrechina vividula (Nylander)	2,4,7,9,10,11,19,20,28,29
Pheidole adrianoi Naves	29
Pheidole bicarinata Mayr	10,21,26,29
Pheidole bicarinata vinelandica Forel	10,14
Pheidole crassicornis Emery	18,19,22,23,25,26
Pheidole dentata Mayr	1,10,14,16,19,21,23,24,29
Pheidole dentigula Smith	10,18,19,23,24,28,29
Pheidole littoralis Cole	23
Pheidole metallescens	23
Pheidole morrisii Forel	29
Pheidole tysoni Forel	10,18,19,21,23
Pogonomyrmex badius (Latreille)	27,29
Polyergus lucidus Mayr	29
Ponera pennsylvanica	1,2,5,6,7,8,9,10,11,14,18,19
Prenolepis imparis (Say)	1,2,3,5,6,7,8,9,10,11,12,14,16,18,19,20,23
Proceratium croceum (Roger)	7
Proceratium pergandei (Emery)	19
Pseudomyrmex ejectus (Smith)	21
Pyramica bunki (Brown)	11
Pyramica carolinensis (Brown)	3
Pyramica ornata (Mayr)	10,18,19,21
Pyramica rostrata (Emery)	18,19
Pyramica wrayi (Brown)	29
Solenopsis geminata (Fabricius)	27
Solenopsis invicta Buren	10,18,19,21,22,24,25,26,28,29
Solenopsis molesta complex[4]	2,4,7,9,10,11,12,14,17,18,19,21,22,23,24,26,29
Stenamma brevicorne (Mayr)	18
Stenamma diecki Emery	1,4,5,6,7,8,9,23
Stenamma impar Forel	18,19
Stenamma schmitti Wheeler	5,6,8,18,19
Stenamma spp. (2 undescribed species)[5]	6
Strumigenys louisianae Roger	18,19,21,23,29
Tapinoma sessile (Say)	3,6,8,9,18,19,21
Trachymyrmex septentrionalis (McCook)	13,14,19,22,23,25

[1]Sites and site information are provided in Table 1.
[2]*Aphaenogaster picea/rudis/texana* complex includes *A. picea* (Wheeler), *A. picea rudis* Enzmann, *A. texana* Wheeler, and *A. texana carolinensis* Wheeler species (S. Cover, personal communication).
[3]Previously undescribed species (S. Cover, personal communication).
[4]*Solenopsis molesta* complex includes *S. carolinensis* Forel, *S. molesta* (Say), *S. pergandei* Forel, *S. texana* Emery, *S. truncorum* Forel species (S. Cover, personal communication).
[5]Two previously undescribed species and first records from Georgia (S. Cover, personal communication).

each sampling time. The total amount of time spent on visual searching was 1.5 h, but varied based on the number of individuals involved in the search. Ants discovered in the visual searches were collected, placed in 70% ethyl alcohol, and transported to the laboratory for processing.

In the laboratory, ant specimens were separated and placed in 95% ethyl alcohol. Identifications were made with keys by Bolton (1994, 2000); Buren (1968); Creighton (1950); Cuezzo (2000); Deyrup et al. (1985); DuBois (1986); Gregg (1958); Holldobler & Wilson (1990); Johnson (1988); MacKay (2000); Smith (1957); Snelling (1973, 1988); Snelling & Longino (1992); Taylor (1967); Trager (1984, 1988); Ward (1985, 1988); Wilson (1955); and Wing (1968), and by comparison with specimens housed in the University of Georgia Natural History Museum (Athens, GA). Stefan Cover (The Museum of Comparative Zoology, Harvard Univ., Cambridge, MA) and Mark Deyrup

TABLE 3. SPECIES OF GROUND-DWELLING ANTS PREVIOUSLY REPORTED TO OCCUR IN GEORGIA BUT NOT COLLECTED IN THE 2000-2002 STATE SURVEY.

Species	Record
Acanthomyops claviger (Roger)	UGANHM[1]
Acanthomyops murphyi (Forel)	UGANHM[1]
Aphaenogaster ashmeadi (Emery)	Wheeler 1913
Aphaenogaster treatae Forel	Wheeler 1913
Camponotus caryae (Fitch)	UGANHM[1]
Camponotus decipiens Emery	Wheeler 1913
Camponotus discolor (Buckley)	Wheeler 1913
Camponotus impressus (Roger)	ABS[2]
Camponotus socius Roger	Wheeler 1913
Crematogaster missuriensis Emery	ABS[2]
Crematogaster pilosa Emery	Wheeler 1913
Crematogaster sp. (undescribed)	ABS[2]
Cryptopone gilva (Roger)	UGANHM[1]
Discothyrea testacea Roger	ABS[2]
Dolichoderus mariae Forel	Wheeler 1913
Dolichoderus pustulatus Mayr	Wheeler 1913
Dorymyrmex grandulus (Forel)	UGA NHM[1]
Formica difficilis Emery	Wheeler 1913
Formica integra Nylander	Wheeler 1913
Formica nitidiventris Emery	Wheeler 1913
Formica obscuriventris Mayr	Wheeler 1913
Leptothorax bradleyi Wheeler	Wheeler 1913
Leptothorax texanus Wheeler	ABS[2]
Monomorium pharaonis (L.)	Wheeler 1913
Myrmica latifrons Starcke	Wheeler 1913
Nievamyrmex carolinensis (Emery)	UGANHM[1]
Nievamyrmex nigrescens (Cresson)	UGANHM[1]
Neivamyrmex opacithorax (Emery)	Wheeler 1913
Paratrechina longicornis (Latreille)	Wheeler 1913
Pheidole pilifera (Roger)	UGANHM[1]
Ponera exotica Smith	ABS[2]
Proceratium creek De Andrade	ABS[2]
Proceratium crassicorne Emery	ABS[2]
Pseudomyrmex pallidus (Smith)	Wheeler 1913
Pyramica abdita (Wesson)	ABS[2]
Pyramica angulata (Smith)	ABS[2]
Pyramica clypeata (Roger)	UGANHM[1]
Pyramica dietrichi (Smith)	UGANHM[1]
Pyramica laevinasis (Smith)	ABS[2]
Pyramica ohioensis (Kennedy & Schramm)	ABS[2]
Pyramica pergandei (Emery)	ABS[2]
Pyramica pilinasis (Forel)	ABS[2]
Pyramica pulchella (Emery)	ABS[2]
Pyramica reflexa (Wesson)	ABS[2]
Solenopsis picta Emery	UGANHM[1]
Solenopsis tennesseensis Smith	ABS[2]
Solenopsis xyloni McCook	Jouvenaz et al. 1977
Tetramorium bicarinatum (Nylander)	UGA NHM[1]

[1]University of Georgia Natural History Museum.
[2]Archbold Biological Station.

(Archbold Biological Station, Lake Placid, FL) confirmed species identifications. Voucher specimens have been deposited in the University of Georgia Natural History Museum and the Museum of Comparative Zoology at Harvard University.

RESULTS AND DISCUSSION

Ninety-six species of ground-dwelling ants representing 33 genera were collected and identified in this 2-year survey (Table 2). Of those collected,

9 species have not been previously reported from Georgia. These are *Myrmica americana* Weber, *M. pinetorum* Wheeler, *M. punctiventris* Roger, *M. spatulata* Smith, *Pyramica wrayi* (Brown), *Stenamma brevicorne* (Mayr), *S. diecki* Emery, *S. impar* Forel, and *S. schmitti* Wheeler.

Of those previously unreported species, *M. americana* was collected from 3 sites, *M. pinetorum* was collected from 1 site, *M. punctiventris* was collected from 7 sites, and *M. spatulata* was collected from 2 sites. Ants of this genus nest in soil and in rotting wood and are primarily carnivorous, but they will feed on plant exudates such as nectar (Creighton 1950). In addition, *P. wrayi* and *S. brevicorne* were each collected from 1 site, *S. diecki* was collected from 8 sites, *S. schmitti* was collected from 5 sites, and *S. impar* was collected from 2 sites. All *Stenamma* species are carnivorous, and *Pyramica* are specialized predators of collembolans (Holldobler & Wilson 1990).

Eleven individuals of *Myrmica* and 3 individuals of *Stenamma*, possibly representing two species, were collected from Amicalola State Park in Dawson Co. (site 6) and represent as yet undescribed species (S. Cover, pers. comm.). Those specimens were collected on 2-V-2000, primarily by pitfall trapping and leaf litter collection.

A review of ant specimens deposited in the Archbold Biological Station (ABS), the University of Georgia Natural History Museum (UGANHM), the lists of ants published by Wheeler (1913), and a survey conducted by Jouvenaz et al. (1977) reveal that 48 species of ground-dwelling ants representing 21 genera have been reported from Georgia but were not collected in the survey reported herein (Table 3). To date, these two lists (Tables 2 and 3) comprise the ground-dwelling ant species reported from Georgia. Species collected within the *Aphaenogaster picea/rudis/texana* complex and the *Solenopsis molesta* complex are footnoted in Table 2.

In terms of occurrence and distribution, *Prenolepis imparis* (Say) was collected from 17 of the 29 sites sampled, the *Aphaenogaster picea/rudis/texana* complex from 21 sites; the *Solenopsis molesta* complex from 17 sites, and *Crematogaster ashmeadi* Mayr from 16 sites in this survey. All other species were collected from less than one-half of the sites. Members of the genus *Pheidole* were most numerous with 2,765 individuals representing 10 species collected at 14 sites. *Dorymyrmex burnei* (Trager), *D. insanus* (Buckley), and *Cyphomyrmex rimosus* (Spinola) were collected only at southern sites, while *Amblyopone pallipes* (Haldeman), *Ponera pennsylvanica* Buckley, and *Tapinoma sessile* (Say) were collected from sites in northern Georgia. *Pseudomyrmex ejectus* (Smith) was collected from pitfall traps at one site. *Pseudomyrmex* spp. are characteristically arboreal in their habits. These specimens most likely dropped to the forest floor, and thus were collected as ground-dwellers. Three species - the seed harvester *Pogonomyrmex badius* (Latreille), the obligate slave raider *Polyergus lucidus* Mayr, and the generalist *Aphaenogaster miamiana* Wheeler—were recovered only on Sapelo Island, a barrier island on Georgia's coast.

The survey reported herein provides a basis for various ecological studies and assessments. Ant assemblages, species composition, and community structure are important in terms of community ecology. For example, in Australia, ants are one of the most functionally important faunal groups (Matthews & Kitching 1984; Anderson 1992) and are model organisms for studies in community ecology (Anderson 1983, 1988, 1991; Greenslade & Halliday 1983). Ants also have been used as bio-indicators in mine site rehabilitation (Majer 1983, 1985).

Schultz & McGlynn (2000) noted the many interactions that occur between ants and other organisms within habitats. They further postulated that if these interactions are understood, one could predict ecological conditions within a given habitat based upon the presence or absence of specific ants. Furthermore, one could correlate the presence of a specific ant species with specific ecological conditions, and these correlations could be used as predictors of ant biodiversity and interactions among ant species (Alonso 2000).

This survey is the first published listing of ground-dwelling ants in Georgia since Wheeler (1913). This compilation will serve to support biodiversity, systematics, and ecological studies for Georgia and surrounding environs.

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INFLUENCE OF DROUGHT STRESS ON SWEETPOTATO RESISTANCE TO SWEETPOTATO WEEVIL, *CYLAS FORMICARIUS* (COLEOPTERA: APOINIDAE), AND STORAGE ROOT CHEMISTRY

LIXIN MAO[1], LOUIS E. JETT[2], RICHARD N. STORY[1], ABNER M. HAMMOND[1], JOSEPH K. PETERSON[3] AND DON R. LABONTE[4]

[1]Department of Entomology, Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center, Baton Rouge, LA 70803 USA

[2]Sweet Potato Research Station, Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center, Chase, LA 71324 USA
Current address: Department of Horticulture, University of Missouri, Columbia, MO 65211 USA

[3]USDA-ARS, US Vegetable Laboratory, 2700 Savannah Highway, Charleston, SC 29414 USA

[4]Department of Horticulture, Louisiana Agricultural Experiment Station
Louisiana State University Agricultural Center, Baton Rouge, LA 70803 USA

ABSTRACT

The effect of drought stress on the resistance of sweetpotato roots to sweetpotato weevil (SPW), *Cylas formicarius* (Fab.), was studied in 1997 and 1998 in two genotypes ("Beauregard" and "Excel") with different SPW susceptibility. Storage roots produced under drought or normal conditions were tested for adult feeding, oviposition, larval survival and pupal weight in the laboratory under no-choice and free-choice test conditions. The levels of sweetpotato resin glycoside and caffeic acid in the periderm tissue of the roots were also determined. Drought-stressed roots received significantly more SPW eggs under no-choice and free-choice conditions and more feeding punctures under free-choice conditions than non-stressed roots in 1997. Larval survival rate was significantly lower on drought-stressed roots. A significant drought effect on feeding, oviposition and larval survival was absent in 1998. Drought stress had no effect on sweetpotato resin glycosides content in both years, but significantly reduced the content of caffeic acid in 1997. Genotype had a significant effect on SPW feeding in 1997 and on feeding and oviposition in 1998 under free-choice test conditions, where Beauregard was preferred for both feeding and oviposition. Beauregard also supported a significantly higher larval survival rate compared with Excel. Resin glycosides or caffeic acid contents were similar for the two genotypes in 1997, while higher level of resin glycosides was detected in Excel than in Beauregard in 1998. The interaction between drought stress and genotype was significant for adult feeding under free-choice conditions and for larval survival, indicating a different response between the two genotypes.

Key Words: host plant resistance, feeding, oviposition, resin glycosides, caffeic acid.

RESUMEN

El efecto de estres causado por la sequía sobre la resistencia de las raices del camote (= batata) al "gorgojo del camote" *Cylas formicarius* (Fab.), se estudio durante 1997 y 1998 en dos genotipos ("Beauregard" y "Excel") con susceptibilidad diferentes al insecto. Las raices almacenadas producidas bajo condiciones de sequía o condiciones normales fueron evaluadas para la alimentación de adultos, la oviposición, la sobrevivencia de las larvas, y el peso de las pupas en el laboratorio bajo condiciones de pruebas de no-alternativa y de selección libre. Los niveles del glucósido de la resina del camote y el ácido cafeico en el tejido del peridermo de las raices tambien fueron determinados. Las raices con el estrés de la sequía recibieron significativamente más huevos del gorgojo de camote bajo las condiciones de no alternativa y de selección libre y más picaduras de alimentación bajo las condiciones de no-alternativa que en las raices sin estrés en 1997. Un efecto significativo de la sequía sobre la alimentación, oviposición, y sobrevivencia de las larvas no se presentó en 1998. El estrés de la sequía no tenia efecto sobre el contenido de los glucósidos de la resina de camote en ambos años, pero redujó significativamente el contenido del ácido cafeico en 1997. El genotipo tuvo un efecto significativo sobre la alimentación y la oviposición en 1998 bajo condiciones de pruebas de selección libre, donde el Beauregard fue preferido para la alimentación y la oviposición. El genotipo Beauregard tambien suportó una tasa de sobrevivencia de larvas significativemente más alta en comparación con el Excel. El contenido de los glucósidos de la resina o del ácido cafeico fueron similares para los dos genotipos en 1997, mientras que niveles más altos de glucósidos fueron detectados en Excel que en Beauregard en 1998.

Sweetpotato weevil (SPW) *Cylas formicarius* (Fab.) is a destructive insect pest of sweetpotato *Ipomoea batatas* (L.) Lam. worldwide (Chalfant et al. 1990). It attacks sweetpotato both in the field and during storage. Adults make feeding and oviposition punctures on root surfaces, reducing root quality and market value. Larvae feed internally and induce terpenoid production in storage roots that imparts a bitter taste and renders even slightly damaged roots unfit for human or animal consumption (Uritani et al. 1975). The search for SPW-resistant sweetpotato cultivars has been conducted for decades, but little success has been achieved partly because of the inconsistent expression of the resistance (Collins et al. 1991). Sweetpotato exhibits a wide variation in a number of traits such as yield, dry matter, intercellular space, nutrient content, flavor components, secondary metabolites, and resistance to microorganisms and insects (Ezell & Wilcox 1958; Hammett 1974; Collins et al. 1987; Woolfe 1991; Clark & LaBonte 1992; Thompson et al. 1992; Marti et al. 1993). Identification of environmental factors that affect the expression of resistance and the knowledge of the magnitude of these variations would assist in the development of cultivars with stable SPW resistance. In addition, secondary plant compounds are often associated with host plant resistance. Sweetpotato resin glycosides and caffeic acid are two such compounds found in the sweetpotato storage roots that have shown insecticidal activities (Peterson & Harrison 1992; Peterson et al. 1998; Jackson & Peterson 2000). Any effect of environmental factors on the level of these two compounds may provide insights on sweetpotato weevil resistance.

Drought stress is a common abiotic environmental factor that induces physical and/or chemical changes in plants and consequently influences the associated herbivorous insects (Holtzer et al. 1988). In this study, both field and laboratory experiments were conducted to determine the impact of drought stress on SPW resistance by measuring adult feeding, oviposition, larval survival, and development (pupal weight) on storage roots. Two genotypes with different levels of SPW susceptibility were used. Sweetpotato resin glycosides and caffeic acid contents also were analyzed.

Materials and Methods

Field Experiment

The experiments were conducted at the Sweet Potato Research Station, Louisiana State University Agricultural Center, Chase, Louisiana, in 1997 and 1998. "Beauregard" and "Excel" were used because Beauregard, a major cultivar in the region, is susceptible to SPW and Excel has shown a moderate level of resistance (Story et al. 1996). The treatments were 2×2 factorial combinations of water treatment (drought stressed and irrigated) by genotype arranged in a randomized complete block design with 4 replications. Each plot consisted of four 25-plant rows. Uniform transplants were mechanically transplanted on 30 June, 1997, and 27 June, 1998 in a Gilbert silt loam with a pH of 5.6 at 0.3-m spacing within rows on 1.0-m centered beds. The fields were fumigated with Telone™ C-17 (1,3-dicholropropene) 2 weeks before transplanting. Standard cultural practices were followed throughout the growing season (Boudreaux 1994).

The drought stress treatment was initiated 50 days after transplant (DAT) by constructing moveable rain shelters over the plots to exclude natural precipitation. The shelters were placed over the plots wherever there was more than 30% chance of precipitation in the local weather forecast. Otherwise, the plots were left open. The irrigated plots were watered starting 3 weeks after transplant with drip tubes (3.8 ml/min). Storage roots were harvested at 120 DAT, cured (30°C, 90% RH for 7 d), and stored at 15 ± 2°C for about 30 d before the bioassays and chemical analyses were started.

Insect Rearing

A SPW colony was established in January of 1997 from a field-collected population (about 500 adult insects) and maintained on storage roots of Beauregard in plastic containers (5.6 L) with screen covers at 28 ± 2°C and 85 ± 10% RH in the laboratory located at Louisiana State University Baton Rouge campus. In preparing experimental insects, 5 fresh storage roots (US #1) were exposed to about 1000 unsexed adults for 5 d and then removed and kept under the conditions described above. Emerging adults were collected weekly and held with fresh storage roots. Female adults 3-4 wk old were used in the bioassays to ensure adequate egg-laying capability (Wilson et al. 1988).

Adult Feeding and Oviposition Bioassay

The bioassay technique was an adaptation of one previously described by Mullen et al. (1980) that has been used in several SPW feeding and oviposition studies (Wilson et al. 1988). The apparatus consisted of a 24-well tissue culture plate ($12.5 \times 8.5 \times 2.0$ cm, Falcon® Model 3047, Becton Dickenson & Co., Lincoln Park, NJ) placed in a rectangular clear plastic container ($17 \times 12 \times 6$ cm, Tri-State Plastic, Dixon, KY). Cores were cut from storage roots with a cork borer (1.6 cm diameter) and were inserted into the wells so that only the periderm was exposed. The diameters of the cores and the wells were the same, providing a close fit. Female adults were kept without food for 3 h before being introduced into the arena at a density of 2 weevils per root core. A moist cotton

ball was placed in the container to prevent desiccation of the cores. After 24 h the number of feeding punctures on each root core was counted, and after 48 h the number of eggs was counted. No-choice tests were conducted by presenting a single root core in the arena. Free-choice tests were conducted by presenting 4 root cores in the arena which were cut from one root (U.S. #1) randomly selected from each treatment combination. Before testing, the roots were gently washed with tap water and allowed to dry. All tests were conducted at 28 ± 5°C, 85 ± 10% RH under total darkness to eliminate light as a variable. For each treatment, the tests were repeated 4 times with 4 roots (sampling units). Roots from four field blocks were tested in 4 consecutive weeks.

Larval Survival and Development Bioassay

SPW were reared individually in Petri dishes by transferring a single egg into a root section (about 1.5 × 1.5 × 1.5 cm) in a cavity (1-2 mm deep, 4.0 mm diameter) cut with a cork borer. Eggs were obtained by exposing Beauregard storage roots to a large number of females for 24 h. A pair of needle-nosed forceps was used to transfer eggs. At 12 d after the eggs were deposited, root sections were examined to determine if eggs had hatched. Nonviable eggs or rotten root sections were discarded. At about 25 d after oviposition, root sections were dissected for pupae. Larval survival and pupal weight were recorded. Two replications of each treatment combination were conducted with sample sizes ranging from 18 to 32 pupae each. The bioassays were conducted under conditions of 28 ± 5°C and 85 ± 10% RH in total darkness.

Chemical Analysis

The chemical analysis was conducted in the USDA-ARS Vegetable Laboratory, Charleston, South Carolina. Storage roots were carefully washed under flowing water and allowed to dry. Periderm tissue was gently scraped off with a scalpel, dried at 50°C, and ground to a fine powder in liquid nitrogen with a mortar and pestle. Subsequently the powder was re-dried at 40°C and stored in vials under nitrogen at -20°C until analysis. Powder samples were weighed (200 mg) into Teflon-lined, screw-capped test tubes, and 2.0 ml of methanol were added containing 0.08 mg of chrysin (recrystallized from amyl alcohol) as an internal standard. Test tubes were ultrasonicated for 20 min while the surrounding water was ice-cooled. The tubes were centrifuged and the supernatant was filtered through Nylon-66 membrane filters (0.20 µm, Pierce Chemical Company, Rockville, IL) into auto injector vials. Resin glycosides and caffeic acid concentrations were analyzed by reverse-phase HPLC with 20 µl of the solution. For resin glycosides, a H_2O/MeOH linear gradient from 60% to 100% MeOH in 15 min was used and held at 100% MeOH for 25 min; flow rate was 1 ml min^{-1} and detection was at 230 nm. For caffeic acid, a second injection of 20 µl was made, with the same sample as was used for the resin glycosides analysis. A H_2O/MeOH linear gradient from 10% to 100% MeOH in 35 min was used and held at 100% MeOH for 25 min; flow rate was 1 ml min^{-1} and detection was at 340 nm. Each solvent contained 0.1% H_3PO_4. The column used was a Beckman Ultrasphere C_{18}, 5 µm (4.6 × 250 mm, Beckman and Coulter, Fullerton, CA). Purified reference substances were used as external standards to determine response factor versus chrysin for quantification. Reference glycoside material was purified by Sephadex column chromatography followed by semi-preparative HPLC as described previously (Peterson et al. 1998). Reference caffeic acid was purchased from Aldrich Chemical Company (Milwaukee, WI).

Data Analysis

The data were analyzed by year using two-way analysis of variance (PROC GLM, SAS 1990). A square-root transformation was used for larval survival data. Year effect was evaluated by analyzing the data by one-way analysis of variance. The significance level was $\alpha = 0.05$.

Results

Adult Feeding and Oviposition

Drought stress significantly increased adult SPW feeding and oviposition in free-choice tests and oviposition in no-choice tests in 1997 (Table 1). In 1998, drought stress had no significant effect on oviposition or on feeding (Table 1). Year effect was significant on feeding (no-choice test: $F = 23.08$, $df = 1,24$, $P < 0.0001$; free-choice test: $F = 10.09$, $df = 1,24$, $P = 0.0041$) and on oviposition under both testing conditions (no-choice test: $F = 50.51$, $df = 1,24$, $P < 0.0001$; free-choice test: $F = 17.50$, $df = 1,24$, $P = 0.0003$). Beauregard received more feeding punctures than Excel in free-choice tests, but not in no-choice tests in 1997. No significant cultivar effect was found on oviposition in 1997. In 1998, cultivar had a significant effect on both feeding and oviposition in free-choice tests where Beauregard was the preferred cultivar, but there was no cultivar effect in no-choice tests (Table 1). The interaction of drought stress and cultivar was significant for feeding in free-choice tests in 1997, but not in 1998 (Table 1).

Larval Survival and Development

Drought stress significantly reduced larval survival rate in 1997, but not in 1998 (Table 2). No significant drought effect was found on pupal

TABLE 1. THE EFFECT OF DROUGHT STRESS AND CULTIVAR ON SWEETPOTATO WEEVIL ADULT FEEDING AND OVIPOSITION UNDER NO-CHOICE AND CHOICE TEST CONDITIONS IN 1997 AND 1998.

		1997				1998			
		No-choice test		Choice test		No-choice test		Choice test	
Cultivar	Water treatment	Feeding punctures[a]	Eggs[b]	Feeding punctures[c]	Eggs[d]	Feeding punctures[e]	Eggs[f]	Feeding punctures[g]	Eggs[h]
Beauregard	Drought	31.0 ± 4.3	9.3 ± 1.1	51.6 ± 5.5	9.6 ± 1.5	38.4 ± 4.1	12.4 ± 0.9	46.6 ± 5.9	12.1 ± 1.1
Beauregard	Irrigated	27.9 ± 2.3	7.1 ± 0.4	30.8 ± 1.4	8.3 ± 0.7	43.7 ± 5.7	13.2 ± 1.3	50.3 ± 1.6	11.9 ± 0.6
Excel	Drought	24.4 ± 1.1	8.8 ± 0.5	26.8 ± 1.9	9.6 ± 1.6	36.3 ± 2.0	12.6 ± 1.2	35.2 ± 4.3	10.6 ± 1.1
Excel	Irrigated	25.3 ± 2.7	6.3 ± 0.5	23.1 ± 1.1	6.1 ± 0.6	32.3 ± 5.2	9.31 ± 0.7	36.4 ± 4.9	8.4 ± 0.9

Mean ± SEM.
[a]Water treatment: $F = 0.15$; $df = 1,9$; $P = 0.7055$. Cultivar: $F = 2.76$; $df = 1,9$; $P = 0.1311$. Interaction: $F = 0.49$; $df = 1,9$; $P = 0.5002$.
[b]Water treatment: $F = 13.32$; $df = 1,9$; $P = 0.0053$. Cultivar: $F = 1.04$; $df = 1,9$; $P = 0.3335$. Interaction: $F = 0.06$; $df = 1,9$; $P = 0.8132$.
[c]Water treatment: $F = 25.83$; $df = 1,9$; $P = 0.0007$. Cultivar: $F = 45.86$; $df = 1,9$; $P < 0.0001$. Interaction: $F = 12.59$; $df = 1,9$; $P = 0.0062$.
[d]Water treatment: $F = 5.90$; $df = 1,9$; $P = 0.0380$. Cultivar: $F = 1.18$; $df = 1,9$; $P = 0.3052$. Interaction: $F = 1.18$; $df = 1,9$; $P = 0.3054$.
[e]Water treatment: $F = 0.03$; $df = 1,9$; $P = 0.8574$. Cultivar: $F = 3.99$; $df = 1,9$; $P = 0.0768$. Interaction: $F = 1.92$; $df = 1,9$; $P = 0.1987$.
[f]Water treatment: $F = 2.90$; $df = 1,9$; $P = 0.1225$. Cultivar: $F = 2.71$; $df = 1,9$; $P = 0.1341$. Interaction: $F = 1.10$; $df = 1,9$; $P = 0.3168$.
[g]Water treatment: $F = 0.75$; $df = 1,9$; $P = 0.4084$. Cultivar: $F = 20.38$; $df = 1,9$; $P = 0.0015$. Interaction: $F = 0.20$; $df = 1,9$; $P = 0.6670$.
[h]Water treatment: $F = 2.72$; $df = 1,9$; $P = 0.1332$. Cultivar: $F = 11.18$; $df = 1,9$; $P = 0.0086$. Interaction: $F = 1.72$; $df = 1,9$; $P = 0.2220$.

weight in both years. Higher larval survival rate was found on Beauregard than on Excel in 1997 and in 1998 (Table 2). Weevils reared on Beauregard had lower pupal weight than that of Excel in 1997, but not in 1998. Drought and cultivar interaction effect was significant for larval survival in 1997, but not in 1998. No significant interaction effect was found with pupal weight (Table 2). The test for year effect was not significant for larval survival ($F = 1.18$; $df = 1,16$; $P = 0.2936$) and pupal weight ($F = 1.34$; $df = 1,16$; $P = 0.2642$).

Resin Glycosides and Caffeic Acid Contents

Drought stress did not have a significant effect on the level of resin glycosides in either year (Table 3). Drought stress significantly reduced the level of caffeic acid in 1997, but not in 1998. Excel tended to have a higher level of resin glycosides than Beauregard, but this difference was statistically significant only in 1998. Both genotypes contained similar levels of caffeic acid (Table 3). No significant interaction effect was found. Year effect was significant for caffeic acid ($F = 88.45$; $df = 1,21$; $P < 0.0001$), but not for resin glycosides ($F = 0.01$; $df = 1,21$; $P = 0.9283$).

Discussion

The impact of drought stress on plants and its consequences on herbivorous insects has drawn much attention. Numerous studies have been reported on the subject with often conflicting results obtained in different insect-host plant systems (Holtzer et al. 1988; Koricheva et al. 1998). Drought is often associated with heavy insect damage (Kelly 1917; White 1969). Several explanations for this ecological consequence have been proposed, including higher plant nutritional quality, more favorable micro-environment, and diminishment of plant defense systems (White 1974; Mattson & Haack 1987). More recent studies regarding the effect of drought stress on insects have focused on evaluating host suitability, and found that drought-stressed plants often have reduced suitability. Many insect species, such as *Pseudoplusia includens* (Lambert & Heatherly 1991), *Epilachna varivestis* (McQuate & Conner 1990), and *Empoasca fabae* (Hoffman et al. 1990, 1991), exhibited a lower feeding and/or oviposition level, longer development time, higher mortalities, and lower fecundities when fed on drought-stressed plants. Our study showed that drought stress seemed to favor SPW feeding and oviposition but reduced larval survival rate. The magnitude of the response of the two genotypes appeared to differ.

Drought stress may alter the production of secondary plant compounds (Gershenzon 1984; Holtzer et al. 1988). Sweetpotato contains numerous secondary compounds, which are produced either constitutively or upon induction by external agents (Kays 1992). Boehmeryl acetate found in the periderm tissue of storage roots was identified as a SPW oviposition stimulant (Son 1989). The results of this study suggest that drought stress may increase the activity of this oviposition stimulant because weevils deposited more eggs on drought-stressed plants. Jackson and Peterson (2000) reported sublethal effects of sweetpotato resin glycosides on *Plutella xylostella*. Caffeic acid showed adverse effects on a generalist herbivore, *Helicoverpa zea* (Summers & Felton 1994) and sweetpotato pathogenic fungi (Harrison et al. 2003a). Recent analyses showed that the levels of resin glycosides and caffeic acid vary between sweetpotato genotypes and within genotypes among years or areas of production (Harrison et al. 2003a, b). This may indicate a relationship between the quantity of these two compounds and the antibiosis of sweetpotato. It also may indicate that the production of these compounds is subject to environmental influence. The results in this study show that drought stress significantly re-

Table 2. The effect of drought stress and cultivar on sweetpotato weevil larval survival and pupal weight reared on storage roots in 1997 and 1998.

		1997		1998	
Cultivar	Water treatment	Larval survival (%)[a]	Pupal weight (mg)[b]	Larval survival (%)[c]	Pupal weight (mg)[d]
Beauregard	Drought	95.4 ± 2.5	7.20 ± 0.1	94.5 ± 1.6	7.44 ± 0.3
Beauregard	Irrigated	97.4 ± 0.8	7.22 ± 0.1	100.0 ± 0.0	7.68 ± 0.2
Excel	Drought	79.4 ± 1.1	7.57 ± 0.2	88.3 ± 4.3	7.61 ± 0.0
Excel	Irrigated	91.4 ± 0.1	7.84 ± 0.6	88.9 ± 4.2	8.06 ± 0.1

Mean ± SEM.
[a]Water treatment: F=12.02; df =1,9; P=0.0071. Cultivar: F=29.04; df =1,9; P=0.0004. Interaction: F=6.26; df =1,9; P=0.0338.
[b]Water treatment: F=1.03; df =1,9; P=0.3363. Cultivar: F=12.05; df=1,9; P=0.0070. Interaction: F=0.80; df =1,9; P=0.3956.
[c]Water treatment: F=1.90; df=1,9; P=0.2014. Cultivar: F=16.27; df=1,9; P=0.0030. Interaction: F=1.23; df =1,9; P=0.2962.
[d]Water treatment: F=1.73; df =1,9; P=0.2209. Cultivar: F=1.06; df=1,9; P=0.3301. Interaction: F=0.15; df =1,9; P=0.7075.

TABLE 3. THE EFFECTS OF DROUGHT STRESS AND CULTIVAR ON RESIN GLYCOSIDE AND CAFFEIC ACID LEVELS IN PERIDERM TISSUE OF SWEETPOTATO STORAGE ROOTS IN 1997 AND 1998.

		1997		1998	
Cultivar	Water treatment	Resin glycoside[a] (% DW)	Caffeic acid[b] (% DW)	Resin glycoside[c] (% DW)	Caffeic acid[d] (% DW)
Beauregard	Drought	0.84 ± 0.150 (3)	0.17 ± 0.071 (3)	0.86 ± 0.100 (3)	0.44 ± 0.021 (3)
Beauregard	Irrigated	0.74 ± 0.212 (4)	0.31 ± 0.025 (4)	0.75 ± 0.081 (4)	0.44 ± 0.037 (4)
Excel	Drought	2.16 ± 1.052 (4)	0.18 ± 0.001 (4)	1.54 ± 0.151 (3)	0.46 ± 0.009 (3)
Excel	Irrigated	1.10 ± 0.125 (4)	0.22 ± 0.015 (4)	1.73 ± 0.155 (4)	0.44 ± 0.019 (4)

Mean ± SEM (sample size); DW = dry weight.
[a]Water treatment: $F = 2.56$; $df = 1,8$; $P = 0.1481$. Cultivar: $F = 1.55$; $df = 1,8$; $P = 0.2483$. Interaction: $F = 0.28$; $df = 1,8$; $P = 0.6133$.
[b]Water treatment: $F = 8.24$; $df = 1,8$; $P = 0.0198$. Cultivar: $F = 1.62$; $df = 1,8$; $P = 0.2389$. Interaction: $F = 2.59$; $df = 1,9$; $P = 0.1464$.
[c]Water treatment: $F = 1.20$; $df = 1,7$; $P = 0.3100$. Cultivar: $F = 62.37$; $df = 1,7$; $P < 0.0001$. Interaction: $F = 2.10$; $df = 1,7$; $P = 0.1908$.
[d]Water treatment: $F = 1.86$; $df = 1,7$; $P = 0.2150$. Cultivar: $F = 0.69$; $df = 1,7$; $P = 0.4342$. Interaction: $F = 0.44$; $df = 1,7$; $P = 0.5290$.

duced the level of caffeic acid but had no effect on the level of resin glycosides, suggesting that the lower larval survival rate observed on drought stressed plants was not due to higher caffeic acid or resin glycoside content. It appears that there is no relationship between the level of these two compounds and sweetpotato weevil resistance. This is possibly because of the feeding behavior of the weevil, in which weevils chew through the periderm and feed primarily on the tissue beneath it, thereby avoiding the periderm layer.

In addition, the effect of drought stress on SPW resistance and on the storage root chemistry was not consistent between years. Significant drought effects in 1997 diminished in 1998. This may be due to the unusual hot and dry conditions in the area in 1998, in which all plots perhaps were stressed.

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SURVEY OF PARASITOIDS OF WHITEFLIES (HOMOPTERA: ALEYRODIDAE) IN CASSAVA GROWING REGIONS OF COLOMBIA AND ECUADOR

H. E. TRUJILLO[1], B. ARIAS[1], J. M. GUERRERO[1], P. HERNANDEZ[1], A. BELLOTTI[1] AND J. E. PEÑA[2]
[1]Centro Internacional de Agricultura Tropical, CIAT, A.A. 6713, Cali, Colombia

[2]University of Florida, Department of Entomology and Nematology
Tropical Research and Education Center, Homestead, FL USA

ABSTRACT

A survey for parasitoids of the whiteflies, *Bemisia tuberculata* Bondar, *Trialeurodes variabilis* Quantaince, *T. vaporariorum* (Westwood), *Aleurotrachelus socialis* Bondar, *Tetraleurodes* sp., *Aleuroglandulus malangae* Russell and *Aleurodicus* sp., was conducted in 6 cassava growing regions of Colombia and Ecuador. In Colombia, the degree of infestation was predominantly high (>29 whiteflies/cm^2) for *A. socialis*, *B. tuberculata* and *T. variabilis* in all cassava growing regions. In Ecuador, levels of infestations were high for *Aleurodicus* sp., *A. socialis*, *B. tuberculata*, *Tetraleurodes* sp. in the coastal region, and for *T. vaporariorum* in the Highlands. The parasitoid fauna of the whiteflies appeared to be more diverse in Colombia than in Ecuador. Eleven species of parasitoids representing 5 genera, 4 families and two superfamilies, as well as 1 hyperparasitoid, were collected from the cassava growing regions of Colombia and 4 species were collected from Ecuador. The parasitoids, *Amitus macgowni* Evans and Castillo, *Encarsia* sp., *E. hispida* De Santis, *E. pergandiella* Howard, *E. bellottii* Evans and Castillo, *E. luteola* group, *E. sophia* (Girault and Dodd), *E. strenua* group, *Eretmocerus* sp., *Metaphycus* sp. and *Euderomphale* sp., were collected. There were notable differences in parasitism among the different geographic regions and whitefly species. In general, *Eretmocerus* was the dominant genus in Colombia and Ecuador, followed by *Encarsia* sp. We found *A. macgowni* in regions characterized by high temperatures and bimodal rainfall. Percent parasitism per region surveyed ranged from 3 to 25% in Colombia and from 12 to 21% in Ecuador.

Key Words: whiteflies, parasitoids, Colombia, Ecuador, cassava, *Manihot*.

RESUMEN

Se efectuó un estudio de reconocimiento de parasitoides de las moscas blancas *Bemisia tuberculata* Bondar, *Trialeurodes variabilis* Quantaince, *T. vaporariorum* (Westwood), *Aleurotrachelus socialis* Bondar, *Tetraleurodes* sp., *Aleuroglandulus malangae* Russell y *Aleurodicus* sp. en regiones productoras de yuca de Colombia y Ecuador. En Colombia, los niveles de infestación fueron altos (>29 moscas blancas/cm^2), particularmente para *A. socialis, B. tuberculata* y *T. variabilis* y en el Ecuador para *Aleurodicus* sp., *A. socialis*, *B. tuberculata, Tetraleurodes* sp., en la región de la costa y *T. vaporariorum* en la región de la sierra. Aparentemente, la fauna de parasitoides fué mas diversa en Colombia que en Ecuador. Se colectaron 11 especies de parasitoides, los cuales representan 5 géneros, 4 familias y dos superfamilias en Colombia y 4 especies de parasitoides en Ecuador. Los parasitoides fueron *Amitus macgowni* Evans y Castillo, *Encarsia* sp., *E. hispida* De Santis, *E. pergandiella* Howard, *E. bellottii* Evans y Castillo, grupo *E. luteola*, *E. sophia* (Girault and Dodd), grupo *E. strenua*, *Eretmocerus* sp., *Metaphycus* sp. y *Euderomphale* sp. Hubo diferencias en parasitismo entre las diferentes regions geograficas y especies de mosca blanca. En general, *Eretmocerus* fué el género que predominó en Colombia y Ecuador, seguido por *Encarsia. A. macgowni* fué encontrado en diferentes regions geograficas caracterizadas por temperatures altas y dos epocas con alta precipitación. El promedio de parasitismo por region fluctuó entre 3 a 25% en Colombia y entre 12 al 21% en Ecuador.

Translation provided by the authors.

Whiteflies (Homoptera: Aleyrodidae) injure valuable agricultural commodities through mechanical feeding and virus transmission. Cassava, *Manihot esculenta* Crantz, is no exception to this rule, acting as a host of several species of whiteflies (i.e., *Bemisia tuberculata* Bondar, *Trialeurodes variabilis* Quantaince, *Aleurotrachelus socialis* Bondar, *Tetraleurodes* sp., and *Aleuroglandulus malangae* Russell (Castillo 1996)) in Colombia and of *Bemisia tabaci* (Gennadius) in Africa and Asia (Bellotti & Vargas 1986) where it vectors African cassava mosaic virus (ACMD).

While this disease has not been reported yet in the Americas (Brown & Bird 1992), in Colombia, other diseases such as 'cassava frog skin disease' (CFSD) and common cassava mosaic are transmitted by *B. tuberculata* Bondar. *Aleurotrachelus socialis* is known to be the most important whitefly in the northern coast, eastern plains and western area of Colombia, but other species of whiteflies (e.g., *T. variabilis*, *Tetraleurodes* sp.) infesting cassava are poorly known (Castillo 1996).

Gold (1987) reported that cassava whiteflies in the area of Nataima, Tolima, Colombia are attacked by a complex group of natural enemies, including parasitoids, predators, and fungi and reported that among the natural enemies, parasitoids were more important mortality factors of cassava whiteflies than predators. Castillo (1996) and Evans and Castillo (1998) reported several cassava whitefly parasitoids in the northern cassava growing areas of Colombia. The parasitoids belong to the genera *Encarsia*, *Eretmocerus* (Hymenoptera: Aphelinidae) and *Amitus* (Hymenoptera: Platygastridae). Specifically, the species are *Encarsia hispida* De Santis, *E. bellottii* Evans and Castillo, and three undescribed species of *Eretmocerus* and *Amitus macgowni* Evans and Castillo.

The objectives of the present study were to determine the frequency of cassava whitefly parasitoid species in different geographical areas of Colombia and Ecuador.

Materials and Methods

This survey was conducted from April 1998 through June 2000 in the cassava growing regions of Colombia and Ecuador. The surveyed areas of Colombia were the Caribbean coast, Andean region, Valle Interandino del Cauca (Cauca), and Valle Interandino del Magdalena (Magdalena); the surveyed Ecuadorean regions were the coastal area and the highlands (Sierra). Geographic and climatic characteristics of each region are addressed in Table 1. In each area, the number of surveys ranged from 1 to 6 depending on cassava crop availability through the years. Each survey was conducted on 2-6-month-old cassava crops during periods of low or no rainfall in each surveyed area.

Sampling for whitefly species consisted of collecting a single leaf from the middle plant canopy from each of 100 randomly selected plants. A disc of 2.54 cm^2 was excised from the leaf lobe that had the highest density of whitefly pupae and then placed in a 5-ml glass vial with 70% alcohol and transported to the laboratory. Whitefly density/cm^2 was grouped into three different categories: high (>29 pupae/cm^2), medium (12-28 pupae/cm^2) and low (<11 pupae/cm^2). Whitefly pupae were identified with the keys of Caballero (1992; 1994) and Martin (1987). For further identification, pupae were sent to A. Hamon (Florida Department of Plant Industry and Consumer Services, Gainesville, FL).

To determine parasitism, 40 additional leaves were collected during each survey. Leaves were inspected for whitefly pupae, and the dominant whitefly species was identified. Once again, 2.54 cm^2 of leaf were excised and those whitefly species with the lowest density in the sample were removed, leaving only the most abundant whitefly species in the sample. Samples were placed individually in 25-ml glass vials and held for 2-3 days at 24.5 ± 4°C and 70 ± 5% RH under laboratory conditions until parasitoids emerged. Emerging parasitoids were identified to genus with the taxonomic keys of Polaszek et al. (1992) for *Amitus*, *Eretmocerus*, *Encarsia*, *Metaphycus* and *Signiphora*; LaSalle & Schauff (1994) for *Euderomphalini*, and Rose & Zolnerowich (1997) for *Eretmocerus*. Each specimen was individually placed in a gel capsule vial and sent for further identification by G. A. Evans (Florida Department of Plant Industry and Consumer Services, Gainesville, FL) and M. Rose (Montana State University, Bozeman, MT).

Results and Discussion

Whitefly Species

Aleurotrachelus socialis, *B. tuberculata*, *T. variabilis*, and *Tetraleurodes* sp. were collected

Table 1. Climatic and geographical range of Cassava growing regions included during the 1998-2000 survey in Colombia and Ecuador.

Country	Region	Elevation (m)	Rainfall (mm)	T (°C)	Latitude	Longitude	No. Surveys
Colombia	Caribbean	12-154	861-1313	25-37	8.53N-10.46N	74.37W-75.48W	3
	Andean	600-1800	1556-2696	18-26	1.48N-2.27N	76.30W-77.10W	4
	Cauca	960-990	1155-1722	19-29	3.01N-3.32	76.16W-76.28W	6
	Magdalena	330-550	1211-2965	26-27	4.01N-10.02N	74.38W-75.02W	3
Ecuador	Coast	25-130	833-2229	22-26	0.54S-2.07S	79.29W-80.44W	1
	Highland	1550	673	19-28	0.24N	77.58W	1

Geographic Information System, CIAT, 2000 (unpublished).

from the cassava growing regions of Colombia, confirming the results of Gold (1987), Arias (1995), and Castillo (1996). In Colombia, the degree of infestation was predominantly high (>29 whiteflies/cm^2) for *A. socialis, B. tuberculata,* and *T. variabilis* in all cassava growing regions, with the exception of the Andean region, where *T. variabilis* was the dominant species (Table 2). The lowest degree of infestation (<11 whiteflies/cm^2) was observed in the Caribbean coast for *Aleurodicus* sp. and *A. malangae*. We did not record *T. vaporariorum* from any of the surveyed cassava regions of Colombia (Table 2).

Levels of infestations were high for *Aleurodicus* sp., *A. socialis*, *B. tuberculata*, and *Tetraleurodes* sp. for the coastal region of Ecuador. We found *T. vaporariorum*, which commonly infests beans, *Phaseolus vulgaris*, for the first time at high infestation levels in cassava in the coastal and highlands regions of Ecuador (Table 2).

In general, *A. socialis*, *B. tuberculata*, *Tetraleurodes* sp., and *Aleurodicus* sp. were distributed in climatic regions characterized by high temperatures and extensive periods of drought (e.g., Caribbean, Magdalena). *Trialeurodes* sp. was found in higher numbers in mountainous regions, characterized by lower temperatures and high rainfall (Andean) (Table 1).

Parasitoids

The parasitoid fauna of whiteflies appeared to be more diverse in Colombia than in Ecuador. Eleven species of parasitoids representing 5 genera, 4 families and two superfamilies, as well as 1 hyperparasitoid, were collected from the cassava growing regions of Colombia and 4 species were collected from Ecuador. Two of the *Eretmocerus* species are undescribed (Table 3) (M. Rose, pers. comm.). All other parasitoid species collected during this study were reported by Castillo (1996) and Evans & Castillo (1998). There were notable differences among the different geographic regions. On the Caribbean coast, *A. socialis* was parasitized by 8 species, with the genus *Eretmocerus* comprising 70% of the parasitoids (Table 4). In the Andean region, *Eretmocerus* sp., parasitized all whitefly species, but *E. pergandiella* was the predominant parasitoid of *T. variabilis*. The hyperparasitoid *Signiphora aleyrodis* Ashmead appeared in high densities in almost all sampled regions, probably reducing the efficacy of the parasitoids of *A. socialis*. In the Magdalena region, 73% of *A. socialis* were parasitized by *A. macgowni*, followed by *Encarsia* sp (26%). In the Caribbean, Andean, and Magdalena sampled regions of Colombia, *B. tuberculata* was parasitized by two undescribed species of *Eretmocerus* in addition to several described and undescribed *Encarsia* species. In the Cauca region the number of parasitoid species on *A. socialis* was almost the same as that collected on the Caribbean coast. However, the dominant genus was *Encarsia* (99%), represented by the species *E. hispida*, *E. sophia*, *E. luteola*, and *E. bellotti*.

The proportion of each parasitoid species collected from *T. variabilis* varied among the sampled geographical areas of Colombia. *Encarsia* was dominant in the Caribbean coast and in Andean region while *Eretmocerus* was dominant in Magdalena. In the Andean region, *E. pergandiella* was more frequent, followed by *Eretmocerus* sp., and *E. hispida* (Table 4).

TABLE 2. NUMBER OF WHITEFLIES RECORDED ON CASSAVA LEAVES IN 6 GEOGRAPHICAL AREAS OF COLOMBIA AND ECUADOR.

	Colombia				Ecuador		
Whitefly species	Caribbean Total (%)[a]	Andean Total (%)	Cauca Total (%)	Magdalena Total (%)	Coastal Total (%)	Highlands Total (%)	Total (%)
Aleurodicus sp.	4 (0.29)	0 (0.00)	0 (0.00)	0 (0.00)	21 (1.54)	0 (0.00)	25 (1.83)
Aleuroglandulus malangae	1 (0.07)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
Aleurotrachelus socialis	141 (10.37)	69 (5.07)	80 (5.88)	215 (15.81)	48 (3.53)	0 (0.00)	553 (40.66)
Bemisia tuberculata	112 (8.24)	50 (3.68)	0 (0.00)	37 (2.72)	5 (0.37)	0 (0.00)	204 (15.01)
Tetraleurodes sp.	16 (1.18)	0 (0.00)	0 (0.00)	0 (0.00)	68 (5.00)	0 (0.00)	84 (6.18)
Trialeurodes variabilis	74 (5.44)	303 (22.28)	0 (0.00)	28 (2.06)	0 (0.00)	0 (0.00)	405 (29.78)
Trialeurodes vaporariorum	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	51 (3.75)	37 (2.72)	88 (6.47)
Total	348	422	80	280	193	37	1360 (100)

[a]Percent each whitefly species at localities.

TABLE 3. PARASITOIDS EMERGING FROM CASSAVA WHITEFLIES COLLECTED IN COLOMBIA AND ECUADOR.

Order	Superfamily	Family	Genus	Species
Hymenoptera	Platygastroidea	Platygastridae	*Amitus*	*macgowni* Evans and Castillo
	Chalcidoidea	Aphelinidae	*Encarsia*	sp.
			E.	*hispida* De Santis
			E.	*pergandiella* Howard
			E.	*bellotti* Evans and Castillo
			E.	*sophia* (Girault and Dodd)
			E.	*luteola* group
			E.	*strenua* group
			Eretmocerus	sp.
		Encyrtidae	*Metaphycus*	sp.
		Eulophidae	*Euderomphale*	sp.
		Signiphoridae	*Signiphora*	*aleyrodis*[a] Ashmead

[a]Hyperparasitoid.

The complex of parasitoids collected from whiteflies in cassava in Ecuador has only been identified to the generic level (G. Evans, M. Rose, pers. comm). In the coastal region, *A. socialis* was parasitized by *Encarsia*, followed by *Amitus* and *Eretmocerus*; *Aleurodicus* was parasitized by *Euderomphale* sp. *Tetraleurodes* and *Trialeurodes* were mostly parasitized by *Eretmocerus* sp., and *B. tuberculata* was parasitized by *Encarsia* sp., and *Euderomphale.* In the highlands region, *T. vaporariorum* was the only whitefly species collected, with approximately 16 pupae/2.54 cm^2. The dominant parasitoids were *Encarsia* spp. representing 98% of the sample (Table 4).

In general, *Amitus macgowni* showed a localized distribution in those areas (e.g., Magdalena) with high temperatures and bimodal rainfall. With the exception of Ecuador, *Eretmocerus* was found both in warm regions and cool regions. Description and identification of the species within this genus will determine if the undescribed species are more frequent in some climatic areas than in others. *Euderomphale* was found in higher numbers in those areas (e.g., Coastal) with a low whitefly density, high temperatures, and minimal rainfall. *E. pergandiella* showed a general distribution among the different climatic regions (Magdalena, Andean), and particularly associated with *Trialeurodes* sp. The species *E. hispida*, *E. sofia*, *E. bellottii*, and *Metaphycus* sp., were found in the Magdalena region, characterized by high temperatures and a yearly average precipitation of 1,000 mm.

These observations indicate that both Colombia and Ecuador have a diverse parasitoid fauna attacking whiteflies on cassava. At the same time, these results indicate that the parasitism trend is influenced by the characteristics of each geographical area. During this study, some parasitoid species were discovered for the first time in some of the geographical areas of Colombia. In the Caribbean coast and in the Cauca region, *A. socialis* was parasitized by *E. sophia*; *B. tuberculata* was parasitized by *E. sophia* and *Metaphycus* sp. in the Caribbean coast, and by *E. pergandiella* and *Euderomphale* sp. in the Andean region. For the first time, *E. pergandiella* and *E. sophia* were collected as parasitoids of *T. variabilis* in the Caribbean coast, while *E. hispida* was the dominant parasitoid in the Andean region.

Because of the temporal and spatial limitation of our collections, parasitism of whiteflies on cassava will probably vary within the year, and data presented here may underestimate parasitism. For instance, high periods of parasitism may have been interspersed with periods of 0% parasitism. The highest frequency of parasitoids was obtained in *A. socialis*, which in general had the highest density in most of the surveyed regions (Table 5). Low levels of parasitism were not uncommon and ranged from 3-5% in the Andean and Cauca regions of Colombia, 10-12% for the Caribbean and Highlands regions of Colombia and Ecuador, respectively, to 21 and 25% in the coastal region of Ecuador and Magdalena region of Colombia, respectively (Table 5). These data suggest that parasitoids are ineffective in reducing cassava whitefly populations in the surveyed areas of Colombia and Ecuador. However, it is necessary to do a more thorough study on those parasitoids that cause higher mortality. For instance, *A. macgowni* was observed as the dominant parasitoid of *A. socials* in the Magdalena region. Therefore, studies toward augmentation and conservation of *A. macgowni* should be encouraged in that region. Life cycle and behavioral studies of *Encarsia pergandiella* as an important parasitoid of *Trialeurodes* sp., should be conducted.

During this study, whitefly densities were low in Ecuador. Therefore, further studies are necessary to properly determine the potential of each parasitoid species in that country.

TABLE 4. FREQUENCY OF PARASITOIDS FROM WHITEFLY SPECIES IN 6 GEOGRAPHICAL REGIONS OF COLOMBIA AND ECUADOR

Country	Region	Whitefly species	Parasitoid species										
			Encarsia sp.	*E. hispida*	*E. pergandiella*	*E. sophia*	*E. luteola group*	*E. bellotti*	*E.Strenua group*	*Eretmocerus sp.*	*Amitus macgowni*	*Metaphycus sp.*	*Euderomphale sp.*	*Signiphora aleyroidis*
Colombia	Caribbean	*Aleurodicus* sp.	1	0	0	0	0	0	0	0	0	0	0	0
		Aelurotrachelus socialis	12	12	0	7	12	4	0	101	0	2	1	45
		Aleuroglandulus malangae	0	0	00	0	0	0	0	0	0	0	0	0
		Trialeurodes variabilis	5	0	5	3	2	0	1	8	0	0	0	0
		Bemisia tuberculata	2	0	1	2	0	0	0	0	0	0	0	0
		Tetraleurodes sp.	0	2	0	0	0	0	0	0	0	0	0	0
	Andean	*Aleurotrachelus socialis*	0	0	0	0	0	5	0	2	0	0	0	11
		Trialeurodes variabilis	0	5	36	0	0	0	0	12	0	0	0	0
		Bemisia tuberculata	0	0	6	0	0	0	0	11	0	0	2	3
	Cauca	*Aleurotrachelus socialis*	28	139	0	54	27	5	0	1	0	0	0	0
	Magdalena	*Aleurotrachelus socialis*	338	0	0	0	0	0	0	0	936	0	0	29
		Trialeurodes variabilis	0	0	0	0	0	0	0	9	0	0	0	0
		Bemisia tuberculata	6	0	0	0	0	0	0	14	0	0	0	0
Ecuador	Coastal	*Aleurodicus* sp.	1	0	0	0	0	0	0	0	0	0	11	0
		Aleurotrachelus socialis	13	0	0	0	0	0	0	3	4	0	0	29
		Tetraleurodes sp.	3	0	0	0	0	0	0	21	0	0	0	3
		Trialeurodes vaporariorum	0	0	0	0	0	0	0	22	0	0	0	0
		Bermisia tuberculata	6	0	0	0	0	0	0	0	0	0	1	0
	Highlands	*Trialeurodes vaporariorum*	92	0	0	0	0	0	0	2	0	0	0	0

TABLE 5. NUMBER OF EMERGING PARASITOIDS FROM CASSAVA WHITEFLIES IN 6 GEOGRAPHICAL AREAS OF COLOMBIA AND ECUADOR.

	Colombia				Ecuador		
Whitefly species	Caribbean Total (TW)	Andean Total (TW)	Cauca Total (TW)	Magdalena Total (TW)	Coastal Total (TW)	Highlands Total (TW)	Total Whitefly (% Parasitism)
Aleurodicus sp.	1 (15)	0 (0)	0 (0)	0 (0)	13 (33)	0 (0)	14 (29)
Aleuroglandulus malangae	0 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aleurotrachelus socialis	146 (1418)	19 (1241)	254 (7411)	2142 (8472)	21 (161)	0 (0)	2582 (14)
Bemisia tuberculata	25 (320)	22 (122)	0 (0)	21 (52)	1 (9)	0 (0)	69 (14)
Tetraleurodes sp.	2 (32)	0 (0)	0 (0)	0 (0)	28 (99)	0 (0)	30 (23)
Trialeurodes variabilis	26 (146)	54 (694)	0 (0)	9 (46)	0 (0)	0 (0)	89 (10)
Trialeurodes vaporariorum	0 (0)	0 (0)	0 (0)	0 (0)	19 (92)	96 (776)	115 (13)
Totals	200 (1932)	95 (2057)	254 (7411)	2172 (8570)	82 (394)	96 (776)	

Total = Total parasitoids emerging.
TW = Total whitefly pupae.

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LABORATORY PARASITISM BY *PHYMASTICHUS COFFEA* (HYMENOPTERA: EULOPHIDAE) UPON NON-TARGET BARK BEETLES ASSOCIATED WITH COFFEE PLANTATIONS

ALFREDO CASTILLO[1], FRANCISCO INFANTE[1], GUILLERMO LÓPEZ[1], JAVIER TRUJILLO[2], LAWRENCE R. KIRKENDALL[3] AND FERNANDO E. VEGA[4]
[1]El Colegio de la Frontera Sur (ECOSUR), Carretera Antiguo Aeropuerto km 2.5. Tapachula 30700 Chiapas, Mexico

[2]Colegio de Posgraduados, Instituto de Fitosanidad, Montecillo, 56230 Edo. de México, Mexico

[3]Institute of Zoology, University of Bergen, Allégaten 41, N-5007 Bergen, Norway

[4]Insect Biocontrol Laboratory, U. S. Department of Agriculture, Agricultural Research Service Beltsville, Maryland, 20705-2350, USA

ABSTRACT

Phymastichus coffea (LaSalle) is an African parasitoid of adults of the coffee berry borer *Hypothenemus hampei* (Ferrari) that has been introduced to Mexico and other Central and South American countries for the biological control of this important pest. The present study assessed the host specificity of this parasitoid in the laboratory. We tested the acceptance and parasitism of *P. coffea* on five species of bark beetle adults commonly found in coffee plantations of Mexico: *Hypothenemus crudiae*, *H. plumeriae*, *H. eruditus*, *Scolytodes borealis* and *Araptus fossifrons*. As a control, we used adults of *H. hampei,* the natural host. *P. coffea* parasitized and successfully completed its life cycle in *H. crudiae* and *H. eruditus*, as well as in *H. hampei*. The degree to which bark beetles were attacked by *P. coffea* was estimated by percent of parasitism, which was 64% for *H. hampei*, 14% for *H. crudiae*, and 6% for *H. eruditus*. The risk of potential deleterious effects of the parasitoid on non-target organisms in coffee agroecosystems is discussed.

Key Words: Host specificity, *Phymastichus*, *Hypothenemus*, *Scolytodes*, *Araptus*, Mexico.

RESUMEN

Phymastichus coffea (LaSalle) es un parasitoide africano de adultos de la broca del café *Hypothenemus hampei* (Ferrari) que ha sido introducido a México y otros países de Centro y Sudamérica para el control biológico de esta importante plaga. El presente trabajo se llevó a cabo con la finalidad de evaluar la especificidad de huéspedes de este parasitoide en el laboratorio. Se probó la aceptación y parasitismo de *P. coffea* sobre adultos de cinco especies de descortezadores comúnmente encontrados en plantaciones de café de México: *Hypothenemus crudiae*, *H. plumeriae*, *H. eruditus*, *Scolytodes borealis* y *Araptus fossifrons*. Como control se usaron adultos de *H. hampei* (hospedero natural). *P. coffea* parasitó y completó exitosamente su ciclo biológico en sólo dos especies de escolítidos, *H. crudiae* y *H. eruditus*, además de *H. hampei*. El grado en el cual los descortezadores fueron atacados por *P. coffea* fue estimado por el porcentaje de parasitismo el cual fue de 64% para *H. hampei*, 14% para *H. crudiae*, y 6% para *H. eruditus*. Es discutido el riesgo de los efectos negativos potenciales de este parasitoide sobre organismos no blanco en agroecosistemas de café.

Translation provided by the authors.

Phymastichus coffea LaSalle (Hymenoptera: Eulophidae) is a primary parasitoid of the coffee berry borer (CBB), *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), the most devastating pest of coffee throughout the world (Baker 1999). This parasitoid, indigenous to Africa, was first recorded in 1987 from Togo parasitizing the CBB (Borbon-Martínez 1989), and subsequently was described as a new species (LaSalle 1990). *Phymastichus coffea* has a pan-African distribution, having been collected from east Africa (Kenya) and west Africa (Togo, Benin, Cameroon, Burundi, and Ivory Coast) (Infante et al. 1992). So far, this parasitoid has been introduced to coffee producing countries, such as Colombia, Guatemala, Honduras, Jamaica, El Salvador, Ecuador, India, Brazil and Mexico for biological control purposes (Castillo, unpublished data). This species is the only known parasitoid of adults of CBB, and is considered to be a potentially useful tool in

integrated pest management programs against *H. hampei* (López-Vaamonde & Moore 1998; Baker 1999).

Eulophidae is one of the largest families in Hymenoptera with nearly 4000 described species (Noyes 1998; Gauthier et al. 2000). The subfamily Tetrastichinae, to which *P. coffea* belongs, has an extraordinarily wide host range and exhibits a great variety of life styles. Members of this subfamily attack over 100 families of insects, as well as mites, spider eggs, and even nematodes (J. LaSalle, pers. comm.). Despite the fact that *P. coffea* has already been imported and released in various countries of the Americas, it is important to determine whether this parasitoid can attack non-target scolytids.

The objective of this study was to test the host specificity of *P. coffea* in the laboratory with six bark beetles species commonly found in coffee plantations of Mexico, with *H. hampei* serving as the control. A previous study in Colombia reported that *P. coffea* was able to parasitize *H. obscurus*, *H. seriatus*, and *Araptus* sp. in the laboratory (López-Vaamonde & Moore 1998). We included different species than those examined in Colombia; thus, this study serves to further elucidate the potential risk of *P. coffea* for non-target scolytids.

Materials and Methods

Parasitoids

We used mated females of *P. coffea*, which were less than 1h old from the time they emerged from CBBs. The parasitoids were obtained from a colony established in the laboratory in March of 2000. The colony was initiated with insects imported from Guatemala with methodology described by Infante et al. (1994). Adult CBB females obtained from the field were used for parasitoid rearing. The insect colony is normally maintained at 26 ± 2°C, 75 ± 10% RH and 8:16 (L:D) photoperiod.

Hosts

We offered six species of bark beetles collected as adults in coffee plantations near Tapachula, Chiapas to adults of *P. coffea* in the laboratory. The parasitoid was never released in the plantations where we collected the bark beetles. To minimize the risk, the CBBs were obtained by dissecting infested coffee fruits from the field, while we collected *Hypothenemus eruditus* Westwood, *Hypothenemus crudiae* (Panzer), *Hypothenemus plumeriae* (Nordlinger), and *Scolytodes borealis* Jordal from petioles of *Cecropia* sp. leaves. The three *Hypothenemus* species we used are widespread and co-occur with *Coffea* spp. where the latter are native (Wood 1982; Wood & Bright 1992). They are extremely polyphagous, and have been collected from dozens of host species. The *Scolytodes* breeds only in fallen *Cecropia* leaves (Jordal 1998). *Araptus fossifrons* Wood, a Mesoamerican species previously collected from various seed pods and lianas (Wood & Bright 1992), was captured using CBB traps (Dufour 2002).

Experimental Procedure

The host specificity of *P. coffea* was evaluated in the laboratory in a non-choice test. Fifty specimens of each species were placed individually in 40 × 10 mm glass vials and immediately afterwards, a *P. coffea* female was introduced. The insects were observed for five hours under a 20W fluorescent lamp. We considered oviposition to have occurred when the female parasitoid adopted a characteristic ovipositing position on the elytra of the scolytids (López-Vaamonde & Moore 1998). Hosts attacked by *P. coffea* were transferred individually into vials containing CBB diet (Villacorta & Barrera 1996) and parasitization was assessed based on the emergence of the progeny, or by dissecting the hosts that did not yield parasitoids. We also recorded the time required for the encounter (defined as the time elapsed between the release of the parasitoid in the arena until it assumed the characteristic ovipositing position on the beetle), and the handling time (defined as the time elapsed between the encounter and the end of parasitization). Environmental conditions of the laboratory during the development of parasitoid's progeny were 26 ± 2°C and 70-80% RH.

Taxonomy

Samples of the species used were compared by Kirkendall with type material, or with specimens in his collection or in the S. L. Wood collection which had been compared with type material. Vouchers for all species are in Kirkendall's collection at University of Bergen, Norway.

Results

Oviposition attempts by *P. coffea* were observed on all scolytid species tested, but parasitization and development of progeny was completed on only two species of bark beetles, in addition to *H. hampei* (Fig. 1). The percentage parasitism for *H. hampei*, *H. crudiae,* and *H. eruditus* was 64%, 14% and 6%, respectively (Table 1). We did not detect any oviposition by *P. coffea* in *S. borealis*, *H. plumeriae,* and *A. fossifrons*, nor did we find adult or immature stages of the parasitoid after hosts were dissected. Sex ratio of progeny produced by *P. coffea* in the three species was 1:1. The shortest encounter time was between *P. coffea* and *H. hampei* (mean = 23 min). Encounter time was 5-6 times longer when para-

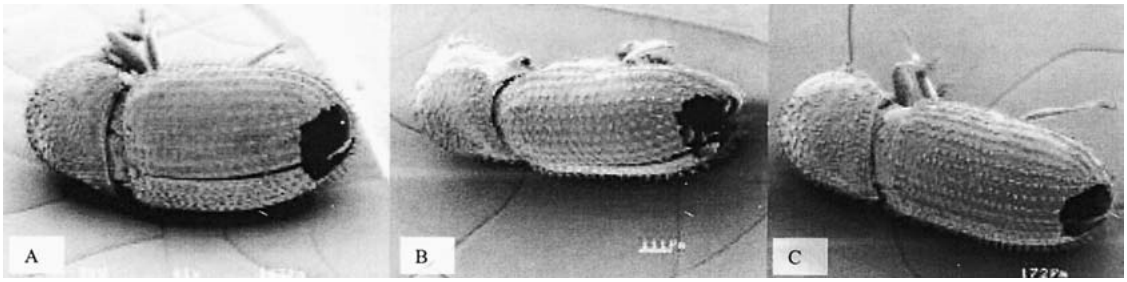

Fig. 1. Adults of three species of scolytids parasitized by *Phymastichus coffea* in the laboratory, showing the hole made by the emerging wasp adult: (A) *H. hampei* (B) *H. eruditus* and (C) *H. crudiae*.

sitization was on *H. crudiae* or *H. eruditus*. However, the handling time was shorter (1.5 min) in *H. eruditus* than in the other hosts. In most cases *P. coffea* allocated more than two eggs per host after a single attack. Development of immature stages of the parasitoid ranged from 39 to 42.6 days (Table 1).

Discussion

Phymastichus coffea parasitized two species of *Hypothenemus* in addition to its natural host, *H. hampei*. Our findings confirm the oligophagic behavior of *P. coffea* reported by López-Vaamonde & Moore (1998). Five species of beetles are now known to serve as hosts for *P. coffea*: *H. obscurus*, *H. seriatus*, *Araptus* sp (Lopez-Vaamonde & Moore 1998), *H. crudiae* and *H. eruditus* (this study), thus indicating that this parasitoid is not specific to the CBB. Host specificity tests under laboratory conditions are the first step in assessing the potential host range of a given species (Orr et al. 2000; Babendreier et al. 2003a, 2003b). Although laboratory tests can overestimate the range of hosts under field conditions (Sands 1997), they are necessary to give an idea of the number of hosts at risk of being attacked in the field. Based on laboratory trials, specificity can be demonstrated in the field later on (Barron et al. 2003; Babendreier et al. 2003b). Accordingly, parasitism by *P. coffea* on *H. crudiae* and *H. eruditus* in the laboratory does not necessarily mean that these beetles will be attacked by this parasitoid in the field. Careful observations under field conditions should be carried out before concluding that these beetles are alternative hosts of *P. coffea*.

There are no reports of parasitism by *P. coffea* on other hosts under field conditions. However, entomologist are not specifically collecting other bark beetles in or around coffee plantations, so parasitism of other hosts is unlikely to be recorded. Encounters between the parasitoid and scolytids in the field are likely, since the five species of polyphagous beetles parasitized by *P. coffea* in the laboratory are common in coffee agroecosystems and the disturbed habitats which often surround them (Atkinson & Equihua-Martínez 1985).

Our results could have important implications in the biological control of CBB in Latin America if further studies demonstrate that *P. coffea* attacks these scolytids in the field. First, with several host species available, there would be a risk of dilution of the parasitism exerted on CBB in the field. Second, one or more of these species might be more easily reared in large numbers in

Table 1. Parasitism by *Phymastichus coffea* on several bark beetles species under laboratory conditions. Fifty specimens of each species were exposed to fifty parasitoid females individually.

Scolytidae species	Parasitism attempts (%)	Time required for the encounter with host (min ± SE)[1]	Handling time (min ± SE)[2]	Parasitism (%)	Progeny production of *P. coffea*	Development of parasitoids (days)
Hypothenemus hampei	78	23.0 ± 4.4	5.8 ± 0.7	64	54	42.6
Hypothenemus crudiae	58	136.2 ± 26.0	4.2 ± 0.8	14	14	40.4
Hypothenemus eruditus	50	123.0 ± 23.0	1.5 ± 0.4	6	4	39
Hypothenemus plumeriae	36	0	—	0	0	0
Scolytodes borealis	8	0	—	0	0	0
Araptus fossifrons	46	0	—	0	0	0

[1]Defined as the time elapsed between the release of the parasitoid in the arena and when it assumed the characteristic oviposition position on the beetle.

[2]Defined as the time elapsed between the encounter and the end of the parasitization.

the laboratory than the CBB, which has more stringent food requirements, for mass rearing of *P. coffea* for augmentative biological control programs against CBB. Third, these scolytids could be important as alternative hosts in the field, increasing survival of the parasitoid during the intercropping season, when the CBB population is at its lowest. Intercropping season in coffee interferes severely with the establishment of natural enemies of CBB (Barrera et al. 1990).

Finally, taking into consideration that parasitism of non-target hosts is usually much higher in the laboratory than in the field (Orr et al. 2000), it is possible that the levels of laboratory parasitism of *H. crudiae* and *H. eruditus* do not reflect a potential risk of this parasitoid for populations of non-target bark beetles in the field. Only careful field observations can answer this important question.

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DESCRIPTIONS OF THE FINAL INSTAR OF *EURYTOMA NODULARIS* AND *E. HERIADI* (HYMENOPTERA: EURYTOMIDAE)

J. TORMOS, J. D. ASÍS, S. F. GAYUBO AND M. A. MARTÍN
Unidad de Zoología, Departamento de Biología Animal, Facultad de Biología,
Universidad de Salamanca, 37071-Salamanca (Spain)

ABSTRACT

The final instars of *Eurytoma nodularis* and *E. heriadi* are described and illustrated. Morphological structures of diagnostic value are discussed. The most salient character shown by the mature larvae of these two species lies in the mandibles, which are simple (unidentate), a feature that, according to current knowledge, is only shared with *E. verticillata*.

Key Words: larva, *Eurytoma,* morphology.

RESUMEN

Se describen, y dibujan, las larvas maduras de *Eurytoma nodularis* y *E.heriadi*. El carácter más relevante, sólo compartido con *E. verticillata*, radica en la presencia, en ambas especies, de mandíbulas unidentadas.

Translation provided by the author.

The family Eurytomidae includes some 1424 species in 88 genera (Noyes 2003). Included species display diverse larval feeding habits and are mainly parasitoids of Diptera, Coleoptera, Hymenoptera, and Lepidoptera (Gauld & Bolton 1988; Zerova & Fursov 1991), although phytophagy (Crosby 1909; Bugbee 1941, 1971, 1967) and entomophytophagy (Phillips 1917, 1927; Claridge 1961; Bugbee 1975) are known.

The genus *Eurytoma* Illiger, 1807, with 692 species, is the largest of the family Eurytomidae (Noyes 2003). This study addresses the larval morphology of two species of this cosmopolitan genus: *Eurytoma nodularis* Boheman, 1836, and *E. heriadi* Zerova, 1984. These species are parasitoids of Hymenoptera Aculeata that nest in hollow stems. Within *Eurytoma*, the main studies of mature larvae have been carried out by Roskam (1982), Henneicke et al. (1992) and Dawah & Rothfritz (1996).

MATERIALS AND METHODS

Material Examined

Eurytoma nodularis: SPAIN: Segovia: Iscar, 2 mature larvae from nest of Eumenidae December 1999, emerg. 1 female May 2000 (one mature larva was fixed and preserved in 70% ETOH for subsequent study and description); Cáceres: Mestas, 4 mature larvae from nest of Eumenidae December 1999, emerg. 2 females, 1 male May 2000 (one mature larva was stored in a vial with 70% ethanol for later study). *E. heriadi*: SPAIN: Ávila: Barco de Ávila, 2 mature larvae from nest of *Trypoxylon* Latreille, 1796 (Hymenoptera, Apoidea: Crabronidae) December 1999, emerg. 1 female May 2000 (one mature larva was stored in a vial with 70% ethanol for later study). Voucher specimens are deposited at the: a) Fundación Entomológica "Torres-Sala" (València, Spain) (larvae), and b) Institute of Zoology of National Ukrainian Academy of Sciences (Ukraine) (adults).

In both cases, the larvae were obtained from nests established in stems of *Phragmites australis* (Cav.) (Poaceae), which had been placed in the field between April-December 1999 when they were collected and transported to the laboratory.

Nests were opened one week after collection to allow the development of possible natural enemies. The contents of each cell were transferred to glass vials and kept at 6-8°C over the winter. During the following spring (May 2000), the vials were transferred to a culture chamber at 28°C, 60-80% RH, to trigger emergence of the imagos, thus making it possible to identify the occupants of the nests and their parasitoids.

The methodology used in the preparation of mature larvae was similar to that employed by Evans (1987). Terminology and organization used in the ensuing descriptions fundamentally follow that of Henneicke et al. (1992). The following abbreviations have been used in the descriptions: A1-9 = abdominal segments; ADP = anterodorsal protuberances; AN = antennae; APP = anterior pleurostomal process; ATR = atrium of spiracle; AS = anal segment; BA = base of mandibles; BL = blade of mandibles; C = ventral articular process of mandible; CA = closing apparatus of spiracle; CLP = clypeus; CLPS = clypeal setae; D = dorsal setae; d

= diameter; DAP = dorsal articular process of mandible; DT = dorsal terminal seta; EPST = epistomal arc; EPX = epipharynx; FI = inferior frontal setae; FS = superior frontal setae; GE = setae on the genae; h = height; HY = hypostomal setae; l = length; LM = labrum; LMS = labral setae; LS = prelabial sensilla; LUM = labium; MD = mandibles; MS = maxillary setae; MX = maxillae; n = number of specimens; P = pleural setae; PLOS = lateral postlabial setae; PLST = pleurostoma; POS = postlabial setae; PPA = maxillary papilla; PPP = posterior pleurostomal process; PRLS = lateral prelabial setae; PRMS = middle prelabial setae; SP = spiracles; ST = spiracular trachea; TH1-3 = thoracic segments; V = ventral setae, and w = width.

Descriptions of Mature Larvae

Eurytoma nodularis Boheman

General aspect (Fig. 1)

Body l = 6.6-7.2 mm ($\bar{x}$ = 6.9), maximum w = 1.7-2.1 mm ($\bar{x}$ = 1.9) (n = 2), shape varying between barrel-shaped and cylindrical, slightly broader in mid region, ADP present on TH3-A9, with three thoracic and ten abdominal segments, tapering anteriorly and more strongly curved posteriorly. Color yellowish. Weakly sclerotized, except for MD, SP and setae. Anus small, subterminal, transverse. Pleural lobes very scarcely developed. Tegument setose, with: a) D (l = 180-410 µm): three pairs on TH1-A2; two pairs on A3-A7; a pair on the A8 and A9; b) DT (l = 90 µm) two pairs; c) P (l = 170-425 µm): four pairs on TH1-AS2; two pairs on A3-A9; d) V (l = 150-420 µm): one pair on TH1-A9. SP (Fig. 3) on TH2, TH3, and on A1-A7; ATR (l = 70 µm, d maximum = 30 µm) funnel-shaped, with approximately fourteen chambers; CA (l = 20 µm; w = 9 µm) adjacent to ATR.

Cranium (Fig. 4)

Cranium 0.5× as high as broad (w = 657 µm, h (from apex of cranium to base of MD) = 335 µm), narrower than TH1, very weakly sclerotized, with four pairs of long setae (l = 11.5-13.5 µm): FI (l = 11.5 µm), FS (l = 13 µm), GE (l = 12.5 µm), HY (l = 13.5 µm). AN approximately 2.5× as long as broad, located below middle of cranium, with three small sensilla on apex. CLP and LM without setae or sensilla; EPX with two pairs of small sensilla (a). Tentorium (Fig. 5) with the PLST and its APP and PPP sclerotized and differentiated. EPST almost indistinct, and very weakly sclerotized.

Mouthparts

Mandibles (MD) (Figs. 4, 5) (l = 10.25 µm, w = 6.25 µm) sclerotized, more heavily sclerotized at their BL, unidentate, with a wide BA, with prominent DAP and C; MX and LUM completely fused (Fig. 6): MX with a pair of short MS (l = 9.5 µm) and a protuberant PPA (9 × 5.5 µm); LUM with a pair of PRMS (l = 20 µm), one pair of PRLS (l = 9 µm), and three pairs of LS (d = 2 µm) on the prelabial membrane, postlabial membrane with one pair of long POS (l = 23.5 µm) at center and two pairs of small PLOS (l = 9 µm).

Diagnosis

The mature larva of *E. nodularis* can be characterized and distinguished from the mature larvae of other known *Eurytoma* spp. by the combination of the following characters: a) antennae located below middle of cranium; b) the presence of more than four rows of setae dorsally and pleurally; c) segment A1 with one pair of ventral setae; d) more than two dorsal setae present on abdominal segments A6-8; e) mandibles simple, crescentic, with one acute tooth.

Eurytoma heriadi Zerova

General aspect (Fig. 2)

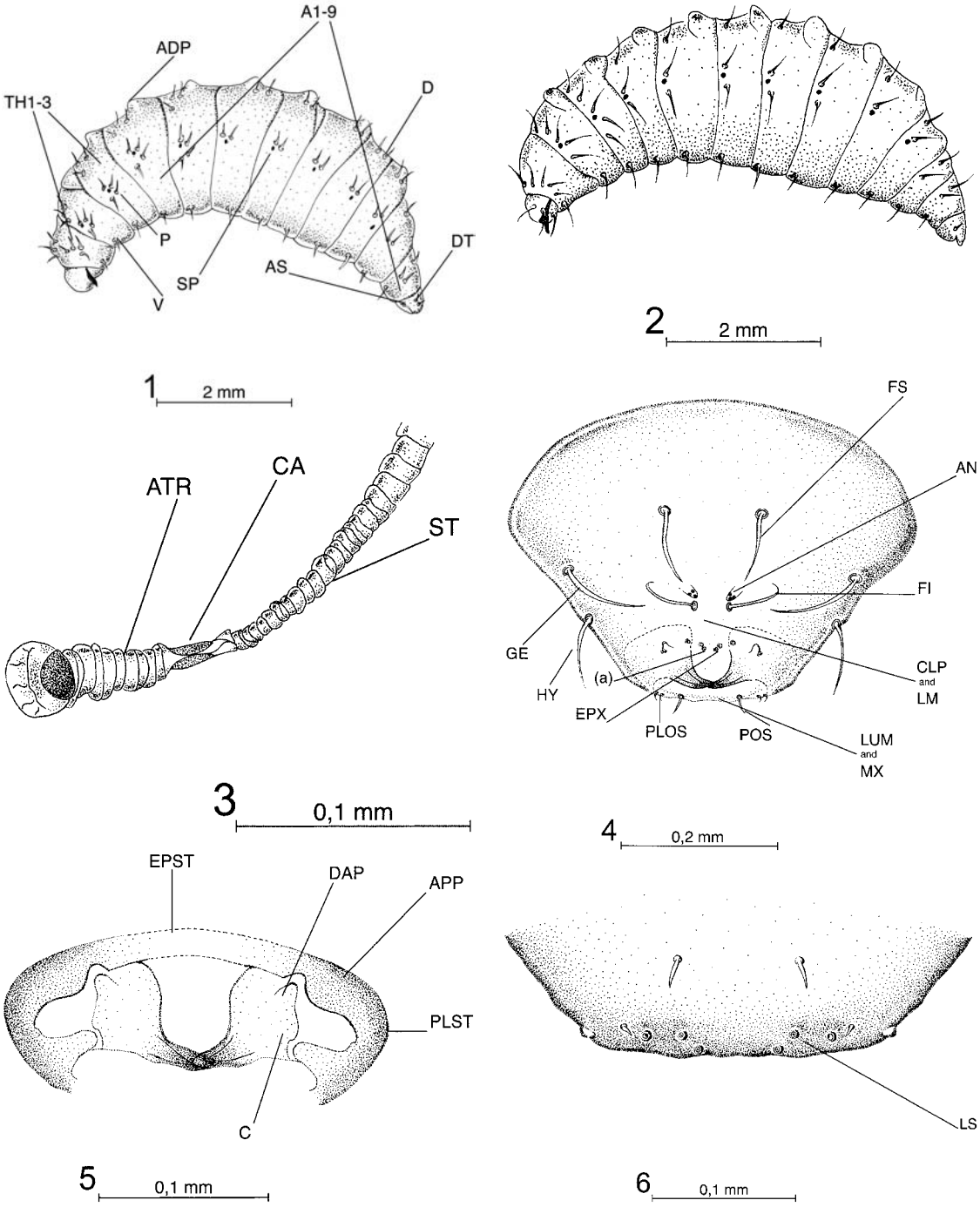
Body (l = 5.2 mm, maximum w = 1.8 mm) shape varying between barrel-shaped and cylindrical, slightly broader in mid region, ADP present on TH3-A8, with three thoracic and ten abdominal segments, tapering anteriorly and more curved posteriorly. Color yellowish. Weakly sclerotized, except for MD, SP and setae. Anus small, subterminal, transverse. Pleural lobes very scarcely developed. Tegument setose, with: a) D (l = 190-415µm): two pairs on each of the TH1-3; a pair on A1-A9; b) DT (l = 85 µm) one pair; c) P (l = 180-440 µm) three pairs on each of the TH1-3; two pairs on A1-A5; a pair on A6-A9; d) V (l = 160-435 µm) one pair on TH1-A9. SP on TH2, TH3, and on A1-A7; ATR (l = 60 µm, d maximum = 22 µm) funnel-shaped, with approximately ten chambers; CA (l = 15 µm; w = 6 µm) adjacent to ATR.

Cranium (Fig. 7)

Wider than high (w = 448 µm, h (from apex of cranium to base of MD) = 255 µm), narrower than TH1, very weakly sclerotized, with three pairs of long setae (l = 10-12 µm): FS (l = 11 µm), GE (l = 10 µm), HY (l = 12 µm). AN approximately 2.5× as long as broad, located in the middle or above the middle of cranium, with three small sensilla on apex. CLP and LM with a pair of short CLPS and LMS, respectively; EPX with two pairs of small sensilla (a) (Fig. 4). Tentorium with the PLST and its APP and PPP sclerotized and differentiated. EPST almost indistinct, and very weakly sclerotized.

Mouthparts

MD (l = 8 µm, w = 4 µm) sclerotized, more heavily sclerotized at their BL, unidentate, with a



Figs. 1-6. Mature larvae of *Eurytoma nodularis* Boheman and *E. heriadi* Zerova. *E. nodularis*: (1) General aspect. (3) Spiracle. (4) Cranium. (5) Tentorium and mandibles. (6) maxillae and labium. *E. heriadi*: (2) General aspect. (Abbreviations: A1-9 = abdominal segments; ADP = anterodorsal protuberances; AN = antennae; APP = anterior pleurostomal process; ATR = atrium of spiracle; AS = anal segment; C = ventral articular process of mandible; CA = closing apparatus of spiracle; CLP = clypeus; D = dorsal setae; DAP = dorsal articular process of mandible; DT = dorsal terminal seta; EPST = epistomal arc; EPX = Epipharynx, (a) = sensilla; FI = inferior frontal setae; FS = superior frontal setae; GE = setae on the genae; HY = hypostomal setae; LM = labrum; LS = prelabial sensilla; LUM = labium; MX = maxillae; P = pleural setae; PLOS = lateral postlabial setae; PLST = pleurostoma; POS = postlabial setae; SP = spiracles; ST = spiracular trachea; TH1-3 = thoracic segments; V = ventral setae, and w = width.)

wide BA, with prominent DAP and C; MX and LUM completely fused: MX with a pair of short MS (l = 8 µm) and a protuberant PPA (8.5 × 5 µm); LUM (Fig. 8) without setae, with a pair of small LS.

Diagnosis

The mature larva of *E. heriadi* can be characterized and distinguished from mature larvae of other known *Eurytoma* spp. by the combination of the following characters: a) cranium without FI setae; b) the presence of more than four rows of setae dorsally and pleurally; c) segment A1 with one pair of ventral setae; d) mandibles simple, crescentic, with one acute tooth.

Taxonomic position. The mature larvae of these two species can be inserted in the key of Henneicke et al. (1992) as follows:

1. Hypostomal setae (Hy) shorter than half the width of labrum ..... *Sycophila mellea* (Curtis, 1831), *Tetramesa* Walker, 1848

–Hypostomal setae longer or about as long as half the width of labrum (Figs. 4, 7) 2

2. More than two dorsal setae (D) present on abdominal segments A6-8 (Fig. 1) 3

–At least one of abdominal segments A6-8 with only two dorsal setae (Fig. 2) 4

3. Mandibles bidentate ..... *E. (Ahtola) atra* (Walker, 1832)

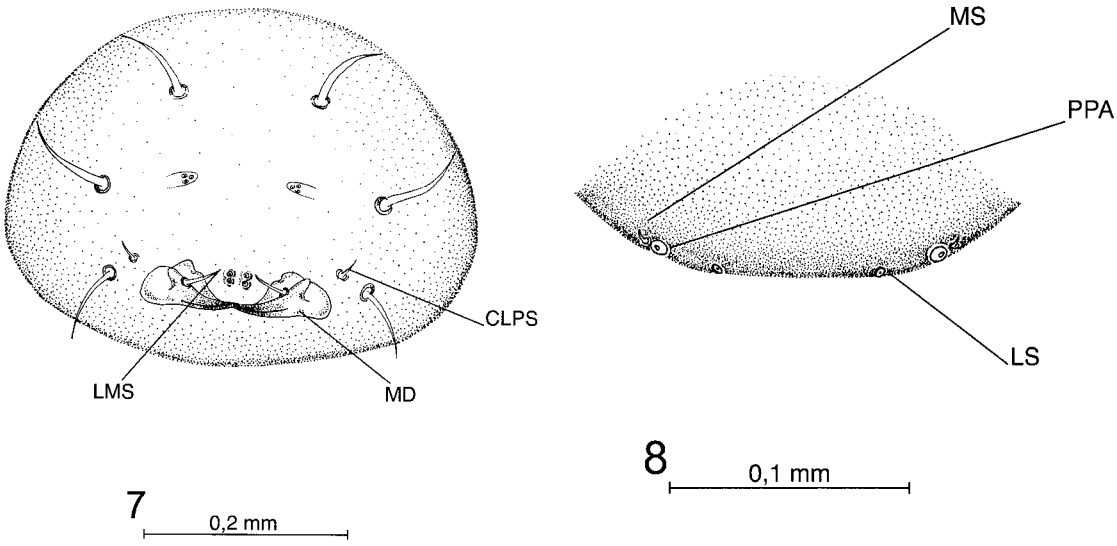
- Mandibles unidentate (Figs. 4, 5) ..... *E. nodularis* Boheman

4. Mandibles bidentate ..... *Eurytoma appendigaster* group

- Mandibles unidentate (Fig. 7) ..... *Eurytoma heriadi* Zerova

DISCUSSION

The mature larvae of *E. nodularis* and *E. heriadi* share the following characters with other *Eurytoma*: a) body mainly barrel-shaped, broader in mid-region; b) head hemispherical, without pronounced clypeus, with hypostomal setae longer or about as long as half the width of labrum, and with inconspicuous and unpigmented craneal sclerites; c) integument with setae arranged in distinct rows along all body segments, and with ventral setae arranged in paired rows; d) atrium of spiracle long. However, the following characters differentiate these larvae from most other known larvae of the genus: a) the presence of more than four rows of setae dorsally and laterally; b) segment A1 with one pair of ventral setae; c) mandibles simple, crescentic, with one sharp/acute tooth. Additionally, *E. nodularis* has the antennae located below middle of cranium, and more than two dorsal setae present on abdominal segments A6-8, and in *E. heriadi* the cranium is without FI setae.



Figs. 7-8. Mature larvae of *E. heriadi* Zerova: (7) Cranium. (8) Maxillae and labium. (Abbreviations: CLPS = clypeal setae; LMS = labral setae; LS = prelabial sensilla; MD = mandibles; MS = maxillary setae; PPA = maxillary papilla.)

The most salient character shown by the mature larvae of these two species lies in the mandibles, which are simple, a feature that, according to current knowledge, is only shared with *E. verticillata* (F., 1798) (Zerova 1983). In this respect, it should be noted that Danks (1970) described a mature larva of an indeterminate species of *Eurytoma*, a parasitoid of rubicolous aculeates, indicating that it was probably *E. nodularis*. This larva had unidentate mandibles.

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ERETMOCERUS RUI N. SP. (HYMENOPTERA: CHALCIDOIDEA: APHELINIDAE), AN EXOTIC NATURAL ENEMY OF *BEMISIA* (*TABACI* GROUP) (HOMOPTERA: ALEYRODIDAE) RELEASED IN FLORIDA

GREGORY ZOLNEROWICH[1] AND MIKE ROSE[2]
[1]Department of Entomology, 123 Waters Hall, Kansas State University, Manhattan, KS 66506-4004

[2]Department of Entomology, Leon Johnson Hall, Montana State University, Bozeman, MT 59717

ABSTRACT

Eretmocerus rui n. sp. imported from Hong Kong and released against *Bemisia* (*tabaci* group) in Florida is described. This thelytokous species was recovered after release, but it is unknown if it is established in Florida.

Key Words: biological control, *Eretmocerus*, Aphelinidae, *Bemisia*, Aleyrodidae.

RESUMEN

Se describe a *Eretmocerus rui* n. sp., especie introducida de Hong Kong y liberada en la Florida para el control de *Bemisia*. Esta especie telitóquica fue recapturada después de su liberación, pero se desconoce si está establecido en la Florida.

Translation provided by the authors.

Numerous populations of exotic parasitic Hymenoptera, primarily in the genera *Encarsia* and *Eretmocerus* (Hymenoptera: Chalcidoidea: Aphelinidae), were introduced during population explosions of *Bemisia* (*tabaci* group) (Homoptera: Aleyrodidae: Aleyrodinae) in the southern United States during the 1980s and 1990s. Introductions of *Eretmocerus* Haldeman (Haldeman 1850) were emphasized, as this genus is composed of primary parasites that attack Aleyrodidae (Rose et al. 1996). Zolnerowich & Rose (1998) characterized and described five of the introduced *Eretmocerus* species that were released in the U.S., while Rose & Zolnerowich (1997a, b) characterized and described species of *Eretmocerus* indigenous to, or naturally occurring in, the United States.

Most of the *Eretmocerus* populations released in the United States were introduced through the USDA-APHIS quarantine laboratory in Mission, Texas (Goolsby 1996; Goolsby et al. 1998). However, biological control researchers in Florida also introduced natural enemies of *Bemisia* (*tabaci* group) through the quarantine laboratory in Gainesville. One of these is an undescribed species of *Eretmocerus* that F. D. Bennett (Nguyen & Bennett 1995) discovered attacking a *Bemisia* species in Hong Kong. This thelytokous species (McAuslane & Nguyen 1996) was consigned from quarantine, reared in culture, and released in Florida. We describe that species here to aid in determination of the *Eretmocerus* species complex attacking *Bemisia* (*tabaci* group) in Florida, efficacy evaluations of the same, and discovery of possible utilization of non-target whitefly hosts by exotic *Eretmocerus* species released in Florida.

MATERIALS AND METHODS

Terminology and measurements follow those used by Rose & Zolnerowich (1997a). Measurements of 61 morphological features were taken from 10 females in the type series that were mounted in balsam or Hoyer's. Measurements were made with a customized data acquisition program written for an Apple Macintosh computer, and linked to a digitizing tablet and Zeiss compound microscope equipped with Nomarski contrast enhancement. In the description, stated lengths are means.

Eretmocerus rui Zolnerowich and Rose, new species (Figs. 1-3, 5)

Diagnosis

Females of *Eretmocerus rui* can be identified by the presence of 3 setae on each parapsis, the presence of 6 setae on the mesoscutum, a very elongate, cylindrical antennal club that is 6.88-8.06× as long as wide (Fig. 1), a long ovipositor approximately the same length as the club, and a long, narrow forewing about 2.7× as long as wide (Fig. 2).

Of the *Eretmocerus* species known from the U.S. (Rose & Zolnerowich 1997a, b; Zolnerowich & Rose 1998) only *E. furuhashii* Rose and Zolnero-

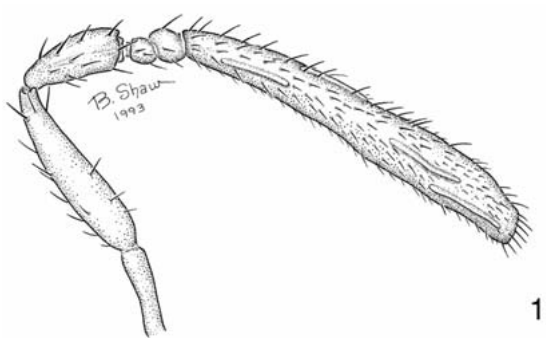

Fig. 1. *Eretmocerus rui*, female antenna.

wich bears 3 setae on the parapsis. However, females of *E. furuhashii* bear 4 setae on the mesoscutum and have much shorter (about 4.3-5× as long as wide) antennal clubs that are clavate with slightly deflected (rostrate) apices.

All other nominal exotic *Eretmocerus* species introduced and released against *Bemisia* (*tabaci* group) in the United States bear 4 setae on the mesoscutum and 2 setae on the parapsis. Those species are *E. emiratus* Zolnerowich and Rose, *E. hayati* Zolnerowich and Rose, *E. melanoscutus* Zolnerowich and Rose, and *E. mundus* Mercet.

Female

Length of specimens in Hoyer's 0.575-0.75 mm (n = 10). Holotype female 0.75 mm. Body light yellow. Head amber. Antennae pale amber. Legs pale yellow. Wings hyaline.

Face and occiput with transverse substrigulate sculpture, interscrobal area vertically substrigulate. Antenna (Figs. 1 and 3) with radicle 3.7× as long as wide; scape 5.08× times as long as wide, 2.31× as long as radicle, 2.01× length of pedicel, 0.55× length of club; pedicel 2.6× as long as wide, 1.12× as long as radicle, 0.50× length of scape. Funicle I triangular, dorsum 0.21× length of venter. Funicle II subquadrate, somewhat compressed, dorsum 0.73× length of venter. Club cylindrical, narrowed at apex, 7.4× as long as greatest width, 14.3× as long as narrowest width, 1.83× length of scape, 3.69× length of pedicel.

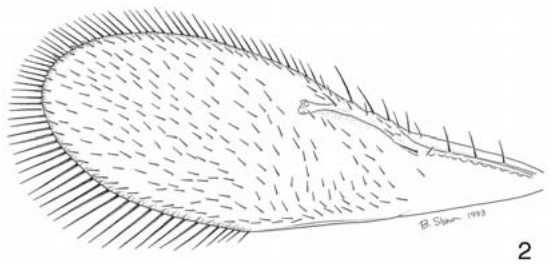

Fig. 2. *Eretmocerus rui*, female forewing.

Mesoscutum trapezoidal with 6 setae, anterior ¼ with cellular reticulate sculpture, remainder with faint elongate reticulations. Parapsis with 3 setae, anterior margins with elongate cellular reticulations; axilla with 1 seta, faintly reticulate. Scutellum with 4 setae, anterior pair shorter, 2 placoid sensilla lateral and closer to the posterior pair of setae, and with faint, elongate reticulations. Metanotum slightly more narrow in longitudinum than propodeum; propodeum with faint transverse reticulations, central lobe broad and smooth, reaching ½ distance into gastral tergite II. Endophragma extending into gastral tergite II.

Forewing (Fig. 2) 3.09× as long as wide between points on wing margin immediately above apex of stigmal vein and at distal apex of frenal fold; 2.73× as long as maximum width of disc (the area distad of an imaginary line extending from the distal apex of the frenal fold to the wing margin immediately above the distal apex of the stigmal vein). Longest anterior alary fringe 0.15× width of disc, longest posterior alary fringe 0.32× width of disc. Base of wing with 1 seta, distal portion of costal cell with 3 setae. Marginal vein with 3 longer setae, 8-16 setae between marginal vein and partial linea calva; setae often interspersed above tubercles and extending about ⅔ distance to proximal advent of frenal fold. Partial linea calva closed posteriorly by setae, including those just described, with 13-14 tubercles on ventral surface near posterior end; a group of 19-29 setae, depending on size of specimen, including those closing the distal margin of the linea calva, generally point toward the anterior margin of the wing; 102-130 setae, depending on size of specimen, in disc (excluding a row of setae around the interior margin) generally point toward distal apex of wing. Submarginal vein 2.78× as long as marginal vein and 3.62× length of stigmal vein. Marginal vein 1.31× length of stigmal vein.

Hind wing 7.31× as long as wide with 0-2 setae in disc.

Gastral tergite I with reticulations on lateral anterior margins, remaining tergites appear smooth; gastral tergites I-IV with paired setae as follows: 1, 1, 2, 2, 2, 2. Syntergum with 4 setae.

Ovipositor prominent, exserted, nearly equal (1.01×) to length of club, 1.85× length of scape, 1.13× length of midtibia.

Male

There is only one male known, and this specimen is uniquely different from nominal exotic biparental *Eretmocerus* species in the United States in its overall paucity of pigment (see Zolnerowich & Rose 1998).

Specimen mounted in Hoyer's with head amber; antennae pale fuscous overall, pedicel and multiporous plate sensilla slightly darker. Pronotum pale fuscous, propodeum and anterolateral

portion of gastral tergite I pale fuscous, remainder of dorsal habitus pale. Forewing with submarginal vein fuscous, remaining veins and proximal ⅓ of costal cell pale fuscous, becoming slightly darker in a narrow band proximal to frenal fold; frenal fold fuscous; all setae in forewing dark; pale fuscous band around forewing margin. Hind wing venation fuscous. Coxa and trochanter pale, with femur, tibia, and tarsi pale brown on all legs. Aedeagus pale fuscous.

Host

Bemisia (*tabaci* group) on *Hibiscus* sp. in laboratory culture in Gainesville, FL.

Etymology

This species is named for our colleague, Ru Nguyen, Research Entomologist for the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville. Nguyen undertook the rearing and release program for *E. rui* in Florida and conducted biological studies on this species. He is currently undertaking field recovery sampling for *Eretmocerus* spp. attacking *Bemisia* (*tabaci* group) and other whitefly genera in Florida. He was instrumental in successful biological control programs directed against the citrus whitefly, *Dialeurodes citri* (Ashmead) (Aleyrodidae: Aleyrodinae) in Florida, and the citrus blackfly, *Aleurocanthus woglumi* (Ashby) (Aleyrodidae: Aleyrodinae) in Florida and elsewhere. Nguyen also facilitated the successful introduction of *Entedononecremnus krauteri* Zolnerowich and Rose (Hymenoptera: Chalcidoidea: Eulophidae) against the invading giant whitefly, *Aleurodicus dugesii* Cockerell (Aleyrodidae: Aleurodicinae) in Florida.

Discussion

Eretmocerus rui was originally reared from *Bemisia* sp. collected on *Emilia* sp. (Asteraceae) in 1992 by F. D. Bennett during searches for natural enemies of *Bemisia* (*tabaci* group) in Hong Kong. Nguyen & Bennett (1995) discussed the importation, release, and recovery of natural enemies of *Bemisia* (*tabaci* group) in Florida. *Eretmocerus rui* was released in 10 Florida counties, and specimens were recovered a few weeks after release. However, it is not known if *E. rui* is permanently established in Florida. Sampling of *Bemisia* (*tabaci* group) and other whitefly genera in Florida to recover *Eretmocerus* species, and to elucidate species and species complexes associated with whitefly species is underway.

McAuslane & Nguyen (1996) discussed the reproductive biology and behavior of *E. rui* (their *Eretmocerus* sp.), and noted the significance of thelytoky to applied biological control. Although we describe the single male specimen of *E. rui*, males are not necessary for reproduction.

De Barro et al. (2000) compared sequences of the D2 expansion segment of the 28S ribosomal RNA gene between an unnamed species of *Eretmocerus* from Hong Kong and *Eretmocerus queenslandensis* Naumann and Schmidt (in De Barro et al. 2000), which was described from *Bemisia tabaci* in Queensland, Australia. Out of 590 positions, they found the sequence from the Hong Kong *Eretmocerus* differed from *E. queenslandensis* by a single mutation at position 206, and suggested the two were conspecific. There is a possibility that the species of *Eretmocerus* from Hong Kong they used is the same as *E. rui*, but unfortunately, preserved specimens or voucher material from their analysis are not available.

However, there are a number of striking morphological differences between *E. rui* and *E. queenslandensis* which lead us to believe that if the *Eretmocerus* from Hong Kong used by De Barro et al. was indeed *E. rui*, the two are not conspecific. The most obvious difference lies in the amount of pigmentation, with unmounted females of *E. queenslandensis* being fuscous, with this dark pigmentation also very evident on cleared, slide-mounted specimens (Fig. 6). Females of *E. rui* have the head and antennae amber, the legs and body are light yellow, and there are no fuscous markings (Fig. 5). Additional morphological differences include: the club of *E. queenslandensis* is 4.8-6.4× as long as wide (Fig. 4), the club of *E. rui* is 6.88-8.06× as long as wide (Figs. 1 and 3); the parapsis of *E. queenslandensis* has 2-3 setae, that of *E. rui* has 3 setae; the placoid sensilla on the scutellum are very close to the posterior pair of scutellar setae in *E. queenslandensis* (Fig. 6), while in *E. rui* they are usually more anterior and lateral to the posterior setae (Fig. 5). There are other morphological differences, but the ones given here are the most obvious to the untrained eye.

Material Examined

Holotype female in balsam with 3 other females on a single slide with a single right-hand label with the following data: Hong Kong, 14 VII 1992, FD Bennett 1312, Bemisia tabaci, lab culture F1, Eretmocerus rui, Zol.& Rose 2003, HOLOTYPE (in red ink, author's note). The left side of the slide is frosted glass upon which is written: 1312, Eret., 4 V. Written in black ink on the glass below the coverslip is: balsam. The coverslip is ringed with red Glypt. The holotype is the bottom left specimen. The specimens are mounted with the heads facing the bottom of the slide.

The paratype series consists of three females mounted in balsam on a single slide with the holotype, 13 females and one male individually mounted on slides in Hoyer's with two labels each that bear the following data: Left label: Name:

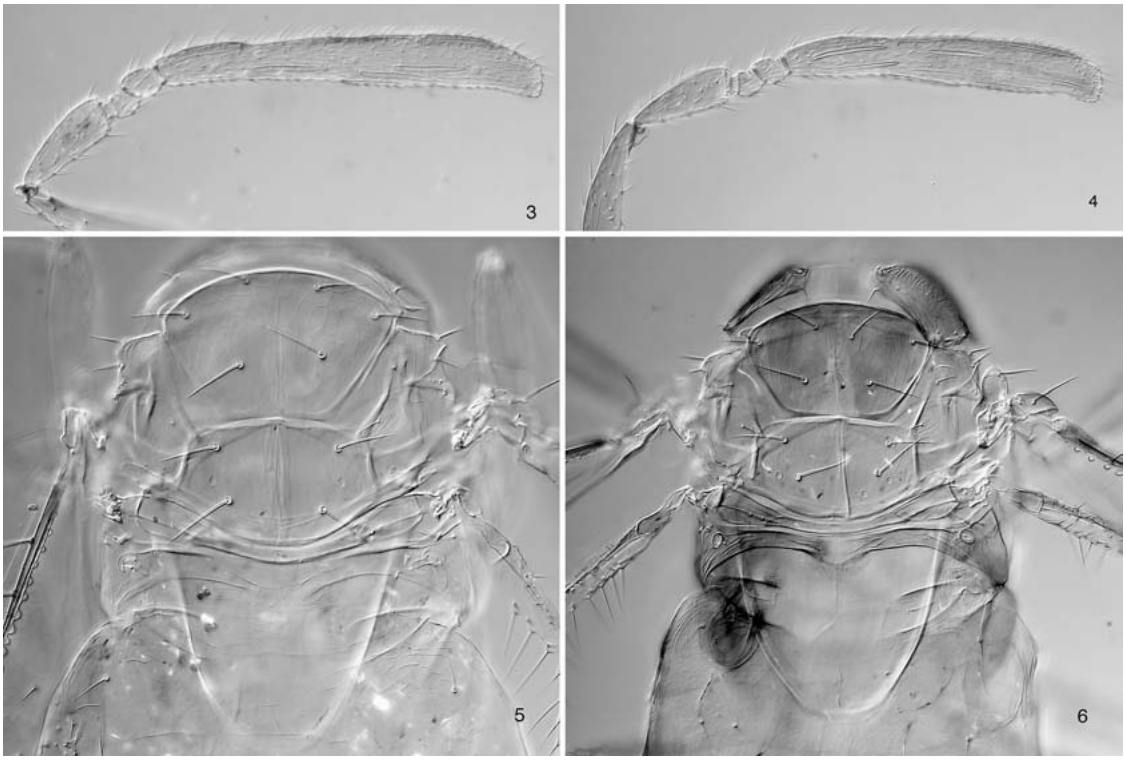

Fig. 3. *Eretmocerus rui*, female antenna.
Fig. 4. *Eretmocerus queenslandensis*, female antenna.
Fig. 5. *Eretmocerus rui*, female thorax and metasoma.
Fig. 6. *Eretmocerus queenslandensis*, female thorax and metasoma.

Eretmocerus rui, paratype (in red ink), Det. Rose and Zol. 2002, Coll. R. Nguyen, No., Corr. R. Nguyen II-18-93. Right label: Loc. (Hong Kong), Univ. Fl. Gainesville, Date: II-18-1993, Host: Bemisia (culture), Det., On: Hibiscus.

The holotype female and three paratype females on a single slide and single male paratype specimen will be deposited in the USDA-ARS Systematic Entomology Laboratory at the U.S. National Museum, Washington, D.C. The remaining type slides and additional specimens will remain with the authors while ongoing systematic studies on *Eretmocerus* species recovered from whitefly in the U.S. are in progress.

Additional Material Examined

USA: Florida: Gainesville DPI, XII.2.1994, lab culture on Hibiscus, R. Nguyen (25 females); USA: Florida: Gainesville, II.15.1995, Bemisia lab culture, cage 2, R. Nguyen (10 females).

Acknowledgments

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FACTORS AFFECTING THE TRAPPING OF MALES OF *SPODOPTERA FRUGIPERDA* (LEPIDOPTERA: NOCTUIDAE) WITH PHEROMONES IN MEXICO

EDI A. MALO[1], FERNANDO BAHENA[2], MARIO A. MIRANDA[3] AND J. VALLE-MORA[1]
[1]Departamento de Entomología Tropical, El Colegio de la Frontera Sur
Apdo. Postal 36, Tapachula, 30700, Chiapas, México

[2]Centro Nacional de Investigaciones para la Producción Sostenible, INIFAP
Apdo. Postal 58260, Morelia, Michoacán, México

[3]Campo Experimental Valle de Apatzingan, INIFAP
Apartado Postal 262, Apatzingan, Michoacán

ABSTRACT

Four commercial sex pheromones and virgin females were tested as attractants for male fall armyworm (FAW), *Spodoptera frugiperda* with Scentry *Heliothis* traps in sorghum fields in Chiapas, Mexico. We observed significant differences among the lures tested. Pherotech, virgin females, and Scentry lures elicited different responses from Chemtica and Trece lures. In another experiment performed in Michoacán, Mexico, we found that Scentry *Heliothis* traps baited with Chemtica lures placed at 1.5 m above ground caught significantly more males than traps placed at a height of 2 m. In contrast, the capture of *S. frugiperda* males with bucket traps placed at 1 m height was not significantly different from that of traps placed at 1.5 and 2 m height. When baited with pheromone, Scentry *Heliothis* traps caught more non-target insects than bucket traps. Apidae was the most prevalent family of non-target insects caught in both experiments.

Key Words: *Spodoptera frugiperda*, trapping, pheromones, Mexico, non-target insects.

RESUMEN

Se evaluaron cuatro feromonas comerciales y hembras vírgenes como atrayentes contra el gusano cogollero *Spodoptera frugiperda* usando trampas tipo *Heliothis* en un campo de sorgo en Chiapas. Las capturas de los machos con las feromonas comerciales Chemtica y Trece fueron significativamente diferentes a las capturas obtenidas con las feromonas Pherotech, Scentry y hembras vírgenes. En otro ensayo realizado en el Estado de Michoacán, México, encontramos que las capturas obtenidas con las trampas tipo *Heliothis* cebadas con feromona de Chemtica y colocadas a una altura de 1.5 m arriba del suelo, fueron significativamente mejores que las capturas de las trampas colocadas a 2 m. Por lo contrario, las capturas obtenidas con las trampas bucket colocadas a 1 m de altura fueron muy similares a las de las trampas colocadas a 1.5 m y 2 m. Las capturas de la entomofauna asociada fueron mucho mayores en las trampas tipo *Heliothis* que las obtenidas con las trampas bucket, siendo Apidae la familia más abundante.

Translation provided by authors.

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), is a major pest of corn, rice, and forage grass (Pashley 1989), and is found in almost all parts of Mexico with the greatest damage occurring in the Southern and Eastern tropical States (Andrews 1980). Control of *S. frugiperda* in maize is achieved by application of methyl parathion, chlorpyrifos, methamidophos, and phoxim, among others insecticides. There are a number of problems related to the habitual use of synthetic pesticides including detrimental effects on the health of farm workers in rural communities in Latin America (McConnell & Hruska 1993; Tinoco & Halperin 1998). For this reason, additional methods of control are desirable for development of a safe system of integrated pest management in the field, including the use of pheromones. Lepidopteran pheromones have been used for insect monitoring, mass trapping, and mating disruption of a great diversity of insect pests (Wyatt 1998). The female-produced sex pheromone of *S. frugiperda*, which is commercially available, has been shown to be a useful tool for monitoring male populations (Adams et al. 1989; Mitchell et al. 1989; Lopez et al. 1990; Gross & Carpenter 1991; Weber & Ferro 1991). How-

ever, commercial sex pheromones lures made in Great Britain and USA can give erratic capture rates in Mexico and Central America (Andrade et al. 2000; Malo et al. 2001).

The population of adult male *S. frugiperda* is frequently monitored with plastic funnel traps (Universal Moth Traps or "bucket" traps or Unitraps) baited with sex pheromones components as lures (Mitchell et al. 1985; Tumlinson et al. 1986). However, this type of trap gave poor results when tested in the coastal plain of Chiapas, Mexico (Malo et al. 2001). Many of the parameters for monitoring FAW with sex pheromone traps have already been described (Mitchell et al. 1985; Mitchell et al. 1989; Pair et al. 1989). However, there exists the possibility that these parameters may differ from one region to another. It is therefore necessary to determine the trap and commercial sex pheromone combination most appropriate for use in southern Mexico. For example, two FAW strains have been reported in Mexico, which are believed to be due to reproductive isolation of the populations arising from geographical isolation (Lopez-Edwards et al. 1999). In this study, we tested a selection of commercial lures. We also report the evaluation of the height of traps placed in the field and three designs of traps with Chemtica lures. These experiments were made in the states of Chiapas and Michoacán, two of the most important maize growing states in Mexico.

Materials and Methods

Chiapas Trial

The first trial was performed at El Manzano in the municipality of Tapachula (14°44'N, 92°19'W, altitude 20 m above sea level), Chiapas, Mexico, in a field planted with sorghum at 20 days post-planting. This area has a humid tropical climate with heavy rain in the summer, with an average annual rainfall of 2,063 mm. The average annual temperature is 26°C, with April and May being the warmest months. Two crop cycles are grown annually in El Manzano; sorghum or maize from January to May, watered by sprinkler irrigation, and soybean during the rainy season from July to October.

Four commercial sex pheromone lures and virgin females as controls were evaluated in a fully randomized plot design with three replicates of each treatment. The replicate plots were arranged in parallel lines approximately 30 m apart in a field planted with sorghum (10 ha). The traps were placed at height of 1.5 m. Lures tested were Scentry (Scentry, Inc., Buckeye, AZ), a gray rubber septum dispenser; Trece (Trece, Inc., Salinas, CA) a red rubber septum dispenser, obtained through Gempler's, Inc. (Belleville, WI); Chemtica, a bubble cup (Chemtica, Heredia, Costa Rica); Pherotech, a red rubber septum dispenser (Pherotech, Delta, BC, Canada) and a virgin female (from the laboratory colony) used as control. The traps used were the Scentry *Heliothis* trap, which is a white double cone collapsible plastic net (Ecogen, Inc., Billings, MT). The traps were placed on 10 February 2001 and they remained in place for one week. The trap captures were recorded daily from 11 to 16 February, a total of 6 observation dates. The virgin female was checked daily and replaced when necessary. On each date, we emptied the traps and recorded the number of *S. frugiperda* males. All non-target insects captured were identified to order (Borror et al. 1989). Voucher specimens were placed in the insect collection held at El Colegio de la Frontera Sur, Tapachula, Chiapas, Mexico.

Michoacán Trial

The second trial was performed in Apatzingán (19°02'N, 102°02'W), Michoacán, Mexico, from 29 July to 26 September, 2001. Apatzingán is at an altitude of 320 m above sea level with a tropical dry climate. Two varieties of maize (V454 and V455) were grown here, at the usual density of 50,000 plants/ha with 80 cm row spacing. A two-factor design was used in the experiment. Two types of traps were used, Scentry *Heliothis* trap and a green reusable bucket trap (Gempler's). The traps were placed at heights of 1, 1.5, and 2 m above the ground. Traps were hung on wooden stakes placed at 30-m intervals along planted rows. We used a bubble cup, commercial sex pheromone from Chemtica. The treatments were arranged in a fully randomized plot design with four replicates of each treatment. All lures were changed monthly. Trap captures were recorded every 3-4 d, and the treatments were rotated after each observation date. On each date, we emptied the traps and recorded the numbers of FAW males and non-target insects captured. All non-target insects captured were identified to family (Borror et al. 1989). Voucher specimens were placed in the insect collection held at Centro Nacional de Investigaciones para la Produccion Sostenible (CENAPROS), Morelia, Mexico.

At the same time that the traps baited with pheromone were being checked, evidence of feeding damage produced by FAW larvae was evaluated in 100 plants chosen at random within the area of trapping. Typically, larvae stay in the whorl, feeding on new leaves, so the damage to the newly expanding leaves and the presence of frass is easily detected by visual examination of the whorls.

Statistical Analysis

The numbers of male FAW captured per trap per sample period were converted to percentages of the total number of moths captured by each trap and lure within each plot (Mitchell et al.

1985). Percentage values were arcsine transformed to increase the homogeneity of variance and normality. Results of the experiment to test lures were analyzed by one-way ANOVA and results of the traps placed at different heights were analyzed by two-way ANOVA (trap × height). Treatment means were compared with the Tukey test ($P = 0.05$). The number of non-target insects caught with the lures in the Chiapas State trial was analyzed as a randomization test (Manly 1994). The effects of trap and height on the families of non-target insects caught at prevalence above 5% in Michoacán State were analyzed by a contingency table involving 2 (traps) × 3 (heights) × 7 (insect families) in the GLIM program (Generalized Linear Interactive Modeling, Numerical Algorithms Group, 1993) in a log-linear model.

RESULTS

Chiapas Trial

The total capture of *S. frugiperda* males for all traps pooled throughout the 6 days was 727. There were significant differences among lures tested ($F = 12.5$, $df = 5,25$, $P < 0.01$). Chemtica and Trece lures elicited a greater capture than virgin females, Scentry, or Pherotech lures (Fig. 1). A very low number of non-target insects ($n = 64$) were captured during the period of trapping, mainly Apidae (bees), representing 64% of the total non-target insects caught. Other orders captured were Coleoptera, Lepidoptera, and Hymenoptera. No significant differences were detected in non-target insects caught in relation to the lures used in a randomization test ($P > 0.05$). Chemtica and virgin females were the lures that attracted the greatest number of non-target insects caught (32 and 31%, respectively). Trece, Scentry, and Pherotech lures captured 20.3%, 14%, and 1.5%, respectively.

Michoacán Trial

The total capture of *S. frugiperda* males was 2397. Of the total number of males captured, 81.1% were caught with the Scentry *Heliothis* trap and 18.9% with bucket traps. The efficiency of Scentry *Heliothis* traps was affected by height, whereas the catch of bucket traps was independent of height, resulting in a significant interaction effect ($F = 4.3$; $df = 2,15$; $P = 0.03$) (Fig. 2).

The number of insects caught with pheromone traps (Scentry *Heliothis* and bucket traps placed at different heights) as well as the feeding damage produced by *S. frugiperda* larvae on the plants was generally higher at the start of the study, but at the end of the first month, the population and the feeding damaged produced by FAW larvae had decreased (Fig. 3). Great variation was observed in the number of insects caught over time and in the feeding damage resulting from the FAW infestation. In this experiment, no chemical insecticide was used to control FAW.

A total of 2352 non-target insects was caught with both traps (Scentry and bucket) during the trial period. Significant differences were observed among the number of non-target insects at the level of family ($\chi^2 = 488$, $df = 5$, $P < 0.01$). The most abundant non-target insects caught were Apidae, followed by Cicadellidae and Tachinidae (Fig. 4). Scentry *Heliothis* traps caught a total of 2000 non-target insects, whereas bucket traps caught 352. Apidae was the most prevalent group caught

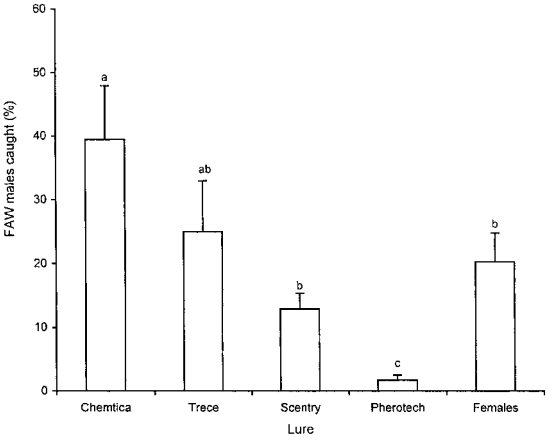

Fig. 1. Percent capture of male fall armyworm (+SEM) with Scentry traps baited with commercial lures in a sorghum field in Chiapas State, Mexico. Significant differences within traps and height are shown by different letters over the bars (Tukey test, $P = 0.05$).

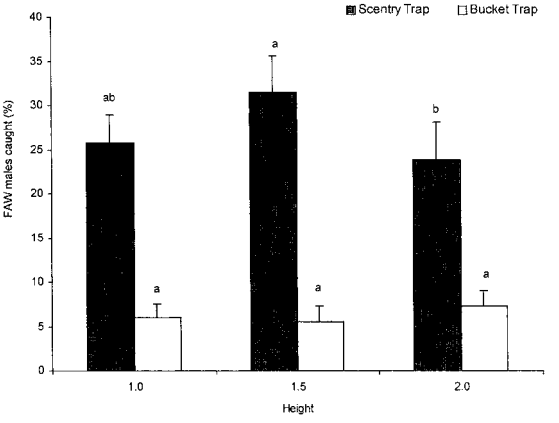

Fig. 2. Percent capture of male fall armyworm (+SEM) with Scentry and bucket traps baited with Chemtica lures and placed at different heights (mm) in a maize field in Michoacán State, Mexico. Significant differences within traps and height are shown by different letters over the bars (Tukey test, $P = 0.05$).

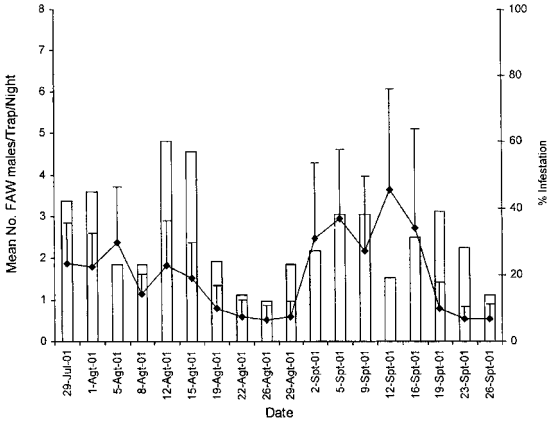

Fig. 3. Seasonal mean number (+SEM) of male *Spodoptera frugiperda* caught with sex pheromone traps in a maize field in Michoacán, Mexico, is in line. Percentage of feeding damage produced by *S. frugiperda* at each observation date is in column.

with Scentry *Heliothis* traps and Carabidae with bucket traps. Carabidae, caught most with bucket traps, was not included in the analysis of the effects of trap and height because few were caught in Scentry *Heliothis* traps. Scentry *Heliothis* traps placed at 1 m height caught a total of 910 non-target insects, whereas traps placed at 1.5 m caught 649 non-target insects and traps placed at 2 m caught 441 non-target insects. Bucket traps placed at 1 m caught a total of 105 non-target insects, traps placed at 1.5 m caught a total of 139 non-target insects and traps placed at 2 m caught a total of 108. Overdispersion was observed in the distribution of data on the non-target insect families and different traps placed at different heights. Overdispersion was corrected by the methods described by Hinkley et al. (1990). No significant interaction was detected among trap type and height of traps (χ^2 = 9.98, *df* = 14, *P* = 0.76), indicating that the number of non-target insects caught in the traps was independent of the height at which the trap was placed. However, a significant interaction was observed between

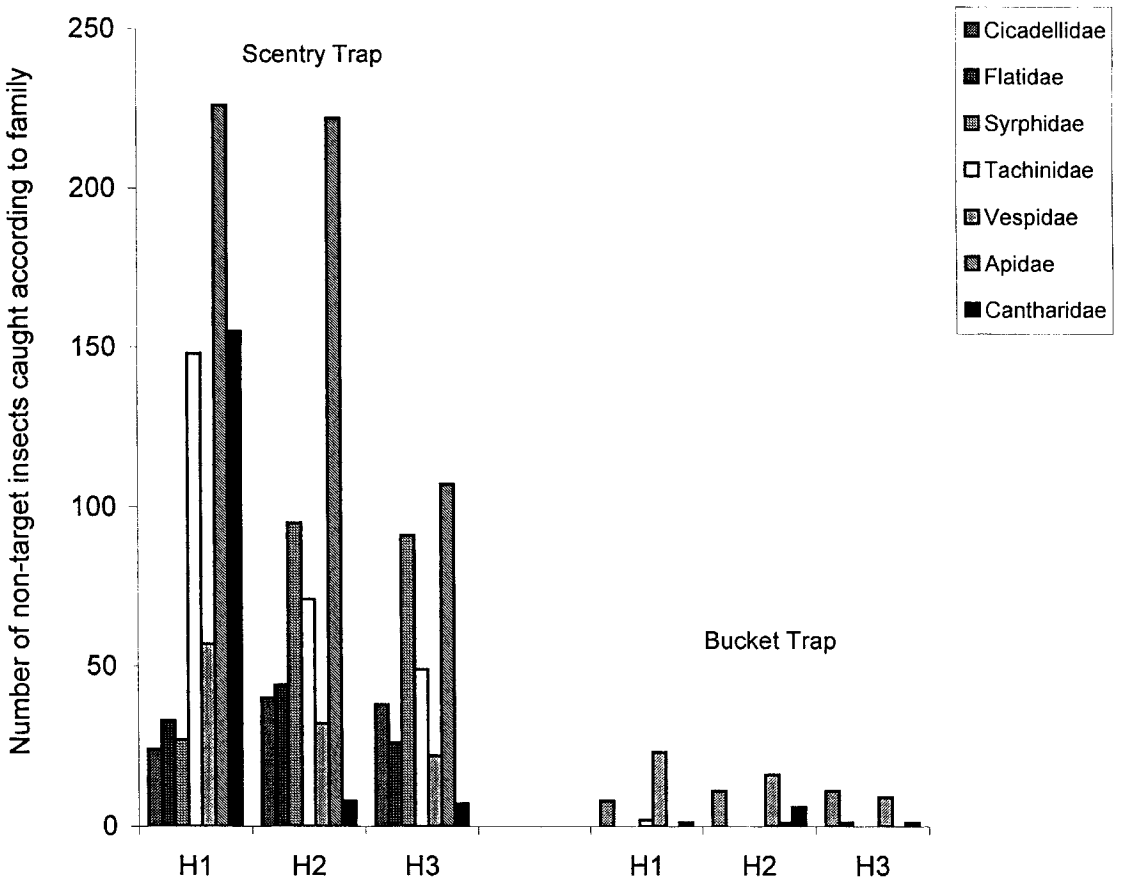

Fig. 4. Number of each non-target insect family caught with Scentry and bucket traps in a maize field in Michoacán State, Mexico. H1 = 1 m, H2 = 1.5 m, and H3 = 2 m height at which traps were placed.

family × type of trap × height of trap indicating that each family of non-target insects responded differently to trap type and height (Table 1).

Discussion

From the results of the commercial lures tested it is clear that the Chemtica and Trece lures can be used for monitoring *S. frugiperda* males in Mexico. Scentry *Heliothis* traps baited with Chemtica lures placed at 1.5 m above ground caught significantly more *S. frugiperda* males than traps placed at a height of 2 m. In contrast, capture with bucket traps was not affected by trap height. The parameters for monitoring FAW males with pheromone traps have been described in studies performed in Florida, USA (Mitchell et al. 1985; 1989; Pair et al. 1989). The results obtained in Florida and the results in Mexico are not markedly different. Trap height is one of the most important aspects of trap deployment, along with trap density and the position of the trap with respect to vegetation (Wall 1989).

Hartstack screenwire cone traps and plastic funnel traps were reported to capture more moths than sticky and electric grid traps (Tingle & Mitchell 1975; Mitchell et al. 1985). However, when the population density of FAW was low, both types of trap designs tested did equally well in capturing *S. frugiperda* males (Mitchell et al. 1985; Adams et al. 1989; Pair et al. 1989). For higher density populations, Hartstack traps generally performed better than unitraps (Mitchell et al. 1985; Pair et al. 1989). Green traps were only minimally attractive when baited with FAW pheromone and insecticide (Gross & Carpenter 1991). Similar results were reported by Malo et al. (2001) in the evaluation of commercial lures and traps; green traps caught a low number of FAW males in the coastal plain of Chiapas, Mexico. Trap color has been reported to be influential to the capture of several noctuids, including *S. frugiperda*. Plastic bucket traps with green canopies, yellow funnels, and white bucket traps collected more *Spodoptera* spp. males than all-green traps in several studies (Mitchell et al. 1989; Pair et al. 1989; Lopez 1998). However, Meagher (2001a) reported that more moths were captured in these standard traps than all-white or all-green traps, as was also reported with *S. exigua* (Lopez 1998). It was suggested that a possible factor responsible for low rates of capture of moths in green traps was the low reflectance at wavelengths where moth vision is most sensitive (Mitchell et al. 1989).

In this study, we caught very few non-target insects in the trial conducted in Chiapas in traps baited with pheromone. In contrast, in the Michoacán trail, the number of FAW males caught with pheromone traps was similar to the number of non-target insects. However, the presence of honeybees, *Apis mellifera* L., was evident in both trials. The fact that bees were caught in both traps also elevated the apparent number of captures (Fig. 4), although bee captures were more common in Scentry *Heliothis* traps. It is possible that a few species of non-target moths may be attracted by certain chemical components of the pheromone of *S. frugiperda*. Weber and Ferro (1991) reported that noctuids *Leucania phragmitidicola* Guenée, *Sideridis rosea* (Harvey) and *Eurois occulta* (L.) were commonly caught in FAW traps in Massachusetts, USA. Others have also reported that baited traps attract non-target and even beneficial insects (Adams et al. 1989; Mitchell et al. 1989; Gauthier et al. 1991; Gross & Carpenter 1991; Meagher & Mitchell 2001; Malo et al. 2001; Meagher 2001a,b). Apparently trap color may play a role in the attraction of the insects, for example white or yellow traps can attract large number of *Bombus* spp. (Hamilton et al. 1971; Mitchell et al. 1989).

In conclusion, Scentry *Heliothis* traps with a Chemtica and Trece lures gave good results for monitoring FAW males in Chiapas, Mexico. Parts of these results were reconfirmed in Michoacán, Mexico and suggest that the traps are best placed at a height of 1.5 m. However, these traps caught a considerable number of non-target insects and

Table 1. Test of significance of the factors involved in a log-linear model of trap design, trap height, and family of non-target insects caught.

Source of variation	χ^2	*df*	*P*
Trap	591.5	19	<0.001
Height	188.4	20	<0.001
Family	301.0	24	<0.001
Trap-Height	9.98	14	0.76
Trap-Family	78.2	12	<0.001
Height-Family	97.8	28	<0.001

Trap used: Scentry type *Heliothis* and bucket.
Height at which traps were placed: 1, 1.5, and 2 m.
Family of non-target insects caught above 5%: Cicadellidae, Flatidae, Syrphidae, Tachinidae, Vespidae, Apidae, and Cantharidae.
Analysis performed in GLIM with Poisson error distribution corrected for overdispersion.

it is possible that one of the chemical compounds from pheromones or the color of the trap may be involved in the attraction of non-target insects.

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CABBAGE LOOPER MOTHS (LEPIDOPTERA: NOCTUIDAE) TRAPPED WITH MALE PHEROMONE

PETER J. LANDOLT[1], RICHARD S. ZACK[2], D. GREEN[1] AND L. DECAMELO[2]
[1]USDA-ARS, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951 USA

[2]Department of Entomology, Washington State University, Pullman, WA 99164

ABSTRACT

Traps in field plots assessed attraction of the cabbage looper moth, *Trichoplusia ni* (Hübner), to lures emitting synthetic chemicals identified as the pheromone of the male; linalool, *p*-cresol and *m*-cresol. Male and female cabbage looper moths were captured in traps baited with racemic linalool, but significantly greater numbers of both sexes were captured in traps baited with the 3-component blend. Virgin and mated female cabbage looper moths were captured, with up to 5 spermatophores per female in mated ones. Pheromone was dispensed from polypropylene vials, and numbers of moths captured in traps increased with the size of the hole in the vial lid, up to the maximum 25-mm diameter hole tested. Rates of release of pheromone from vials with 25-mm diameter holes in the laboratory decreased from 4 to 3 milligrams per h over a four-week duration. This is the first evidence in the field of cabbage looper response to the chemicals identified as pheromones of the male.

Key Words: pheromone, attraction, trap, behavior, cabbage looper, linalool, *p*-cresol, *m*-cresol.

RESUMEN

Mediante trampas de campo se estimó la atracción de la palomilla del falso medidor, *Trichoplusia ni* (Hübner), a cebos que emiten linalool, *p*-cresol y *m*-cresol, químicos sintéticos identificados como la feromona del macho. Se atraparon machos y hembras de la palomilla del falso medidor en trampas cebadas con sólo linalool racémico, pero se atraparon números significativamente mayores de ambos sexos con la mezcla de los tres componentes. Se capturaron palomillas vírgenes y apareadas con hasta cinco espermatóforos por hembra. La feromona se expuso en viales de polipropileno y el número de palomillas capturadas por trampa se incrementó con el tamaño del orificio en la tapa del vial, hasta el máximo probado de 25 mm de diámetro. La tasa de liberación de la feromona de los viales con orificio de 25 mm en laboratorio disminuyó de 4 a 3 miligramos por hora en un periodo de cuatro semanas. Esta es la primera evidencia de campo de la respuesta de la palomilla del falso medidor a los químicos identificados como la feromona del macho.

Translation provided by the authors.

The cabbage looper moth, *Trichoplusia ni* (Hübner), uses two mate-finding strategies (Landolt & Heath 1990; Lenczewski & Landolt 1991). One strategy involves male attraction to the female-produced sex pheromone which includes the major component *Z*-7-dodecenyl acetate (Berger 1966), and several other structurally related compounds (Bjostad et al. 1984). The other strategy involves female attraction to the male pheromone composed of the major component *S*-(+)-linalool, as well as *p*-cresol and *m*-cresol (Heath et al. 1992a; Landolt & Heath 1989; Landolt 1995). *S*-(+)- or racemic linalool alone and the 3-component male pheromone blend of linalool, *p*-cresol, and *m*-cresol attracted females in a laboratory flight tunnel assay (Heath et al. 1992a). However, these chemicals have not been tested in the field for their attractiveness to cabbage looper moths.

A synthetic lure for females could be developed for use in monitoring the activities of females and also for reducing reproduction of this pest in agricultural crops. To date, however, there have been no demonstrations in the field of female cabbage looper moth attraction to synthetic male pheromone, although female and male attraction to chemicals from flowers is well documented (Cantelo & Jacobson 1979; Haynes et al. 1991; Heath et al. 1992b). We report here the results of trapping experiments that tested the hypothesis that cabbage looper moths are attracted to the male pheromone compounds. Because pure *S*-(+)-linalool was not available in amounts sufficient for these experiments, we used racemic linalool, both alone and in combination with *p*- and *m*-cresol.

MATERIALS AND METHODS

Universal moth traps, (UniTraps, IPM Technologies, Portland, OR) were used in all tests. These traps had white buckets, yellow cones, and

green tops, and included a 6.5-cm^2 piece of Vaportape (Hercon Environmental Inc., Emigsville, PA) stapled to the inside of the bucket wall to kill captured insects. Traps were hung from fences or stakes in or adjacent to irrigated fields of alfalfa, *Medicago sativum*, or corn, *Zea mays*, at a height of 0.5 m, and were 10 to 15 m apart. Pheromone chemicals were dispensed from polypropylene narrow mouth bottles (vials) (#2006 9125 for 4 ml vials, #2118 9050 for 15 ml vials, Nalge Nunc International, Rochester, NY). These pheromone dispensers (vials) were suspended vertically with wire inside the UniTrap buckets.

The first experiment tested attractiveness of the 3-component blend of racemic linalool, *p*-cresol and *m*-cresol (Aldrich Chemical Co., Milwaukee, WI) to cabbage looper moths. Two ml of a 90:5:5 mixture of linalool, *p*-cresol, and *m*-cresol were added to a 2.5-cm diam cotton ball inside of a 4-ml vial. Vials had a 3-mm diameter hole in the lid for pheromone emission. Control traps had no lures. Five pairs of treated and control traps were maintained from 20 to 28 August 2001. Traps were checked for moths three times (every 2 to 3 days), providing 15 samples. Treatment and control traps were alternated in position each time that traps were checked.

The second experiment tested for a role of the cresols in cabbage looper moth attraction to the 3-component pheromone blend of linalool, *p*-cresol, and *m*-cresol. The 3 treatments were (1) a trap with no lure as a control, (2) a trap with a 4-ml vial containing 2 ml of racemic linalool on a cotton ball, and (3) a trap with a 4-ml vial containing 2 ml of a 90:5:5 mixture of racemic linalool, *p*-cresol, and *m*-cresol on a cotton ball. Each vial had a 3-mm diameter hole in the lid for pheromone emission. A randomized complete block experimental design was used, and the ten replicate blocks were maintained from 17 July to 4 September 2003. Traps were checked and treatments randomized each week for 7 weeks, providing 70 samples. Lures were replaced every two weeks.

The third experiment evaluated a range of release rates of the 3-component blend of racemic linalool, *p*-cresol, and *m*-cresol. The objective was to determine if attractiveness of the pheromone to cabbage looper moths increased with increasing amounts of pheromone released and to determine an optimum lure for trapping cabbage looper moths with male pheromone. Lure release rate was altered by changing the diameter of the hole in the vial lid. Treatments were 15-ml vials, each with 2 ml of an 90:5:5 mixture of racemic linalool, *p*-cresol, and *m*-cresol, and with holes 1.5, 3, 6, 12.5, and 25 mm in diameter. The larger vials were used to accommodate holes of a greater diameter in the vial lid. A randomized complete block design was used, and the 5 blocks were maintained from 4 to 25 September 2003. Traps were checked and treatments randomized each week, providing 15 samples. Lures were replaced every week.

Female moths captured in traps baited with the 3-component blend in experiments two and three were stored in 70% ethanol and then dissected under a binocular microscope for determination of their reproductive status. The presence of fat in the abdomen was noted, the numbers of mature eggs in the ovaries were counted, and the number of spermatophores in the bursa copulatrix was recorded. This information was used to categorize the reproductive state of female moths captured in the system of Hitchcox (2000). Moths in category I were unmated and immature, with no spermatophore, abundant fat, and fewer than 10 mature eggs present. Moths in category II were mated and immature, with one or more spermatophores, fat in the abdomen, and fewer than 10 mature eggs present. Moths in category III were mated and mature, with one or more spermatophores, 10 or more mature eggs and with some fat present. Moths in category IV were senescent, with one or more spermatophores present, no fat, and fewer than 10 mature eggs.

Release rates of male pheromone from vials with 25-mm diameter holes were determined as weight lost over time. Ten 15-ml polypropylene vials were each loaded with 5 ml of male pheromone (90:5:5 ratio of racemic linalool, *p*-cresol, and *m*-cresol) on 3 cotton balls. Dispensers were then weighed one day after loading, then daily until 28 days after loading. Daily weight loss was determined by subtracting the vial weight from the weight of the vial the day before. Hourly weight loss was calculated by dividing daily weight loss by 24. The weight lost was then attributed to emission of male pheromone from the dispensers.

Trap catch data for treatments in experiments number 1 and 2 above were compared with a paired *t*-test. Data for experiment 3 were subjected to a quasilinear (with a square root transformation) regression analysis to determine if numbers of moths captured varied with pheromone release rate (vial hole size). Daily weight loss data were subjected to a regression analysis to determine if dispenser weight loss per day changed with time. All statistical analyses were performed with the Statmost software (DataMost 1995).

Results

In the first experiment, cabbage looper moths were captured in traps baited with the mixture of racemic linalool, *p*-cresol, and *m*-cresol, while no moths were captured in unbaited traps (Table 1). Numbers of moths in baited traps were significantly greater than in unbaited traps ($t = 3.45$, $df = 14$, $P = 0.002$). These moths were not sorted by sex, and were not dissected to determine reproductive status. A total of 143 cabbage looper moths were captured in traps in this experiment.

TABLE 1. MEAN (± SE) NUMBERS OF CABBAGE LOOPER MOTHS CAPTURED IN TRAPS BAITED WITH VIALS LOADED WITH THE RACEMIC LINALOOL, *P*-CRESOL, AND *M*-CRESOL.

Test 1.		Moths/trap	
	Control	0.0 ± 0.00 a	
	Linalool, *p*-cresol, *m*-cresol	9.5 ± 2.80 b	
Test 2.		Females/trap	Males/trap
	Control	0.0 ± 0.00 a	0.0 ± 0.00 a
	Linalool	0.2 ± 0.08 b	0.5 ± 0.13 b
	Linalool, *p*-cresol, *m*-cresol	0.5 ± 0.14 c	0.9 ± 0.15 c

Means followed by the same letter are not significantly different by paired *t*-test at $P < 0.05$.

In the second experiment (Table 1), numbers of both sexes of the cabbage looper moth were greater in traps baited with racemic linalool alone ($t = 3.65$, $df = 84$, $P < 0.001$ for females, $t = 4.75$, $df = 84$, $P < 0.001$ males) or with the combination of linalool and cresols ($t = 4.33$, $df = 84$, $p < 0.001$ for females; $t = 5.53$, $df = 84$, $P < 0.001$ for males) compared to unbaited traps. Numbers of both sexes captured in traps baited with racemic linalool, *p*-cresol, and *m*-cresol were significantly greater than the numbers of both sexes trapped with racemic linalool alone ($t = 2.18$, $df = 84$, $P = 0.016$ for females; $t = 1.62$, $df = 84$, $P = 0.05$ for males). Totals of 79 female and 141 male cabbage looper moths were captured in this test.

In the third experiment, numbers of both sexes of the cabbage looper moth increased with the increases in diameter of hole in the vial lid (Fig. 1), with greatest numbers of males and greatest numbers of females in traps baited with pheromone in vials with a 25-mm hole in the lid. For females, there was a significant regression of numbers of moths captured by vial hole diameter ($r^2 = 0.80$, $P = 0.01$, $Y = -01.36 + 16.6 * SQRT X$, where Y is vial hole diameter, and X is numbers of moths per trap). For males there was also a significant regression of numbers of moths captured by vial hole diameter ($r^2 = 0.97$, $P = 0.0004$, $Y = 1.35 + 13.0 * SQRT X$). Totals of 48 females and 61 males were captured in this test.

Seventy-one female cabbage looper moths that were captured in traps baited with linalool, *p*-cresol and *m*-cresol were dissected in order to categorize their reproductive condition. The greatest number of females were in category III, mated and mature (n = 31). The smallest number of females dissected were in category II, mated and immature (n = 3). Category I, unmated and immature, and category IV, senescent, were represented by 19 and 18 moths respectively. Fifty-one female moths were mated (71.8%), and those that were mated, possessing from one to 5 spermatophores (mean = 2.5 ± 0.15 spermatophores per female). Twenty female moths of the 71 dissected (28.2%) were unmated.

There was a significant negative regression of daily vial weight loss in relation to the age of vials ($r^2 = 0.14$, $P = 0.03$, $Y = 3.87 - 0.0386X$, where Y is weight lost, and X is days in age). Daily weight loss of vial dispensers loaded with cabbage looper male pheromone was near 4 milligrams per h during the first several days after loading of the vials with pheromone, dropping to near 3 milligrams per h near the end of the 4 week evaluation (Fig. 2)

DISCUSSION

Previous studies have demonstrated attraction of female and male cabbage looper moths to male pheromone. Females were attracted to male cabbage looper moths in a flight tunnel (Landolt & Heath 1989) and a field cage (Lenczewski & Landolt 1991), and both sexes were attracted to males in cotton fields (Landolt 1995). Female cabbage looper moths in a flight tunnel were attracted to the combination of *S*-(+)- or racemic linalool and *p*-cresol and *m*-cresol, 3 compounds isolated from hairpencils of male cabbage looper moths (Heath et al. 1992a). The flight tunnel attraction response was significantly reduced with the omission of the cresols from the blend or the omission of *S*-(+)-linalool from the blend. These studies did not address moth attraction to male pheromone compounds under field conditions or the possible use of male pheromone as a lure for trapping female cabbage looper moths.

This is the first demonstration in the field of male and female cabbage looper moth attraction to the chemicals identified as the male pheromone of *T. ni* by Heath et al. (1992a). We interpret captures of the moths in traps as evidence of orientation responses to the chemicals used as lures in the trap. Males and females were attracted then to racemic linalool and to the 3-component blend of linalool and the two cresols. Numbers of male or female cabbage looper moths in traps were higher when the cresols were present in the lure, indicating some importance of these compounds in male attractiveness to females. Additional testing in the field will be necessary to

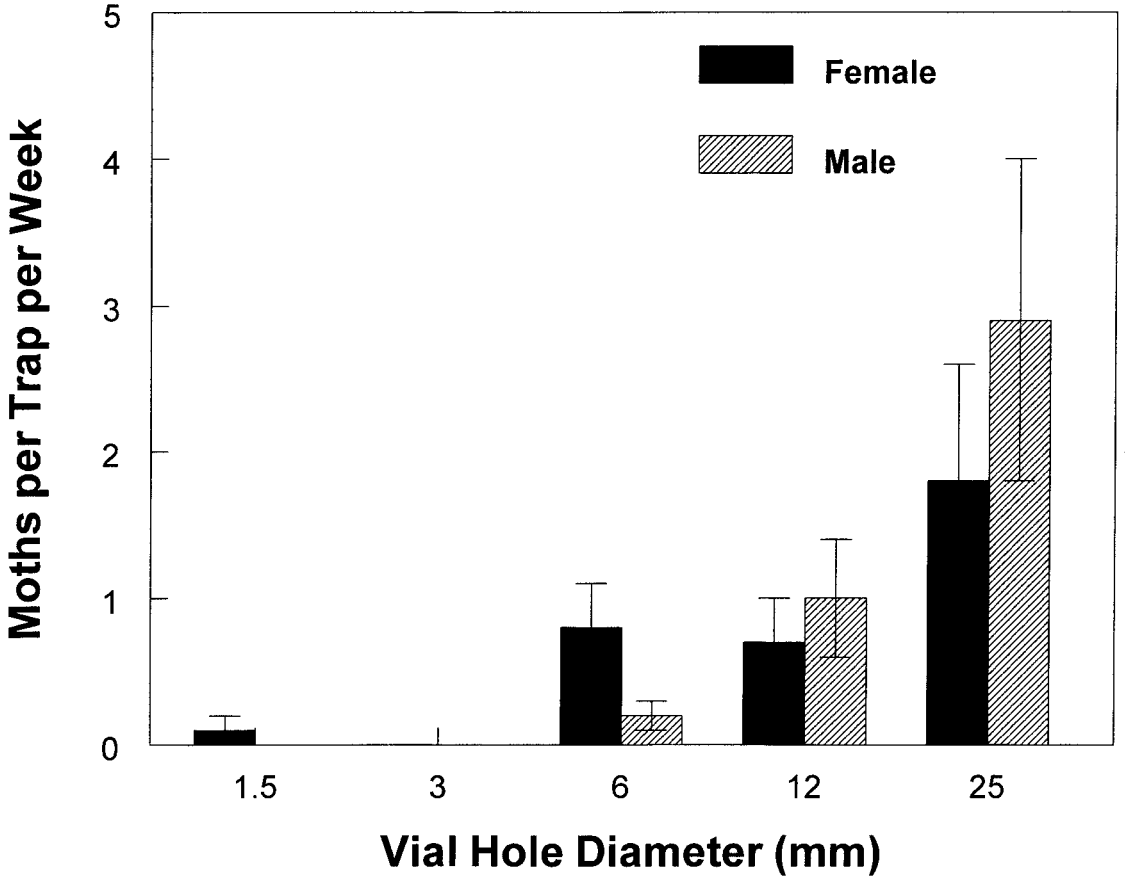

Fig. 1. Mean (± SE) numbers of female and male cabbage looper moths captured in traps baited with linalool, *p*-cresol, and *m*-cresol in a polypropylene vial with a hole in the lid for pheromone release. Vials had different hole diameters (1.5, 3, 6, 12, and 25 mm) to alter the rate of release of pheromone.

determine if attraction of moths to pheromone is stronger when *S*-(+)-linalool is used instead of racemic linalool, and to determine if both *p*-cresol and *m*-cresol increase attractiveness of the pheromone to cabbage looper moths.

Other species of moths were not captured in traps in this study, and the cabbage looper male pheromone chemicals are not reported as attractants for other species of insects. Linalool was tested previously as a possible floral lure for alfalfa looper moths, with no indication of attractiveness to either alfalfa looper or cabbage looper moths (Landolt et al. 2001). At that time (summer 2000) there were many alfalfa looper moths but few cabbage looper moths in the area; thus, the lack of cabbage looper moths captured did not indicate a lack of attractiveness of chemicals tested. Specificity of this lure in attracting only or primarily cabbage looper moths would be desirable for monitoring applications, because responses of other species of moths might be interpreted as false positives for cabbage looper moths.

The attraction of cabbage looper moths to male pheromone in the field may be a mate-finding or a food-finding response, or both. In addition to its presence in, and release by, male cabbage looper moths (Heath et al. 1992a), linalool is present in the odor of honeysuckle flowers which are visited by cabbage looper and other moths in search of nectar (Pair 1994; Schlotzhauer et al. 1996). Cabbage looper females that are deprived of sugar are more strongly attracted to males, suggesting responses to male pheromone may be based in part on food-finding needs (Landolt et al. 1996). Females attracted to males (Landolt 1995) and synthetic male pheromone (herein) include both mated and unmated individuals. Some possessed 5 spermatophores, indicating mating up to 5 times before responding to the male pheromone in the study. Perhaps male cabbage looper moths contribute nutritional material in the spermatophore and mimic flowers by releasing chemicals characteristic of certain moth-visited flowers, as a strategy of luring females.

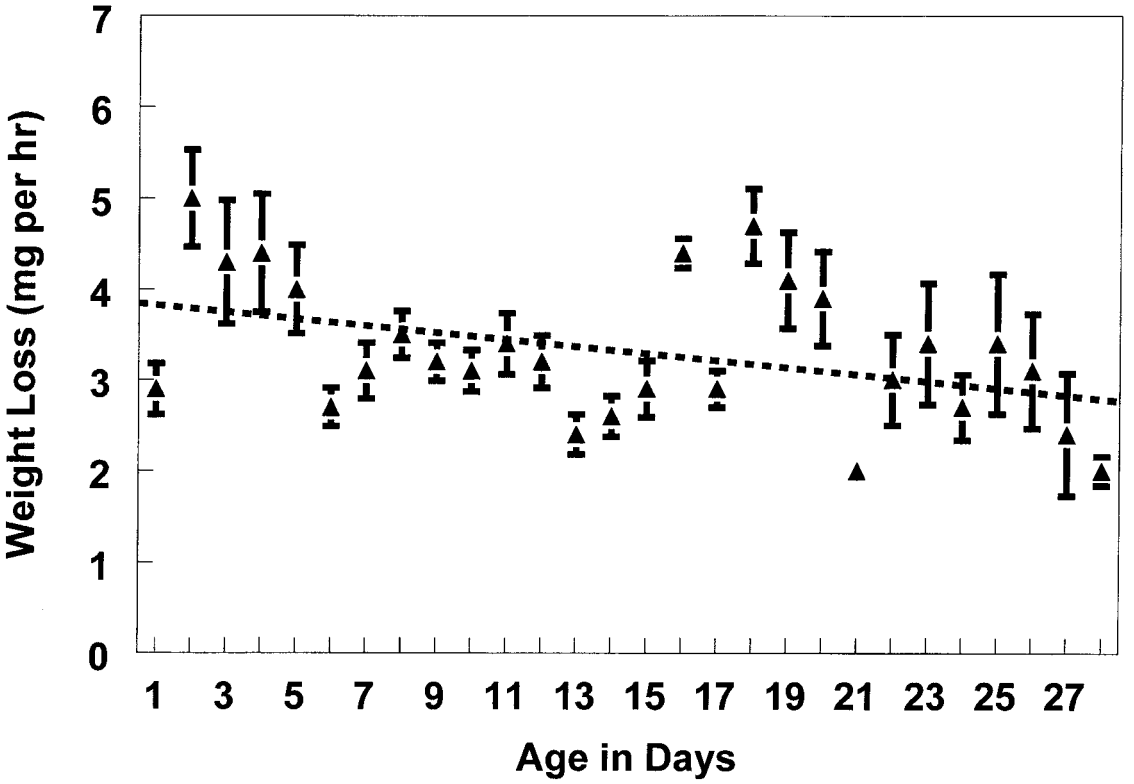

Fig. 2. Mean (± SE) loss of weight per hour over time, for 15-ml polypropylene vials loaded with 5 ml of male cabbage looper pheromone, with a 25-mm diameter hole in each vial lid.

Males of other noctuid moths produce chemicals in hairpencils that are also present in the odors of flowers (Birch & Hefetz 1987), but are not known to be attractive to conspecific moths. For example, 2-phenylethanol is present in the volatiles of the moth-visited flowers *Abelia grandiflora* (Haynes et al. 1991) and *Gaura drummondii* (Shaver et al. 1997). This compound is also found in the hairpencils of *Mamestra configurata* (Walker) (Clearwater 1975), *Polia nebulosa* (Hufnagel), and *Peridroma saucia* (Hübner) (Birch 1972; Birch et al. 1976). Birch & Hefetz (1987) suggested that the tendency for male scents to resemble plant odors is because females likely already have receptors for these chemicals and have behavioral responses to those food plant odors.

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SURVIVAL AND INFECTIVITY OF *STEINERNEMA SCAPTERISCI* (NEMATODA: STEINERNEMATIDAE) AFTER CONTACT WITH SOIL DRENCH SOLUTIONS

KATHRYN A. BARBARA AND EILEEN A. BUSS
University of Florida, Entomology and Nematology Department, Gainesville, FL 32611

ABSTRACT

After a nematode application, mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) are frequently assayed to confirm nematode establishment and infectivity. However, the standard soap flush was suspected of providing false negatives under field conditions. Thus, we examined the effect of several potential flushing solutions on the survival and infectivity of *Steinernema scapterisci* Nguyen and Smart (Nematoda: Steinernematidae) as well as flushing ability under field conditions. Seventy percent of *S. scapterisci* died in lemon dish detergent solution, confirming that assays for nematode infection of soap-flushed mole crickets are likely to be inaccurate. When sampling for mole crickets in areas where *S. scapterisci* has been applied, a potential alternative to the standard soap drench is a dilute permethrin drench.

Key Words: Sampling, soap flush, soil drench, entomopathogenic nematode, biological control, integrated pest management.

RESUMEN

Después de una aplicación de nemátodos, los grillos topo (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) son evaluados frecuentemente para confirmar el establecimento y la capacidad de infectar de los nemátodos. Sin embargo, la lavada con jabón que es el proceso usado usualmente, es sospechada de proveer datos negativos falsos bajo condiciones de campo. Por ello, nosotros examinamos el efecto de diferentes soluciones para la lavada sobre la sobrevivencia y la capacidad de infectar de los *Steinernema scapterisci* Nguyen y Smart (Nematoda: Steinernematidae) así como la habilidad para lavar bajo condiciones de campo. Setenta por ciento de los *S. scapterisci* mueren en una solución de detergente con limon para platos, confirmando que los ensayos para determinar la infección de nemátodos de grillo topos lavados con jabón son probablemente imprecisos. Cuando recolectan los grillos topo en áreas donde *S. scapterisci* ha sido aplicado, una alternativa a la lavada basica con jabón es una lavada con una solución diluida de permetrina.

Mole crickets (Orthoptera: Gryllotalpidae: *Scapteriscus* spp.) are subterranean pests of turfgrass in Florida and much of the southeastern United States (Walker & Nickle 1981; Walker 1985). Mole cricket damage and cost of control in Florida in 1986 was estimated at $45 million with an additional $33 million in Alabama, Georgia, and South Carolina combined (Frank & Parkman 1999). Estimates of annual expenditure in 1996 were over $18 million for insecticides in Florida turf, and over $12 million in control costs (Hudson et al. 1997). Mole crickets damage turf by their tunneling in the soil, which exposes and dries out roots and by direct root feeding. As a result, the turfgrass thins and bare patches appear. The tunneling and mounds that mole crickets make also disrupt the playing surface on golf courses, especially the roll of the golf ball on greens. Superintendents and golf course members have little tolerance for damage (Frank & Parkman 1999). Insecticides are usually targeted against the most destructive, nymphal stage. A more sustainable, environmentally friendly management approach for mole cricket control is needed.

Several biological control agents have been investigated for control of *Scapteriscus* spp. mole crickets in Florida (Hudson et al. 1988). One of these biological control agents is an entomopathogenic nematode, *Steinernema scapterisci* Nguyen and Smart. *Steinernema scapterisci* was originally collected in Uruguay in pitfall-trapped *Scapteriscus* mole crickets in the 1980s (Nguyen & Smart 1990). The nematode was cultured and released in several Florida counties in 1985, where it established a population, and was spread from the release site by infected *Scapteriscus* mole crickets (Hudson et al. 1988; Parkman & Frank 1992). The nematode kills the adult and late instar nymphs of *Scapteriscus borellii* Giglio-Tos and *S. vicinus* Scudder, and to a lesser extent *S. abbreviatus* Scudder. Fewer small to medium-sized nymphs of *S. borellii* and *S. vicinus* become infected (Nguyen 1988).

Several techniques have been used to sample mole crickets including counts of dead nymphs and adults after insecticide applications (Short & Koehler 1979), estimation of surface burrowing (Walker et al. 1982; Cobb & Mack 1989), pitfall

trapping (Lawrence 1982; Adjei et al. 2003), removal with a tractor mounted soil corer (Williams & Shaw 1982), sound trapping (Walker 1985) and soil drenching (Short & Koehler 1979; Walker 1979; Hudson 1989). However, results from each of these techniques are often inconsistent (Short & Koehler 1979; Lawrence 1982, Hudson 1988). Comparisons of different methods have indicated that soil drenching with soap solutions is the most practical and consistent at obtaining direct counts of mole crickets (Short & Koehler 1979; Hudson 1988).

Soil drenching with a solution of 15 ml of lemon dishwashing detergent in 3.8 L of water is inexpensive and commonly used by turfgrass managers to sample soil pests. Soil drenches with soap solutions irritate mole crickets and force them out of the soil. Soap flushes are often used for monitoring mole crickets to determine the size, age, and species present, the relative population density over time, and for control timing. However, it was suspected that soap flushes, when used to monitor mole crickets potentially infected with *S. scapterisci*, might be lethal to the nematodes because nematodes are rarely found in soap-flushed mole crickets (our observations, and K. B. Nguyen & G. C. Smart, Entomology and Nematology Dept., University of Florida, pers. comm.). Solutions such as pyrethroids, ammonia, vinegar, Lysol®, and other soap detergents have previously been tested as potential soil drench solutions (Short & Koehler 1979).

This study was conducted to determine if a standard soap detergent solution affects *S. scapterisci* survival and infectivity in pest mole crickets. Potential alternatives to the standard soap drench solution were also evaluated.

Materials and Methods

Nematodes and Mole Crickets

Steinernema scapterisci (Nematac S®, Becker Underwood, Ames, IA) were stored at 7°C in a cold room until used (<3 mo). Nematode viability was tested before each application by dissolving a pinch (~10 mg) of Nematac S® into water and observing nematode shape and mobility under a light microscope. Healthy nematodes were opaque in color and S-shaped with oscillating movements. Dead or unhealthy nematodes were translucent, straight, and lacked movement. The product was used if viability was >50% and discarded if <50% viable.

Scapteriscus vicinus were collected from pitfall traps or sound traps in Alachua Co., FL, and returned to the laboratory. Each mole cricket was placed in a 120-ml plastic vial (Thorton Plastics Salt Lake City, UT) with sterilized sand and held for ≥14 d to ensure health. Surviving mole crickets were used in this study. Mole crickets were maintained at 23°C with a photoperiod of 12:12 (L:D) and fed commercial cricket chow (Purina®, St. Louis, MO).

Bioassay

Nematode viability and infectivity were assessed after exposure to various drenching materials. *Steinernema scapterisci* were extracted from Nematac S® using a modified Baermann technique (K. B. Nguyen, pers. comm.). *Steinernema scapterisci* were kept at a density of 10,000 infective juveniles in solutions of water (control), lemon dishwashing detergent (Joy®), insecticidal soap (Safer Soap®, Woodstream Corporation, Litiz, PA), and permethrin (Spectracide Bug Stop®, Spectrum Brands, St. Louis, MO) for test 1. The mixtures were kept at room temperature (24°C) in a 125-ml Erlenmeyer flask with 125-ml per flask on a shaker at 65 rpm. There were five replicates for each treatment. Concentrations (Table 1) were selected based on recommendations for flush extraction of mole crickets in the field (Short & Koehler 1979) and label rates for mole cricket control. After 24h, 10-µl samples were taken from each treatment and placed on a microscope slide. The number of living and dead nematodes were counted with a dissecting microscope (10×); three 10-µl counts were taken and averaged to determine percent mortality for each replicate. Immobile nematodes were touched with a probe to determine survival.

Table 1. Mean nematode mortality and percent of mole crickets infected with *Steinernema scapterisci* after exposure for 24 h to various drenching solutions.

Treatment	Rate	Mean % nematode mortality (± SEM)[1]	% Mole crickets infected with *S. scapterisci*[2]
Water	n/a	6.2 ± 3.95	16.7
Lemon Joy	15 ml/ 3.79 L	70.6 ± 4.52*	8.3
Insecticidal Soap	15 ml/ 3.79 L	90.0 ± 7.82*	0
Permethrin	18 ml/ 3.79 L	35.6 ± 1.97*	16.7

*Statistically significant values using Dunnett's method comparing treatments to water.
[1]n = 20, $F = 54.68$, $df = 19, 3$, $P = < 0.0001$.
[2]n = 12, $R^2 = 0.2971$, $df = 11, 3$, $\chi^2 = 4.843$ (likelihood), $P = 0.18$.

A second test was initiated to further test potential drench materials. Treatments for test 2 included water (control), azadirachtin (Safer® Brand BioNeem, Woodstream Corporation, Litiz, PA), citrus oil (Green Sense®, Garland, TX), garlic extract (Garlic Barrier®, Garlic Research Labs, Inc., Glendale, CA), lemon juice (Realemon®, Rye Brook, NY), permethrin (Spectracide Bug Stop®, Spectrum Brands, St. Louis, MO) and cyfluthrin (Bayer Advanced Lawn and Garden®, Bayer Environmental Sciences, Montvale, NJ). Concentrations (Table 2) were selected based on label and half label rates for mole cricket control. Methods from test 1 were repeated.

Nematode infectivity was assessed by filtering nematodes from above solutions and adding 50 living infective juveniles to 120-ml plastic cups (Fisher Scientific, Pittsburgh, PA) containing 20 g sterilized sand, 4% deionized water, and one *S. vicinus* adult. Dead mole crickets were dissected and the presence or absence of nematodes was recorded.

The above solutions were tested for their effectiveness at flushing mole crickets at the University of Florida G. C. Horn Turfgrass Research Unit in Gainesville, FL, on 20 and 28 May 2003. Each treatment from tests 1 and 2 (3.8 L of each solution) was applied to areas of bermudagrass (*Cynodon dactylon* [L.] Persoon) that had mole cricket damage (75 cm^2). The numbers of adult and first instar mole crickets emerging from the soil within 3 min were counted. Five replicates for each solution were completed. Any turfgrass phytotoxicity was noted at 1 h and 1 wk posttreatment.

The effect of nematode infected crickets exposed to soap solutions was also tested. *Scapteriscus abbreviatus* adults were obtained from a lab colony at the University of Florida Entomology and Nematology Department, Gainesville, FL and were inoculated with about 10,000 nematodes by applying predetermined amount (approximately 150 µl) of concentrated nematode solution onto a piece of filter paper (Fisher #P8, 5.5 cm) inside a petri dish with one *S. abbreviatus* adult. The mole cricket was allowed to incubate in the petri dish for 1, 5, 8, 12, or 24 h (five mole crickets per treatment). *Scapteriscus abbreviatus* was used because *S. vicinus* adults were unavailable at the time of the test. All infected mole crickets were then dipped into a 118-ml Solo soufflé cup (Gainesville Paper Co., Gainesville, FL) containing the soapy water or soapy water followed by a deionized water rinse for 5 sec. Untreated controls were healthy, uninfected mole crickets dipped in water. Mole crickets were placed into 20-dram plastic scintillation vials (Fisher Scientific, Pittsburgh, PA) and observed every 24 h for 10 days. On day 10, mole crickets were dissected and the presence of nematodes was noted.

Statistical Analysis

Nematode mortality and field test data were subjected to an analysis of variance (SAS Institute 2001). Treatments were compared to the control (water) by Dunnett's means comparison method ($\alpha = 0.05$). Nematode infectivity data were subjected to Chi-square analysis (SAS Institute 2001). Treatments were compared to the control (water) and the standard soap flush solution (15 ml lemon dish detergent/3.8 L water) by Dunnett's means comparison method ($\alpha = 0.05$). Nematode mortality data were transformed by arcsine-square root transformation before statistical analysis; nontransformed data are presented. Effects of nematode infected crickets exposed to soap solutions data were subjected to PROC GLM (SAS Institute 2001) procedure.

Results and Discussion

Insecticidal soap, lemon dishwashing soap, and permethrin at the label rate for mole cricket control caused significantly more nematode mortality than water (Table 1). Nematodes exposed to

Table 2. Mean nematode mortality and infectivity after exposure for 24 h to various drenching solutions.

Treatment	Rate	Mean % nematode mortality (± SEM)[1]	% Mole crickets infected with *S. scapterisci*[2]
Water	n/a	12.9 ± 7.89	3.7
Citrus Oil	15 ml/ 3.79 L	32.1 ± 9.47	3.7
Cyfluthrin	8 ml/ 3.79 L	6.4 ± 6.44	3.7
Cyfluthrin	15 ml/ 3.79 L	4.1 ± 4.12	3.7
Garlic Extract	111 ml/ 3.79 L	0	3.7
Lemon Juice	15 ml/ 3.79 L	6.8 ± 6.76	0
BioNeem	60 ml/ 3.79 L	4.4 ± 4.38	0
Permethrin	9 ml/ 3.79 L	10.8 ± 6.73	3.7
Permethrin	18 ml/ 3.79 L	0	3.7

[1]n = 45, $F = 2.70$, $df = 44, 8$, $P = 0.193$.
[2]n = 27, $R^2 = 0.1349$, $df = 26, 8$, $\chi^2 = 4.170$ (likelihood), $P = 0.18$.

all treatments showed similar infectivity in mole crickets (R^2 = 0.2971; *df* = 2,11; χ^2 = 4.843; *P* < 0.184) (Table 1). Nematode mortality was similar among all treatments in test 2 (Table 2). Nematodes surviving all treatments except azadirachtin and lemon juice, demonstrated a low percentage infectivity of mole crickets, no significant treatment differences were observed (R^2 = 0.1349; *df* = 2,26; χ^2 = 4.170; *P* < 0.842) (Table 2).

In the field, insecticidal soap and the higher rate of permethrin flushed significantly more mole crickets than water (Table 3). However, when all treatments were compared to the standard lemon dish detergent, insecticidal soap and permethrin brought a similar number of mole crickets to the surface (n = 55; *F* = 2.88; *df* = 10,54; *P* = 0.008). None of the mixtures tested produced any noticeable phytotoxicity to the turf.

Soil drenches with a mixture of lemon dish detergent and water are commonly used to monitor turfgrass insects such as mole crickets, chinch bugs (*Blissus* spp.), big-eyed bugs (*Geocoris* spp.), and several species of caterpillars (Short & Koehler 1979; Hudson 1989). Soil drenches are inexpensive and are not labor intensive when compared with other methods of monitoring mole cricket populations. These other methods include large pitfall traps (Lawrence 1982; Adjei et al. 2003), an emitter producing a synthetic song of male mole crickets (Parkman & Frank 1993), and a soil-coring device (Williams & Shaw 1982). Each method requires more than one person, are labor intensive or costly (Lawrence 1982; Williams & Shaw 1982).

Seventy percent of *S. scapterisci* died in the lemon dish detergent solution. Assays for nematode infection of soap-flushed mole crickets, the method currently used by many turfgrass managers, are likely to be inaccurate. Krishnayya & Grewal (2002) reported a toxic effect of a common soap surfactant (Ajax®) on *S. feltiae* Bovien nematodes. They found 24% mortality of nematodes when incubated at 4, 24, 72, and 120 h (Krishnayya & Grewal 2002). Kaya et al. (1995) reported an insecticidal soap (M-Pede®) adversely affected *S. carpocapse* (Weiser) and *Heterorhabditis bacteriophora* Poinar survival and infectivity. However, infectivity may not be affected if the nematodes are combined with an insecticidal soap and applied immediately (Kaya et al. 1995). Nematodes cannot be stored in an insecticidal soap solution because without aeration, nematode survival can be adversely affected (Kaya et al. 1995). The toxicity of metal ions present in soap may be responsible for the high mortality in soap solutions (Jaworska et al. 1994; Krishnayya & Grewal 2002).

Tests of exposure of nematode infected mole crickets to soap solutions show that soap flush solution does not greatly affect nematode infection at least 8 h post infection (Table 4). The soap flush solutions may potentially kill nematodes in certain areas of the body (i.e., mouth) and further testing should be done to determine this. Immediately rinsing flushed mole crickets with clean water may potentially increase the accuracy of determining nematode infection. The unavailability of *S. vicinus* at the time of experimentation may have also led to inconsistent, low levels of infection. It is known that *S. scapterisci* does not infect *S. abbreviatus* as successfully as *S. vicinus* or *S. borellii* (Nguyen 1988).

Although permethrin solutions killed some nematodes in our experiments, *S. scapterisci* infectivity was not compromised and field flushes successfully extracted mole crickets from the soil. The field data concur with Short & Koehler (1979) who reported that pyrethrins were the most effective material, flushing a mean of 11.5 mole crickets/0.6 m^2. Hudson (1988) compared three sampling tech-

TABLE 3. MEAN NUMBER OF MOLE CRICKETS EMERGING FROM BERMUDAGRASS WITH VARIOUS DRENCHING SOLUTIONS IN MAY 2003.

Treatment	Rate	Mean number of mole crickets flushed (± SEM)
Water	n/a	0
Citrus oil	15 ml/ 3.79 L	2.6 ± 1.6
Cyfluthrin	8 ml/ 3.79 L	0.2 ± 0.2
Cyfluthrin	15 ml/ 3.79 L	4.0 ± 2.1
Garlic extract	111 ml/ 3.79 L	0.4 ± 0.2
Lemon juice	15 ml/ 3.79 L	0.6 ± 0.4
BioNeem	60 ml/ 3.79 L	3.2 ± 1.2
Permethrin	9 ml/ 3.79 L	2.6 ± 1.1
Permethrin	18 ml/ 3.79 L	5.8 ± 1.4*
Insecticidal soap	15 ml/ 3.79 L	5.4 ± 1.3*
Lemon Joy	15 ml/ 3.79 L	4.6 ± 2.1

*Means statistically significant values by Dunnett's method comparing treatments to water.
n = 54, *F* = 2.88, *df* = 59,10, *P* = 0.01.

TABLE 4. PERCENT NEMATODE INFECTION FROM MOLE CRICKETS EXPOSED TO TREATMENT SOLUTIONS 1, 5, 8, 12, OR 24-H POST INFECTION.

Time Post Infection	1 H	5 H	8 H	12 H	24 H
Joy (15 ml/ 3.79 L)	0	40	60*	60*	100*
Joy (15 ml/ 3.79 L) + H_2O rinse	40	40	100*	80*	100*
Control[†]	0	0	0	0	0

n = 75, $F = 6.77$, $df = 14, 2$, $P < 0.0001$.
*Means within columns statistically significant values when compared to control.
[†]Control = uninfected, healthy mole crickets immersed in water.

niques, soil flushing with lemon dish detergent or synergized pyrethrins, and a tractor mounted soil corer. None of the methods were significantly different. Our results from the field test show drenching solutions of permethrin are useful in determining if mole crickets collected in the field are infected with *S. scapterisci* nematodes. A soil drench containing permethrin may be the best monitoring tool to flush mole crickets to determine the presence of *S. scapterisci*.

However, there are disadvantages to pyrethroids as soil drenches for mole crickets. Pyrethroid drenches at the half or full label rate may cause more mole cricket mortality than a soap solution. Subsurface mortality of mole crickets can be as high as 65% with pyrethroids or similar insecticides (Ulagaraj 1974; Walker 1979; Hudson 1988). Applicator exposure to insecticides is increased with a pyrethroid soil drench.

Soil drenches are effective, non labor-intensive methods to sample soil insect populations. Soap detergent solutions, although inexpensive, may not accurately indicate mole crickets infected with *S. scapterisci*. Permethrin solutions are less cost effective (Short & Koehler 1979), but are effective at flushing mole crickets potentially infected with nematodes.

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A NEW SPECIES OF *ANTRUSA* AND THREE NEW SPECIES OF *CHOREBUS* (HYMENOPTERA: BRACONIDAE) FROM THE IBERIAN PENINSULA

M. FISCHER[1], J. TORMOS[2], I. DOCAVO[3] AND X. PARDO[4]
[1]Naturhistorisches Museum Wien, Zweite Zoologische Abteilung (Insekten)
Burgring 7, A-1014 Wien, Postfach 417, Austria

[2]Unidad de Zoología, Facultad de Biología, Universidad de Salamanca, 37071-Salamanca, Spain

[3]Fundación Entomológica "Torres-Sala", Paseo de la Pechina, 15, 46008-Valencia, Spain

[4]Universitat de València, Institut Cavanilles de Biodiversitat i Biologia Evolutiva,
Apartat Oficial 2085, 46071 València, Spain

ABSTRACT

Antrusa curtitempus, Chorebus liliputanus, *C. propediremptum*, and *C. vicinus*, four new species of Dacnusini from the Iberian Peninsula, are described, illustrated, and compared with allied species. Keys for their discrimination are provided. The taxonomic rehabilitation of the genus *Antrusa* is proposed.

Key Words: new species, *Antrusa*, *Chorebus*, Alysiinae, Dacnusini, Braconidae.

RESUMEN

Se describen, e ilustran, cuatro nuevas especies de Dacnusini de la Península Ibérica: *Antrusa curtitempus, Chorebus liliputanus*, *C. propediremptum*, y *C. vicinus*. Se discuten sus afinidades filogenéticas, y se propone la rehabilitación taxonómica del género *Antrusa*.

Translation provided by the authors.

In this work, four species of Dacnusini (Hymenoptera: Braconidae: Alysiinae)—*Antrusa curtitempus*, *Chorebus liliputanus*, *C. propediremptum*, and *C. vicinus*—from the Iberian Peninsula, are described as new, illustrated, and compared with allied species. Keys for their discrimination are provided. The genus *Antrusa* Nixon is rehabilitated taxonomically.

The terms for body morphology and wing venation, together with the criteria for collecting biometric data, follow Fischer (1973, 2002) with the two following modifications: (a) mesosoma vs. thorax, and (b) setae vs. hairs. All the material examined is deposited at the Museo del Medio Ambiente (Valencia, Spain). The following abbreviations have been used in the descriptions: a2 = lower vein of B (brachius); B = brachial cell; cq1 = first cubital cross-vein; cu2 = 2nd abscissa of cu (= cubital vein); cu2' = second abscissa of cubital vein of hind wing; cu1b = lower cubital-anal cross vein (3rd discoideal segment); d = discoidal vein; F, Fl, F2, etc.= flagellomere (s), flagellomere 1, 2, etc.; Fm, Fp = middle flagellomere (s), penultimate flagellomere; M = medial cell of hind wings; np = parallel vein (nervus parallelus); r' = radiellus (radial vein of hind wing); nr = recurrent vein (nervus recurrens); nr' = recurrent vein of hind wing; nv = nervulus; R = radial cell; r, rl, r2 = radial vein, first, second abscissa of radius; st = pterostigma; SM' = submedial cell of hind wings; T, T1, T2, T3, T2+3 = tergite (s), first, second, third tergite, second + third tergite.

Genus *Antrusa* Nixon

The genus was described by Nixon (1943). Later, it was sunk into synonymy with *Exotela* Foerster by Griffiths (1964) based on his interpretation of characters in the light of phylogenetic systematics. E. Haeselbarth (Zoologische Staatssammlung, Munich) regarded *Antrusa* Nixon as justified (unpublished notes). The reason for the *Exotela*-dilemma was the fact that the decisive character of *Exotela*, the postfurcal nr, does not apply to all species. Another difficulty is the diagnostic separation from *Dacnusa* Haliday, with which it shares most characters. *Antrusa* can be characterized and delimited from *Exotela*, *Dacnusa* and *Chorebus* Haliday by a combination of the following characters: (a) mandibles three-dentate; (b) nr antefurcal; (c) T1 with medial longitudinal keel; (d) no sexual dimorphism of the pterostigma. The latter character is significant for separation from *Dacnusa*, but cannot be easily applied without having both sexes available. The longitudinal carina of T1 may be helpful. The three-dentate mandibles separate *Antrusa* from *Chorebus*, and the antefurcal nr separates it from *Exotela* in the restricted sense.

Antrusa curtitempus **sp. nov**. (Figs. 1-4)
Female—Body length: 1.5 mm.

Head (Fig. 1): Twice as wide as long, twice as wide as face, 1.33 times as wide as mesoscutum, eyes at least 1.8 times as long as temples, protruding, eyes narrowed behind, eyes and temples rounded in a common bow; toruli in normal position, occiput bayed inwards; upper side with scattered setae on the sides, occiput and in the ocellar area, epicranial suture between ocelli; distance between ocelli greater than ocelli width, distance between an ocellus and eye as long as width of ocellar area. Face 1.5 times as wide as high, only slightly and evenly convex, middle elevation nearly missing (only faintly visible in a certain oblique position), with rather evenly distributed, scattered setae, seta points discernable, edges of eyes only slightly converging below, nearly parallel sided. Clypeus slightly convex, 3 times as wide as high, with few outstanding setae. Tentorial pits round, their diameter as great as the distance from eyes. Labrum triangular, protruding, with inconspicuous setae. Mandible (Fig. 2) slightly longer than wide, lower edge straight, upper edge slightly directed upwards, tooth 1 rounded, tooth 2 pointed and only slightly protruding, tooth 3 broadly rounded, an incision between tooth 2 and 3, outer surface shiny to uneven and a few scattered setae. Antennae 23-segmented, scarcely longer than body, the basal flagellar segments about 2.5 times as long as wide, the following slightly shorter, Fp about 1.5 times as long as wide; the setae as long as the segment width, in lateral view 3 sensillae visible.

Mesosoma: 1.4 times as long as high, upper side convex. Mesoscutum about 1.25 times wider than long, evenly rounded anteriorly, notauli developed on declivity and crenulate, merging into the anteriorly crenulated lateral rim, central lobe and declivity setose, dorsal slit reaching middle of disc. Prescutellar furrow rectangular, with 3 longitudinal ridges. Scutellum triangular. Postaxillae and metascutum glabrous. Propodeum reticulate, with pentagonal area, a longitudinal carina inside, with basal carina and costulae. Furrows of sides of pronotum crenulate below. Prescutellar furrow broad, irregularly striated, tapering anteriorly and reaching edge, not reaching middle coxa, prepectal furrow narrow, passing into the crenulate anterior mesopleural furrow, posterior mesopleural furrow simple, epicoxal area of middle coxa with a few scattered setae only. Metapleuron glabrous, uneven, with long scattered setae, delimited from propodeum by an irregular lamella. Hind femur 5 times as long as wide, hind tarsus hardly shorter than hind tibia.

Wings (Fig. 3): st parallel-sided, reaching beyond middle of R, r arising from base of st by a distance as long as r1, the latter slightly longer than the width of st when infolded, distal half of r2 almost straight, R not reaching tip of wing, cu2 developed by a distance greater than cq1 long, nr clearly antefurcal, d slightly longer than nr, nv postfurcal, B about twice as long as wide, closed by vein cu1b, np arising from middle of B; r' and cu2' indicated only as folds, nr' absent.

Metasoma: T1 (Fig. 4) 1.5 times as long as wide, apically 1.5 times as wide as basally, evenly narrowed towards base, dorsal keels converging and uniting near middle to a longitudinal median keel, the remainder smooth, laterally a lamella which is medially slightly angulated (lateral view), the spiracle outside the lamella. Ovipositor sheath as long as hind basitarsus, reaching slightly beyond tip of metasoma.

Color: Black. Yellow: anellus, labrum, mouth parts, tegulae, wing venation, legs, and parts of the lower side of the metasoma.

Male—Unknown.

Host—Unknown.

Material examined: Holotype: female, SPAIN: Castellón: Alcora, 7-VII-1990. Paratype: SPAIN: Castellón: Alcora, 7-VII-1990, 1 female.

Etymology: The specific name "curtitempus" means "short temple" and refers to the narrowed and shortened part of the head behind the eyes (in dorsal view).

Taxonomic position: The west-Palearctic species may be separated as follows:

1. Head (Fig. 1) behind eyes strongly narrowed; temples about half as long as eyes; eyes and temples rounded in a common curve. Body length: 1.5 mm. Spain ..... *A. curtitempus* **sp. nov.** (female)

—Head behind eyes as wide as at eyes or wider; eyes about as long as temples 2

2. Head behind eyes widened. T2+3 setose all over, T2 weakly sculptured. Scape and pedicel yellow. Antennae 29-32 segmented. Body length: 2.5 mm. England, Germany, Central Russia ..... *A. vaenia* Nixon

—Head at temples not or only slightly wider than at eyes. Setae of T2+3 not distributed over the entire surface; a broad, bare area between T2 and T3. Only T1 sometimes longitudinally striated 3

3. r2 nearly evenly bent 4

—r2 distally bisinuate; tegulae dark 5

4. T1 longitudinally striated, weak points between the striae, shiny. Hind femora 5.5 times as long as wide. Mesoscutum setose only anteriorly, the rest predominantly bare. T2 glabrous, bare. T1 brownish; T2+3 dark brown. Antennae 31-segmented. Body length: 2.2 mm. Central Russia ..... *A. chrysotegula* (Tobias)

—Hind femora 4.5 times as long as wide. Mesoscutum nearly entirely covered with short, fine semi-appressed setae. T2 weakly longitudinally rugose at base. Metasoma yellow, T2 dark brown, T1 black. Antennae 30-segmented. Body length: 2.3 mm. Moldavia . . . . . *A. chrysogastra* (Tobias)

5. Antennae 23-36-segmented, F up to 28-segmented. Head behind eyes clearly widened. T1 narrow, folds stronger. T2 smooth. Body length: 2.2-2.3 mm. Western Europe; North-West and Central Russia; Azerbaijan . . . . . *A. melanocera* (Thomson)

—Antennae 28-34-segmented. Head behind eyes not widened. T1 somewhat wider, the folds rather weak. T1 sometimes weakly sculptured. 2.0-2.4 mm. Western Europe; North-, Central and South-West Russia; Azerbaijan; Siberia (Irkutsk) . . . . . *A. flavicoxa* (Thomson)

Genus *Chorebus* Haliday

Chorebus liliputanus **sp.nov**. (Figs. 5-6)

Male—Body length: 1.2 mm.

Head: Twice as wide as long, 1.4 times as wide as the mesoscutum, 1.9 times as wide as the face, 2.5 times as wide as T1, at eyes as wide as behind them; eyes 1.2 times as long as the temples, toruli not especially prominent, occiput slightly bayed inwards, upper side with very few setae on sides and occiput; ocelli small, distance between them greater than their diameter, epicranial suture not visible. Face 1.33 times as broad as high, setose all over, seta points present, middle elevation only feebly indicated and bare, edges of eyes slightly rounded. Clypeus trapezoidal, 3 times as wide as high. Tentorial pits small. Mandible (Fig. 5) as wide as long, lower edge straight, upper edge only slightly directed upwards, distally only very little broader than at base; tooth 2-pointed, not very prominent, tooth 1 round on tip, an obtuse angle between tooth 1 and tooth 2, tooth 3 and tooth 4 pointed, positioned one behind the other, from tooth 1 and tooth 4 arise keels, which unite to a faint, round carina separating a distal smooth area, from the lower part of the inner surface arise long, bent setae which surpass teeth 3 and 4 and the lower edge; labrum triangular, prominent, setose; maxillary palpi as long as height of head. Antennae as long as body, 22-segmented; F1-F3 about 3 times, Fm 2.5 times, Fp twice as long as wide, the F closely lined up, the numerous setae shorter than the F width, in lateral view 2 sensillae visible.

Mesosoma: 1.3 times as long as high, considerably higher than head, upper side strongly arched. Mesoscutum 1.4 times as wide as long, anteriorly round, notauli on declivity irregularly lamellate, crenulate, reaching lateral edge in right angle, absent on disc; setae on the declivity, along the imaginary course of the notauli, near lateral and posterior rim, seta points developed on declivity. Prescutellar fovea deep, narrow, densely crenulate. Axillae with long white setae. Postaxillae crenulate behind. Metascutum with short central lamella, lateral areas covered with white setae. Propodeum densely covered with dirty white tomentum. Sides of pronotum covered with long, white setae, bare only above. Prepectal furrow narrow, densely crenulate, passing into the crenulate epicoxal furrow, precoxal furrow densely crenulate, shortened behind. Metapleuron densely covered with white setae, with a central swelling. Hind femora 5 times as long as wide, hind tarsi at most slightly shorter than hind tibia.

Wings (Fig. 6): st mostly parallel-sided, narrowed only towards the end, r arising behind base of st by a distance equal to the length of r1, r2 evenly curved, R ending considerably before tip of wing, nr antefurcal, cu2 scarcely developed, d 1.1 times as long as nr, nv postfurcal, B open distally and below, cu1b absent, a2 mostly absent; r' and cu2' practically absent, nr' absent, SM' half as long as M'.

Metasoma: T1 1.5 times as long as wide, apically straight-lined and narrowed, spiracles on small tubercles, densely rugose, dorsal carina only near base, with dense white tomentum, fewer setae only along middle line. The rest of metasoma oval, depressed, smooth and bare, except for single rows of setae near the hind edges of the T.

Color: Black. Antennae dark including base. Yellow: anellus, mouthparts, the entire legs, tegulae, wing venation and T2+3. Wing membrane hyaline.

Female—Unknown.

Host—Unknown.

Material examined: Holotype: male, SPAIN: Castellón: Burriana, 30-VI-1990. Paratype: SPAIN: Castellón: Burriana, 30-VI-1990, 1male.

Etymology: The name refers to the very small size of the species.

Taxonomic position: The species is nearest to *Chorebus melanophytobiae* Griffiths, 1968 from which it can be distinguished as follows:

Chorebus melanophytobiae: (a) from tooth 1 of the mandible arises a lamella which runs to the base of the mandible; (b) r2 unevenly bent, distally rather straight; (c) distal part of st wedge-shaped; (d) base of antennae brown. Body length: 1.4-1.6 mm.

Chorebus liliputanus: (a) from tooth 1 and tooth 4 of the mandible (Fig. 5) arise keels, which unite to a faint, round carina separating a distal smooth area; (b) r2 (Fig. 6) evenly curved; (c) st mostly parallel-sided, narrowed only towards the end; (d) base of antennae dark like the remainder. Body length: 1.2 mm.

Remarks: According to the original description of *Chorebus melanophytobiae* (Griffiths 1968a), there are also specimens with the base of the antennae dark, like the remaining F. On the other hand, a contrasting yellow color of the antennal base is often taken as an important specific character. In spite of there being several, although only small, differences between *Chorebus melanophytobiae* and *Chorebus liliputanus*, we do not believe that the two taxa are conspecific.

Chorebus propediremptum **sp. nov**. (Figs. 7-9)
Male—Body length: 1.4 mm.

Head: 1.8 times as wide as long, 1.7 times as wide as face, 1.33 times as wide as mesoscutum; between eyes as broad as between temples, eyes as long as temples, distance of toruli from eyes and between them as great as their diameter, upper side only with very few setae on vertex and occiput and in the ocellar area, ocelli small, the distance between them greater than their diameter; epicranial suture distinct on occiput, fading away between ocelli. Face 1.4 times as broad as high, moderately setose with distinct seta points, edges of eyes very weakly rounded, median elevation very weak. Clypeus trapezoidal, 3 times as wide as high, with some faint, long setae. Tentorial pits as wide as their distance from eyes. Labrum projecting, apically round, with long setae. Mandible (Fig. 7) 1.1 times as long as wide, tooth 2 protruding and pointed, tooth 1 as long as tooth 2 and apically rounded, a right angle between tooth 1 and 2, tooth 3 resembling an intercalar swelling on lower edge of tooth 2, tooth 4 retracted, lower edge slightly bulged downwards, short keels arising from teeth 1 and 4, distal area slightly excavated. Maxillary palpi not longer than the head high. Antennae at most slightly longer than body, 30-segmented, most F equally wide, only the F towards the apex slightly narrower; F1 3 times, F2 2.5 times, F10 2 times, Fp 2 times as long as broad, the F beyond the middle clearly separated from each other, but not very much, the numerous setae shorter than the F width, in lateral view 3 sensillae visible.

Mesosoma: 1.33 times as long as high, upper side very weakly rounded, nearly flat, strongly bent downwards behind. Mesoscutum 1.4 times as broad as long, round on lateral lobes, anteriorly nearly straight, notauli distinct on declivity, crenulate here, passing in a bow into the marginal furrow which is anteriorly crenulate, faintly indicated on disc near to the elongate dorsal fovea, with many setose hollows on declivity, with a row of setae along notauli. Prescutellar fovea deep, crenulate. Axillae with white setae. Postaxillae clearly smooth. Metascutum and propodeum covered with dense, dirty white setae obscuring the surface, spiracles of propodeum on small tubercles. Sides of pronotum without any sculpture, bare. Precoxal furrow narrow, bisinuate, crenulate, shortened behind, reaching the anterior edge, presternal furrow crenulate, posterior mesopleural furrow simple. Metapleuron with central swelling and radiating setae, covered with dirty white pubescence obscuring the surface. Hind femora 5 times as long as wide, hind tarsi as long as hind tibiae.

Wings: st nearly parallel sided, wedge-shaped in distal third, r arising from base of st by a distance equal to length of r1, r2 unevenly bent, nearly straight distally, R ending considerably before tip of wing, nr antefurcal, d scarcely longer than nr, nv postfurcal throughout its width, B open on distal corner; a2 weak, cu1b absent, np very weak, or indicated only as a fold.

Metasoma: T1 (Fig. 8) 1.5 times as long as wide, parallel sided, narrowed near base, dorsal carinae only near base, surface irregularly and densely net-like rugose, the few white setae not obscuring the surface, the setae longer and more numerous on apical corners.

Color: Black. Yellow: base of antennae as far as F1, mouth parts, all legs, tegulae, wing venation and T2+3. Clypeus brownish. Wing membrane hyaline.

Female—Unknown.

Host—Unknown.

Material examined: Holotype: male, SPAIN: Castellón: Almenara, 5-VII-1990. Paratype: SPAIN: Castellón: Almenara, 25-VII-1990, 1 male.

Etymology: The name indicates that the species stands near *Chorebus diremtus* (Nees von Esenbeck, 1834).

Taxonomic position: The species is, according to Griffiths's key (1968b) and Tobias's key (1986), nearest to *Chorebus flavipes* (Goureau, 1851) or *Chorebus diremtus*. The species can be differentiated from each other as follows:

Chorebus diremtus: Mesosoma about 1.45 times as long as high vs. *Chorebus propediremptum*: Mesosoma 1.33 times as long as high.

Chorebus flavipes: (a) sides of pronotum bare and smooth centrally, with a little fine pubescence mainly below the oblique suture; (b) T1 caudally broadened; (c) mesosoma 1.4 times as long as high. Body length: 1.8 mm vs. *Chorebus propediremptum*: (a) sides of pronotum predominantly shiny, without setae; (b) T1 (Fig. 8) parallel sided; (c) mesosoma 1.33 times as long as high.

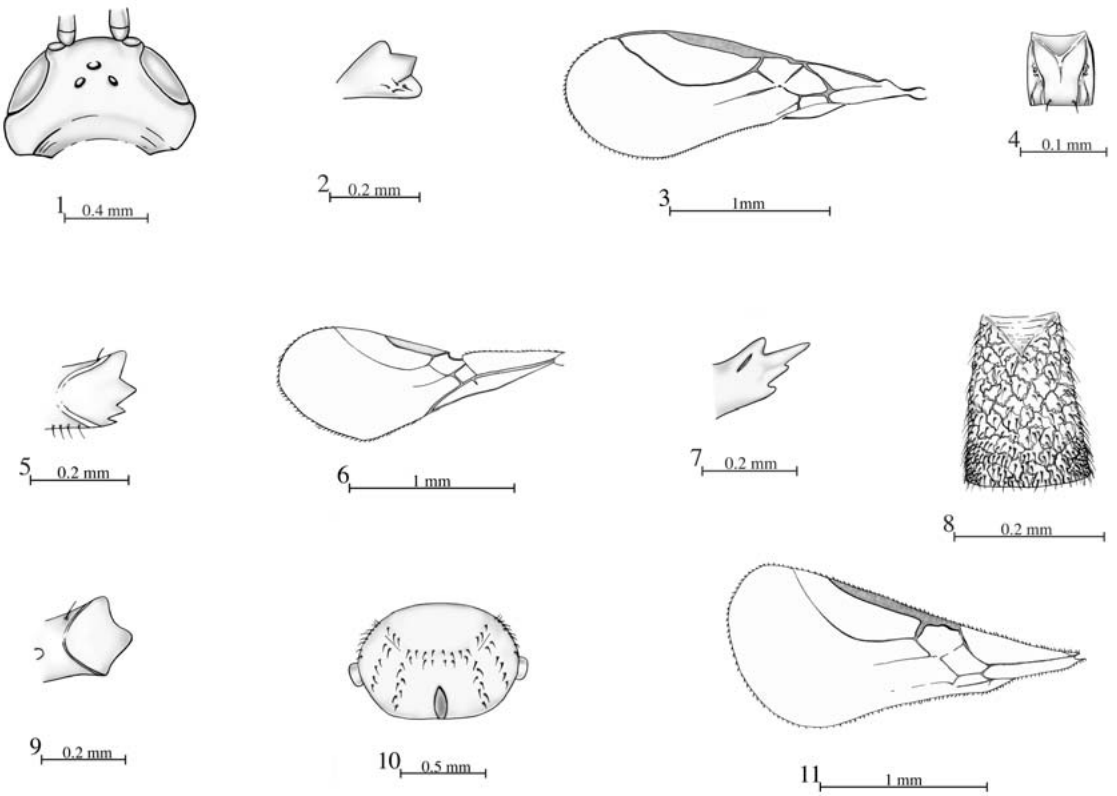
Chorebus vicinus **sp.nov**. (Figs.9-11)
Female—Body length: 1.7 mm.

Head: 1.9 times as wide as long, 1.4 times as wide as mesoscutum, 1.7 times as wide as face, 4 times as wide as T1; eyes as long as temples, behind eyes scarcely wider than between eyes. Upper surface with scattered, very fine setae on the sides, the occiput, and in the ocellar area, seta

points not recognizable. Ocelli small, the distance between them greater than their diameter, epicranial furrow very weak. Face 1.2 times as wide as high, with short, white, not very dense setae, only near eyes a few longer, erect setae, seta points very fine, middle elevation scarcely developed, edges of eyes weakly rounded. Clypeus three times as wide as high, glabrous, trapezoidal, slightly standing out from face. Tentorial pits small. Mandibles (Fig. 9) as long as wide, apically only a little wider than at base, 3-dentate, the teeth blunt and of about equal width, edges rounded between teeth, keels arising from teeth 1 and 3, forming a round lamella, which separates a smooth, inward sloping area distally. Labrum broad, prominent. Palpi of the present example damaged. Antennae slightly longer than body, 24-segmented; F1 4 times as long as wide, F2 as long as F1, the following very gradually shorter, Fm about 2.5 times, Fp twice as long as wide; the F clearly separated from each other, the setae shorter than the F width, in lateral view 3 sensillae recognizable.

Mesosoma: 1.33 times as long as high, upper side arched. Mesoscutum wider than long, round anteriorly, notauli (Fig. 10) only on anterior declivity, upper surface with moderately long, white setae, only areas on side lobes and a small area on the disc bare, seta points especially visible anteriorly, dorsal fovea oval. Prescutellar furrow crenulate at depth. Axillae dirty white, setose. Postaxillae hardly sculptured. Sides of metascutum hidden by white setae. Propodeum densely covered with short white setae hiding the punctate surface, spiracles small. Sides of pronotum bare. Precoxal furrow densely crenulate, shortened behind, reaching the anterior edge of mesopleuron, prepectal furrow narrow, crenulate, passing over to the broader crenulate epicoxal furrow, posterior mesopleural furrow simple. Metapleuron with a rosette of dirty white setae around a central swelling. Hind femora 5 times as long as wide, hind tarsi as long as hind tibiae.

Wings (Fig. 11): st parallel sided, metacarp less than half as long as the distal part of st, r arises from base of st by a distance as long as r1, r2 unevenly curved, R ending long before tip of wing, nr strongly antefurcal, d 1.2 times as long as nr, nv postfurcal throughout its length, B laterally open, cu1b absent, a2 (lower vein of B) grad-



Figures. 1-4. *Antrusa curtitempus* (female). Fig. 1. Head in dorsal view. Fig. 2. Mandible. Fig. 3. Anterior right wing. Fig. 4. T1: First tergite of metasoma. Figures 5-6. *Chorebus liliputanus* (male). Fig. 5. Mandible. Fig. 6. Anterior right wing. Figures 7-8. *Chorebus propediremptum* (male). Fig. 7. Mandible. Fig. 8. T1. Figures 9-11. *Chorebus vicinus* (female). Fig. 9. Mandible. Fig. 10. Notauli. Fig. 11. Anterior right wing.

ually extinct distally, np not developed; r' and cu2' developed only as folds.

Metasoma: T1 twice as long as wide, sides slightly converging anteriorly, rugose all over, evenly covered with white setae, dorsal lamellae short, spiracles on small tubercles. T2 with few scattered setae on basal half. Ovipositor sheaths hardly projecting beyond the apex of metasoma.

Color: Black. Yellow: anellus, mouth parts, all legs, tegulae, and wing venation. Wing membrane hyaline.

Male—Unknown.

Host—Unknown.

Material examined: Holotype: female, SPAIN: Castellón: Alcora, 17-VII-1990. Paratype: SPAIN: Castellón: Alcora, 19-VII-1990, 1 female.

Etymology: The epithet means "neighbor" to indicate the morphological similarity between *Chorebus vicinus* and *Chorebus transversus* (Nixon, 1954).

Taxonomic position: It runs in the key of Griffiths (1968b) and also in the key of Tobias (1986) to *Chorebus transversus*. The latter also has 3-dentate mandibles. The species are separated from each other by several characters:

Chorebus transversus (male, female): Antennae of female 28-36-segmented, of male 34-36-segmented, base of antennae to F2 yellow-brown, the apical F 2.5 times as long as wide. Mandible longer than wide. Sides of pronotum densely setose below and sculptured. Notauli developed as rugose furrows reaching as far as middle of mesoscutum. Metacarp as long as distal part of st.

Chorebus vicinus (female): Antennae of female 24-segmented, dark including the base. The apical F twice as long as wide. Mandibles (Fig. 9) as long as wide. Sides of pronotum bare. Notauli (Fig. 10) present only on anterior declivity, absent on disc. Metacarp (Fig. 11) about half as long as distal part of st.

Remarks: This species is ascribed to the genus *Chorebus* Haliday in spite of the 3-dentate mandibles, because of the dense, nearly dirty white tomentum of the propodeum and the metapleuron (rosette of radiating setae around a central swelling).

Acknowledgments

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FEEDING EFFECTS OF *ISCHNODEMUS VARIEGATUS* (HEMIPTERA: BLISSIDAE) ON PHOTOSYNTHESIS AND GROWTH OF *HYMENACHNE AMPLEXICAULIS* (POACEAE)

WILLIAM A. OVERHOLT[1], SHARON M. L. EWE[2], RODRIGO DIAZ[1], ERIC C. MORGAN[1] AND ONOUR E. MOERI[1]
[1]University of Florida, IFAS, 2199 South Rock Road, Fort Pierce, FL 34945

[2]Florida International University, Southeast Research Center, Miami, FL 33199

ABSTRACT

The influence of *Ischnodemus variegatus* feeding on photosynthesis and growth of the invasive semi-aquatic grass, *Hymenachne amplexicaulis*, was investigated in field and greenhouse environments. In the field, carbon dioxide assimilation of infested plants was approximately 35% less than that of non-infested plants, and the rate of assimilation was related to *I. variegatus* density. The relative growth rate of infested plants in the greenhouse was 77% of that of non-infested plants, and biomass of infested plants was significantly less than for non-infested plants 79 days after infestation. The value of *I. variegatus* as a fortuitous biological control agent of *H. amplexicaulis* is discussed.

Key Words: invasive plants, biological control, photosynthesis, wetlands.

RESUMEN

La influencia de la alimentación de *Ischnodemus variegatus* sobre la fotosíntesis y el desarrollo del pasto invasivo semi-acuatico *Hymenachne amplexicaulis*, fue investigado en ambientes de campo e invernadero. En el campo, la asimilación del dióxido de carbono por plantas infestadas fue aproximadamente 35% menos que en las plantas no infestadas y la tasa de asimilación fue relacionada con la densidad de *I. variegatus*. La tasa de crecimiento relativo de plantas infestadas en el invernadero fue 77% menos que las plantas no infestadas y la biomasa de plantas infestadas fue significativamente menos en plantas no infestadas a los 79 días después de la infestación. Se discute sobre el valor de *I. variegatus* como un agente de control biológico fortuito.

Hymenachne amplexicaulis Rudge (Nees) (Poaceae), commonly referred to as West Indian Marsh Grass, is an exotic, perennial, semi-aquatic grass which was first seen in Florida in 1957 (University of Florida Herbarium 2003). The native range of the grass is tropical Central and South America (Bogman 1977). *Hymenachne amplexicaulis* reproduces and grows from stolons or seeds in areas with fluctuating water levels. It can survive long periods of flooding, but will only persist along the edges of permanent deep water (Tejos 1980). Csurhes et al. (1999) stated that the plant preferred low lying fresh water wetlands and flood plains, and grew most prolifically in wetlands which receive high nutrient and sediment influx.

In the 1970s and 80s, *H. amplexicaulis* began invading wetlands in southern and central Florida (Langeland & Craddock-Burks 1998). The Florida Exotic Pest Plant Council has listed *H. amplexicaulis* as a Category I invasive plant (FLEPPC 2003), and it is included on the Florida Department of Environmental Protection's list of noxious plants (DEP 2003). Although no quantitative studies have yet been conducted to examine the effect of *H. amplexicaulis* on wetland biodiversity, it has clearly displaced native plant species in some areas, particularly in marshes in Myakka River State Park where large monocultures of the grass occur (Langeland & Craddock-Burks 1998; R. Diaz unpublished data).

In 2000, a biologist at Myakka River State Park noticed an insect causing considerable damage to *H. amplexicaulis* in the park (P. Benshoff, Park Naturalist, Myakka River State Park, pers. comm.). Specimens of the insect sent to the Florida Department of Agriculture and Consumer Services were identified as *Ischnodemus variegatus* Signoret (Hemiptera: Blissidae), a new record for Florida (Halbert 2000). Previously, *I. variegatus* had been reported from several countries in Central and South America (Baranowski 1979; Slater 1987). *Hymenachne amplexicaulis* is the only host mentioned for *I. variegatus* in South America, although Baranowski (1979) cites a 'sitting' record on *Thalia geniculata* L. (Marantaceae) from Suriname. The objective of the present study was to quantify the effect of *I. variegatus* feeding on photosynthesis and growth of *H. amplexicaulis*.

Materials and Methods

Field Measurements

A portable infra-red gas exchange system (CIRAS-1, PP Systems, Massachusetts, USA) was used to measure leaf photosynthesis (i.e., net carbon dioxide (CO_2) assimilation). Gas exchange was measured on plants growing along the banks of Fisheating Creek (Glades Co.) (26.95°N, 81.14°W) on the western side of Lake Okeechobee on three days (28/8, 4/9, 18/9) during August and September 2003. On each sampling date, photosynthesis was measured on 27-66 *H. amplexicaulis* plants. In order to increase the chance of having both infested and non-infested plants in the sample, plants were selected according to leaf color. Previous observations (W. Overholt, unpublished data) on laboratory infested plants indicated that feeding by *I. variegatus* induced a change in color of *H. amplexicaulis* leaves from green to dark red. On each sampling date, an approximately equal number of plants were sampled from patches of *H. amplexicaulis* showing no damage symptoms and patches with red leaves. After measuring gas exchange, culms were excised at water level and dissected to count nymphs and adults of *I. variegatus*.

Plants

Stolons and seeds of *H. amplexicaulis* were collected in Myakka River State Park from October to December, 2002, and used to propagate plants in a greenhouse in Fort Pierce. Plants were grown in 1-L plastic pots in commercial potting soil from rooted stolon cuttings or seeds. Pots were placed in solid-bottomed trays, to which water was added and maintained at a depth of 4-6 cm.

Insects

Adults and nymphs of *I. variegatus* were collected in Myakka River State Park and taken to the laboratory. *Hymenachne amplexicaulis* plants (~30 cm in height) were inoculated with 10 *I. variegatus* adults/nymphs. Plants in pots were placed in trays with water, and maintained inside a PVC framed cage covered with fine organdy cloth.

Greenhouse Bioassay

The bioassay was conducted under ambient conditions (22.2-37.2°C, mean temperature of 27.1°C, natural lighting) in a greenhouse at the University of Florida's Indian River Research and Education Center in Fort Pierce. Plants used for the bioassay were grown from seed planted on the same day, and grown under the same conditions for 80 days. All plants were grown in 1-L plastic pots containing commercial potting soil, and at the time of infestation were approximately the same size. Six plants were randomly selected from this group, and infested with 20 *I. variegatus* second instars, and six plants were selected as non-infested controls. In addition, six plants were removed from their pots, dried, and weighed to estimate initial biomass. Infested and non-infested plants were caged in open-bottomed acrylic cylinders (46 cm × 14.5 cm, height × diameter) in which the top was covered with 300 µ mesh nylon cloth. Holes (8.5 cm diameter) were cut at 6 locations in the sides of the tubes and replaced with the same nylon cloth to allow ventilation. Plants and their cages were placed in trays and water was added to a depth of 4-6 cm. Water was replenished daily to maintain this depth throughout the course of the experiment.

Net CO_2 assimilation was measured once or twice a week on the second fully expanded leaf from the top of the plant with the CIRAS-1 instrument. The first measurements were taken just prior to infestation, and the last measurements were made at 79 days after infestation. After 79 days, infested plants were dissected and all *I. variegatus* individuals removed, classified by age (nymphal instars 1-5 or adult), and counted. Eggs were difficult to locate due to their small size and cryptic coloration, and were not counted.

At harvest, the numbers of leaves and culms were counted. Basal diameter of each culm and the length and width of the largest leaf of each plant were measured with a micrometer. All growth medium was then washed off the plants. Leaves were separated from the roots and all leaves digitally photographed. Leaf area was determined from the digital images with ImageJ software (ImageJ shareware, NIH, Bethesda, MD). The proportion of damaged area also was assessed by this method. Plants were then dried in an oven at 65°C for two weeks and weighed to obtain measurements of biomass.

Data Analysis

CO_2 assimilation in the field was analyzed with two-way analysis-of-covariance (ANCOVA) with date and infestation status (infested vs. non-infested, where infested plants had one or more nymphs and/or adults of *I. variegatus*) as main effects and light level as a covariate (PROC GLM, SAS Institute 2001). The covariate was included in the model because light intensity varied greatly among observations, ranging from 502 to 1964 µmol photons $m^{-2}s^{-1}$. The number of *I. variegatus* was also regressed on CO_2 assimilation (PROC REG, SAS Institute 2001). Greenhouse gas exchange was analyzed with repeated measures analysis-of-variance (ANOVA) (PROC GLM, SAS Institute, 2001). We used *t*-tests to compare leaf area, morphometric parameters and growth rates between infested and control plants.

RESULTS

Field Measurements

Mean CO_2 assimilation was different between sampling dates ($F = 53.1$, $df = 2, 132$, $P < 0.0001$) and between infested plants (8.5 ± 0.5 µmol CO_2 $m^{-2}s^{-1}$) and non-infested plants (12.9 ± 0.6 µmol CO_2 $m^{-2}s^{-1}$) ($F = 42.3$, $df = 4, 132$, $P < 0.0001$). The covariate light was also significant ($F = 5.12$, $df = 1, 132$, $P < 0.025$). A regression of CO_2 assimilation on number of insects showed a decline in photosynthesis as insect numbers increased ($F = 21.5$, $df = 1, 135$, $P < 0.0001$, $R^2 = 0.13$) (Fig. 1).

Greenhouse Bioassay

The number of *I. variegatus* found on plants at harvest ranged from 50 to 149, with a mean of 97 ± 13 (SE). Approximately 86% of the individuals removed from the plants were either first (70.1%) or second (15.9%) instars. As the plants were infested with 20 second instars, this represented a population increase of about 2.5 to 7.5 times during the 79-day experiment. A generation of *I. variegatus* is completed in approximately 60 days (R. Diaz, unpublished), and therefore, the insects found at harvest were most likely the F_1 progeny of those released.

Growth patterns of the infested plants were different from those of the control plants (Fig. 2, Table 1). The amount of damage, as quantified by the amount of red leaf area, in the infested plants was higher ($F = 4.722$, $df = 1, 10$, $P < 0.01$) than non-infested plants (Table 1).

The relative growth rate of infested plants was 77% that of the control plants, which resulted in much larger control plants (Table 1). Both root and shoot biomass were greater in the controls relative to the infested plants (roots: $F = 3.437$, $df = 1,10$, $P < 0.01$; shoots: $F = 1.968$, $df = 1,10$, $P < 0.05$) (Fig. 2). On average, control plants were more than 1.5 times larger than the infested plants. Neither infested nor non-infested plants flowered during the experiment, and no plants died.

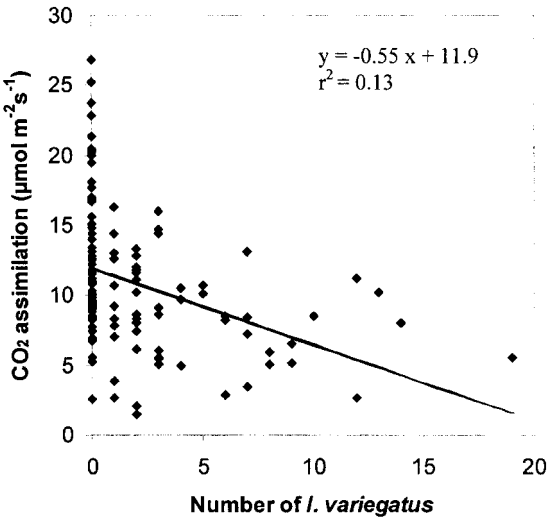

Figure 1. Carbon dioxide assimilation of plants in the field with variable densities of *I. variegatus*.

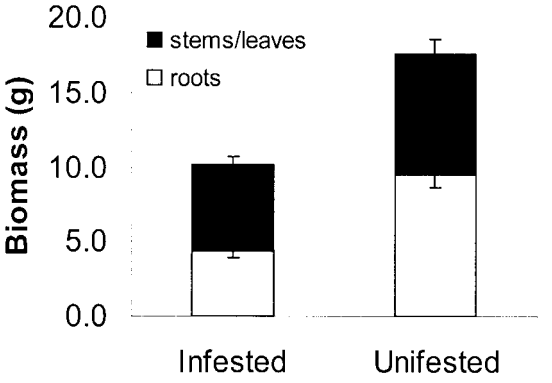

Figure 2. Average biomass (± SEM) allocated to roots and shoots for treatment and control plants.

Morphometric differences were observed between the two treatments. Control plants had larger total leaf area, longer leaves and thicker culms than the infested individuals (Table 1). The number of culms, however, was not different between treatments.

Carbon dioxide assimilation rates were higher in the control plants during the first seven sampling periods (days 4-29), but were not different after day 29 (Fig. 3). Repeated measures ANOVA indicated a difference between treatments ($F = 6.92$, $df = 1,10$, $P < 0.05$, power = 0.660).

DISCUSSION

Feeding by *Ischnodemus variegatus* adults and nymphs clearly affected the overall growth of *H. amplexicaulis*. Both above and below ground parts of infested plants were smaller than those of uninfested plants. Infested plants also had a higher proportion of damaged leaves relative to the controls. In addition to the effect of infestation on biomass, the majority of leaves of infested plants were red or brown at harvest. The reddish discoloration of the leaves, suggestive of a breakdown and/or disruption in the function of plant photosynthetic pigment complexes, is similar to damage symptoms on corn (*Zea mays* L.) and buffalograss (*Buchloë dactyloides* (Nuttall)) caused by feeding of *Blissus* spp. (Hemiptera: Blissidae) (Negron & Riley 1990; Baxendale et al. 1999).

In the greenhouse bioassay, CO_2 assimilation was lower in infested plants for the first 29 days after infestation. It was somewhat surprising

TABLE 1. AVERAGE (± SEM) VALUES OF MEASURED PARAMETERS IN PLANTS WITH *I. VARIEGATUS* AND CONTROL PLANTS. SIGNIFICANT *P*-VALUES FROM *T*-TEST COMPARISONS ARE SHOWN IN BOLD.

	Infested	Control	*P*-value
Leaf area (cm^2):			
Damaged	69 ± 9	17 ± 1	0.000
Undamaged	201 ± 30	441 ± 42	0.001
Total	270 ± 29	458 ± 43	0.005
Largest leaf (cm):			
Length	18.8 ± 0.7	22.3 ± 0.5	0.003
Width	1.2 ± 0.1	1.16 ± 0.0	0.429
Culm:			
Number	6.8 ± 0.4	8.5 ± 1.1	0.171
Thickness (mm)	3.4 ± 0.1	4.0 ± 0.1	0.002
Relative growth rate (g/g/week)[1]	0.167 ± 0.01	0.215 ± 0.01	0.008

[1]Grams biomass gained/grams initial plant biomass/week.

that CO_2 assimilation was not different between infested and non-infested plants from 32 days after infestation until the experiment was terminated on day 79. We suspect that the size of the acrylic tubes covering the plants may have negatively influenced growth. Both infested and non-infested plants grew to the top of the tubes within the first month, after which their growth may have been impaired by the size of the containers. The small chamber size may also explain the color changes in leaves of non-infested plants (Table 1).

Insect herbivores access plant resources either through consumption of foliage or other solid materials (chewing insects) or by ingesting plant sap (piercing/sucking insects). Piercing/sucking insects, such as blissids, feed in phloem, xylem, epidermal, or mesophyll paraenchyma tissues (Walling 2000). Xylem feeding is reported to inhibit photosynthesis more than other types of herbivory (Meyer & Whitlow 1992). Johnson & Knapp (1996) concluded that photosynthetic inhibition of *Spartina falicus* (Link) (Poaceae) caused by *Ischnodemus falicus* (Say) was consistent with xylem feeding. Our field and greenhouse results clearly demonstrated that *I. variegatus* decreased photosynthetic capacity of *H. amplexicaulis*. Although the tissues accessed during feeding were not identified, we are doubtful that this insect is a xylem feeder. Press & Whittaker (1993) stated that there is little evidence to support xylem feeding of insects other than cercopids, cicadids, and some cicadellids. Moreover, we saw no evidence of copious amounts of excreted liquids typically associated with xylem feeding (Press & Whittaker 1993).

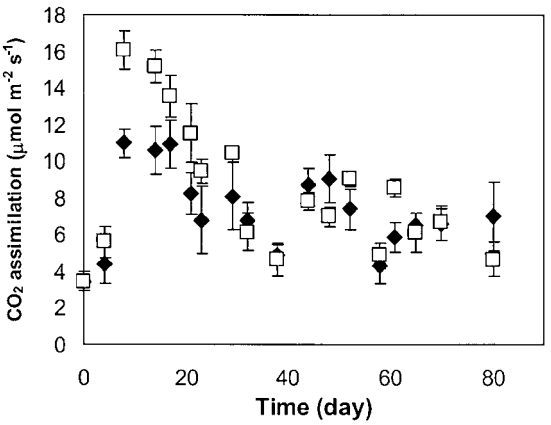

Figure 3. Average rates of carbon dioxide net assimilation (± SEM) in infested (◆) and control (□) plants for each sample day.

Determining the value of *I. variegatus* as a biological control agent of *H. amplexicaulis* requires additional research. The insect undoubtedly affected photosynthesis and plant growth, but even at relatively high initial densities of 20 *I. variegatus* per plant, which increased to an average of 97 insects per plant during the course of the experiment, plants were not killed. Densities of *I. variegatus* monitored in the field in 2002/2003 rarely surpassed 20 insects per plant, and were most often much lower (Overholt, unpublished data). However, in the field, plants are stressed by a variety of factors, including climate, water, soil nutrient levels, pathogens, herbivores, and competition with other plants. *Hymenachne amplexicaulis* has low drought tolerance (Medina & Motta 1990) and thus a combined effect of low water availability and insect damage may have additive negative effects on *H. amplexicaulis* growth. Additionally, *I. variegatus* may influence flowering and seed production, which were not measured in our experiments. A negative impact on seed production could conceivably slow the spread of *H. amplexicaulis* in Florida's wetlands.

Finally, the value of *I. variegatus* to natural resource management and agriculture in Florida will depend on its host range. In South America, *I. variegatus* has been recorded only from *H. amplexicaulis* (Baranowski 1979), and there are no native members of this genus in Florida or the USA (reference). However, no detailed studies of the insect's biology have been conducted. We are currently investigating the host range of *I. variegatus* by measuring survivorship on a large number of native and economically important grasses. If *I. variegatus* is restricted to *Hymenachne*, there is little threat of its shifting to more distantly related grasses (Pemberton 2000).

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THYSANOPTERA RECORDED FROM CALIFORNIA, U.S.A.: A CHECKLIST

MARK S. HODDLE[1], LAURENCE A. MOUND[2] AND SUEO NAKAHARA[3]
[1]Department of Entomology, University of California, Riverside, CA 92521, U.S.A.

[2]Honorary Research Fellow, CSIRO Entomology, GPO Box 1700, Canberra, ACT 2601, Australia
and Scientific Associate, The Natural History Museum, London

[3]Systematic Entomology Laboratory, USDA, Agricultural Research Service, Beltsville, MD 20705-2350, U.S.A.

ABSTRACT

In California U.S.A., 238 named species of the insect Order Thysanoptera, in 87 genera and eight families, are listed as having been found in this state. Inspection of museum collections indicates many undescribed species of thrips exist. Little is known of the host plants of native California thrips species, due to imprecise collecting methods such as sweep netting swaths of mixed vegetation. At least 40 (~17%) of the listed species in California are introduced from other parts of the world. Two terebrantian families (Adiheterothripidae and Fauriellidae), and one genus (*Orothrips*) in a third terebrantian family (Aeolothripidae), have a remarkably disjunct distribution between California and the European Mediterranean region.

RESUMEN

En el estado de California EEUU, estan listadas 238 especies descritas en el ordén de insectos Thysanoptera, pertenecientes a 87 géneros y ocho familias, apuntadas de haber sido encontrada en este estado. Una revisión de las colecciones en los museos indica que existe más especies de trips no descritas. Las plantas hospederas de las especies de trips nativas de California son poco conocidas, debido a los métodos imprecisos de recolectar, tal como pasar una red sobre vegetación mezclada. Por lo menos 40 (~17%) de las especies mencionadas en California son introducidas de otras partes del mundo. Dos familias terebrantianas (Adiheterothripidae y Fauriellidae), y un género (*Orothrips*) en una tercer familia terebrantiana (Aeolothripidae), tienen una distribución desarticulada entre California y la región mediterránea europea.

The foundation publication concerning Thysanoptera diversity was by Heinrich Uzel from Bohemia in 1895. Uzel's (1895) publication was followed by Dudley Moulton's (1907) first paper on Californian thrips at the start of his long career in agriculture in this State. Moulton's taxonomic studies culminated 61 years later in a posthumous publication (Arnaud & Lee 1973). Stanley Bailey, at the University of California, Davis, published extensively on thrips between 1937 and 1968, and encouraged contributions from two students, H. E. Cott (1956) and A. G. Gentile (Gentile & Bailey 1968). Although primarily involved in pest control, Bailey produced several valuable bibliographic treatments concerning the publications of other North American thrips workers. Tokuwo Kono was employed at the California Department of Food and Agriculture in Sacramento to study thrips, aphids and mites. Kono prepared a large slide collection of thrips and published an introduction to the more common species (Kono & Papp 1977). In contrast, William Ewart, a Professor at the University of California, Riverside developed an extensive knowledge of thrips together with a beautifully prepared collection of slide-mounted specimens and library. Unfortunately, Ewart's published contribution on Thysanoptera was negligible.

Given this extensive and prolonged investment of expertise in the study of Thysanoptera in California, it is surprising that as many as 12 new species of Thysanoptera have been described from California in the past 10 years, particularly since the authors involved carried out no new field studies but merely described taxa available in museum collections. It seems that earlier Californian workers lacked the opportunity, or the passion, for the extensive field studies that are so evident in the work of J. D. Hood at Cornell University (Ithaca, New York, U.S.A.). Certainly, the technical expertise of Hood in preparing microscope slides of thrips was rarely matched. The slides of Moulton and Bailey are often inadequate for critical study of structural details. This lack of technical expertise, together with the lack of devotion to field biology, is presumably the origin of the very high synonymy rate of some early workers on this group of insects. Moulton, for example, published 510 species-group names for thrips from around the world, but 178 of these (35%) are now considered synonyms.

The two most recent revisionary studies of the Californian thrips fauna, Bailey (1957) on the Terebrantia and Cott (1956) on the Tubulifera, are now totally inadequate due to the generic classification that they used having been greatly

changed in recent years, and many species now being placed in synonymy. Moreover, neither publication gives any indication of the extraordinary biological interest in these insects, the remarkable biogeographical distributions, the host plant associations, or the seasonality of various species. Both papers are essentially "descriptive taxonomy" in the most pedestrian sense, concerned with statements of fact with no attempt to convey the fascination of the biological diversity exhibited by this group of insects.

The checklist provided here is intended to lay the foundations for a modern approach to the study of California Thysanoptera. The insect fauna of this state is diverse, as is the range of ecosystems that thrips inhabit. But the lack of adequate field surveys for thrips in different ecosystems and at appropriate times of year results in it being impossible to gauge how accurately this list reflects the native and exotic thrips fauna. Despite this, the substantial number of sorted but unidentified species in the Ewart Collection at the University of California at Riverside suggests that the Thysanoptera of California is considerably richer than the following list might suggest.

Host Plant Relationships

The host plant relationships of Californian thrips are poorly known, many species being known from too few specimens for any serious information to be available on their host plants and seasonality. Three of the species of *Dactuliothrips* and both species of *Orothrips* are known from the flowers of *Ceanothus* (Rhamnaceae), but whether these species breed in (or even only in) these flowers is unclear. In many species of thrips, even wingless adults may be transported readily by winds, and adults can be taken commonly from plants on which they are not able to breed. Many of the "host plants" recorded in the published literature cannot be relied upon as indicating that a thrips species can reproduce on a particular plant, let alone that it is dependent on that plant species to maintain its populations. It is essential to find and recognize the larvae of thrips species, and to collect repetitively at different localities, in order to establish the biological dependence of such insects on particular plant species.

Two genera listed below, *Aeolothrips* and *Leptothrips*, involve species that are presumed to be predatory, but it seems unlikely that so many closely related predatory species should co-exist in this area. If these species prove to be both valid and obligate predators, then niche apportionment among them will be an interesting field for study. However, it seems likely that at least some of the *Aeolothrips* species are actually phytophagous, and probably facultative predators with some level of host-specificity.

Biogeographic Relationships

The thrips fauna of the southwestern U.S.A. includes taxa with distributions that are remarkably disjunct. *Orothrips* in the Aeolothripidae is known from three species; one from European Mediterranean countries and two from California. The Adiheterothripidae includes three genera, one from the eastern Mediterranean to India and two from California. The Fauriellidae includes four genera, one Mediterranean, one Californian, and two from southeastern Africa. At least 17% of the species in this checklist appear to be non-native to California, and most of the native species appear to be found in the southern and eastern areas of the state. However, the available locality information and "host plant" records for collected species are not adequate to make any broad generalizations concerning the thrips fauna of California.

Thrips Collections in California

Several of the most important reference collections of world Thysanoptera are housed within California research institutes. One of the smallest collections, but in terms of the quality of slide-mounted material, the most useful, is the Ewart Collection at the University of California at Riverside. The Ewart Collection has approximately 450 named species and many flagged and group-organized but unnamed species from California. The Moulton Collection at the Californian Academy of Sciences, San Francisco, is particularly rich, with type material of 640 species and over 25,000 slides. The Bailey Collection at the University of California at Davis includes representatives of almost 750 named species. The Cott Collection at the University of California at Berkeley has about 170 species. Finally, the collection at the California Department of Food and Agriculture, Sacramento California, largely developed by Kono, has about 630 species.

Checklist

Although based largely on published literature, this list includes a considerable number of previously unpublished State records, derived primarily from the Ewart Collection at the University of California at Riverside, but also from the United States Museum of Natural History collections, held at USDA, Beltsville, Maryland. Some of the literature records seem likely to involve misidentifications, particularly that of *Oxythrips quercicola*, and some of the museum records are based on few and old specimens, eg. *Frankliniella tritici* and *Fr. williamsi*. Other problems for future work include the currently unsatisfactory distinctions among several genera of leaf-feeding phlaeothripine species, e.g., *Liothrips*, *Rhynchothrips*

and *Pseudophilothrips*. The objective of this list is to provide a starting point for further work.

Within the Order Thysanoptera, two suborders are recognized, Terebrantia and Tubulifera, and both of these major groups are well represented in California. The Terebrantia includes eight families worldwide, of which only the monotypic, tropical, Uzelothripidae has not been found in California. The Tubulifera includes a single large family, the Phlaeothripidae, with two subfamilies, the Idolothripinae and Phlaeothripinae, both of which are represented in California.

In the checklist below, an asterisk (*) indicates species that presumably are not native to California. The original generic combination, when this differs from the current one, is indicated in square brackets [ ] after a species entry.

Merothripidae

This family of three genera, with about 15 fungus-feeding species that live on dead twigs and in leaf-duff, is found mainly in the Neotropics.

Merothrips Hood, 1912: 132
- *floridensis* Watson, 1927: 60
- *morgani* Hood, 1912: 132

Melanthripidae

The four genera now placed in this family until recently were considered to be members of the Aeolothripidae. They are found in the northern and southern hemisphere temperate regions, and include a total of about 65 flower-feeding species.

Ankothrips Crawford, 1909: 100
- *aequalis* Moulton, 1926: 20
- *gracilis* Moulton, 1926: 19
- *notabilis* Bailey, 1940: 101
- *robustus* Crawford, 1909: 100
- *yuccae* Moulton, 1926: 119

Melanthrips Haliday, 1836: 450
- *digitus* Bailey, 1954: 79

Adiheterothripidae

The three genera in this family have a remarkably disjunct distribution, one with four species breeding in the flowers of date palms in western Asia, and the two listed here from California.

Heratythrips Mound & Marullo, 1998: 88
- *sauli* Mound & Marullo, 1998: 89

Oligothrips Moulton, 1933: 139
- *oreios* Moulton, 1933: 139

Fauriellidae

Four genera and five species are placed in this family, one Mediterranean with two species, and two from southeastern Africa together with the one listed here.

Parrellathrips Mound & Marullo, 1998: 83
- *ullmani* Mound & Marullo, 1998: 85

Heterothripidae

The four genera and 70 species recognized in this family are all from the New World. These species are almost all flower-feeding, 65 of them being placed in *Heterothrips*, but one Brazilian species that is placed in a monobasic genus is ectoparasitic on an homopteran.

Heterothrips Hood, 1908: 361
- *pectinifer* Hood, 1915: 5
- *prosopidis* Crawford, 1943: 93
- *salicis* Shull, 1909: 220
- *vitifloridus* Bailey & Cott, 1954: 616

Aeolothripidae

Most of the 190 species in this family of about 23 genera are found in the temperate areas of the northern and southern hemispheres, although most of the genera are from tropical countries. The tropical species, in genera such as *Franklinothrips* and *Stomatothrips*, are mainly obligate predators of other arthropods, whereas most of the temperate area species in the genus *Aeolothrips* are flower-living facultative predators.

Aeolothrips Haliday, 1836: 451
- *albicinctus* Haliday, 1836: 451
- *auricestus* Treherne, 1919: 184
- *brevicauda* Hood, 1935: 105
- *brunneipictus* Bailey, 1951: 53
- *clarus* Bailey, 1951: 53
- **collaris* Priesner, 1919: 119
- *crucifer* Hood, 1935: 104
- *duvali* Moulton, 1927: 186
- **ericae* Bagnall, 1920: 60
- *fasciatus* (Linnaeus), 1758: 266 [*Thrips*]
- *fuscus* Watson, 1931: 340
- *hartleyi* Moulton, 1927: 185
- *hesperus* Bailey, 1951: 58
- *kuwanaii* Moulton, 1907: 47
- **melaleucus* (Haliday), 1852: 1117 [*Coleothrips*]
- *metacrucifer* Bailey, 1951: 61
- *montanus* Bailey, 1951: 62
- *nasturtii* Jones, 1921: 2
- *nitidus* Moulton, 1946: 59
- *occidentalis* Bailey, 1951: 63
- *terrestris* Bailey, 1951: 64
- *vittipennis* Hood, 1912: 129

Dactuliothrips Moulton, 1931: 173
- *boharti* Bailey, 1937: 122
- *diversus* Bailey, 1939: 170
- *spinosus* Moulton, 1931: 174
- *xerophilus* Bailey, 1937: 122

Erythrothrips Moulton, 1911: 34
arizonae Moulton, 1911: 21
fasciculatus Moulton, 1929: 224
keeni Moulton, 1929: 226

Franklinothrips Back, 1912: 75
orizabensis Johansen, 1974: 249
vespiformis Crawford, 1909: 109

Orothrips Moulton, 1907: 45
kelloggii Moulton, 1907: 43
yosemetii Moulton, 1911: 13

Rhipidothrips Uzel, 1895: 66
**brunneus* Williams, 1913: 216
**gratiosus* Uzel, 1895: 46

Stomatothrips Hood, 1912: 63
flavus Hood, 1912: 64

Thripidae

More than 2000 species in 290 genera are placed in this family worldwide. Most of them are phytophagous on higher plants, with a few species on ferns. A few species are obligate predators (e.g., *Scolothrips sexmaculatus*), but some polyphagous pest thrips can behave as facultative predators (e.g., *Frankliniella occidentalis*). Four subfamilies within the Thripidae are currently recognized worldwide, and each of these is represented in California.

Thripidae—Panchaetothripinae

Wilson (1975) provided an account of the members of this subfamily that is now considered to include 125 species in 35 genera. The name *Hercothrips* occurs in earlier literature in California, but this is a synonym of *Caliothrips*.

Caliothrips Daniel, 1904: 296
fasciatus (Pergande), 1895: 391 [*Heliothrips*]
marginipennis (Hood), 1912: 136 [*Heliothrips*]
(*bromi* Moulton, 1927: 31 [*Heliothrips*])
phaseoli (Hood), 1912: 113 [*Heliothrips*]

Heliothrips Haliday, 1836: 443
**haemorrhoidalis* (Bouche) 1833: 42 [*Thrips*]

Hercinothrips Bagnall, 1932: 506
**femoralis* (Reuter), 1891: 166 [*Heliothrips*]

Monilothrips Moulton, 1929: 93
**kempi* Moulton, 1929: 94

Parthenothrips Uzel, 1895: 170
**dracaenae* (Heeger), 1854: 365 [*Heliothrips*]

Thripidae—Dendrothripinae

More than 90 species, in 10 genera, are recognized worldwide in this subfamily. All of the species live on young leaves, and they are usually small and jump when disturbed.

Asprothrips Crawford, 1938: 109
**seminigricornis* (Girault), 1926: 2 [*Euthrips*]

Dendrothrips Uzel, 1895: 159
**ornatus* (Jablonowski), 1894: 93 [*Thrips*]

Leucothrips Reuter, 1904: 107
furcatus Hood, 1931: 153
**nigripennis* Reuter, 1904: 108
piercei (Morgan), 1913: 19 [*Microthrips*]

Thripidae—Sericothripinae

This subfamily includes worldwide at least 90 species in 10 genera. Most of these genera are subdivisions of the genus *Sericothrips* that have been recognized relatively recently. Moreover, in contrast to earlier authors, the genus *Scirtothrips* is not considered now to be related to *Sericothrips*. The species are all phytophagous in flowers and on leaves.

Neohydatothrips John, 1929: 33
albus (Jones), 1912: 6 [*Sericothrips*]
catenatus (Hood), 1957: 51 [*Sericothrips*]
collaris (Hood), 1936: 91 [*Sericothrips*]
chrysothamni (Hood), 1936: 85 [*Sericothrips*]
moultoni (Jones), 1912: 7 [*Sericothrips*]
opuntiae (Hood), 1936: 88 [*Sericothrips*]
setosus (Hood), 1927: 135 [*Sericothrips*]
variabilis (Beach), 1896: 220 [*Thrips*]

Thripidae—Thripinae

This is a large group of over 1700 species in 235 genera, although almost 50% of these genera remain monotypic. The species exhibit a wide range of biologies, and most of the pest thrips are included in this subfamily.

Anaphothrips Uzel, 1895: 142
**obscurus* (Muller), 1776: 96 [*Thrips*]

Apterothrips Bagnall, 1908: 185
apteris (Daniel), 1904: 295 [*Sericothrips*]
(=*stanfordi* Moulton 1907: 43 [*Sericothrips*])
secticornis Trybom, 1896: 620 [*Thrips*]

Aptinothrips Haliday, 1836: 445
**rufus* (Haliday), 1836: 445 [*Thrips*]
**stylifer* Trybom, 1894: 43

Arorathrips Bhatti, 1990: 194
mexicanus (Crawford), 1909: 114 [*Chirothrips*]
spiniceps (Hood), 1915: 12 [*Chirothrips*]

Arpediothrips Hood, 1927: 197
mojave Hood, 1927: 198

Baileyothrips Kono & O'Neill, 1964: 1
arizonensis (Morgan), 1913: 12 [*Anaphothrips*]
(=*minutus* Moulton: 1929, 127 [*Anaphothrips*])

Bregmatothrips Hood, 1912: 66
venustus Hood, 1912: 67
(=*sonorensis* Stannard, 1956: 71)

Chaetanaphothrips Priesner, 1926: 204
**orchidii* (Moulton), 1907: 52 [*Euthrips*]

Chilothrips Hood, 1916: 119
occidentalis Stannard, 1973: 110
pini Hood, 1916: 120
rotrameli Stannard, 1973: 114

Chirothrips Haliday, 1936: 444
**aculeatus* Bagnall, 1927: 567
falsus Priesner, 1925: 312
**manicatus* (Haliday), 1836: 444 [*Thrips*]
patruelis Hood, 1940: 550
secalis Moulton, 1936: 173
simplex Hood, 1927: 128

Drepanothrips Uzel, 1895: 213
**reuteri* Uzel, 1895: 213

Echinothrips Moulton, 1911: 37
americanus Morgan, 1913: 14

Ewartithrips Nakahara, 1996: 233
californicus Nakahara, 1996: 236
dispar Nakahara, 1996: 239
ehrhornii (Moulton), 1907: 52 [*Euthrips*]
flavidus Nakahara, 1996: 244
longirostrum (Jones), 1912: 10 [*Euthrips*]
salviae Nakahara, 1996: 248

Frankliniella Karny, 1910: 46
davidsoni Sakimura & O'Neill, 1979
deserticola Sakimura & O'Neill, 1979
ewarti Sakimura & O'Neill, 1979
fusca (Hinds), 1902: 154 [*Euthrips*]
fuscicauda Hood, 1927: 197
gossypiana Hood, 1936: 68
insignis Moulton, 1936: 170
insularis (Franklin), 1908: 715 [*Euthrips*]
minuta (Moulton), 1907: 56 [*Euthrips*]
occidentalis (Pergande), 1895: 393 [*Euthrips*]
(=*conspicua* Moulton, 1935: 173)
tenuicornis (Uzel), 1895: 99 [*Physopus*]
tritici (Fitch), 1855: 385 [*Euthrips*]
tuttlei Sakimura & O'Neill, 1979: 30
williamsi Hood, 1915: 19
yuccae Moulton, 1936: 171

Kurtomathrips Moulton, 1927: 187
morrilli Moulton, 1927; 188

Limothrips Haliday, 1836: 444
**angulicornis* Jablonowski, 1894: 45
**cerealium* (Haliday), 1836: 445 [*Thrips*}

Microcephalothrips Bagnall, 1926: 113
**abdominalis* (Crawford), 1910: 157 [*Thrips*]

Mycterothrips Trybom, 1910: 158
albus (Moulton), 1911: 39 [*Euthrips*]
(=*corni* Moulton, 1927: 34 [*Rhopalandrothrips*])
(=*albipennis* Moulton, 1929: 129 [*Taeniothrips*])
aureus (Moulton), 1946: 59 [*Taeniothrips*]

Odontanaphothrips Moulton, 1926: 24
tricolor (Moulton), 1911: 17 [*Anaphothrips*]

Odontothrips Amyot & Serville, 1843: 642
**loti* (Haliday), 1852: 1108 [*Thrips*]

Oxythrips Uzel, 1895: 133
**quercicola* Bagnall, 1926: 282

Plesiothrips Hood, 1915: 129
perplexus (Beach), 1897: 217 [*Sericothrips*]

Proscirtothrips Karny, 1921: 237
zeae (Moulton), 1911: 28 [*Anaphothrips*]
(=*longipennis* Crawford, 1910: 150
[*Anapho thrips*])

Prosopoanaphothrips Moulton, 1926; 22
reticulatus (Moulton), 1907: 50 [*Sericothrips*]

Pseudanaphothrips Karny, 1921: 242
**achaetus* (Bagnall), 1916: 398 [*Pseudothrips*]

Psilothrips Hood, 1927: 198
pardalotus Hood, 1927: 198
priesneri (Moulton), 1926: 123 [*Anaphothrips*]

Scirtothrips Shull, 1909: 222
aceri Moulton, 1926: 122
albus (Jones), 1912: 15 [*Anaphothrips*]
citri (Moulton), 1909: 119 [*Euthrips*]
ewarti Bailey, 1964: 341
**inermis* Priesner, 1933: 186
**longipennis* (Bagnall), 1909: 173 [*Euthrips*]
**perseae* Nakahara, 1997: 189
solaris, Bailey, 1964: 344
tehachapi Bailey, 1964: 345

Scolothrips Hinds, 1902: 157
**longicornis* Priesner, 1926: 239
pallidus (Beach), 1896: 226 [*Thrips*]
sexmaculatus (Pergande), 1890: 539 [*Thrips*]

Taeniothrips Amyot & Serville, 1843: 644
**inconsequens* (Uzel), 1895: 117 [*Physopus*]
orionis Treherne, 1924: 86

Tenothrips Bhatti, 1967: 18
**frici* (Uzel), 1895: 126 [*Physopus*]

Thrips Linnaeus, 1758: 457
albogilvus Nakahara, 1994: 28
**australis* (Bagnall), 1915: 592 [*Isoneurothrips*]
brevipilosus Moulton, 1927: 194
graminae Moulton, 1936: 106
**hawaiiensis* (Morgan), 1913: 3 [*Euthrips*]
helvolus Nakahara, 1994: 67
heraclei Moulton, 1926: 25
konoi Nakahara, 1994: 77
madronii Moulton, 1907: 57
magnus Moulton, 1911: 36
**nigropilosus* Uzel, 1895: 198
paramadronii Nakahara, 1994: 97

pruni Nakahara, 1994: 105
sierrensis Gentile & Bailey, 1968: 45
**simplex* (Morison), 1930: 12 [*Physothrips*]
**tabaci* Lindeman, 1888: 61
**trehernei* Priesner, 1927: 356
(=*hukkineni* Priesner, 1937: 108)
**vulgatissimus* Haliday, 1836: 447
(=*lemanis* Treherne, 1924: 87)

Toxonothrips Moulton, 1927: 30
gramineae Moulton, 1927: 30

Trichromothrips Priesner, 1930: 9
**cyperaceae* (Bianchi), 1945: 283 [*Taeniothrips*]
**xanthius* (Williams), 1917: 59 [*Physothrips*]

Xerothrips Nakahara, 1996: 209
dissimilis Nakahara, 1996: 210

Phlaeothripidae

This is the only family recognized in the Tubulifera and includes more than 3200 species worldwide, primarily in the warmer parts of the world. Two subfamilies are recognized, and both are well represented in California.

Phlaeothripidae—Idolothripinae

The smaller of the two subfamilies includes at least 700 species in about 80 genera, mainly in tropical countries. All of these species feed by imbibing whole fungal spores, as is evident from their gut contents. The larger species can be particularly common on dead leaves that remain hanging on broken branches, but many smaller species live on the ground in leaf duff.

Allothrips Hood, 1908: 372
aureus Stannard, 1955: 155

Bactrothrips Karny, 1912: 131
hesperus (Moulton), 1907: 65 [*Megalothrips*]

Bolothrips Priesner, 1926: 90
rachiphilus Cott, 1956: 181

Compsothrips Reuter, 1901; 214
hookeri (Hood), 1916: 64 [*Oedaleothrips*]
jacksoni (Hood), 1925: 137 [*Oedaleothrips*]
tristis (Cott), 1956: 186 [*Oedaleothrips*]
yosemitae (Moulton), 1929: 135 [*Formicothrips*]

Cryptothrips Uzel, 1895: 228
carbonarius Hood, 1908: 376
rectangularis Hood, 1908: 307
sordidatus Hood, 1927: 199

Megalothrips Uzel, 1895; 224
picticornis Hood, 1927: 204

Megathrips Targioni-Tozzetti, 1881: 124
timidus Cott, 1956: 177

Priesneriella Hood, 1927: 198
citricauda Hood, 1927: 199

Phlaeothripidae—Phlaeothripinae

Worldwide, more than 2500 species in 350 genera are placed in this subfamily, although 50% of these genera remain monotypic. Probably about half of the species are fungus-feeding on dead wood or in leaf duff. However, species of a few genera live in flowers, and a large number of tropical species are leaf-feeding, some inducing galls.

Acanthothrips Uzel, 1895: 259
albivittatus Hood, 1908: 374
argentifer (Cott), 1956: 141 [*Notothrips*]
nodicornis (Reuter), 1880: 16 [*Phloeothrips*]

Adraneothrips Hood, 1925: 54
ephippium Stannard, 1956: 24
faustus Stannard, 1956: 21
saturatus Cott, 1956: 82
vacuus Stannard, 1956: 23

Amynothrips O'Neill, 1968: 175
**andersoni* O'Neill, 1968: 179

Bagnalliella Karny, 1920: 41
desertae Hood, 1927: 201
mojave Hood, 1927: 200
yuccae (Hinds), 1902: 194 [*Cephalothrips*]

Cephalothrips Uzel, 1895: 244
hesperus Hood, 1941: 197
**monilicornis* (Reuter), 1880: 21 [*Phloeothrips*]

Goniothrips Hood, 1927: 202
denticornis Hood, 1927: 202

Gynaikothrips Zimmermann, 1900: 13
**ficorum* Marchal, 1908: 252 [*Phloeothrips*]

Haplothrips Amyot & Serville, 1843: 640
halophilus Hood, 1915: 29
**leucanthemi* (Schrank), 1781: 298 [*Thrips*]
(=*niger* Osborn, 1883: 154 [*Phloeothrips*])
malifloris Hood, 1916: 121
**robustus* Bagnall, 1918: 209
ruber (Moulton), 1911: 42 [*Trichothrips*]
**verbasci* (Osborn), 1897: 228 [*Phloeothrips*]

Hoplandrothrips Hood, 1912: 145
armiger (Jones), 1912: 23 [*Phloeothrips*]
costano Hood, 1942: 567
lissonotus Hood, 1942: 561
salicacearum Hood, 1942: 564

Hoplothrips Amyot & Serville, 1843: 640
baileyi Cott, 1956: 40

Karnyothrips Watson, 1923: 23
flavipes (Jones), 1912: 18 [*Anthothrips*]
longiceps (Hood), 1908: 364 [*Zygothrips*]

Leptothrips Hood, 1909: 249
distalis (Hood), 1925: 103 [*Haplothrips*]
fasciculatus (Crawford), 1909: 105 [*Phyllothrips*]
heliomanes Hood, 1927: 202
larreae Hood, 1939: 207

mali (Fitch), 1855: 807 [*Phloeothrips*]
oribates Hood, 1939: 205
purpuratus (Hood), 1925: 101 [*Haplothrips*]

Liothrips Uzel, 1895: 261 (=*Rhynchothrips*)
brevitubus Kono, 1964: 4
corni Moulton, 1926: 124
cunctans (Cott), 1956: 68 [*Rhynchothrips*]
dumosa (Moulton), 1907: 3 [*Trichothrips*]
eremicus Cott, 1956: 60
gaviotae (Moulton), 1929: 132 [*Haplothrips*]
ilex (Moulton), 1907: 62 [*Trichothrips*]
invisus (Cott), 1956: 65 [*Rhynchothrips*]
lepidus Cott, 1956: 62
monoensis Kono, 1964: 6
**vaneeckei* Priesner, 1920: 211
varicornis Hood, 1912: 74
xanthocerus Hood, 1927: 203

Macrophthalmothrips Karny, 1922: 34
**argus* (Karny), 1920: 38 [*Ophthalmothrips*]

Neurothrips Hood, 1924: 315
apache Hood, 1957: 58
magnafemoralis (Hinds), 1902: 199 [*Acanthothrips*]

Phlaeothrips Haliday, 1836: 442
karnyi Hood, 1914: 20 [*Trichothrips*]
**coriaceus* Haliday, 1836: 442

Plectrothrips Hood, 1908: 370
crocatus Cott, 1956: 80

Poecilothrips Uzel, 1895: 264
**albopictus* Uzel, 1895: 264
dens (Moulton), 1907: 60 [*Trichothrips*]

Scopaeothrips Hood, 1912: 70 (=*Rhopalothrips*)
bicolor (Hood), 1912: 72 [*Rhopalothrips*]

Stephanothrips Trybom, 1912: 42
bradleyi Hood, 1927: 204

Stictothrips Hood, 1925: 295
maculatus (Hood), 1909: 250 [*Phloeothrips*]

Trachythrips Hood, 1930: 317
astutus Cott, 1956: 196

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MORTALITY OF ANT (HYMENOPTERA: FORMICIDAE) PEST SPECIES EXPOSED TO SODIUM HYDROGEN CARBONATE

MARK A. BRINKMAN AND WAYNE A. GARDNER
Department of Entomology, University of Georgia, College of Agricultural and Environmental Sciences
Griffin Campus, 1109 Experiment Street, Griffin, GA 30223-1797 USA

ABSTRACT

Laboratory bioassays enabled us to determine the mortality of Argentine ant (*Linepithema humile* [Mayr]) workers, and red imported fire ant (*Solenopsis invicta* Buren) workers exposed to sodium hydrogen carbonate ($NaHCO_3$, sodium bicarbonate). The median lethal concentration (LC_{50}) of $NaHCO_3$ for Argentine ants was 5.64 mg per cm^2 after 5 d exposure and 3.96 mg per cm^2 after 6 d. Cumulative mortality for Argentine ants exposed to 28 mg $NaHCO_3$ per cm^2 was 89.5% on day 6. Workers of both species were exposed to concentrations of 9.92, 17.70, or 152.00 mg $NaHCO_3$ per cm^2 in separate tests. Mortality of Argentine ants was significantly higher than that of fire ants following exposure to 9.92 mg $NaHCO_3$ per cm^2, while mortality for the two species did not differ following exposure to the two higher concentrations. Mortality of both species treated with the highest concentration exceeded 99% at 6 d. In tests with equivalent amounts of sodium in $NaHCO_3$ and NaCl treatments, mortality for fire ants exposed to $NaHCO_3$ was about 46% after 6 d. Mortality for fire ants exposed to NaCl was about 15% and was similar to that for untreated ants. Argentine ants were provided sugar water baits containing a range of $NaHCO_3$ concentrations. Argentine ant mortality after 6 d exposure to 5% $NaHCO_3$-sugar water treatment was about 50%. Mortality was not higher for workers exposed to higher concentrations of $NaHCO_3$ in sugar water baits. Enzymatic dysfunction caused by unfavorable increases in internal pH is the most likely explanation for worker mortality following exposure to $NaHCO_3$.

Key Words: Sodium bicarbonate, bicarbonate of soda, Argentine ant, red imported fire ant, pest ant, laboratory bioassays, Gut pH.

RESUMEN

Los bioensayos del laboratorio nos permiten determinar la mortalidad de las trabajadoras de la hormiga Argentina (*Linepithema humile* [Mayr]), y la hormiga de fuego roja importada, (*Solenopsis invicta* Buren) expuestas al hidróxido carbonato del sodio ($NaHCO_3$, hidrogenocarbonato del sodio). La concentración media letal (LC_{50}) del $NaHCO_3$ para la hormiga Argentina fue 5.64 mg por cm^2 después de exponerlas por 5 dias y fue 3.96 mg por cm^2 después de 6 dias. La mortalidad acumulativa de las hormigas Argentinas expuestas a 28 mg de $NaHCO_3$ por cm^2 fue 89.5% en el día 6. Las trabajadoras de ambas especies fueron expuestas a concentraciones de 9.92, 17.70, o 152.00 mg de $NaHCO_3$ por cm^2 en pruebas separadas. La mortalidad de las hormigas Argentinas fue significativamente más alta que la mortalidad de las hormigas de fuego roja importada después de exponerlas al 9.92 mg de $NaHCO_3$ por cm^2, mientras que la mortalidad de las dos especies no fue diferente después de exponerlas a las dos concentraciones más altas. La mortalidad de ambas especies tratadas con la más alta concentración alcanzo el 99% al dia 6. En pruebas con cantidades equivalentes de sodio en los tratamientos de $NaHCO_3$ y NaCl, la mortalidad de las hormigas de fuego roja importadas expuestas al NaCl fue aproximadamente 15% y fue similar a la mortalidad en las hormigas no tratadas. Las hormigas Argentinas fueron proveidas con cebos de agua azucarada que tenian varias concentraciones de $NaHCO_3$. La mortalidad de la hormiga Argentina después de exponerlas por 6 dias al tratamiento del agua azucarada con 5% de $NaHCO_3$ fue aproximádamente 50%. La mortalidad no fue mas alta para las trabajadoras expuestas al concentraciones mas altas de $NaHCO_3$ en cebos de agua azucarada. La disfunción enzimática causada por aumentos no favorables en el pH es la explicación mas probable para la mortalidad de las trabajadoras después de exponerlas al $NaHCO_3$.

The Argentine ant, *Linepithema humile* (Mayr), and red imported fire ant, *Solenopsis invicta* Buren, are indigenous to South America. Both have become important pests in urban and agricultural areas in the southern United States (Callcott & Collins 1996; Suarez et al. 1999). Fire ants infest lawns and are nuisances as well as dangerous pests because of their aggressive behavior and sting. In surveys of South Carolina residents conducted by Lemke & Kissam (1989), 87% of respondents felt that they had a severe fire ant problem on their property, and 89% reported having one or more members of their immediate family stung by fire ants. Although Argentine

ants do not sting humans and livestock, they are considered a nuisance pest because they invade homes in search of food and nesting sites. Argentine ants are opportunistic feeders and will forage in garbage receptacles and pet food dishes (Rust et al. 2003).

Ant control in urban environments usually is accomplished with chemical insecticides (Pereira & Stimac 1997). Argentine ant control strategies have focused on the use of baits and the application of contact and barrier sprays and granules (Rust et al. 2003); however, most toxic or repellent barriers fail to provide long-term control, and commercial baits are not always accepted by foraging Argentine ants (Rust et al. 2003). Retreatments are often necessary, adding to the expense of ant control. Many homeowners find extensive use of insecticides in and around the home undesirable. Therefore, additional control methods with low toxicity are needed for urban pest ant management (Klotz et al. 1997a).

Brinkman et al. (2004) previously determined that *S. invicta* workers were susceptible to sodium hydrogen carbonate ($NaHCO_3$, also known as sodium bicarbonate) placed on surfaces and in liquid baits. Workers were not repelled by concentrations of $NaHCO_3$, and mortality was over 78% in treated arenas with liquid bait. They further reported that the median lethal concentration (LC_{50}) decreased from 9.66 mg per cm^2 on day 5 to 8.16 mg per cm^2 on day 6. Vinson (1970) tested the preferences of fire ants (*S. richteri* Forel) for various electrolytes (including $NaHCO_3$) in solution, but did not report on potential mortality following ingestion of those electrolytes. If effective against *S. invicta* and Argentine ants, $NaHCO_3$ could prove to be a safe alternative to conventional insecticides. The objective of this research, therefore, was to compare the mortality of Argentine ants and red imported fire ants after exposure to $NaHCO_3$ in simultaneous laboratory tests.

Materials and Methods

Fire ant workers used in this study were obtained from monogyne field populations in Griffin, GA (Spalding Co.), and were removed from soil by procedures described by Jouvenaz et al. (1977). Argentine ants were collected from nests in logs and leaf litter on the Georgia Experiment Station campus. Although these ants were collected from different areas on the campus, they likely belonged to the same unicolony (Giraud 2002). These laboratory colonies were maintained in plastic trays containing artificial nests constructed of plastic Petri dishes (150 × 10 mm) with dental plaster on the bottom to maintain moisture (Stimac et al. 1993). Fluon® (Northern Products Inc., Woonsocket, RI) was applied to the inside walls of trays to prevent ant escape. Ants were fed 10% sugar water (v/v) and tuna in oil.

The LC_{50} of $NaHCO_3$ against Argentine ants was established in laboratory bioassays. Test arenas were clear 35-ml plastic cups. Each cup had a 5-mm diam hole in the bottom and contained dental plaster to about 10% of total cup volume. Lids for the cups were plastic and had a 1.2-cm diam hole to allow for air exchange. Fluon was applied to the inside walls of cups and undersides of lids. Ten cups were randomly assigned to each of the treatments.

Treatment concentrations were 0, 0.85, 1.7, 3.5, 7.0, 14.0, and 28.0 mg $NaHCO_3$ per cm^2. The $NaHCO_3$ was deposited as powder on the surface of dental plaster in the appropriate cups. Cups were lightly tapped to evenly distribute the material on the surface. Ten Argentine ant workers were placed in each container with a small quantity of sugar water for food. Cups were placed on a wet foam pad to maintain moisture within cups over the duration of the tests. Initially, the $NaHCO_3$ treatments were dry, but as water was drawn up through the dental plaster, they became slightly moistened. Mortality was checked daily for 6 d; dead workers were removed each day. Treatments were replicated 10 times in a randomized complete block design (RCBD). These tests were conducted four times between 03 October and 15 December 2003. Data were subjected to probit analysis (SAS Institute 1985) to obtain estimates of lethal concentrations and associated parameters. Concentration of $NaHCO_3$ was transformed by log (x + 1) prior to regression analysis and graphing of ant mortality data (SPSS Inc. 1998).

Potential differences in mortality of the two ant pest species from $NaHCO_3$ exposure also were determined in laboratory tests. Test arenas, application of treatments, and maintenance of ants and arenas were the same as previously described. In this test, fire ants and Argentine ants were exposed to 9.92 mg $NaHCO_3$ per cm^2, a concentration that approximated the LC_{50} for fire ant workers following 5 d of exposure (Brinkman et al. 2004). Groups of 10 workers of each species were placed in the appropriate arenas and maintained as previously described. Mortality was checked daily for 7 d in the treatment and control arenas. Dead ants were removed each day. Treatments were replicated 5 to 10 times in a RCBD with each arena being a replicate. These experiments were repeated five times between 21 July and 5 August 2003.

Two higher concentrations were tested in separate assays by methods previously described. Both were compared to an untreated control. The concentration of 17.7 mg $NaHCO_3$ per cm^2 was evaluated three times between 18 December 2003 and 15 January 2004. The highest concentration of 152.0 mg $NaHCO_3$ per cm^2 was evaluated in five experiments between 16 January and 27 January 2004. Data resulting from these experiments were analyzed by the PROC MIXED procedure

with repeated measures in SAS (Littell et al. 1996); means were separated with LSD ($P = 0.05$).

A range of concentrations of $NaHCO_3$ was tested in sugar water baits on Argentine ants. Test arenas and maintenance of ants and arenas were the same as previously described. A stock solution of sugar water was prepared by mixing 8.37 g (10 ml) of granulated sugar with 90 ml sterile distilled water. Concentrations of 0, 1, 5, 7.5, and 10% $NaHCO_3$—sugar water (v/v) were prepared (Table 2). Treatments were pipetted into 0.65-ml microcentrifuge tube lids, and these were individually placed on the dental plaster in cups. Treatments were replicated 10 times in a RCBD. Mortality was checked daily for 6 d. Dead ants were removed from cups each day. These tests were conducted three times between 20 February and 8 March 2004. Data were analyzed by the PROC MIXED procedure, and means were separated with LSD ($P = 0.05$).

Tests were conducted with equivalent amounts of sodium in the form of $NaHCO_3$ and sodium chloride (NaCl) to determine whether or not fire ant mortality would be similar for the two compounds. The total amount of either $NaHCO_3$ or NaCl placed in each test arena was 84.0 mg (11.898 mg per cm^2) of $NaHCO_3$ and 58.0 mg (8.215 mg per cm^2) of NaCl. Formula weight of $NaHCO_3$ is 84.00687 and is 58.44277 for NaCl (Whitten & Gailey 1981). Untreated arenas (control) were also included in these tests. Test arenas, treatment application, and maintenance of ants were the same as previously described. Treatments were replicated 10 times in a RCBD. Mortality was checked daily for 6 d, and dead ants were removed from cups each day. These experiments were repeated five times between 30 October 2003 and 18 January 2004 using fire ants from four different colonies. Data were analyzed by the PROC MIXED procedure with repeated measures in SAS (Littell et al. 1996), and means were separated with LSD.

Results and Discussion

A positive linear relationship ($R^2 = 0.3665$; $F_{1,278} = 160.81$; $P < 0.0001$) occurred between $NaHCO_3$ concentration on surfaces and Argentine ant mortality (Fig. 1). Probit analysis of the concentration-mortality response of workers after 5 d exposure to $NaHCO_3$ yielded a LC_{50} of 5.64 mg per cm^2 and 3.96 mg $NaHCO_3$ per cm^2 after 6 d (Table 1). This LC_{50} is lower than that obtained for red imported fire ants on day 5, as reported by Brinkman et al. (2004). Argentine ant LC_{50} followed a similar trend as that observed with fire ants by decreasing over time. Furthermore, fire ant mortality following exposure to 28 mg $NaHCO_3$ per cm^2 for 6 days was 66.0% (Brinkman et al. 2004), while Argentine ant mortality following 6 days of exposure to 28 mg $NaHCO_3$ per cm^2 was 89.5%. These results suggest that less $NaHCO_3$ is required to kill Argentine ant workers than fire ant workers.

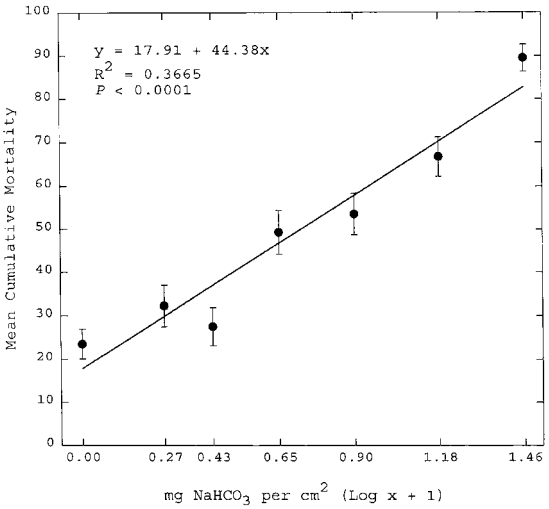

Fig. 1. Linear regression for $NaHCO_3$ concentration effects on Argentine ant mortality in test cups (Day 6). Vertical lines represent ± SEM. Treatments were an untreated control, 0.85, 1.70, 3.50, 7.00, 14.00, and 28.00 mg $NaHCO_3$ per cm^2. Concentration of $NaHCO_3$ was transformed by log (x + 1) prior to regression analysis and graphing of ant mortality data.

Cumulative mortality for Argentine ants exposed to 9.92 mg $NaHCO_3$ per cm^2 was ($F = 9.85$; $df = 3,6$; $P = 0.0001$) higher than mortality of fire ants exposed to the same concentration over the 7 d of exposure. On day seven, cumulative mortality among Argentine ants exposed to $NaHCO_3$ was 38.0% (±5.5) and was 35.6% (±4.9) for fire ants exposed to $NaHCO_3$ (Fig. 2A).

Table 1. Concentration—mortality of worker Argentine ants after exposure to $NaHCO_3$ for 5 to 6 d (n = 400 in each treatment).

	mg per cm^2				
Day	LC_{50} (95% CL)	LC_{95} (95% CL)	Slope ± SE	χ^2	$P > \chi^2$
5	5.64 (2.74-13.10)	210.0 (50.0-26930.0)	1.05 ± 0.21	24.6	0.0001
6	3.96 (1.84-7.60)	150.0 (40.0-7700.0)	1.04 ± 0.20	28.2	0.0001

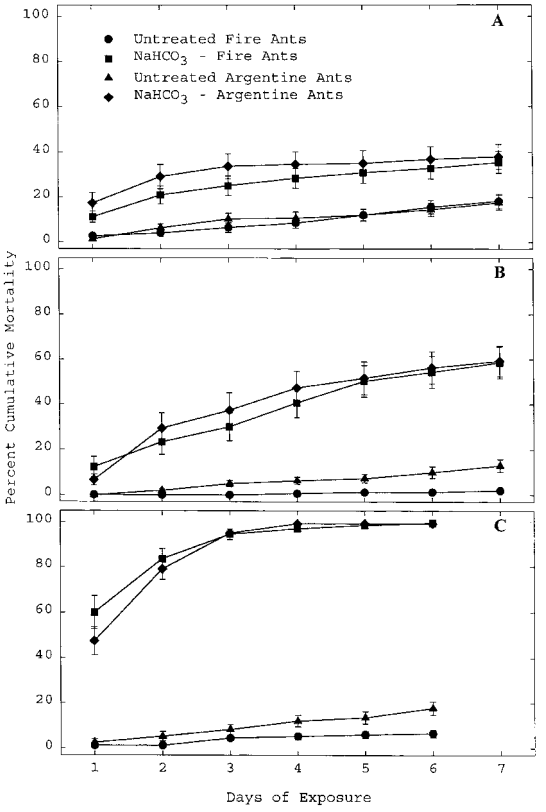

Fig. 2. Mortality of fire ants and Argentine ants exposed to a concentration of (A) 9.92, (B) 17.7, (C) 152.0 mg $NaHCO_3$ per cm^2 on dental plaster in test cups. Untreated workers of both species also were kept as controls.

Mortality of Argentine ants and fire ants exposed to 17.7 mg $NaHCO_3$ per cm^2 was (F = 24.20; df = 3,6; P = 0.0001) higher than mortality for their respective untreated controls over the 7 d of the test (Fig 2B). Argentine ant mortality did not differ ($P > 0.05$) from fire ant mortality following exposure to 17.7 mg $NaHCO_3$ per cm^2. Cumulative mortality at day 7 for Argentine ants was 59.3% (±6.9) and 58.7% (±7.1) for fire ants. This concentration was the predicted LC_{75} for Argentine ants after 6 d of exposure to $NaHCO_3$, but mortality for workers of both species was lower than 75%. The reason for the lower mortality is not known, but it was consistent for both ant species at this concentration.

Mortality for Argentine ants and fire ants exposed to 152.0 mg $NaHCO_3$ per cm^2 was (F = 592.02; df = 3,5; P = 0.0001) higher than mortality for their respective untreated controls over the 6 d of the test. Following correction for control mortality (Abbott 1925), Argentine ant mortality was 99.09% and fire ant mortality was 99.47% after 6 days of continuous exposure to 152.0 mg $NaHCO_3$ per cm^2 (Fig. 2C, non-corrected mortality). This concentration was the predicted LC_{95} for Argentine ants following 6 d exposure to $NaHCO_3$. However, mortality for both species was higher than 95% in less than 6 d. In fact, almost all workers of both species were killed by this concentration after only 4 d of exposure. These results suggest that, at lower concentrations, there may be small differences in mortality of workers of these two species; however, as concentration of $NaHCO_3$ is increased, the mortality of the two species is similar.

Cumulative mortality for fire ants provided untreated sugar water was (F = 14.46; df = 4,5; P = 0.0001) lower than for the three highest concentrations of $NaHCO_3$ in sugar water at 6 d. The highest corrected mortality was 49.56% in the 5.0% $NaHCO_3$-sugar water treatment (Table 2, non-corrected mortality). However, this was not ($P > 0.05$) different from ant mortality for the 7.5% $NaHCO_3$-sugar water treatment. Corrected Argentine ant mortality in the highest concentration of $NaHCO_3$ in sugar water was 29.20% after 6 d. A range of concentrations of $NaHCO_3$ mixed in sugar water was tested, yet the greatest mortality was observed in the 5% $NaHCO_3$-sugar water treatments. A concentration-dependent relationship did not occur beyond this level. A similar trend was observed with fire ants provided sugar water treatments containing $NaHCO_3$ (Brinkman et al. 2004). Brinkman et al. (2004) concluded that excess $NaHCO_3$ had settled out of solution in the higher concentrations and was not available for ant consumption. In this study, some precipitate was observed in the bottoms of lids

TABLE 2. CUMULATIVE MORTALITY FOR ARGENTINE ANTS (N = 100 PER TREATMENT PER TEST) PROVIDED SUGAR WATER AND $NaHCO_3$.

Food Treatment	Mean # Dead (day 6)
Untreated sugar water	24.67 ± 3.31a
0.113 g $NaHCO_3$ in 9.9 ml sugar water (1%)	30.33 ± 3.47a
0.563 g $NaHCO_3$ in 9.5 ml sugar water (5%)	62.00 ± 4.11c
0.844 g $NaHCO_3$ in 9.25 ml sugar water (7.5%)	55.67 ± 4.86c
1.125 g $NaHCO_3$ in 9.00 ml sugar water (10%)	46.67 ± 4.53b

Means (± SEM) followed by same letter are not different (LSD, $P > 0.05$).

containing 10% $NaHCO_3$-sugar water treatment, yet mortality for Argentine ants exposed to this concentration was significantly higher than mortality for Argentine ants provided untreated sugar water. Therefore, we concluded that workers were not repelled by the higher concentrations of $NaHCO_3$ in sugar water. Vinson (1970) found that fire ants workers preferred $NaHCO_3$ in solution over NaCl in solution. Preferential response to sodium was variable in comparison with other cations (Vinson 1970). Brinkman et al. (2004) conducted tests in arenas in which fire ants could feed on sugar water or sugar water containing $NaHCO_3$. In those tests, fire ant mortality was much higher in arenas with both sugar water and sugar water containing $NaHCO_3$, than it was in arenas with sugar water only. Brinkman et al. (2004) concluded that fire ants were not repelled by $NaHCO_3$ in food.

Fire ant mortality following exposure to NaCl was (F = 22.76; df = 2,5; P = 0.0001) lower than mortality occurring among those workers exposed to $NaHCO_3$ (Fig. 3). Worker mortality in response to NaCl was 15.4% at 6 d and did not differ (P > 0.05) from mortality of untreated ants. Fire ant mortality following 6 d exposure to $NaHCO_3$ was 46.2%. Brinkman et al. (2004) found that whole-body pH of fire ant workers exposed to $NaHCO_3$ increased with increasing concentration of $NaHCO_3$. They theorized that fire ants ingested $NaHCO_3$ while cleaning appendages and that the resultant changes in internal pH were unfavorable to normal enzymatic functions. According to Tortora & Grabowski (1996), sodium hydrogen carbonate contributes hydroxide ions (OH^-) to solutions causing increases in pH. However, Bigner et al. (1997) attributed the alkalinizing action of $NaHCO_3$ to Na^+ and they based this on the strong ion difference theory. In the theory of strong ion difference in acid-base physiology (Stewart 1983), addition of non-metabolizable, positively charged cations to a body fluid compartment raises the pH of that compartment. Bigner et al. (1997) tested three Na compounds to determine which was best for treating metabolic acidosis in dairy cattle. Blood pH and blood HCO_3^- increased in the $NaHCO_3$ treatment, and was much higher than that observed for NaCl. They assumed, based on the theory, that the NaCl treatment did not alter blood pH because both the positively charged cation Na^+ and the negatively charged anion Cl^- were absorbed equally well and added no net charge to the blood fluid compartment. This suggests that Na^+ may have played a role in killing fire ants by raising pH, but only when delivered in the form of $NaHCO_3$, and not as NaCl. According to Audesirk et al. (2002), salts dissociate into ions in solution, and may then form bonds with enzymes and interfere with the enzymes' normal three-dimensional structure. Also, changes in pH may modify the structure of enzymes and strongly alkaline solutions can denature enzymes (Conn & Stumpf 1976).

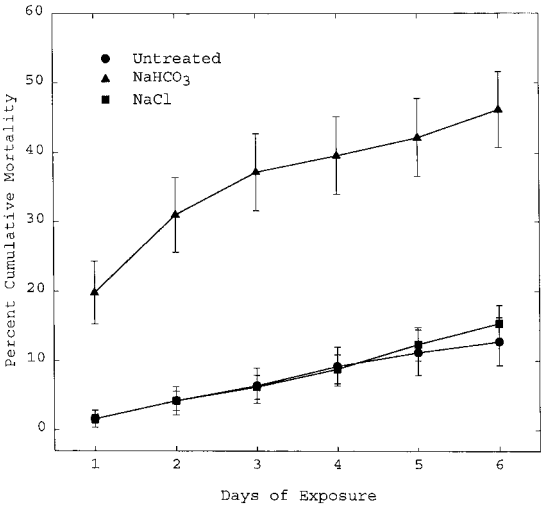

Fig. 3. Mortality of fire ants exposed to $NaHCO_3$ and NaCl treatments. Untreated workers of both species also were kept as controls. $NaHCO_3$ and NaCl treatments that were placed in respective arenas contained equal amounts of sodium.

Hertel (1997) tested NaCl and KCl as protectants for pinewood against attack of a longhorn beetle species [*Hylotrupes bajulus* (L.)]. Both compounds were effective, but NaCl provided better protection than KCl. Dehydration of beetle larvae after feeding on salt treated wood was offered as a possible explanation for mortality. In our study, the role of dehydration in deaths of fire ants exposed to NaCl and $NaHCO_3$ on surfaces was minimal because workers had unrestricted access to untreated sugar water.

Optimal procedures for use of $NaHCO_3$ as an ant control treatment in the home have yet to be determined. According to Klotz et al. (1997a), dusts are an excellent formulation for insecticides because ants readily pick up dusts that are applied to their trails. This may be an acceptable application strategy for NaHCO3 in that Brinkman et al. (2004) determined that fire ants were not repelled by $NaHCO_3$ and would readily forage over treated areas. Crust will develop on $NaHCO_3$ powder if it is exposed to moisture and then dries. Therefore, retreatment may be necessary on unprotected ant trails. Klotz et al. (1997a) suggest that dusts could be applied during home construction when there is easy access to wall voids. Knight & Rust (1990) reported that repellency often determines how much contact an insect will have with a toxicant and that very low repellency treatments may produce high kill, even with only intermediate toxicity, because of increased contact with the treatment.

Sugar water has been used as a bait carrier for boric acid against fire ants (Klotz et al. 1997b) and Argentine ants (Klotz et al. 2002). Sucrose solutions are attractive to Argentine ants and are a means of transporting toxicants into the colony (Hooper-Bui & Rust 2000). Sodium hydrogen carbonate is inexpensive, easy to handle, and generally recognized as safe (GRAS) for use in foods (Montville & Goldstein 1987). Thus, sugar water baits containing $NaHCO_3$ could be used safely in the homes with children or pets. Toxicants that are effective in baits exhibit delayed action, are readily transferred between ants and kill the recipient, and are not repellent (Stringer et al. 1964). Perhaps an ant trail treatment with $NaHCO_3$ powder, sucrose baits containing $NaHCO_3$, or a combination of both, may provide safe methods of Argentine ant control in and around homes.

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ASIAN CITRUS PSYLLIDS (STERNORRHYNCHA: PSYLLIDAE) AND GREENING DISEASE OF CITRUS: A LITERATURE REVIEW AND ASSESSMENT OF RISK IN FLORIDA

SUSAN E. HALBERT[1] AND KEREMANE L. MANJUNATH[2]
[1]Division of Plant Industry, Florida Department of Agriculture and Consumer Services
P.O. Box 147100, Gainesville, FL 32614-7100

[2]University of Florida Department of Plant Pathology, Gainesville, FL 32611

ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, was discovered in Florida in 1998. It can be one of the most serious pests of citrus if the pathogens that cause citrus greening disease (huanglongbing) are present. Citrus greening recently has been reported in Brazil by Fundecitrus, Brazil. The establishment of *D. citri* in Florida increases the possibility that the disease may become established. *Diaphorina citri* can be separated from about 13 other species of psyllids reported on citrus. The biology of *D. citri* makes it ideally suited to the Florida climate. Only two species, *D. citri* and *Trioza erytreae* (del Guercio), have been implicated in spread of citrus greening, a disease caused by highly fastidious phloem-inhabiting bacteria. The disease is characterized by blotchy mottle on the leaves, and misshapen, poorly colored off-tasting fruit. In areas where the disease is endemic, citrus trees may live for only 5-8 years and never bear usable fruit. The disease occurs throughout much of Asia and Africa south of the Sahara Desert, on several small islands in the Indian Ocean, and in the Saudi Arabian Peninsula. Transmission of citrus greening occurs primarily via infective citrus psyllids and grafting. It is transmissible experimentally through dodder and might be transmitted by seed from infected plants and transovarially in psyllid vectors. Citrus greening disease is restricted to *Citrus* and close citrus relatives because of the narrow host range of the psyllid vectors. Management of citrus greening disease is difficult and requires an integrated approach including use of clean stock, elimination of inoculum via voluntary and regulatory means, use of pesticides to control psyllid vectors in the citrus crop, and biological control of psyllid vectors in non-crop reservoirs. There is no place in the world where citrus greening disease occurs that it is under completely successful management. Eradication of citrus greening disease may be possible if it is detected early. Research is needed on rapid and robust diagnosis, disease epidemiology, and psyllid vector control.

Key Words: *Diaphorina citri* Kuwayama, Asian citrus psyllid, citrus greening disease, huanglongbing, citrus psyllids, *Citrus*.

RESUMEN

El psílido Asiático de cítricos, *Diaphorina citri* Kuwayama, fue descubierto en Florida en 1998. Esta puede ser una de las plagas de cítricos mas serias si los patógenos que causan la enfermedad "greening" de los cítricos (huanglongbing) están presentes. Recientemente, la entermedad "greening" de los cítricos ha sido reportada en Brasil por fundecitrus (Brasil). El establecimiento de *D. citri* en Florida aumenta la posibilidad que la enfermedad pueda establecerse. *Diaphorina citri* puede ser separado de aproximadamente 13 otras especies de psílidos reportadas en cítricos. La biología de *D. citri* lo hace idealmente adaptable al clima de Florida. Solamente dos especies, *D. citri* y *Trioza erytreae* (del Guercio), han sido implicadas en la dispersión del "greening" de cítricos, una enfermedad causada por una bacteria altamente fastidiosa que habita el floema. La enfermedad se caracteriza por causar áreas moteadas en las hojas, y frutas mal formadas, mal coloradas y con sabor anormal. En áreas donde la enfermedad es endémica, los árboles de cítricos pueden vivir por solamente 5-8 años y nunca dar fruta provechosa. La enfermedad ocurre en la mayor parte de Asia, en Africa al sur del Desierto Sahara, en varias islas pequeñas del Océano Índico, y en la Península de Saudi Arabia. La transmisión de "greening" de cítricos ocurre principalmente por medio de psílidos infectados y por injertar las plantas. Puede ser transmisible experimentalmente a través de cuscuta, posiblemente transmitida por la semilla de plantas infectadas y transovariolmente en los vectores psílidos. La enfermedad de "greening" de cítricos es restringida al *Citrus* y sus relativos cercanos debido al rango estrecho de hospederos de los vectores psílidos. El manejo de la enfermedad de "greening" de cítricos es difícil y requiere una estrategia integrada incluyendo el uso de plantas no contaminadas, la eliminación de inóculo por medios voluntarios y regulatorios, el uso de pesticidas para controlar los vectores psílidos en los huertos de cítricos, y el control biológico de los vectores psílidos en depósitos de plantas

que no son cultivos. No hay ningún lugar en el mundo donde ocurre la enfermedad de "greening" de cítricos que esté completemente bajo un manejo exitoso. La erradicación de la enfermedad de "greening" de cítricos puede ser posible si la enfermedad está detectada tempranamente. Se necesita investigación sobre un diagnóstico rápido y robusto, la epidemiología de la enfermedad, y el control del vector psílido.

Asian citrus psyllid (*Diaphorina citri* Kuwayama, Sternorrhyncha: Psyllidae) may be the most serious pest of citrus in the world if any of the pathogens that cause citrus greening also are present. If none of the pathogens are present, the psyllids usually are minor pests. Citrus greening was reported in Brazil in July 2004 by Fundecitrus. This is the first report of the disease in the Western Hemisphere (Anon. 2004).

The Asian citrus psyllid causes damage to the crop primarily by transmission of the pathogen that causes greening, or "huanglongbing" (黄龙病), which means "yellow dragon disease" in Chinese. Huanglongbing has been translated loosely as yellow shoot disease in English language publications because of characteristic yellow shoots caused by the disease. In addition to yellow shoots, the disease also causes mottling, chlorosis resembling zinc deficiency, twig dieback and reduced fruit size and quality. Fruit do not color properly, leading to the name greening. Fruit from diseased trees have a bitter taste. Other names include citrus vein phloem degeneration, and 立枯病 (likubin), which means immediate withering disease. Australian citrus dieback, a disease of unknown etiology, is suspected to be caused by a similar psyllid-transmitted pathogen (Broadbent 2000). Although the official name of the disease is huanglongbing (anon. 1996), we use the name "citrus greening disease" throughout this review because it is the name commonly used in the United States, and by our audience for this paper.

Citrus greening probably is the worst disease of citrus caused by a vectored pathogen. The dynamics, epidemiology, and molecular characteristics of the complex are poorly understood. *Trioza erytreae* (del Guercio) (Sternorrhyncha: Triozidae) in Africa and *Diaphorina citri* Kuwayama (Sternorrhyncha: Psyllidae) in Asia are the only known vectors of the two forms of the disease, namely African and Asian citrus greening, respectively. Two species of fastidious phloem-limited bacteria (*Candidatus* Liberibacter africanus and asiaticus), are thought to be the causal organisms, but Koch's postulates have not been fulfilled because the bacteria have not been cultured yet. (The term *Candidatus* is used for bacteria species that cannot be cultured. If *Candidatus* is used, the actual genus and species are not italicized.) The pathogens prevalently transmitted by the two psyllids are different, but both psyllid species will transmit either pathogen under experimental conditions (Lallemand et al. 1986). The pathogens at present are extremely difficult to detect and characterize, although great strides have been made in recent years with development of detection methods based on polymerase chain reaction (PCR) and DNA hybridization.

Excellent reviews of citrus greening disease complex have been published (da Graça 1991; Garnier & Bové 1993, 2000a; Mead 1977; Roistacher 1991; Viraktamath & Bhumannavar 2002). We do not intend to duplicate these earlier efforts. The recent introduction of *D. citri* into Florida (Halbert 1998) has greatly increased the threat of citrus greening disease for North American citrus. The intent of this paper is to provide a convenient summary of information that is relevant to risk assessment for the Florida citrus industry and possible eradication of citrus greening disease. The emphasis will be on recognition of citrus psyllids, host range information, detection, epidemiology, and management.

Biology of Asian Citrus Psyllid

Systematics and Recognition of Psyllids Reported on Citrus

Diaphorina citri (=*Euphalarus citri* (Kuwayama 1908)) was described from citrus in Shinchiku, Taiwan in 1907 and published in a double volume in 1908. Species of *Diaphorina* usually are separated based on the pattern of maculation in the forewings and the shape of the genal cones. *Diaphorina citri* has a distinct pattern on the forewings and can be separated easily from most of the other species reported on citrus and its relatives.

There are six other obscure species of *Diaphorina* also reported from citrus and other closely related plants: *Diaphorina amoena* Capener 1970b (reported on citrus by da Graça 1991), *Diaphorina auberti* Hollis 1987a (reported on citrus in original description), *Diaphorina communis* Mather 1975 (reported on citrus in original description) and Aubert (1987), *Diaphorina murrayi* Kandasamy 1986 (reported on citrus in original description), *Diaphorina punctulata* (Pettey 1924) (reported on citrus in original description), and *Diaphorina zebrana* Capener 1970b (reported on citrus by Catling & Atkinson 1974).

Diaphorina auberti was described (Hollis 1987a) from the Comoro Islands. The host is citrus, on which nymphs concentrate on the upper surfaces of young leaves near the midribs. This causes the lateral margins of the leaves to curl upwards and inwards, sometimes forming an en-

closed leaf-roll (Dr. B. Aubert, pers. comm., in Hollis 1987a). Hollis (1987a) placed *D. auberti* in the *D. amoena* species group. The wing patterns of *D. auberti* are very similar to those of *D. amoena* (see Fig. 10 and Fig. 11 (*amoena*) compared with Fig. 22 and Fig. 23 (*auberti*) in Hollis (1987a)); however, the genae of *auberti* are much shorter than those of *amoena* (see Fig. 1 and Fig. 7 of Hollis (1987a)). The host for *D. amoena* is *Strychnos innocua* Delile (Loganiaceae (Strychnaceae)). It is not reported from citrus or citrus relatives either in Hollis (1987a) or in the original description (Capener 1970b). Thus, reports of *D. amoena* on citrus probably can be attributed to *D. auberti* or possibly *D. citri*. Both *D. amoena* and *D. auberti* can be separated from *D. citri* by the pattern on the forewings.

Diaphorina communis was described from a long series of specimens collected in Uttar Pradesh, India (Mather 1975). It is reported to be common on *Murraya koenigii* and occurs occasionally on citrus. It is mentioned as a possible species on citrus by Burckhardt (1994a). The forewings of *D. communis* are almost totally dark, making it easily separable from *D. citri*.

Diaphorina murrayi was described by Kandasamy (1986) from 11 specimens taken on *Murraya exotica* L. in Madras (now Chennai, Tamilnadu), India. According to the original description, it is closely related to *D. citri* but differs in having a slightly different wing pattern and slightly different tarsal spine formula. So far, it is reported only from *M. exotica*. Further study is needed to determine whether it differs sufficiently from *D. citri* to be considered a separate species.

Diaphorina punctulata (=*Euphalarus punctulatus* Pettey 1924) was described from a female specimen collected in southern Africa. The host was *Sclerocarya caffra* Sond. (Anacardiaceae) Capener (1970a). The original description says that the species also has been found on *Chorda* [sic] *caffra* Sond. (Boraginaceae) and *Clausena inaequalis* (DC.) Benth. (=*anisata* (Willd.) Hook, S. ex Benth.) (Rutaceae). The description is very brief (barely 13 lines long) and includes no figures or designation of paratypes. Capener (1970a), apparently with access to Pettey's notes, said that the species was described from seven specimens - 1 male, 6 females. Seven specimens (evidently not entirely from the type series because sexes do not match, but determined by Pettey) still exist in the National Collection of Insects, Pretoria. There is some doubt as to whether all of this material is the same species. Catling & Atkinson (1974) mention that *D. punctulata* was found on citrus in Swaziland (Mead 1977) but was a non-vector of citrus greening. If the specimens reported from *Clausena* (above) are in fact *D. punctulata*, it is plausible that this species could infest citrus occasionally. Pettey (1924) (original description), Capener (1970a), and Catling & Atkinson (1974) do not include a thorough description or figure of *D. punctulata*, so it is not possible to explain from the descriptions how to separate it from *D. citri*. Photos of identified specimens of *D. punctulata*, including a paratype, kindly provided by Ian Millar, ARC-Plant Protection Research Institute, South African National Collection of Insects, Queenswood, Pretoria, South Africa, and by Dr. G. J. Begemann, Transvaal Sugar Ltd, Komatipoort, South Africa, show that *D. punctulata* looks very similar to *D. citri*. The two species can be separated by a difference in the maculation pattern on the wings. Both species have a dark band around the edge of the wings, with a clear area in the middle containing irregular spots. The dark outer band on *D. citri* has a definite break near the terminus of the Rs vein. The band on *D. punctulata* lacks that break. In addition to the wing pattern, the wing shape is more angular in *D. punctulata*, and the genal processes of *D. punctulata* are more massive and irregularly tapered than those of *D. citri* (Daniel H. Burckhardt, Naturhistorisches Museum, Basel, Switzerland, pers. comm.).

Diaphorina zebrana was described from *Ozoroa paniculosa* (Sond.) R.& A. (Anacardiaceae). Additional specimens that varied slightly in the intensity of wing banding were collected from *Ozoroa reticulata* (Bak. f.) R. & A. Rernandes. *Diaphorina zebrana*, like *D. punctulata*, was mentioned as a citrus infesting species and a non-vector of greening in Swaziland (Mead 1977) by Catling & Atkinson (1974). There is no mention of infestations on citrus or related plants in the original description. *Diaphorina zebrana*, as its name suggests, has striped forewings and thus is readily separable from *D. citri*.

In addition to the seven species of *Diaphorina* reported on citrus, there are six other psyllid species reported to occur on citrus: *Mesohomotoma lutheri* (Enderlein 1918) (=*Udamostigma lutheri* Enderlein), *Psylla citricola* Yang & Li 1984, *Psylla citrisuga* Yang & Li 1984, *Psylla murrayi* Mathur 1975, *Trioza citroimpura* Yang & Li 1984, *Trioza erytreae* (del Guercio 1918) (= *Aleurodes erytreae* del Guercio, = *Trioza citri* Laing, = *Trioza merwei* Pettey, = *Spanioza merwei* (Pettey), = *Spanioza erythreae* (del Guercio) (Hollis 1984)), and *Trioza litseae* Bordage 1898 (=*Trioza eastopi* Orian 1972).

Mesohomotoma lutheri was described from Peradeniya, Ceylon (Sri Lanka) from collections made in 1910 by Dr. Luther (Enderlein 1918). It was re-described carefully by Mathur (1975). The host was *Urena lobata* L. (Malvaceae), and it also may infest *Hibiscus* (also Malvaceae). There is some doubt about the synonymy for this species. Hollis (1987b) said that there is confusion about the validity of the species currently placed in *Mesohomotoma* because of variation in size and color among collections of the same species. The varia-

tion does not correlate well with host plant and distribution data. Hollis (1987b) suspects that several of the species, including *M. lutheri*, may in fact all be synonyms of *Mesohomotoma hibisci* (Froggatt). Aubert & Quilici (1984) reported that adults of *M. lutheri* were seen on citrus leaves for short feeding periods, but that this was extremely rare, and no eggs were laid. They reported that the preferred host of *M. lutheri* was *Hibiscus*.

Psylla citricola and *P. citrisuga* were described from *Citrus grandis* (L.) Osbeck (now *Citrus maxima* (Burm.) Merr.) and *Citrus medica* L. in Yunnan Province in China (Yang & Li 1984). The two species are very similar and apparently occur together in mixed colonies on the hosts. Yang & Li (1984) suggest that a report of *Psylla alni* on citrus in Sichuan Province may actually be *P. citrisuga*. There is a report by Cen et al. (1999) that *P. citricola* occurs in Guangzhou. Further study is needed to determine whether these are distinct species.

Psylla murrayi normally feeds and reproduces on leaves of *Murraya koenigii* (L.) Sprengel (Mather 1975), but adults have been observed on citrus in Malaysia (Lim et al. 1990). Based on illustrations in the descriptions, the male genitalia appear to be distinct from those of the two Chinese *Psylla* spp.

Trioza citroimpura also was described from Yunnan Province in China. The host was *Citrus reticulata* Blanco. Both male and female genitalia appear distinct from those of *T. erytreae*, based on figures given in the description.

Trioza erytreae is the well-known African citrus psyllid. It was described as a whitefly, *Aleurodes erytreae* del Guercio 1918; however, the drawing of the nymph is clearly a psyllid, and the photograph of the damage is that of *T. erytreae*. It is part of a species group that includes at least ten species that are very difficult to separate morphologically but have different host plant preferences (Hollis 1984). Hosts of *T. erytreae* are listed as *Clausena anisata* (Willd.) Oliv. (=*Clausena inaequalis* (DC.) Benth.), *Citrus* spp., *Vepris undulata* (Thunb.) Verdoorn & C.A. Smith (=*Toddalia lanceolata* Lam.) and *Fagara* spp. There is extensive literature about this serious pest, and particularly about its relationship to African citrus greening disease. *Trioza erytreae* can be found throughout much of Africa south of the Sahara, in Saudi Arabia and Yemen in the Saudi Peninsula, in Mauritius and Réunion, and in Madeira (Toorawa 1998). A key to African *Trioza*, along with additional characters useful in separating species in the *T. erytreae* group can be found in Hollis (1984). It is easily separated from *D. citri* because it has clear forewings that are pointed at the tips. Nymphs of *T. erytreae* live in individual depressions on the undersides of citrus leaves, whereas nymphs of *D. citri* tend to colonize the stems of new growth and never produce individual pits on the leaves.

Trioza litseae Bordage was described based on its damage to vanilla and *Litsea* (Lauraceae) on Réunion Island (Bordage 1898). There has been some confusion about the synonymy with *T. eastopi*. Orian (1972) determined that *T. litseae* was a *nomen dubium* because the description was sketchy; however, the International Code of Zoological Nomenclature (2000) (Articles 1.2.1, 12.2.8, 23.3.2.3, and 72.5.1) allows descriptions based on the "works" (e.g., damage) of an animal if the description was published prior to 1931. Therefore, Bordage's (1898) description, which includes details of the economic damage to vanilla, and also damage to *Litsea*, which he considers the original host, qualifies, and *T. litseae* Bordage is a good species. Thus, *T. eastopi* Orian 1972 becomes a junior synonym of *T. litseae* Bordage. There is some further question as to whether this species actually occurs on citrus. Hollis (1984) reported that the usual host is *Litsea glutinosa* C.B. Robinson (=*L. laurifolia* Cordem) (Lauraceae), where nymphs damage floral parts of the plant. Adults can damage *Vanilla planifolia* Andrews (Orchidaceae). Aubert & Quilici (1984) reported both adults and nymphs of *T. litseae* (as *T. eastopi*) on leaves of citrus, avocado, papaw, and vanilla leaves. Apparently, the favored host was *Litsea chinensis* Jacq., a common weed. When populations on these weeds reached very high levels, the insects began to infest young flush of other plants, including citrus. Nymphs formed pits similar to those of *T. erytreae*. *Trioza litseae* can be separated from *T. erytreae* with the key in Hollis (1984).

Trioza is an extremely large and difficult artificial genus. There are no keys to world fauna. Thus, host plant association is of utmost importance in determining the species. The thorough re-description and key in Hollis (1984) would be useful for diagnosis of *T. erytreae*, along with Burckhardt (1994b), which keys psyllid genera that occur in Chile, along with various potential exotic pests including *T. erytreae*. The *Trioza* spp. can be separated from *D. citri* because the radius, media and cubitus veins in the forewings diverge at the same point (trifurcate) in *Trioza* spp., whereas the media and cubitus share a common stem in *D. citri*.

Psylla loranthi Capener 1973 is not reported to feed on citrus or citrus relatives, but it feeds on *Loranthus zeyheri* Harv., a parasitic plant that sometimes attacks citrus. There is a small possibility that *P. loranthi* might potentially transmit citrus greening bacteria via the parasitic plant, so the species is included in this list. However, we note that no phloem connection may exist between the parasitic plant and its host (Sallé 1983). It can be distinguished from other *Psylla* species associated with citrus by the long slender genitalia, especially of the female (Capener 1973), and by the presence of immatures on its parasitic host plant. *Psylla loranthi* would not be found where its host does not occur.

Finally, *Leuronota fagarae* Burckhardt 1988, the wild lime psyllid, showed up in Florida in July 2001. Its only known host is *Zanthoxylum fagara* (L.) Sarg., a citrus relative. The psyllid is native to South America. Damage consists of rolled leaf edges that enclose the nymphs. We have surveyed citrus growing near infested *Z. fagara* and have not found any *L. fagarae* on citrus. *Leuronota fagarae* is very slender and has dark wings. It is not likely to be confused with *D. citri* or any other citrus psyllids.

Life Cycle

Mead (1977) has an excellent annotated summary of the life cycle of *D. citri*. Eggs are laid on "feather flush" and hatch in 2-4 days (Chavan & Summanwar 1993). There are five nymphal instars (Aubert 1987), which are completed in 11-15 days (Chavan & Summanwar 1993). The total life cycle takes 15-47 days, depending upon the temperature. Adults may live several months and the females lay as many as 800 eggs in a lifetime (Mead 1977). Catling (1970) provided further information on life cycle and biology. Life table parameters at different temperatures have been studied for Florida *D. citri* (Liu & Tsai 2000). Time for completion of the life cycle was the same as Mead (1977) reported. The optimum development temperature range was found to be 25-28°C. Liu & Tsai (2000) found that the maximum average number (748.3) of eggs produced per female occurred at 28°C.

Climatic Requirements

Aubert (1987) states that *Diaphorina citri* does not tolerate frost very well; however, we have observed that populations have overwintered in Gainesville, FL, where temperatures dropped to at least -5°C on several nights. Populations of *D. citri* in the Florida panhandle have been limited so far to *Murraya* and *Citrus* plants for sale at discount outlets, so it is not known whether *D. citri* can overwinter north of Gainesville. Aubert (1987) also states that populations of *D. citri* do not tolerate humidity close to the saturation point because it promotes fungal epizootics, to which the nymphs are very susceptible; however, high humidity in Florida has not prevented extremely high summer populations of *D. citri* in local groves and backyards. Similarly, few *D. citri* regulatory samples sent to Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) have cadavers resulting from fungal infection. *Diaphorina citri* was not found above 1300-1500 m in elevation in various places searched in Asia, presumably because of occasional frosts (Aubert 1987). Populations of *D. citri* moved north in China in the 1980s as a result of more citrus plantings and higher winter temperatures (Qiu et al. 1996).

Distribution and Possible Source of the Florida Infestation

Diaphorina citri can be found in all of southeast Asia and the Indian subcontinent, the islands of Réunion and Mauritius, Saudi Arabia, Brazil (da Graça 1991), southern Iran near the border with Pakistan (Danet, pers. comm., ex Toorawa 1998), Venezuela (Cermeli et al. 2000), and Argentina (DPI records). In early 1998, it was discovered in the island of Guadeloupe in the Caribbean (Étienne et al. 1998). It also was discovered in Florida in Palm Beach, Broward, and Martin Counties in June 1998 and has since spread throughout the state, wherever citrus occurs (Halbert et al. 2002). We have seen specimens from Texas (French et al. 2001), Cayman Islands, and several Bahamian islands (Halbert & Núñez 2004). There is a report in the literature that *D. citri* is present in Honduras (Burckhardt 1994b), which is based upon an interception of *D. citri* in France on citrus trees from Honduras in 1989 (Burckhardt & Martinez 1989). This reported Central American infestation has been difficult to substantiate in Honduras itself. We do not doubt that the insects intercepted were *D. citri*, but the actual source of the infested plants remains an open question.

There are two likely scenarios for the introduction of *D. citri* into Florida. First, *D. citri* has been established in South America for many years. Therefore, it could have spread naturally through Central America and the Caribbean, and ultimately found its way to Florida. If the interception record from Honduras is true, it provides support for the gradual spread of *D. citri* throughout the Western Hemisphere. A USDA/APHIS/PPQ record of an interception of *D. citri* from Mexico in April, 1996, if true, also lends credence to gradual spread from South America. If the Florida *D. citri* population came from Latin America, it is very likely to be free of the greening pathogen.

Alternatively, *D. citri* could have been introduced directly from Asia. The USDA/APHIS/PPQ database has records of 170 interceptions of live *D. citri* from Asian countries at ports in the USA between 1985 and November 2003. There are an additional 73 records of interceptions of live *Diaphorina* spp. on rutaceous plants from Asia. Many of these populations probably were *D. citri*. In most cases, there were only one or two specimens found, but one collection intercepted in Des Plaines, Illinois contained 46 live *D. citri* from India. In most cases, these insects were intercepted on *Murraya* plant material, especially *M. koenigii*, but infested citrus also has been observed.

Interception reports for the most part reflect the known distribution of *D. citri*. An interception report in the USDA/APHIS/PPQ database of *D. citri* from roots of *Colocasia esculenta* (L.) Schott (Araceae) from Cameroon probably is a misidenti-

fication rather than an indication that *D. citri* is established in Western Africa. Several interceptions reported from the Caribbean Basin indicate that *D. citri* already is moving in cargo within five years of known establishment. If citrus greening disease ever became established anywhere in the Caribbean Basin, the potential for movement is high.

Direct Plant Damage

Direct plant damage occurs as a result of high populations of psyllids. Copious amounts of honeydew and moderate leaf distortion have been observed on infested plants (Aubert 1987). In Florida, after the initial invasion of *D. citri*, new growth on some citrus plantings was severely damaged. Feeding by Asian citrus psyllid caused leaves to be curled and notched. In cases of severe infestation, newly emerged sprouts were killed. Lateral leaf notching is particularly characteristic of *D. citri* damage. In dry weather, we have observed curled waxy secretions from nymphs. Heavy oviposition or larval activity sometimes will kill developing terminals or cause abscission of leaves or entire terminals (Michaud 2004).

Populations can reach extremely high levels. A survey technique reported by Ahmad (1961) consisted of spraying citrus trees in West Pakistan with insecticide and collecting the psyllids on a white sheet beneath the tree. This method yielded an average of 41,561 adults per tree! On *Murraya paniculata* hedges in Réunion, catches of 200 adults per m^2 were obtained with a D-VAC machine (Aubert 1987).

Biology of the Greening Pathogens

Nature and Classification of the Pathogens

The greening pathogens are thought to be highly fastidious phloem-inhabiting bacteria in the genus *Candidatus* Liberibacter. Although the bacteria have not been cultured for completion of Koch's postulates, circumstantial evidence points strongly to a bacterial disease agent because citrus greening symptoms abate temporarily when trees are injected with antibiotics (Buitendag & von Broembsen 1993; Lim et al. 1990; Su et al. 1986). The isolate from South Africa has been named *Candidatus* Liberibacter africanus, and the isolate from Asia has been named *Candidatus* Liberibacter asiaticus (Garnier et al. 2000). A subspecies of *Candidatus* L. africanus, *Candidatus* L. africanus subsp. capensis, has been described from the Western Cape Region of South Africa from *Calodendrum capensis* Thunb., a native South African plant. This subspecies also infects citrus (Garnier et al. 2000). Garnier et al. (2000) changed the generic name from Liberobacter to Liberibacter, following the International Code of Nomenclature of Bacteria, which states that since "bacter" is of masculine gender and "Liber" is of Latin origin, the connecting vowel should be an "i."

It is widely accepted that both species of bacteria multiply in both of the psyllid vectors, but this has not been demonstrated with molecular evidence. However, Moll & Martin (1973) noticed marked increases in the number of citrus greening bacteria in *T. erytreae* vectors over 9 days time, and concluded that the bacteria were multiplying in the vectors. Neither species of citrus greening bacteria has been cultured on artificial media. Molecular analysis indicates genetic differences between the two species, and specific DNA probes have been developed for each (Bové et al. 1993, 1996; Garnier & Bové 1996; Harakava et al. 2000; Tian et al. 1996).

African greening manifests symptoms primarily under cool conditions (below 25°C), whereas Asian greening does well under hot conditions (Garnier & Bové 1993). African greening does not show symptoms above 27°C under glasshouse conditions. In South Africa, the greening symptoms are more pronounced in winter than in summer. Similarly, the African citrus greening symptoms are severe in elevations above 700 m, whereas they are absent in low-lying hot areas. Indian greening does well in hot conditions, above 25°C. Asian citrus greening symptoms are less pronounced and disappear above 1500 m, possibly because the vector is absent (Aubert 1987). In a laboratory study, Bové et al. (1974) showed that symptoms of African citrus greening were moderate to severe at 22° to 24°C and disappeared at 27° to 32°C, whereas symptoms of Asian citrus greening from India and Philippines were expressed strongly at both temperature regimes.

Candidatus Liberibacter asiaticus is presumed to be Asian and may have developed with citrus, while *Candidatus* L. africanus probably came from native African rutaceous plants, since citrus is an introduced species in Africa. A native plant, *Toddalia lanceolata*, was found to be a good host of both *Candidatus* L. africanus and its natural vector, *T. erytreae* (Garnier & Bové 1996). Lin & Lin (1990) postulate that Asian citrus greening originated in the northeastern part of Guangdong Province in China. It is also possible that the Asian greening pathogen has a geographical origin similar to that of its primary vector, *D. citri*, which probably evolved with similar species of *Diaphorina* in the Indian subcontinent.

Distribution

It is important to keep an updated file on the known distribution of citrus greening disease because rutaceous plants or citrus psyllids from those locations may harbor the pathogens. Toorawa (1998) compiled a summary of countries known to have citrus greening in his Table 3. Each

entry in his table is referenced by literature citation, and he notes the laboratory that did the molecular confirmation. Locations listed below are from Toorawa (1998) unless noted otherwise. Asian countries include: China (including Hong Kong), Indonesia, southern islands of Japan, Malasia, Philippines, Taiwan, Thailand, and Vietnam. Evidently citrus greening disease is spreading in Japan. Subandiyah et al. (2000) have confirmed citrus greening using molecular diagnosis in four places in Okinawa. Prior to this survey, citrus greening was known only from the southernmost island of Iriomote. Countries with citrus greening in the Indian subcontinent include Bangladesh, Bhutan, India, Nepal, and Pakistan. In the Indian Ocean, citrus greening disease is found in Sri Lanka, the Comoros Islands, Madagascar, Mauritius, and Réunion. All of these places have established populations of *D. citri* and Asian citrus greening. Mauritius and Réunion also have African citrus greening and *T. erytreae*. Similarly, in the Saudi Arabian peninsula, Saudi Arabia and Yemen have both species of vectors and both pathogens. In Africa, Burundi, Cameroon, Central African Republic, Ethiopia, Kenya, Malawi, Rwanda, Somalia, South Africa, Swaziland, Tanzania, and Zimbabwe all have African citrus greening and *Trioza erytreae*. *Diaphorina citri* is not known to be established in the African mainland. Although *D. citri* has been observed in Iran (Danet, pers. comm., ex Toorawa 1998), it is not known if citrus greening disease occurs there. Additionally, Varma & Atiri (1993) reported that over 50% of plants in some areas of Nigeria show symptoms of citrus greening. Symptoms have been observed all over Nigeria, but presence of the pathogen has not been confirmed by molecular analysis. The CABI map 766 (1998) additionally lists Laos and Myanmar as positive for *Candidatus* L. asiaticus. It states that there is an unconfirmed report for Syria. Garnier & Bové (2000b) added Cambodia to the list of countries where citrus greening is present. Citrus greening disease was found in Papua New Guinea in 1999 (Lee 2002). The status of citrus greening in Afghanistan, Brunei, and Singapore is unknown. In July 2004, as this paper was in press, citrus greening disease was reported in Brazil by Fundecitrus (Anon. 2004).

Plant Damage

Citrus greening is a very destructive disease. A survey conducted over an 8-year period in Réunion Island indicated that 65% of the trees were badly damaged and rendered unproductive within 7 years after planting (Aubert et al. 1996). In Thailand, citrus trees generally decline within 5-8 years after planting due to citrus greening (Roistacher 1996). Roistacher (1996) showed that groves must live for a minimum of about 10 years in order to make a profit. Infected trees are stunted and sparsely foliated. The symptoms can resemble nutritional stress, especially zinc deficiency symptoms on recent growth; however, a more diagnostic mottle usually occurs on slightly older leaves that resembles symptoms of luteoviruses in dicots (e.g., *Potato leafroll luteovirus*). The mottle differs from nutrition-related mottling in that greening induced mottling usually crosses leaf veins, whereas nutrition-related mottling usually occurs between or along leaf veins. Off-season bloom, fruit drop, and twig dieback are other symptoms. Fruit are small, lopsided, hard, and have a bitter flavor. Seed abortion is common (Capoor et al. 1974). Citrus greening disease may predispose plants to other pest problems such as the citrus longhorned beetle, *Anoplophora chinensis* Forster (Aubert 1990b). A combination of citrus greening, citrus longhorned beetle, and associated *Phytophthora* fungi are common in advanced citrus greening epidemics (Aubert 1990b).

Toorawa (1998) attempted to compile global infection statistics. He estimated 50 million trees infected in south and southeast Asia, three million trees infected in Indonesia, and ten million trees infected in Africa. In India and Saudi Arabia, there has been a marked decline in citrus industries as a result of citrus greening disease.

Detection

Vector

In low numbers, *Diaphorina citri* is an inconspicuous pest of citrus. The adults are the most easily observable stage. They are about 3-4 mm long. The wings have distinct bars on the top and bottom, giving the insects a flattened X-pattern when viewed laterally. Characteristically, they sit at a 45° angle to the shoot or leaf on which they feed. Adults jump readily when approached. It is best to collect them either by using an aspirator, or by bagging the entire shoot. Another way to collect specimens in excellent condition is to place an inverted empty test tube above an infested shoot while disturbing the colony. The adults will jump up into the tube and remain there.

Nymphs are difficult to see. They are flat and tend to wrap themselves around the shoot where they feed. Superficially, they look similar to scale insects. Nymphs may be green or orange in color, but, unlike scale insects, they have large wing pads. Eggs, bright yellow or orange and shaped like a pointed football, are attached in the plant tissue at one end. Eggs are deposited on the "feather flush" of the host. It is very difficult to see eggs without a hand lens.

Pathogen

It is only within the last few years that reliable detection of the greening pathogens has been

available. DNA probes now have been used successfully to detect *Candidatus* Liberibacter spp. both in infected plants and in psyllid vectors (Bové et al. 1993; Tian et al. 1996). The bacteria also can be detected with an electron microscope, ELISA (Garnier & Bové 1993), and by biological assay. Roistacher (1991) gives a detailed methodology for preparing specimens for electron microscopy.

Unfortunately, infected trees may be overlooked if symptoms alone are used for detection. Aubert (1990b) estimated that 15% to 20% of the infected plants are overlooked by nursery inspectors who rely only on visual inspection.

Lafleche & Bové (1970) using a transmission electron microscope observed a "mycoplasma-like organism" in citrus phloem tissue infected with citrus greening disease. The organisms were about 2000 nm long and 100-200 nm in diameter. Similar bodies soon were observed in both vectors of the citrus greening disease, *T. erytreae* (Moll & Martin 1973) and *D. citri* (Chen et al. 1973). A further comparison of the greening organism (Saligo et al. 1971) with citrus stubborn, a spiroplasma, showed that the outer membrane of the greening organism was much thicker (25 nm) than that of the spiroplasma (10 nm).

Further studies showed the bacterial nature of the greening organism, and a peptidoglycan- containing outer membrane of gram negative bacterial type was identified (Garnier et al. 1984). Molecular information provided the basis for accurate nomenclature for the two species. The bacterium was recognized as a new '*Candidatus*' genus Liberibacter, in the alpha subdivision of proteobacteria (Jagoueix et al. 1994).

Monoclonal antibodies raised against proteins purified from infected greening tissue from Africa, China, and India reacted selectively with the source antigens and a few other isolates of citrus greening, demonstrating the existence of several serotypes of greening (Garnier et al. 1987; Gao et al. 1993). These monoclonal antibodies are too isolate-specific to be used for general detection of greening.

Molecular approaches such as PCR and strain-specific DNA probes now have been used successfully to detect and differentiate *Candidatus* Liberibacter spp. both in infected plants and in psyllid vectors (Bové et al. 1993; Jagoueix et al. 1996; Tian et al. 1996). Unfortunately, detection is not always reliable. Sometimes trees with classic greening symptoms test negative with PCR (Toorawa 1998).

Molecular detection methods have been difficult to develop since the greening organism has not yet been cultured. Villechanoux et al. (1992) isolated total DNA from periwinkle plants infected with Indian greening, and digested it with restriction enzyme, HindIII. The digested DNA was cloned and the clones were screened by differential hybridization with DNA from both healthy and infected tissues. They identified three clones with 2.6, 1.9, and 0.6 kb inserts to be specific to the greening bacterium. The two larger clones reacted with all the Asian forms, but not with the African isolates, while the 0.6 kb clone reacted only with the Indian greening. Villechanoux et al. (1993) sequenced and analyzed the three greening specific clones. The larger 2.6 kb clone contained the genes of the nusG-rplKAJL-rpoBC operon, confirming the eubacterial nature of the greening organism at the molecular level. The 1 kb insert contained sequences for a bacteriophage-type DNA polymerase. The sequences from the 0.6 kb insert did not match anything in the database of known sequences.

Since the bacterial nature of the greening organism was established, Jagoueix et al. (1996) used universal primers for general amplification of prokaryotic 16S rDNA. Based on sequence information, primers have been developed to amplify a 1,160 bp region of ribosomal DNA for detection of greening by PCR. Further differentiation of Asian and African forms of greening can be achieved by restriction enzyme XbaI digestion. The XbaI digestion of an 1160 bp fragment from L. africanus yields three fragments of 520 bp, 506 bp, and 130 bp, while the Asian greening, L. asiaticus, yields only two fragments of 640 bp and 520 bp.

Ribosomal DNA primers have been used widely for detection of both forms of greening. These primers have been shown not to amplify 16S ribosomal sequences of other citrus pathogens (Jagoueix et al. 1996).

Additionally, some citrus species, such as sweet oranges and mandarins, produce a compound (gentisic glucoside) as a result of infection. Gentisic acid glows violet under UV light and can be seen directly in the fruit albedo of sweet oranges. A bark extract procedure (Roistacher 1991) can be used with other commercial citrus species (mandarin and tangelo). Results are not consistent for lemon, lime, pummelo, and grapefruit. Gentisic acid analysis sometimes produces false negatives and false positives, so it cannot be used alone for definitive diagnosis. However, results are fairly consistent for sweet oranges, so the technique can be useful in conjunction with other more reliable (but also more expensive and time-consuming) tests (Hooker et al. 1993).

For many years, biological indexing has been used for citrus greening diagnosis. Miyakawa (1980) found that ponkan (*Citrus reticulata* Blanco) and Orlando tangelo (*Citrus tangelo* J. Ingram & H. Moore) are the best indicators, particularly if severe forms of *Citrus tristeza virus* (CTV) are present and may confound symptom expression.

Detection of citrus greening pathogens from asymptomatic tissue is inconsistent by any known method. Similarly, the molecular assays sometimes are complicated to run, and results are not always believable. Clearly, more accurate, timely, and robust detection methodologies are needed.

EPIDEMIOLOGY

Since it has only been in the last several years that citrus greening pathogens could be detected reasonably reliably by means of molecular methods, some of the basic characteristics of transmission and epidemiology are poorly understood. There are reports of transmission via psyllid vectors, grafting, dodder, and seed.

Psyllid Transmission

Psyllid transmission is the primary means of spread in the field. Acquisition times of 30 min for Asian psyllids (Roistacher 1991) and 24 h for African psyllids (Buitendag & von Broembsen 1993) have been reported. In some experiments, acquisition feeding of 5-7 h was sufficient to transmit citrus greening pathogens, while feeding periods of 1-3 h were not (Xu et al. 1988). The pathogen probably multiplies in the vector (Aubert 1987; Moll & Martin 1973; Xu et al. 1988), but this has not been demonstrated by molecular experiments. It is not known if psyllids can be infected simultaneously by both bacteria species (Garnier et al. 1996), although both psyllid species transmit both pathogens experimentally (Lallemand et al. 1986; Massonie et al. 1969). Adults and fourth and fifth instar Asian citrus psyllids are able to transmit the pathgen after a latent period as short as one day or as long as 25 days (Roistacher 1991; Xu et al. 1988). Fourth and fifth instars were able to retain the pathogen as adults, which were able to transmit the disease immediately after emergence (Xu et al. 1988). First through third instars were unable to transmit citrus greening (Xu et al. 1988). A latent period of 24 h has been reported for African greening (Buitendag & von Broembsen 1993). Transmission is thought to occur via salivary secretions (Aubert 1987). Serial transfer experiments by van den Berg et al. (1992) suggest that young nymphs of *T. erytreae* can acquire the bacteria even though they do not transmit them.

Candidatus Liberibacter spp. potentially should be considered pathogens of the insect as well as the plant, if in fact they multiply in the psyllid vectors, as suggested by Xu et al. (1988) and Moll & Martin (1973). There are conflicting reports as to whether *Candidatus* Liberibacter spp. are transmitted transovarially (Buitendag & von Broembsen 1993; Roistacher 1991; van den Berg et al. 1992; Xu et al. 1988). Xu et al. (1988) reported that there is no evidence for transovarial transmission, because *D. citri* nymphs collected immediately after hatching on diseased plants did not transmit citrus greening disease to indicator plants. The most extensive studies on transovarial transmission of citrus greening pathogens were done with *T. erytreae*. van den Berg et al. (1992) allowed immature psyllids to develop on heavily infected plants. When adults emerged, they were allowed to feed and mate on infected plants. After 14 days, the mouthparts of 100 of the females were severed. Ten of these females were placed on each of ten healthy indicator plants, where they laid eggs. Adults from those eggs were allowed to feed on the same plants for 30 days after emergence. Plants were later sprayed, kept insect-free, and tested for citrus greening disease after six months. One of the ten plants developed citrus greening disease. In another experiment, oviposition was allowed to occur on the infected plants. Crawlers were removed immediately after hatching and prior to feeding and placed on indicator plants. Five of the 24 plants on which these psyllids completed development became infected with citrus greening disease. The most logical explanation for these infections is transovarial transmission; however, the authors postulate that the plant in the first experiment could have been infected via oviposition, and those in the second experiment could have been infected as a result of absorption of greening bacteria from the infected host by the egg. These experiments should be repeated with *D. citri*. To our knowledge, there are no studies on sexual transmission of *Candidatus* Liberibacter spp. in psyllids. Similarly, it is not known if parasites that develop in infected psyllids can transmit the pathogen to the hosts of their offspring. Hoy et al. (2001) has a good review of relevant literature about transmission of plant pathogens via parasites of vectors.

Candidatus Liberibacter spp. can be detected in single psyllids (Bové et al. 1993). Experimental data showing detection of citrus greening pathogens in psyllids indicate that percent transmission by psyllids that feed on infected trees may be variable. Thirty-nine percent of psyllids collected in Malaysia in September 1991 tested positive for the pathogen, whereas less than 1% of those collected in February 1992 in India had positive DNA hybridization reactions for citrus greening (Bové et al. 1993). Toorawa (1998) also found a higher percentage of *D. citri* that had positive DNA hybridization reactions in the fall. The relationship between positive detection of the pathogen in the psyllids and ability to infect indicator plants is not known. Field infectivity experiments, in which adult psyllids are trapped alive and allowed to feed on indicator plants, are badly needed. These kinds of experiments, though labor intensive, would provide valuable information on infectivity and seasonality of transmission.

Graft Transmission

The citrus greening pathogens are graft transmitted (Bové et al. 1996; van Vuuren 1993); however, graft transmission of *Candidatus* Liberibacter spp. is variable, depending upon the plant part used for grafting, the amount of tissue used, and the

pathogen isolate. With single buds, graft transmission of African greening varied from 0 to 50%, depending upon the isolate used (van Vuuren 1993). Side grafts with twigs were even more efficient at transmitting the pathogen, whereas fruit stems and bark strips were not effective (van Vuuren 1993).

Lin & Lin (1990) reported some early experiments performed but apparently not previously published by Chen Qi-bao. Seven months after grafting diseased buds on healthy rootstock, 58% of grafts had survived, and of those, 20% showed citrus greening symptoms. In another experiment, 10-16% of grafts with buds from asymptomatic branches on diseased trees developed symptoms, while 40% of grafts from symptomatic branches developed symptoms of citrus greening in 3-9 months.

Seed Transmission

There is little information on seed transmission. Most fruit is lost, and that which remains has a high proportion of aborted seed; however, Tirtawidjaja (1981) collected normal and greening-affected (very small) fruit and harvested normal-looking seeds from each. No symptoms were observed on seedlings from seed taken from normal fruit, even when they were collected from infected plants; however, seeds derived from smaller, greening-affected fruit produced some stunted chlorotic seedlings. Three of the seedlings had the same appearance as insect-inoculated seedlings. This experiment bears repeating. Miyakawa et al. (1990) reported that greening-infected Troyer citrange trees showed few leaf symptoms and bore a good crop, although there were relatively high numbers of aborted seeds. If seed transmission occurs in cultivars like citrange that are used for citrus rootstocks, spread could occur through liners as well as by budding.

Timing, Patterns, and Rates of Spread

Many of the parameters necessary to develop epidemiological models of citrus greening disease spread are not known. Little is known about how soon after infection by psyllids the pathogens can be detected in the infected plant; however, symptoms of African greening developed 40 cm back towards the trunk of the tree from the vector feeding site on infected shoots within 12 months (van Vuuren 1993). Pathogens became detectable in shoots between 2.5 and 3.5 months after leaves emerged from buds on diseased trees, and symptoms expressed themselves in a similar period of time (Su & Huang 1990). Pathogens could be detected in the root system of Luchen seedlings five months after graft inoculation (Su & Huang 1990). The time interval between the infection of a citrus shoot and the possibility of subsequent acquisition of the pathogen by new vectors is not known.

Percent citrus greening disease transmission by psyllids raised on infected plants is variable. Efficiency may vary from around 1% to 80% for single insects (Xu et al. 1988). Xu et al. (1988) list several conditions that enhance transmission efficiency, including psyllid-inoculated source plants, young (3-4 leaves) indicator plants, psyllids raised on infected plants in the laboratory, and control of shade and temperature in the greenhouse where indicator plants are kept. The genetic makeup of the pathogen and vector also may account for some of the variation in that some populations of citrus psyllids may be inherently better vectors, and some populations of citrus greening bacteria may be inherently more transmissible.

Although patterns of spread in groves have been studied (Gottwald et al. 1991a,b), there has not been an attempt to match disease spread information with a reproducible measurement of vector abundance. It is significant that Gottwald et al. (1991a) found that the source of infection was a small planting near a farmhouse of 24 trees with severe disease in a study in China. The incidence of detectable citrus greening disease rose to 14% in the first 5 years after planting. They estimate that the disease would have reached its asymptote in the next 2-4 years, and thus, the productive life of the grove would be less than 10 years, even with a clean start.

In another Chinese study in Shantou, Guangdong, ingress into densely planted groves showed a typical edge effect. Twenty percent of the plants were lost to greening by the fifth year, and the groves lost their commercial value by the time they were seven to eight years old (Aubert 1990b).

There are robust experimental data for dispersal and infestation patterns for *T. erytreae*, the African citrus psyllid; however, we could not find similar data for *D. citri*. Samways & Manicom (1983) severely pruned a citrus grove and inspected it carefully for initial psyllid infestation in the spring. The initial distribution was random on a tree-to-tree basis, but the side of the grove closest to the neighbor's infested grove had a higher density of *T. erytreae*, suggesting that the source of the infestation was the neighboring grove. Results showed that even after the initial invasion, there was considerable tree-to-tree movement, which potentially can spread the pathogens further. *Trioza erytreae* readily invaded the whole grove within days.

There is good experimental evidence for flight distance of *T. erytreae*, but not for *D. citri*. van den Berg & Deacon (1988) released clean *T. erytreae* in an area that had no citrus. Yellow traps were placed in a grid at various distances from the release site. From these data, it was determined that *T. erytreae* could fly at least 1.5 km in the absence of their host plants. A similar estimate was made for *D. citri*, but it was not based on experimental evidence (Tolley 1990).

Infected plants were clustered within groves, possibly indicating that most *D. citri* normally do not fly very far (Aubert et al. 1996; Gottwald et al. 1991a,b). Similarly, Toorawa (1998) says that in Mauritius, *D. citri* are not very mobile. This assertion is based on the very low percentage (0.33%) of *D. citri* captured on *M. paniculata* that were contaminated with the citrus greening pathogen. An estimate of maximum flight distance for infective vectors is needed for determining safe isolation distance for quarantine and eradication purposes, and this is not well known. A 30-km separation was sufficient in Nepal (Regmi et al. 1996) but not in Vietnam (Bové et al. 1996).

Although the distance *D. citri* can fly is poorly known, experiments on height of capture and color preference have been done. Aubert & Hua (1990) reported that "brown yellow" traps worked better for collecting *D. citri* than other colors on cloudy days. However, plain yellow traps worked better on sunny days. Maximum catch occurred at 1.5 m above the ground.

Host Range and Vector Specificity

The host range of *D. citri* includes many of the close citrus relatives (Table 1). DPI's Citrus Arboretum in Winter Haven, Florida provided a good opportunity to determine which plants serve as field hosts of *D. citri* in Florida. Two species of native *Zanthoxylum* are represented at the facility. No *D. citri* were ever found on *Zanthoxylum clava-hercules* L; however, *Zanthoxylum fagara* (L.) Sarg. may be an occasional host. *Zanthoxylum fagara* nearly always has suitable flush, sporting a nearly year-around population of *Toxoptera citricida* (Kirkaldy), the brown citrus aphid; however, we found *D. citri* nymphs present only once, and in very low numbers. Similarly, no *D. citri* were found on *Z. fagarae* growing next to an infested lime grove in South Florida. *Zanthoxylum* spp. may be non-hosts, or as in the case of *Z. fagara*, very poor hosts, of *D. citri*. Another apparent non-host, based on our observations at the DPI Citrus Aboretum, is *Casimiroa edulis* Llave & Lex., white sapote. Both *Zanthoxylum* and *Casimiroa* are of Western Hemisphere origin, suggesting a preference by *D. citri* for Old World Rutaceae.

There are many observations about preferred hosts of *D. citri*, but only one comparative laboratory study (Tsai & Liu 2000). In their study, Tsai & Liu (2000) tested *Murraya paniculata* (L.) Jack (orange jasmine), *Citrus jambhiri* Lushington (rough lemon), *Citrus aurantium* L. (sour orange), and *Citrus* × *paradisi* Macfad. (grapefruit). Grapefruit was the best host, followed by the other hosts, among which there was no statistical difference.

There is not much information on the host range of the pathogens because of the difficulty in measuring the presence of the pathogen with certainty (Table 2). Most, if not all, *Citrus* spp. apparently are susceptible to some degree, although some species (*Citrus indica* Tan. and *Citrus macroptera* Montr.) remained symptom-free under heavy inoculum pressure (Bhagabati 1993). *Citrus limetta* remained symptom-free after laboratory inoculation (paper does not specify means of inoculation) (Nariani 1981).

Murraya paniculata is a preferred host of *D. citri*; however, there is not agreement on whether it is a host for the greening pathogens. Careful work using dot hybridization by Hung et al. (2000) indicates that Asian greening pathogens from Taiwan will not multiply in *M. paniculata* or *M. koenigii*. Toorawa (1998), who worked in Mauritius, concurs. On the other hand, Tirtawidjaja (1981) was able to observe consistent external and internal symptoms on 25-33% of inoculated *M. paniculata* plants. Asian greening may be caused by a population of bacterial strains with somewhat differing host ranges. The fact that *Murraya* spp., which are native to the Indian subcontinent (Coile 1995) and are good hosts of *D. citri*, are resistant or at least tolerant to citrus greening disease further supports a South Asia origin for the pathogens.

Several citrus relatives have been shown with modern detection methods to harbor greening pathogens, including *Severinia buxifolia* (Poiret) Ten. (Hung et al. 2000), *Limonia acidissima* L. (Koizumi et al. 1996; Su et al. 1995; Hung et al. 2000), and *Toddalia lanceolata* Lam (Korsten et al. 1996). Many other citrus relatives are implicated as hosts of citrus greening pathogens, but symptoms have been the only criteria (Table 2).

Citrus greening has been transmitted experimentally to several hosts outside the Rutaceae. Greening can be transmitted by dodder (*Cuscuta* sp.) (Convolvulaceae (Cuscutaceae)) to non-Rutaceous plants such as *Catharanthus roseus* L. G. Don (Apocynaceae) (periwinkle) (Tirtawidjaja 1981) and *Nicotiana tobacum* L. cv. 'Xanthii' (Solanaceae) (tobacco) (Garnier & Bové 1993), suggesting a wide physiological host range for the pathogens. The pathogens even multiplied in the dodder itself (Ghosh et al. 1977; Su & Huang 1990).

As with plant hosts, vector specificity of *Candidatus* Liberibacter spp. may be low, in that both Asian and African citrus psyllids (two different psyllid families) can transmit both species of greening organisms. The relatively narrow host and vector associations observed in the field may be determined by the restricted host ranges of the psyllid vectors rather than by the potential host and vector associations for the pathogens. After obtaining successful transmission of greening to *Catheranthus* by dodder, Tirtawidjaja (1981) attempted to inoculate *Catharanthus* with *D. citri* and was unsuccessful, presumably because the psyllids would not feed normally on a non-host.

TABLE 1. HOST LIST FOR *DIAPHORINA CITRI* KUWAYAMA.

Species	Source	Comments
Aegle marmelos (L.) Corr.	Viraktamath & Bhumannavar 2002	
Aeglopsis chevalieri Swingle	Koizumi et al. 1996	
Afraegle gabonensis Engl.	DPI Citrus Arboretum survey	
Afraegle paniculata (Schaum.) Engl.	DPI Citrus Arboretum survey	
Artocarpus heterophyllus Lamarck (Moraceae)	Shivankar et al 2000	
Atalantia missionis Oliver	Tirtawidjaja 1981	
Atalantia monophylla (L.) Corr.	DPI Citrus Arboretum survey	
Atalantia sp.	Koizumi et al. 1996; Aubert 1990a	adult feeding only (Aubert)
Balsamocitrus dawei Stapf	Koizumi et al. 1996	
Citropsis gilletiana Swingle & M. Kellerman	DPI Citrus Arboretum survey	
Citropsis schweinfurthii (Engl.) Swingle & Kellerm.	Chavan & Summanwar 1993	good host
Citrus aurantifolia (Christm.) Swingle	Aubert 1987, 1990a; Florida surveys	preferred host
Citrus aurantium L.	Florida surveys	
Citrus deliciosa Tenore	Aubert 1987	common
Citrus grandis (L.) Osbeck	Aubert 1987	occasional, *C. grandis* is considered a junior synonym of *C. maxima*
Citrus hystrix DC.	Aubert 1987; Lim et al. 1990	occasional
Citrus jambhiri Lushington	Florida surveys	
Citrus limon (L.) Burm. f.	Aubert 1987, 1990a	common
Citrus madurensis Loar.	Aubert 1990a	
Citrus maxima (Burm.) Merr.	Aubert 1990a	occasional, but observed nymphal development
Citrus medica L.	Aubert 1987, 1990a	common
Citrus meyeri Tan	Florida surveys	
Citrus × *nobilis* Lour.	Aubert 1987; Florida surveys	common
Citrus obovoidea Hort. ex Tanaka cv 'Kinkoji'	Florida surveys	
Citrus × *paradisi* Macfad.	Aubert 1987; Florida surveys; Tsai & Liu 2000	common; a preferred host in Florida (DPI); best host in laboratory assays (Tsai & Liu)
Citrus reticulata Blanco	Aubert 1987, 1990a, Koizumi et al. 1996; Florida surveys	common
Citrus sinensis (L.) Osbeck	Aubert 1987, 1990a; Florida surveys	common
Citrus spp.	Aubert 1990a; Florida surveys	common host
Clausena anisum-olens Merrill	Aubert 1990a	occasional host, observed nymphal development
Clausena excavata Burm. f.	Aubert 1990a; Lim et al. 1990	
Clausena indica Oliver	Aubert 1990a	adult feeding in laboratory
Clausena lansium (Lour.) Skeels	Koizumi et al. 1996; Aubert 1990; Florida surveys	poor host (Koizumi et al.); common host (Aubert); population highly variable (FL surveys)
Eremocitrus glauca (Lindley) Swingle	Koizumi et al. 1996	poor host, but plant died
Eremocitrus hybrid	DPI Citrus Arboretum Survey	
Fortunella crassifolia Swingle	DPI Citrus Arboretum Survey	
Fortunella margarita (Lour.) Swingle	DPI Citrus Arboretum Survey	
Fortunella polyandra (Ridley) Tanaka	DPI Citrus Arboretum Survey	
Fortunella spp.	Aubert 1987, 1990a	occasional; nymphal development, laboratory only (Aubert 1990)
Limonia acidissima L.	Koizumi et al. 1996	
Merrillia caloxylon (Ridley) Swingle	Lim et al. 1990; Aubert 1990a	cage in laboratory only (Lim et al.); adult feeding in laboratory (Aubert)
Microcitrus australasica (F.J. Muell.) Swingle	Koizumi et al. 1996; Aubert 1987, 1990a; DPI Citrus Arboretum survey	common; observations in laboratory (Aubert 1990a)
Microcitrus australis (Planch.) Swingle	DPI Citrus Arboretum survey	
Microcitrus papuana H.F. Winters	DPI Citrus Arboretum survey	

TABLE 1. (CONTINUED) HOST LIST FOR *DIAPHORINA CITRI* KUWAYAMA.

Species	Source	Comments
Microcitrus sp. 'Sidney'	DPI Citrus Arboretum survey	
Murraya exotica L.	Aubert 1990a	adult feeding in laboratory
Murraya koenigii (L.) Sprengel	Koizumi et al. 1996; Aubert 1987; 1990a; Lim et al. 1990; Florida surveys	good host (Koizumi); occasional host; no eggs observed (Aubert 1987); good host with nymphal development (Aubert 1990a); not an excellent host but will support a small population, including eggs (FL surveys)
Murraya paniculata (L.) Jack	Koizumi et al. 1996; Aubert 1987, Florida surveys	a preferred host
Naringi crenulata (Royb.) Nicholson	DPI Citrus Arboretum survey	
Pamburus missionis (Wight) Swingle	DPI Citrus Arboretum survey	
Poncirus trifoliata (L.) Raf.	Koizumi et al. 1996; Aubert 1987, 1990a)	occasional; eggs, but no nymphs (Aubert 1987, 1990a)
Severinia buxifolia (Poiret) Ten.	Koizumi et al. 1996; Florida surveys	
Swinglea glutinosa (Blanco) Merr.	Garnier & Bové 1993; Florida surveys	
Toddalia asiatica (L.) Lam	Aubert 1987, 1990a	occasional; no eggs observed
Triphasia trifolia (Burm. f.) P. Wilson	Koizumi et al. 1996; Aubert 1987; DPI Citrus Arboretum survey; Aubert 1990a	poor host (Koizumi); occasional host (Aubert); all stages and damage evident (FL surveys)
Vepris lanceolata G. Don	Aubert 1987, 1990a	occasional; no eggs observed
Zanthoxylum fagara (L.) Sarg.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; very few *D. citri* found; possible non-host.
Apparent non-hosts:		
Casimiroa edulis Llave & Lex.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; no *D. citri* found
Zanthoxylum clava-herculis L.	DPI Citrus Arboretum Survey	plenty of suitable new shoots; no *D. citri* found

The potentially wide physiological host range of the pathogens, combined with low vector-pathogen specificity, has potential implications for the epidemiology of the disease. A naturally-occurring native *Candidatus* Liberibacter sp., normally spread by native psyllids in plants unrelated to citrus, could be inoculated into citrus in a rare event. This scenario has been postulated for Australian citrus dieback, a disease of unknown etiology (Miyakawa & Yuan 1990; Broadbent 2000; Broadbent et al. 1976). Once in citrus, the citrus psyllids might spread the pathogen further within the crop, causing major damage.

MANAGEMENT

Control of *D. citri* and citrus greening disease will involve all aspects of an integrated pest management program. The following is a summary, based on a standard IPM approach.

Chemical Control

If both the insect and the pathogen are present, the world literature is basically in agreement that it is necessary to control psyllids with pesticides (Tolley 1990). It is important to control psyllids, even on apparently disease-free plants (Aubert 1990b). Aubert (1987) recommended protecting spring flush. Populations are considered high when they reach three nymphs and five adults per twig. A Chinese program to rehabilitate citrus production in an area affected with citrus greening disease requires 10-13 sprays per year during flush periods (Roistacher 1996). In Thailand, procedures to monitor for psyllids and spray when necessary are recommended (Roistacher 1996). Su et al. (1986) recommended spraying at 10-20 day intervals during critical infection periods. Gonzales & Viñas (1981) recommend spraying young trees at weekly intervals during the rainy season and every ten days during the dry season. Aubert (1990b) indicates that it is very important for farmers in a citrus production area to synchronize chemical applications.

Timing of pesticides is critical. Aubert (1988) suggests using yellow sticky cards for monitoring *D. citri* in order to time control action. In Florida, this method may be too slow. Based on our trapping collections in Florida, the peak spring flights

TABLE 2. HOST LIST FOR *CANDIDATUS* LIBERIBACTER SPP.

Species	Source	Comments
Aeglopsis chevalieri Swingle	Koizumi et al. 1996	questionable symptoms
Atalantia missionis Oliver	Tirtawidjaja 1981	symptoms only, vector transmission
Balsamocitrus dawei Stapf.	Koizumi et al. 1996	symptoms only; vector transmission
Calodendrum capensis Thunb.	Garnier et al. 2000	molecular characterization
Catharanthus roseus (L.) G. Don (Apocynaceae)	Tirtawidjaja 1981	symptoms, electron microscopy; (dodder transmission only)
X *Citroncirus webberi* J. Ingram & H. E. Moore	Miyakawa & Yuan 1990; Nariani 1981	symptoms (few) stunting, seed abortion (Miyakawa & Yuan); symptoms fairly intense (Nariani)
Citrus amblycarpa Ochse	Tirtawidjaja 1981	
Citrus aurantifolia (Christm.) Swingle	Miyakawa & Yuan 1990; Tirtawidjaja 1981	mild symptoms
Citrus aurantium L.	Miyakawa & Yuan 1990	symptoms
Citrus depressa Hayata	Miyakawa & Yuan 1990	symptoms
Citrus grandis (L.) Osbeck	Miyakawa & Yuan 1990; Su & Huang 1990	symptoms; pomelo-infecting strain prevalent since 1970s (Su & Huang). *C. grandis* is considered a junior synonym of *C. maxima*
Citrus hassaku Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus hystrix DC.	Miyakawa & Yuan 1990	symptoms
Citrus ichangensis Swingle	Miyakawa & Yuan 1990	symptoms
Citrus jambhiri Lushington	Tirtawidjaja 1981	
Citrus junos Sieb. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus kabuchi Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus limon (L.) Burm. f.	Miyakawa & Yuan 1990	symptoms, presence of putative pathogen in tissue; plant reported tolerant to disease, but source of vectors (Lee 1996)
Citrus × *limonia* Osbeck	Miyakawa & Yuan 1990; Tirtawidjaja 1981	symptoms
Citrus × *nobilis* Lour. 'Ortanique'	Koizumi et al. 1996	symptoms
Citrus × *nobilis* Lour.	Koizumi et al. 1996	symptoms
Citrus oto Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus × *paradisi* Macfad.	Miyakawa & Yuan 1990	symptoms
Citrus reticulata Blanco	Miyakawa & Yuan 199; Tirtawidjaja 1981	symptoms
Citrus sinensis (L.) Osbeck	Miyakawa & Yuan 1990	symptoms, presence of putative pathogen in tissue
Citrus sunki Hort. ex Tanaka	Miyakawa & Yuan 1990	symptoms
Citrus unshiu (Mack.) Marc	Miyakawa & Yuan 1990	symptoms
Citrus sp. (mandarins)	Miyakawa & Yuan 1990	symptoms
Citrus sp. (pomelo/shaddock)	Miyakawa & Yuan 1990	symptoms
Clausena indica Oliver	Miyakawa & Yuan 1990	symptoms (stunting)
Clausena lansium (Lour.) Skeels	Tirtawidjaja 1981; Koizumi et al. 1996	symptoms only, vector transmission
Cuscuta australis R. Br. (Convolvulaceae (Cuscutaceae))	Su & Huang 1990	observed to multiply in stems, haustoria and flower stalks
Fortunella spp.	Miyakawa & Yuan 1990	symptoms
Limonia acidissima L.	Koizumi et al. 1996; Su et al. 1995; Hung et al. 2000	symptoms only; vector transmission; DNA hybridization (Su et al.); infection apparently temporary (Hung et al.)
Microcitrus australasica (F. J. Muell.) Swingle	Koizumi et al. 1996	stunting
Murraya koenigii (L.) Sprengel	Hung et al. 2000	no detection by dot hybridization after attempted graft transmission; no symptoms (Hung et al.)
Murraya paniculata (L.) Jack	Tirtawidjaja 1981; Aubert et al. 1985; Miyakawa 1980; Hung et al. 2000, Koizumi et al. 1996; Toorawa 1998	Mixed results: symptoms only (external and internal), vector transmission (Tirtawidjaja); can harbor greening organism (Aubert et al.). EM negative (Miyakawa); No detection by dot hybridization after attempted graft transmission (Hung et al.); no symptoms (Koizumi et al.); not a host (Toorawa)
Nicotiana tabacum L. 'Xanthii' (Solanaceae)	Garnier & Bové 1993	symptoms, dodder transmission only

TABLE 2. (CONTINUED) HOST LIST FOR *CANDIDATUS* LIBERIBACTER SPP.

Species	Source	Comments
Poncirus trifoliata (L.) Raf.	Miyakawa 1980; Miyakawa & Yuan 1990; Nariani 1981; Koizumi et al. 1996	back inoculations (Miyakawa, Miyakawa & Yuan)
Severinia buxifolia (Poiret) Ten.	Hung et al. 2000; Koizumi et al. 1996	DNA hybridization with specific probe; symptoms
Swinglea glutinosa (Blanco) Merr.	Tirtawidjaja 1981	symptoms only, vector transmission
Toddalia lanceolata Lam	Korsten et al. 1996	DNA/DNA hybridization, PCR
Triphasia trifolia (Burm. f.) P. Wilson	Koizumi et al. 1996	severe stunting, vector transmission
Possible non-hosts:		
Citrus indica Tanaka	Bhagabati 1993	no symptoms in the field in endemic area
Citrus limetta Risso	Nariani 1981	no symptoms; laboratory inoculation (does not specify how)
Citrus macroptera Montrons	Bhagabati 1993	no symptoms in the field in endemic area

of *D. citri* occur very suddenly, and without prior incremental increase in numbers of adults collected. In Florida, it would be better to monitor buildup of nymphs on shoots, or to sample overwintered adults and observe when they become gravid. (Their abdomens turn orange when egg-laying is imminent.) In our opinion, scouting in the spring should focus on nymphs, because by the time adults emerge, the disease is already spreading. This is true particularly in the case of adults that emerge from nymphs that fed on infected plants, because they can transmit citrus greening bacteria immediately after emergence (Xu et al. 1988).

As is the case with other phloem-sucking Sternorrhyncha, systemic pesticides are particularly efficacious against *D. citri*. Trunk applications have proven useful (Aubert 1988; Buitendag & von Broembsen 1993). Two patent applicators are available in South Africa that calibrate the dose based on the diameter of the tree (Buitendag & von Broembsen 1993). Supriyanto & Whittle (1991) and Shivankar et al. (2000) also had success with trunk applications. The best time to apply was just prior to spring flush.

A computer model indicated that even with careful attention to inoculum reduction, at least 70% reduction in transmission is needed to delay the epidemic significantly (Supriyanto & Whittle 1991). Unfortunately, there was no standard measurement of psyllid abundance, so it is unclear what constitutes 70% reduction; however, it is clear that a pesticide with high efficacy is essential.

There has been little research with "soft" (environmentally friendly) pesticides, but Deacon et al. (1989) have found that certain chitin synthesis inhibitors work for eggs and first instars. Shivankar et al. (2000) report about 90% control for several botanicals, including neem formulations. Use of these materials alone may not be wise in an eradication effort, but they might be used effectively against spring populations if timing were just right.

Antibiotics injected into infected citrus trees provide temporary remission of symptoms (Buitendag & von Broembsen 1993; Lim et al. 1990; Su et al. 1986). Injection with antibiotics is recommended as part of an integrated management program in India (Nariani 1981). It is not known whether the titre of citrus greening bacteria is reduced sufficiently to impact transmission by insects or grafting. Symptoms reappear 1-1.5 years after injection (Zhou 1981). Tetracycline also can be used to treat budwood. The budwood is immersed in 1,000 µg/ml tetracycline hydrochloride for two h, or 500 µg/ml for three h (Zhou 1981).

Biological Control

Aubert (1987) indicated that pathogenic fungi may be the most important mortality factor for *D. citri*. Nymphal mortality of 60-70% could be expected where minimum daily relative humidity exceeded about 87.9% in Réunion Island (Aubert 1987). Two fungal pathogens were reported, including *Cladosporium* sp. nr. *oxysporum* Berk. & M.A. Curtis and *Capnodium citri* Mont. (Aubert 1987). Étienne et al. (2001) said that the fungus *Hirsutella citriformis* Speare was common during periods when humidity was greater than 80%. Use of insect pathogenic fungal sprays has not been reported. In Florida, fungal cadavers of *D. citri* have not been common in the hundreds of submitted regulatory samples of *D. citri* that we have seen in the past five years, in spite of the high relative humidity that characterizes our subtropical climate.

There are two well-known primary parasites of *D. citri*. One is a eulophid ectoparasite, *Tamarixia* (=*Tetrasticus*) *radiata* (Waterston). The other is

an encyrtid endoparasite, *Diaphorencyrtus aligarhensis* (Shaffee et al.). *Tamarixia radiata* apparently is more efficient at parasitizing *D. citri* than *D. aligarhensis* (Tang 1989). In surveys conducted in Réunion, *T. radiata* attacked 60-70% of *D. citri* nymphs, whereas, *D. aligarhensis* parasitism did not exceed 20% (Aubert 1987). Both parasites can be subject to high mortality due to hyperparasitism (Aubert 1987; Garnier & Bové 1993). There is considerable Asian literature about the parasite complex attacking *D. citri*, including life cycle studies (Tang & Wu 1991; Xu & Tang 1993), identification guides (Tang 1990; Tang & Aubert 1990), and survey information. Introduction of *T. radiata* into Réunion has improved citrus production significantly on the island (Aubert et al. 1996). While the success on Réunion is spectacular, it has occurred in the peculiar circumstances of an island environment in the absence of hyperparasites. Experience in Southeast Asia has shown that the same parasites are not able to similarly reduce transmission (Supriyanto & Whittle 1991).

In Mauritius, biological control of *T. erytreae* was much more effective than biological control of *D. citri* (Toorawa 1998). Toorawa (1998) postulates several reasons for this. First, the initial population of *T. erytreae* was much lower than that of *D. citri*. *Trioza erytreae* reproduces principally on citrus, which is regularly treated with pesticide, whereas *D. citri* utilizes *M. paniculata*, which is unsprayed and is ubiquitous as an ornamental throughout the island. The climate on much of the island also is more suitable to *D. citri* than to *T. erytreae*. Second, the parasite of *T. erytreae* (*Tamarixia dryi* (Waterston)) has an alternate host in the common psyllid *T. litseae*, whereas *T. radiata*, the parasite of *D. citri*, has no alternate host (Toorawa 1998).

Tamarixia radiata also was introduced into Guadeloupe in January of 1999. The parasites were imported from Réunion and released immediately on arrival. The parasite apparently has been quite successful (Étienne et al. 2001), although release of any insects without prior quarantine is not recommended, particularly when vectored pathogens may be present in the host population.

Both *T. radiata* and *D. aligarhensis* were released into Florida (McFarland & Hoy 2001), but with mixed results. Apparently only *T. radiata* has established in Florida (Michaud 2002). Field data reported by Michaud (2004) indicate that parasitism by *T. radiata* contributed 1.3%, 0.2%, and 1.0% mortality of psyllid nymphs in three cohorts, respectively, observed in central Florida. The low rate of parasitism was due at least partially to intraguild predation by coccinellids. Exclusion of large predators from some of the observed citrus terminals of cohort 3 increased mummy formation about 20-fold (Michaud 2004).

Viraktamath & Bhumannavar (2002) list several more parasites of *D. citri* in their Table 1 and in the text on the preceeding page. *Psyllaephagus diaphorinae* Lin & Tao probably is a primary parasite. *Syrphophagus taiwanus* Hayat & Lin, *Syrphophagus* (= *Aphidencyrtus*) *diaphorinae* Myartseva & Tryapitsyn, and *Marietta* sp. nr. *exitiosa* Compere probably are hyperparasites. *Diaphorencyrtus diaphorinae* Lin & Tao is listed (Viraktamath & Bhumannavar 2002) as a hyperparasite, but it may be primary.

Predators of *D. citri* are known from wherever the psyllid occurs. One species of *Scymnus* (Coccinellidae) has been reported in Brazil (Gravena et al. 1996). Syrphids in the genus *Allograpta* have been found in Réunion and Nepal (Aubert 1987) and in Florida (Michaud 2002). Several coccinellids and chrysopids also have been reported (Aubert 1987), but there is no information about how much they actually reduce psyllid populations. In Florida, the most abundant predators are *Harmonia axyridis* Pallas and *Olla v-nigrum* Mulsant (Michaud 2001; Michaud 2002; Michaud 2004). *Olla v-nigrum* was a relatively rare species prior to the arrival of *D. citri*, but it exhibited a marked functional response to the establishment of *D. citri* (Michaud 2001). Coccinellid predators are by far the most important sources of biological control for *D. citri* in Florida (Michaud 2002; Michaud 2004). Both Michaud (2002, 2004) in Florida and Al-Ghamdi (2000) in Saudi Arabia have observed that spiders may be important predators for *D. citri*. In Saudi Arabia, spiders accounted for 33.6% of total predators (Al-Ghamdi 2000). Several other predators, including a histerid beetle, *Saprinus chalcites* Illiger and the predaceous carabid, *Egapola crenulata* Dejean, were important in Saudi Arabia (Al-Ghamdi 2000). A similar complex of predators, including Coccinellidae, Chrysopidae, and Syrphidae exists in Cuba (González et al. 2003).

Biological control of vectors of pathogens may have limited value in some circumstances, particularly in the case of a perennial tree crop like citrus. Population fluctuations are inherent in any predator-prey relationship. Some years the natural enemies predominate, and sometimes the pest is more abundant. If citrus greening is present, in years when citrus psyllids predominate, the entire grove may be infected and subsequently destroyed by citrus greening disease. Similarly, if pesticides are needed for some other pest problem such as whiteflies, mealybugs, scales, etc., natural enemies could be killed, and psyllid populations would increase dramatically. Once again, if citrus greening disease were present, the entire grove could be lost.

Biological control of the pathogen (cross-protection) has not been well-studied. It is known that plants can become infected with both Asian and African greening bacteria (Garnier et al.

1996). In South Africa, van Vuuren et al. (2000) found that a population of several strains of CTV was able to cross-protect citrus from African greening. The isolate and its aphid transmitted sub-isolates are under further study for potential use in cross protection. Naidu & Govindu (1981) did a greenhouse study on cross protection. Mild isolates did not provide complete cross protection when graft-inoculated sweet orange plants were challenged with severe isolates. Moreover, isolates that were mild in sweet orange were severe in grapefruit in a subsequent host range test.

Host Plant Resistance

Although there is no real resistance in *Citrus* spp. to citrus greening disease, some species and cultivars are somewhat tolerant. Koizumi et al. (1993) did extensive field surveys showing that some cultivars were less susceptible to decline than others. Most of the sweet orange trees became infected with the pathogen and subsequently declined, while grapefruit was more tolerant. In general, sweet oranges, mandarins and tangelos are most susceptible, grapefruit and lemon are more resistant, and limes, *Poncirus trifoliata* and citranges are the most tolerant (Lee 1996).

Cultural Control

Management of citrus greening in areas where the disease is endemic depends largely upon cultural control. Infected limbs and trees should be removed as symptoms appear. The pathogen apparently moves fairly slowly within the plant after infection, so severe pruning can be helpful. For African greening, Buitendag & von Broembsen (1993) make the following recommendations: If the infected tree is 5 years old or less, remove the tree. If it is between 6 and 10 years, remove it if it is 75% infected; otherwise remove branches. If it is more than 10 years old, remove affected branches up to 40% of the tree. Do not plant young resets in old groves affected by greening. The tendency for suckers that sprout after pruning to be infected with greening depends upon the diameter of the branches. Branches 10-19 mm in diameter grew no suckers. Among branches 20 mm in diameter or more, the smallest ones were most likely to produce infected suckers (86% for branches 20-29 mm, as compared with 29% for those that were 40-60 mm) (van Vuuren 1993).

Roistacher (1996) cited a Chinese program for rehabilitation of citrus in Fujian Province in which cultural control played a major part. Windbreaks were established to protect plants from psyllid vectors (although the efficacy of barriers for protection from a persistently transmitted pathogen is questionable). Trees were examined regularly for citrus greening disease, and all infected trees were immediately removed and replaced with healthy trees from a certified citrus stock program at the Fujian Academy of Agricultural Sciences (Ke & Xu 1990). In another Chinese program, part of the control program involved hand-removal of summer flush in high density citrus plantings following rice cultivation (Aubert 1990b).

Regulatory Measures

If no citrus greening pathogen is present, *D. citri* is not a major pest, and regulatory measures are unnecessary; however, regulation of host material is an essential part of managing citrus greening disease. Initially, all citrus budwood and liners should be tested and certified free of the greening pathogens. Propagation material must be kept isolated from psyllids and potential sources of *Candidatus* Liberibacter spp. Lin & Lin (1990) recommend eliminating all citrus and citroids within 5-8 km of propagating nurseries. In the Chinese program in Fujian discussed above (Ke & Xu 1990), it was necessary to eradicate all backyard citrus trees, as well as *Murraya* and *Clausena* plants. The introduction of any citrus or other Rutaceous plants into the area was strictly forbidden. Additionally, all newly planted trees were supplied by the Fujian Academy of Sciences and certified free of greening.

Integrated Management

There is no place in the world where citrus greening disease occurs that it is under completely successful management. In every place where the disease occurs, life expectancy of citrus trees is vastly reduced, and production losses are significant to total. That having been said, in many areas of the world, citrus production has had to adapt to the presence of citrus greening. The most successful management efforts combine production of clean stock with psyllid control, both within the grove and on alternative host plants, and inoculum suppression after groves are established. Taiwan is a case in point, where Hung et al. (2000) and Su et al. (1986) state that there are three main aspects to managing citrus greening disease: Propagation of clean nursery stock, psyllid control, and removal of potential inoculum sources. Many aspects of citrus greening management are costly both in cash outlay, and in lost production. The economic viability of citrus production in a greening-endemic environment, even with the best current management practices, certainly is not assured.

Can Citrus Greening be Eradicated?

The success of any eradication program depends upon the extent of the problem. Thus, early detection is essential to the success of an eradica-

tion effort. Once the disease becomes widespread, there is little hope of eradication, particularly if the pathogen has become established in an unknown number of native or ornamental non-citrus hosts. Given an ideal situation with early detection and very limited spread of the disease, it may be possible to eradicate citrus greening disease successfully, especially in an island situation, but the outcome depends on a number of factors.

Detection Capabilities

The success of any eradication program depends on rapid and accurate diagnosis, preferably in the field. It is unknown whether reliance on symptoms, even by personnel with extensive training in citrus greening symptom recognition, will be sufficient to contain the problem. Certainty of diagnosis is vital, given today's legal climate (Gottwald et al. 2002; Schubert et al. 2001). It is not known if vectors are able to transmit greening pathogens from infected, but asymptomatic plants. If so, rapid diagnostic methods are needed that can detect the presence of the pathogens prior to symptom expression in infected plants. In any case, reliable, robust diagnostic methods are needed to confirm infection with certainty if control action (i.e., tree removal) is necessary. Research is needed in the area of rapid, reliable diagnostics for citrus greening disease.

Detection of citrus greening disease in plants is difficult because of the irregular distribution of the pathogen in the host. While the currently available molecular detection methods have provided us with a better understanding of the taxonomy of the organism and confirmation of the disease in various parts of the world, there is a need for robust universal diagnostic methods for quarantine and eradication purposes. The presence of the vector in Florida and the increasing numbers of interceptions of illegal plant materials have made it impossible to neglect the need for development of sensitive detection techniques for citrus greening disease. The new PCR methods or hybridization techniques should be sensitive enough to detect very low levels of the pathogen in both host and the vector, and not give false positives. This requires development of sequence information that is unique to citrus greening pathogens. Citrus greening isolates are available at present in the USDA quarantine facility, Beltsville, MD. Genome sequencing of citrus greening should be given a high priority. This should enable us to develop better strategies for both detection and control.

Efficacy of Psyllid Control

Removal of infected and exposed host plants could be extensive and expensive. It is not known how far Asian citrus psyllid vectors can fly, but informal estimates suggest that they might fly several kilometers. Removal of all citrus trees within a radius of several kilometers, especially in an urban environment, is likely to provoke extreme public outcry. If psyllid vectors can be controlled successfully, only infected plants would have to be removed. Thus, safe and extremely effective psyllid control probably is needed for successful eradication. Depending upon the efficiency and seasonality of transmission, it may not be necessary to control citrus psyllids 100% all the time, but research is needed on field infectivity of vectors and seasonality of transmission.

Determining the host range of the psyllid vectors is relatively easy compared with determining the host range of the pathogens. Knowledge of both, however, is needed for successful eradication. Hosts of vectors at least would need to be treated to eliminate the insects within the regulated area. Hosts of the pathogens would need to be tested and removed if found to be infected.

Quarantines

Citrus greening pathogens are transmitted only by psyllid vectors, grafting, dodder, and possibly seed. Dodder transmission probably can be discounted in the field. Thus, only grafting, seed, and vectors need to be considered in quarantine regulations. Nurseries that produce hosts of either pathogens or vectors would have to be quarantined very strictly, similar to citrus nurseries in citrus canker quarantine areas (Florida Department of Agriculture and Consumer Services 2000). Our experience has shown that *D. citri* can move on unprocessed fruit. Numerous *D. citri* were intercepted in boxes of grapefruit picked in the Bahamas and shipped to Ft. Pierce, Florida for packing (Halbert & Núñez 2004). Similarly, *D. citri* certainly can move on leaf and twig material. Thus, it may be necessary to quarantine all citrus plant material within the regulated area, including both citrus yard trash and fruit. Quarantine treatments may be feasible for commercially produced fruit.

D. citri was able to colonize much of the state of Florida as a result of shipments of orange jasmine (*M. paniculata*) plants produced in southern Miami-Dade County and distributed through discount chain stores (Halbert et al. 2002). During the time that the distribution of *D. citri* in Florida was classified as limited, the DPI required pesticide treatment of *M. paniculata* if *D. citri* was found in a nursery. However, the actual producers of the plants in Miami-Dade County frequently were hard to find and proved impossible to regulate. It may be necessary to prohibit movement of all potential hosts of citrus greening pathogens or their vectors within the quarantine area. *Murraya paniculata* now is considered a Category II

invasive plant (Florida Exotic Pest Plant Council 2003), which might give additional leverage to a program to regulate its sale and distribution.

Conclusions

Citrus greening is a devastating disease, and the prospects for maintaining an economically viable citrus industry at current production levels in Florida if citrus greening disease becomes established are very poor, given current knowledge. Keeping citrus greening pathogens out of Florida should be given very high priority. In the meantime, surveys are necessary to find infected plants as soon as possible if the disease becomes established. Given a limited epidemic, eradication may be possible, depending on circumstances. Scientific research on the disease complex is greatly needed, particularly in the areas of rapid field diagnosis, disease epidemiology and efficacy of psyllid vector control.

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HOW EFFECTIVE IS GF-120 FRUIT FLY BAIT SPRAY APPLIED TO BORDER AREA SORGHUM PLANTS FOR CONTROL OF MELON FLIES (DIPTERA: TEPHRITIDAE)?

RONALD J. PROKOPY[1,3], NEIL W. MILLER[2], JAIME C. PIÑERO[1], LESLIE ORIDE[2], NANCY CHANEY[2], HANNAH REVIS[2] AND ROGER I. VARGAS[2]
[1]Department of Entomology, University of Massachusetts, Amherst, MA 01003

[2]U.S. Pacific Basin Agricultural Research Center, USDA, ARS, P.O. Box 4459, Hilo, HI 96720

[3]Deceased, 14 May 2004

ABSTRACT

Application of bait spray to non-host sorghum plants bordering host plants of melon flies, *Bactrocera cucurbitae* Coquillet, is a common practice for melon fly control in Hawaii. In a field study conducted in 2003 in Hawaii, we first asked whether GF-120 Fruit Fly bait spray applied to sorghum plants that bordered only two (opposite) sides of a patch of cucumbers was as effective in protecting cucumbers against melon flies as similar spray applied to sorghum plants that bordered all four sides of a cucumber patch. Second, we asked whether mature melon fly females carrying a high egg load but deprived of protein during the previous 24 h were more responsive to bait spray than mature females having continuous access to protein. Color-marked melon fly females were released outside of patches of sorghum-bordered cucumbers. We found no significant differences between two-sided and four-sided patches of sorghum or between protein-deprived (for 24 h) and protein-fed (continuously) mature females in percentages of released females that found cucumbers in bait-sprayed plots. Moreover, none of these percentages was significantly less than percentages of released females that found cucumbers in unsprayed plots, indicating an overall ineffectiveness of bait spray application. During the 24 h after alighting on cucumbers, released females that were captured alive on cucumbers and placed in cups with cucumbers laid on average almost as many eggs (insignificantly fewer) when taken from bait-sprayed plots as when taken from unsprayed plots. An overriding factor may have been the presence of just a narrow swath of sorghum (arising from a single row of plants), which may have permitted females easy access to cucumbers and masked potential differences among treatments. Bait spray applied to broader swaths of sorghum may be more effective.

Key Words: *Bactrocera cucurbitae*, GF-120 Fruit Fly bait, bait spray, spinosad, sorghum, cucumbers.

RESUMEN

La aplicación de cebos rociados sobre plantas de sorgo (no hospederas) que rodean plantas hospederas de la mosca del melón, *Bactrocera cucurbitae* Coquillet, es una práctica común para el control de esta especie de mosca en Hawaii. En un estudio de campo realizado en 2003 en Hawaii, nos preguntamos primero si el cebo para moscas de la fruta GF-120 aplicado a plantas de sorgo ubicadas en sólo dos lados (opuestos) de un parche de pepinos era tan efectivo protegiendo pepinos contra el ataque de moscas del melón como una aplicación de cebo en plantas ubicadas en los cuatro lados del parche. En segundo término nos preguntamos si hembras maduras conteniendo una alta carga de huevos pero privadas de proteína por 24 horas respondían más al cebo aplicado que hembras maduras mantenidas con acceso continuo a proteína. Estas preguntas fueron contestadas liberando hembras marcadas en la orilla de cada uno de estos tipos de parche que rodeaban el área conteniendo pepinos. No encontramos diferencias significativas entre parches de sorgo teniendo dos o cuatro lados, o entre hembras privadas de proteína por 24 horas y hembras que tuvieron acceso continuo a proteína en cuanto al porcentaje de hembras liberadas que encontraron pepinos en los parches que tuvieron aplicación de cebo. Además, ninguno de estos porcentajes fue significativamente menor que los porcentajes de hembras liberadas que encontraron pepinos en parches no aplicados, lo que indica una general inefectividad del cebo aplicado. Durante las 24 horas posteriores al contacto con los pepinos, hembras liberadas que fueron capturadas vivas y colocadas posteriormente en recipientes de plástico conteniendo pepinos ovipositaron en promedio casi el mismo número de huevos (insignificantemente menos) cuando fueron tomadas de parches aplicados con cebo en comparación con parches no aplicados. Un factor determinante posiblemente fue la presencia de solamente una franja angosta de sorgo (originada por una sola hilera de plantas) lo cual pudo haber permitido a las hembras acceder fácilmente a

los pepinos, enmascarando posibles diferencias entre tratamientos. Una aplicación de cebo en franjas de sorgo más anchas pudiera ser más efectiva.

Translation provided by the authors.

The melon fly, *Bactrocera cucurbitae* Coquillet, is an important pest of cucurbits in Asia, several islands in the Pacific ocean, and Africa (White & Elson-Harris 1992). Beginning in the 1950s, bait sprays containing protein (as an attractant and feeding stimulant) plus an insecticide (as a toxicant) have been used widely for control of melon flies (e.g., Steiner 1955; Nishida et al. 1957; Gupta & Verma 1982; Stonehouse et al. 2002). Recently, GF-120 Fruit Fly bait (Dow Agrosciences, Indianapolis, IN) containing spinosad as toxicant has emerged as an effective and environmentally safe alternative to traditional bait sprays (containing organophosphorus insecticide) for control of several different pest tephritid flies (e.g., King & Hennessey 1996; Peck & McQuate 2000; Burns et al. 2001; Vargas et al. 2001).

In 2002 in Hawaii, we investigated the effectiveness of GF-120 Fruit Fly bait spray applied to border area plants (*Sorghum* sp.) that completely surrounded (on all four sides) patches of cucumbers (*Cucumis sativus* L.), a favored host of melon flies. Application of bait spray to sorghum or other non-host vegetation surrounding host plants of melon flies is a common practice for melon fly control in Hawaii. The intent (after Nishida et al. 1957; Nishida 1958) is to attract (with bait spray droplets) immigrating melon fly females to sprayed sites, where they ingest feeding stimulant and insecticide before entering cultivated fields of hosts. As shown by Nishida (1953), most gravid melon fly females overnight on favored non-host plants in border areas adjacent to cultivated hosts before entering cultivated fields during the day to oviposit. In our 2002 study, we found that GF-120 Fruit Fly bait spray applied to a broad and dense swath of sorghum (50 cm deep) was very effective in preventing 4-week-old released protein-deprived (since eclosion) melon fly females from entering cucumber patches. It was significantly less effective, however, against 4-week-old released protein-fed (since eclosion) melon fly females (Prokopy et al. 2003).

On some Hawaiian islands, fields of cucurbit crops are more frequently bordered by sorghum or other vegetation on two (opposite) sides rather than on all four sides. As our first question here, we asked are bait sprays applied to sorghum plants that border only two (opposite) sides of a patch of cucumbers as effective as bait spray applied to sorghum plants that border four sides of a cucumber patch?

Under certain field conditions, newly-eclosed melon fly females could encounter absence of sufficient protein (to support egg development) for extended periods, possibly even several weeks. Under other field conditions, feral females might encounter sufficient protein to develop a full complement of mature eggs during the 3-4 weeks that precede maturity but after reaching maturity fail to find protein during the course of a given day. As our second question, we asked are melon fly females carrying a high egg load but deprived of protein during the previous 24 h more responsive to bait spray than mature females having continuous access to protein?

In tests conducted in Hawaii in 2003, our objective was to answer these two questions. Our experimental approach was similar to that used in our 2002 investigation (Prokopy et al. 2003).

Materials and Methods

Fly Origin and Maintenance

All melon fly females evaluated here were of the F_2 generation. Grand-parental flies oviposited in field-collected fruit of papaya, *Carica papaya* L. Parental flies and flies used here originated from papaya held in laboratory containers together with flies of the preceding generation. Following eclosion, F_2 adults were held in groups of ~150 females and ~150 males for 28-32 days at ~25°C, ~60% RH and ~13 h of natural light in 30 × 30 × 30 cm laboratory cages to permit mating. During this time, all flies were provided continuously with sucrose, USB enzymatic yeast hydrolysate (United States Biochemical, Cleveland, OH) and water (but no fruit).

Test Plots

A large open area of mowed grass (~70 × ~170 m), bordered by woods and located on the grounds of the Hawaii Agricultural Experiment Station at Kainaliu on Hawaii Island, was selected as the site for establishment of rotatable test plots (Fig. 1). For two of the test plots, on each test day we arranged potted sorghum plants in a square measuring 6 × 6 m (Fig. 1). Three sides of the square consisted of a single row of abutting pots of sorghum (24 plants per pot, 25 pots per row) that gave rise to a swath of sorghum ~25 cm wide × ~150 cm tall. The fourth side consisted of two fewer pots, thus allowing a 50-cm gap at the end of the row through which an observer could enter the plot. Sorghum plants were held upright by sandwiching them between strands of rope (50 cm apart; 75 and 125 cm above ground) attached to

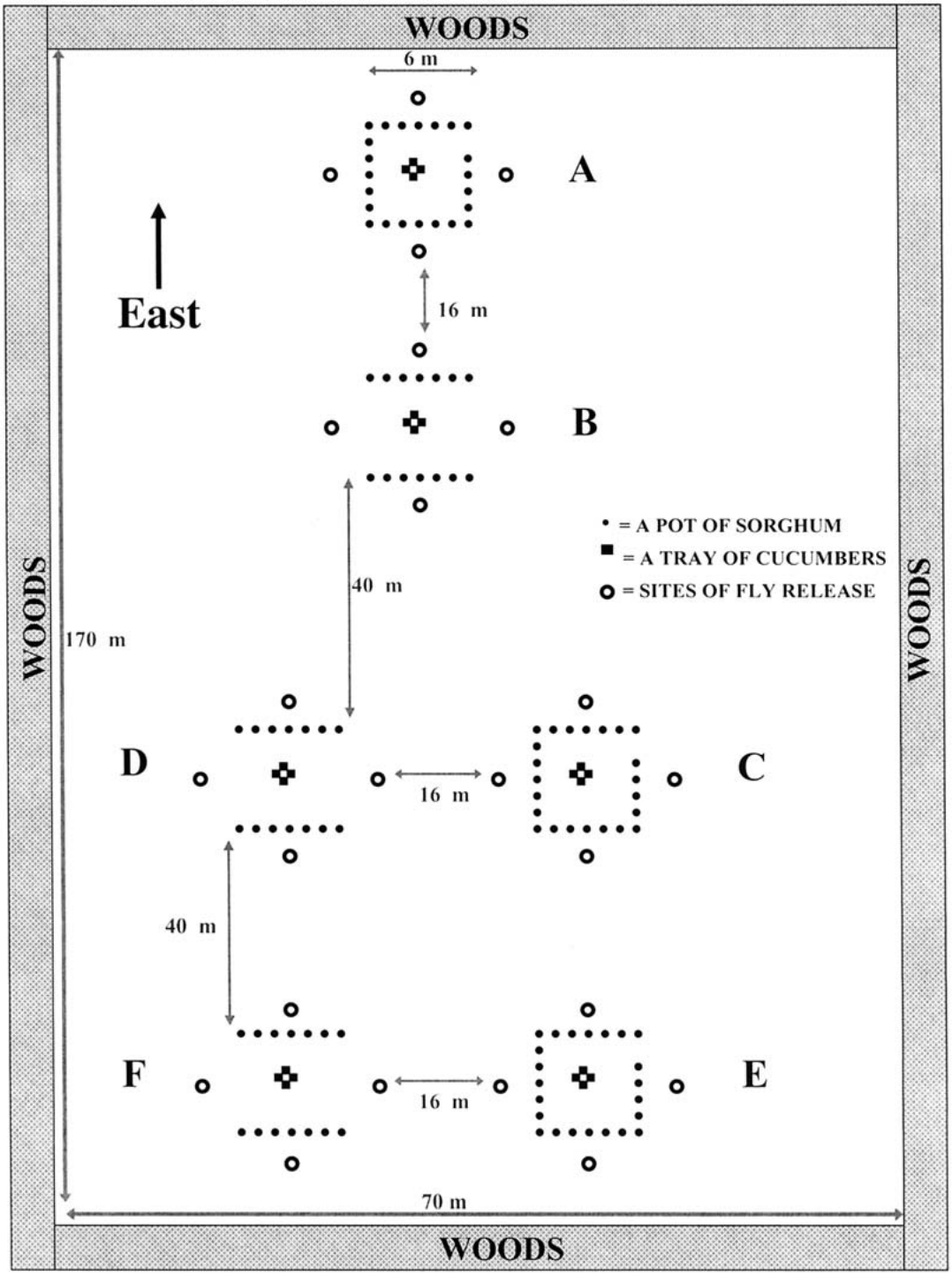

Fig. 1. Schematic arrangement of field test plots. Plots A, C, and E were bordered by a row of potted sorghum plants on four sides. Plots B, D, and F were bordered by a row of potted sorghum plants on two sides. Rows of sorghum plants in Plots A and B (in replicates 1 and 2) and Plots E and F (in replicates 3 and 4) received bait spray. Rows of potted sorghum plants in Plots C and D (in replicates 1 and 2) and Plots A and B (in replicates 3 and 4) did not receive bait spray. Rows of sorghum plants in Plots A and B were switched with those in Plots E and F after the second replicate. The entire area circumscribed by woods was mowed. Distances between plots and between plots and woods are not drawn to scale.

metal stakes. For two other test plots, on each test day we arranged sorghum plants (as above) in two parallel rows, 6 m long, that bordered the east and west sides of a plot, leaving the north and south sides open (Fig. 1).

For each test plot, we established four positions at which flies were released (Fig. 1). Each position was 5 m from the center of the plot (2 m outside of a row of sorghum) and received six pots of sorghum, arranged in a tight circle around a central stake. The plants were enveloped with rope so as to form a dense canopy of foliage that offered flies resting places after departure from release containers attached 80-120 cm above ground to the central stake.

For each test plot, we placed four black plastic trays (50 × 50 cm) on the ground 1 m from the center of the plot (Fig. 1). Each tray received eight cucumbers (purchased at a local supermarket and washed thoroughly before use) that served as potential ovipositional sites for released melon flies. A narrow slice (~5 mm thick) was cut from one end of each cucumber at 0830 h (the time of fly release) and every 30 min thereafter until 1630 h to enhance the emission of fresh odor.

Spray of GF-120

Each test day, two of the four test plots (one bordered by four rows of sorghum, the other by two rows of sorghum) received bait spray at label-recommended amount per hectare applied in the same manner as described in Prokopy et al. (2003). Briefly, using a hand-pumped back-pack sprayer, we applied 60 ml of freshly-made aqueous solution of GF-120 Fruit Fly bait (containing 80 ppm of spinosad) in a continuous swath 50 cm wide (75-125 cm above ground) to the outer perimeter (6 m long) of sorghum plants comprising each row of a plot. The batch of GF-120 Fruit Fly Bait used here was manufactured 21 months before our tests and was considered by the manufacturer to be fully potent at the time of use.

All bait spraying was done at 0815 h, 15 min before fly release. Because our supply of sorghum was limited, we could not apply spray to a new set of sorghum plants for each replicate. Hence, the day after completing a replicate, we thoroughly hosed all sprayed sorghum plants with an amount of water equivalent to ~20 mm of rainfall that, according to Prokopy et al. (2003), effectively removed any residual bait spray. We then waited 4 d before commencing the next replicate. To guard against any possible lingering effects of bait spray on released females, we chose the same set of sprayed sorghum plants for all replicates requiring sprayed plants and a second set of washed but unsprayed sorghum plants for all replicates requiring unsprayed plants. In all, there were four replicates. No rain fell during the conduct of any of the replicates.

Marking and Release of Flies

Two days before release, 640 females were marked on the pronotum with a dot of paint (Gloss Enamel, Tester Corp., Rockford, IL). Different two-color combinations were used to mark each of 16 sets of 20 females designated as protein-fed and each of 16 sets of 20 females designated as protein-deprived. To ensure flight capability of released females, only females that were observed to fly just after marking were used. After marking, 20 same-colored females were placed in a polyethylene box (12 cm wide × 18 cm tall × 5 cm deep) provided with sucrose, enzymatic yeast hydrolysate, and water. An opening (8 × 8 cm) was cut into the lid of the box and covered with removable netting to permit introduction of flies and their departure after release. At 0800 h on the day before release, yeast hydrolysate was removed from boxes containing flies designated to be protein-deprived for 24 h. It remained in boxes containing flies designated to be protein-fed. Dissections revealed that average loads of fully developed eggs for protein-deprived and protein-fed females at time of release were 38.8 ± 2.0 (SEM) and 36.5 ± 2.1 (SEM) eggs per female, respectively (n = 30 females per type).

At 0820 h each test day, one box of protein-deprived and one box of protein-fed females was attached, in vertical orientation, to each of the four stakes positioned 5 m from the center of each of four test plots. The opening of each box faced the center of the plot. In all, each test plot received 80 distinctively colored protein-deprived females and 80 distinctively colored protein-fed females. At 0830 h, netting was removed from each box to permit fly exit. At 1700 h, we censused each box and subtracted the number of flies therein from the number (20) originally intended for release. Across all treatments and replicates, only 3.5% of marked flies failed to exit from the boxes (Table 1). The majority exited by 1000 h.

Censusing Fly Presence in Test Plots

Beginning at 0900 h and every 30 min thereafter until 1700 h (when very few flies were observed), one observer stationed at each of the four simultaneously operative test plots carefully censused the number of color-coded melon flies observed on the foliage of sorghum and on cucumbers. For each census, each of the 6 m rows of sorghum was examined for 3 min and the cucumbers in each plot were examined for 15 min. Dead flies on sorghum and alive flies on cucumbers (none were found dead on cucumbers) were removed. Each of the first 16 females to arrive on cucumbers in a plot was aspirated into a separate net-covered plastic cup supplied with sucrose, water, and a piece of cucumber (1 × 2 × 2 cm) wrapped in parafilm except for a parafilm-free area (1 × 1 cm) punc-

TABLE 1. PERCENTAGES OF RELEASED PROTEIN-DEPRIVED FOR 24 H (P-DEP) AND PROTEIN-FED (P-FED, CONTINUOUSLY) MELON FLY FEMALES OBSERVED DEAD ON SORGHUM PLANTS AND ALIVE ON CUCUMBERS IN PLOTS BORDERED ON TWO OR FOUR SIDES BY BAIT-SPRAYED OR UNSPRAYED SORGHUM PLANTS (ACROSS ALL CENSUS PERIODS).

Flies	Sorghum sprayed	No. plot sides with sorghum	Total no. flies released	Mean percentages (± SEM)[1]	
				Dead on sorghum[2]	Alive on cucumbers[2]
P-DEP	Yes	4	313	5.4 ± 2.9 a	17.5 ± 7.9 a
		2	309	0.3 ± 0.3 b	16.5 ± 1.3 a
P-FED	Yes	4	306	1.0 ± 0.7 b	17.0 ± 8.6 a
		2	303	0.3 ± 0.3 b	12.9 ± 4.2 a
P-DEP	No	4	312	0.0 ± 0.0 b	19.7 ± 5.1 a
		2	302	0.0 ± 0.0 b	21.7 ± 6.5 a
P-FED	No	4	311	0.0 ± 0.0 b	26.2 ± 7.4 a
		2	313	0.0 ± 0.0 b	21.2 ± 8.4 a

[1]Means in each column not followed by the same letter are significantly different according to ANOVA and LSD tests at $P = 0.05$.
[2]During each census, all females observed dead on sorghum or alive on cucumbers were removed.

tured with a needle to create a hole suitable for egg deposition. Cups with flies were returned to the laboratory. At 24 h after fly capture, pieces of cucumber were removed and eggs were counted. At 24 and 72 h after fly capture, females were assessed for mortality. Ambient temperature in field plots was recorded every 30 min from 0830-1700 h each test day and averaged 26°C (range 22-30°C).

Data Analysis

Proportions of each type of female released in each of the four replicates of each of the four test plot treatments (eight treatments in all) that were found dead on sorghum or alive on cucumbers were submitted to arcsin transformation before analysis of variance (ANOVA), which was followed by least significant difference (LSD) tests ($P = 0.05$) for comparison of treatment means where appropriate (significant P value from ANOVA).

Numbers of eggs laid by females of each type in cucumber over a 24-h period in cups subsequent to their capture in each test plot treatment were submitted to square root transformation ($\chi + 0.5$) before ANOVA. Proportions of such females that died during 24 h after capture were submitted to arcsin transformation before ANOVA. For all ANOVAS performed, "replicate" was used as random factor. For a given treatment in each analysis, we included only those females released within 5 m of plot center and subsequently observed in that plot. No females originating from another plot were included.

RESULTS

For females found dead on sorghum, ANOVA revealed a significant effect of treatment on mortality ($F = 3.32$; $df = 7, 21$; $P = 0.015$). Mortality was significantly greater among protein-deprived females in four-sided bait-sprayed plots than for any other treatment (Table 1). Even though numerically more protein-deprived and protein-fed females were found dead on sorghum in four-sided and two-sided plots that were bait-sprayed compared with unsprayed plots, percent mortality per treatment in sprayed plots was low (range = 0.3-5.4% of released flies) (Table 1).

For females found alive on cucumbers, ANOVA revealed no significant effect of treatment on numbers observed ($F = 1.08$; $df = 7, 21$; $P = 0.41$). There was a consistent numerical trend toward fewer numbers of both protein-deprived and protein-fed females observed on cucumbers in each type of bait-sprayed plot (range = 12.9-17.5% of released flies) than in comparable unsprayed plots (range = 19.7-26.2% of released flies) (Table 1).

For females captured alive on cucumbers and transferred to cups with food, water, and a piece of cucumber for oviposition for 24 h after capture, ANOVA revealed a significant effect of treatment on mortality ($F = 5.76$; $df = 7, 21$; $P < 0.002$). For protein-deprived as well as protein-fed females taken from sprayed four-sided as well as sprayed two-sided plots, mortality after 24 h in cups was significantly greater (range = 25.0-38.1%) than for females of either type taken from either type of unsprayed plot (range = 0.0- 6.5%) (Table 2). For the last two of our four replicates, we observed mortality after 72 h but found no additional death among any flies.

For captured females transferred to cups, ANOVA revealed no significant effect of treatment on numbers of eggs laid per female during the 24 h after capture ($F = 1.92$; $df = 7, 21$; $P = 0.07$). For all four treatments involving flies taken from cucumbers in bait-sprayed plots, oviposition averaged numerically less (range = 20.7-27.1 eggs laid per female) than for flies taken from comparable unsprayed plots (range = 28.6-34.2 eggs laid per female) (Table 2).

TABLE 2. DURING THE 24 H AFTER CAPTURE OF RELEASED MELON FLY FEMALES ALIVE ON CUCUMBERS IN FIELD PLOTS BORDERED BY BAIT-SPRAYED OR UNSPRAYED SORGHUM PLANTS, PERCENTAGES OF CAPTURED FEMALES THAT DIED AND AMOUNT OF OVIPOSITION BY CAPTURED FEMALES INTO CUCUMBER.

Flies	Sorghum sprayed	No. plot sides with sorghum	Total no. flies captured[1]	Mean percent dead (± SEM)[2]	Mean no. eggs laid (± SEM)[2,3]
P-DEP	Yes	4	26	34.6 ± 9.7 a	27.1 ± 3.5 a
		2	30	33.3 ± 11.1 a	20.7 ± 4.4 a
P-FED	Yes	4	21	38.1 ± 14.0a	23.3 ± 5.1a
		2	24	25.0 ± 8.4 a	24.2 ± 4.9 a
P-DEP	No	4	22	4.5 ± 4.0 b	28.6 ± 4.0 a
		2	31	6.5 ± 6.3 b	32.0 ± 4.0 a
P-FED	No	4	30	0.0 ± 0.0 b	28.8 ± 3.6 a
		2	26	3.8 ± 3.8 b	34.2 ± 3.7 a

[1]Across all four replicates, 32 females per treatment were captured, placed in netted cups, and returned to the laboratory. While there, some inadvertently escaped from cups, accounting for the reduction from 32 per treatment.
[2]Means not followed by the same letter are not significantly different according to ANOVA and LSD tests at $P = 0.05$.
[3]Means are based on total numbers of females placed in cups, regardless of whether females were alive or dead after 24 h in cups.

Of the 2,560 color-marked females originally placed in release boxes, 2,469 (96.4%) left the boxes during test periods. Of these 2,469, 477 (19.3%) were observed on cucumbers in plots immediately adjacent to sites of release, 50 (2.0%) were observed on cucumbers in the nearest of the other three plots, and 11 (0.4%) were observed on cucumbers in the two most distant plots.

DISCUSSION

Our findings indicate that GF-120 Fruit Fly Bait containing spinosad at 80 ppm in aqueous solution applied as a spray to a single row of potted sorghum plants that surrounded a patch of cucumbers on all four sides (north, east, south, west) was not effective against released melon fly females deprived of protein for 24 h. Only 5.4% were observed dead on sprayed sorghum, whereas 17.5% were found alive on cucumbers. It was equally ineffective against released protein-fed females. Only 1.0% were observed dead on sprayed sorghum, whereas 17.0% were found alive on cucumbers. Furthermore, the same type of spray applied to single rows of potted sorghum plants that bordered a patch of cucumbers on two sides (east, west) was even less effective against melon fly females: only 0.3% of released protein-deprived and 0.3% of released protein-fed females were observed dead on sprayed sorghum. For neither type of female (protein-deprived or protein-fed) and neither structure of plot (a row of sorghum on four or two sides) was the percentage of released females found on cucumbers significantly less for bait-sprayed than unsprayed plots.

For two of the treatments (protein-fed females released adjacent to plots surrounded on all four sides by bait-sprayed or unsprayed sorghum), the experimental protocol here was identical to that used for these two treatments in our 2002 test (Prokopy et al. 2003), except in one respect. Here, only a single row of potted sorghum plants (foliage 25 cm wide) surrounded each plot, whereas in 2002 two abutting rows of sorghum plants (foliage 50 cm wide) surrounded each plot. For plots with unsprayed sorghum, results for each year were similar: 0% of released females observed dead on sorghum each year and 31.2% (2002) vs. 26.2% (2003) observed alive on cucumbers. For plots with bait-sprayed sorghum, however, results were quite different between years: 14.0% (2002) vs. 1.0% (2003) of released females observed dead on sorghum and 10.9% (2002) vs. 17.0% (2003) observed alive on cucumbers. If we presume (as affirmed by the manufacturer) that the GF-120 Fruit Fly Bait used in 2003 was as potent as that used in 2002, we are left to conclude that the much-reduced mortality of flies released adjacent to baited-sprayed plots in 2003 and the greater percentage of released flies observed on cucumbers in 2003 was due principally (or exclusively) to the presence of only a single row rather than a double row of potted sorghum plants. Compared with a double row of potted sorghum plants, a single row could have permitted a greater amount of attractive odor from cucumbers to flow through the sorghum to fly-release sites or afforded less shelter to foraging females (thereby reducing the amount of time females would spend in the presence of bait spray). Evidence from a study in 2004 by Revis et al. (unpublished data) supports the latter explanation.

We anticipated substantially greater mortality than observed of protein-deprived (for 24 h) compared with protein-fed (continuously) females on bait-sprayed sorghum based on the expectation that 24 h of protein deprivation would enhance hunger for protein. We also anticipated observation of substantially more (not fewer) females of each type on cucumbers in bait-sprayed plots

with sorghum on two sides compared with four sides based on presence of half as many bait-sprayed rows of sorghum. These expectations were not met, perhaps because the strong influence of cucumber odor relative to the bait spray odor masked or overrode anticipated effects of fly hunger and test plot structure.

One could argue that the effect of spinosad on target insects may not be immediate mortality but delayed mortality (24, 48, or 72 h later) and that following ingestion of spinosad, target insects may be subjected to sub-lethal effects. Among released females captured on cucumbers at interiors of bait-sprayed plots and held for 24 h in cups, there was indeed substantial mortality (33.3-34.6% for protein-deprived females and 25.0-38.1% for protein-fed females) above that observed on sorghum during the 8 h after fly release (there was no additional mortality from 24-72 h). Even so, on average only 23% fewer eggs were laid by such females during 24 h after capture compared with eggs laid by females captured on cucumbers at interiors of unsprayed plots (Table 2).

In conclusion, our findings here suggest that GF-120 Fruit Fly Bait spray may not be an effective control measure against melon flies if applied only to a narrow or thin swath of sorghum bordering a cultivated field of attractive melon fly hosts, such as cucumbers. This could be true regardless of whether immigrating females have fed on protein within the previous 24 h or not, and regardless of whether cultivated host fields are bordered by sorghum on two or four sides. If sorghum is to be used effectively as a site for bait spray application, we suggest that it be planted in a broad or dense swath that could provide effective shelter for foraging flies, thereby enhancing the probability of local encounter with bait spray droplets.

ACKNOWLEDGMENTS

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CHROMOSOMES OF THE CARIBBEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

VARSOVIA E. CEVALLOS AND JAMES L. NATION
Department of Entomology & Nematology, University of Florida, Gainesville, FL 32611-0620

ABSTRACT

Larval tissues of *Anastrepha suspensa* (Loew) (Diptera: Tephritidae) were examined to determine the optimal tissue and stage for chromosomal preparations and to determine the karyotype. Tissues were dissected in saline, stained in 2% aceto-orcein for 45 minutes, and squashed on a coverglass by thumb pressure. The compound eye imaginal discs from 6-day-old larvae yielded the best preparations of dividing cells. Mitotic figures also can be obtained from larval brain tissue, ventral nerve cord, and leg imaginal discs. In larvae 6 days old, many cells in the tissues examined were dividing. Cell division appears to be synchronized in the different tissues examined, with most cells in interphase or dividing at the same time during all instars. The male is heterogametic (XY) and the female is homogametic (XX). The chromosome number is 12 (10 autosomes + XX or XY). There are 3 pairs of subtelocentric and 2 pairs of submetacentric chromosomes. The X chromosome is subtelocentric and the Y chromosome is submetacentric. The two X chromosomes tend not to pair like the other chromosomes, and in males the Y chromosome often sticks to the short arm of the X chromosome.

Key Words: *Anastrepha suspensa*, karyotype, imaginal discs, mitotic figures.

RESUMEN

Los tejidos de larvas de *Anastrepha suspensa* (Diptera: Tephritidae) fueron examinados para determinar el tejido y la etapa óptima para las preparaciónes de cromosomas. Los tejidos fueron disectados en una solución salina, teñidos en 2% de aceto-orceino por 45 minutos, y aplastados debajo un cubre objeto por la presión del dedo pulgar. Los discos imaginales del ojo compuesto de larvas de 6 dias de edad resultaron ser las mejores preparaciones para dividir las cellulas. Figuras mitóticos también puede ser obtenidas del tejido del celebro de las larvas, la cuerda ventral de los nervios, y los discos imaginales de las patas. En larvas de 6 dias de edad, muchas de las celulas de los tejidos examinados estuvieron dividiendose. La división de celulas aparece ser sincronizada en los diferentes tejidos examinados, con la mayoria de las celulas en interfase o dividiendose al mismo tiempo durante todos los estadios. El macho es heterogamético (XY) y la hembra es homogamética (XX). El número de cromosomas es 12 (10 autosomas + XX o XY). Hay 3 pares de cromosomas subtelocentricas y 2 pares de cromosomas submetacentricas. La cromosoma X es subtelocentrica y la cromosoma Y es submetacentrica. Las dos cromosomas X suelen no aparearse como las otras cromosomas, y en los machos la cromosoma Y a menudo se pega al brazo corto de la cromosoma X.

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), is an important immigrant in Florida, and has a significant economical impact on citrus (Greany & Riherd, 1993). No data are available on the cytogenetics of *A. suspensa*, but there is limited cytogenetic information on other species in the genus *Anastrepha.* Mendes (1958) published chromosomal studies on *Anastrepha fraterculus* (Wied.). Bush (1962) described the karyotypes of nine species of *Anastrepha,* including *A. ludens* (Loew), *A. fraterculus* (Wied), *A. distincta* Greene, *A. mombinpraeoptans* Sein, *A. zuelaniae* Stone, *A. spatulata* Stone, *A. striata* Schiner, *A. serpentina* (Wied), and *A. aphelocentema* Stone. All of the *Anastrepha* species studied by Bush (1962) have 12 chromosomes except males of *A. serpentina*, which has 11. The Mediterranean fruit fly, *Ceratitis capitata* (Weid.) also has 12 chromosomes (Radu et al. 1975). Our objectives were to determine the optimum stages and tissues for display of chromosomes, and to determine the karyotype of *A. suspensa*. Here we report 12 chromosomes as the karyotype of *A. suspensa*, comprising 10 autosomes and a pair of sex chromosomes, and that imaginal disk tissues in larvae are suitable for examining dividing cells and mitotic figures.

MATERIALS AND METHODS

Eggs of *A. suspensa* were obtained from the mass rearing facility at the Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Larvae were reared at 28 ± 1°C and 80% relative humidity (RH) until the 5th or 6th day, and then larvae were moved to a cooler room at 22 ± 1°C and 80% RH. Under these conditions, development from hatching to pupation takes 8-9 days. Various imaginal disc tissues and brain tissue from 1-day-old to 7-day-old larvae were examined to determine optimal stages and tissues showing cell division and mitotic figures. Tissues observed were brain imaginal disc tissue, ventral nerve cord, compound eye imaginal discs, and imaginal discs attached to the nerve cord that develop into the first two pairs of legs.

Larvae were dissected in saline solution (9 g NaCl, 0.42 g KCl, and 0.25 g $CaC1_2$ in 1 liter of water) and the tissue to be examined was cleaned as much as possible from unwanted tissue. Dissected tissues were transferred immediately to a saturated solution of coumarin, as suggested by Bush (1962), but the time of exposure was reduced to 3 minutes. Following coumarin treatment, tissues were rinsed for 30 seconds in 1N HC1. The tissues were stained in 2% aceto-orcein for 45 minutes, and squashed on a coverglass by thumb pressure.

Cell suspension technique also was used in order to get more expanded chromosomes. Freshly dissected tissues were fixed in methanol:acetic acid (3:1) for 30 min, and then treated with 60% acetic acid for about 30 sec. The suspension of tissue was pulled into an eye dropper pipet, and drops were allowed to fall from several centimeters height onto a clean slide. The suspension on the slide was dried at 40-50°C on a hot plate. The cells and chromosomes were stained for 10-15 minutes by flooding the slide with Giemsa stain in 0.1M Sörensen buffer, pH 6.8.

Suitable preparations were photographed with a Zeiss III RS microscope and oil immersion with a 100x objective lens and a 10x ocular lens. Images of chromosomes were cut from the best photographs for the construction of the karyotype. The nomenclature for chromosome morphology and the centromeric index is that of Levan et al. (1964). The relative length of chromosomes was calculated by expressing the length of each chromosome as a percent of the summed length of all chromosomes. The centromeric index and the relative length of chromosomes were calculated from the mean of 19 measured metaphase preparations. The pairs of chromosomes were identified from their relative length and morphology.

Results and Discussion

Data on observations of dividing cells from the various ages of larvae and in different tissues examined are shown in Table 1. Cell division in the different tissues appears to be occurring at the same time, for example, brain and ventral nerve cord cells divide at the same time during the 1st day (instar 1), 4th day (instar 2) and the 6th day (instar 3). Eye imaginal discs begin cell division and growth earlier than the leg imaginal discs, but cell division in the leg imaginal discs occurred in synchrony with the brain and nerve cord cells. During the 6th day, when larvae are in the late 3rd instar, all tissues tested were dividing and this is the best stage of development in which to find workable chromosomes. Bush (1962) also reported good chromosomal preparations from mature 3rd instars.

Only a few dividing cells could be found in brain tissue of 1-day-old larvae. Brain cells in 2-day-old larvae tended to be in interphase and dividing cells were difficult to find. Three-day-old larval cells showed mostly interphase nuclei and a few prophase plates. Some cell division was observed in 4-day-old larvae, and metaphasic cells were found. Almost all cell nuclei from 5-day-old larvae were in interphase again, with few in methaphase. Tissue from 6-day-old larvae showed many dividing cells, and this was the best larval age class to observe the chromosomes. Dividing cells still could be observed in 7-day-old larvae that had not begun pupation. In these older larvae, however, dividing cells were few in number, cell nuclei were small, the chromosomes often remained bunched, and structural details were difficult to observe. By the 7th day, most of the larvae were in the wandering stage, had crawled out of the food, and were seeking a dry pupation site. The observations of distinct times of cell division in the brain are consistent with data showing that the brain does not grow in a linear manner (Nation et al. 1995), but is described by a sigmoid growth curve from day 1 (hatching) through day 8 (prepupa).

Table 1. Cell division in different tissues of *Anastrepha suspensa* larvae.

	Instar						
	I		II		III		
	Larval age (days)						
Tissues	1	2	3	4	5	6	7
Brain	+	—	—	+	—	++	+
Nerve cord	+	—	—	+	—	++	—
Eye discs			—	+	—	++	+
Leg discs						+	+

+Metaphase nuclei present; the number of plus signs indicates the relative number of metaphase nuclei found.
—Nuclei in interphase; mitotic figures not present.

Nerve cord cells in 1-day-old larvae were dividing, and some cells were observed in metaphase. With 2-and 3-day-old larvae, cells were in interphase. Cells in prophase and metaphase were found in 4-day-old larvae. Cell division stopped in 5-day-old larvae, and interphasic nuclei were observed, but at 6 days, cell division was evident and many metaphasic plates were observed. Cells in metaphase were difficult to find in the ventral nerve cord of 7-day-old larvae, and most of the cells had returned to interphase (Table 1).

Interphase and prophase nuclei were observed in cells from the compound eye imaginal disc in 3-day-old larvae. In 4-day-old larvae, cells were actively dividing, but in 5-day-old larvae, most cells were in interphase again. At 6 days, many cells in the tissue were in division. By the 7th day, few cells were in metaphase and most were in interphase. The nuclei of cells in the compound eye imaginal

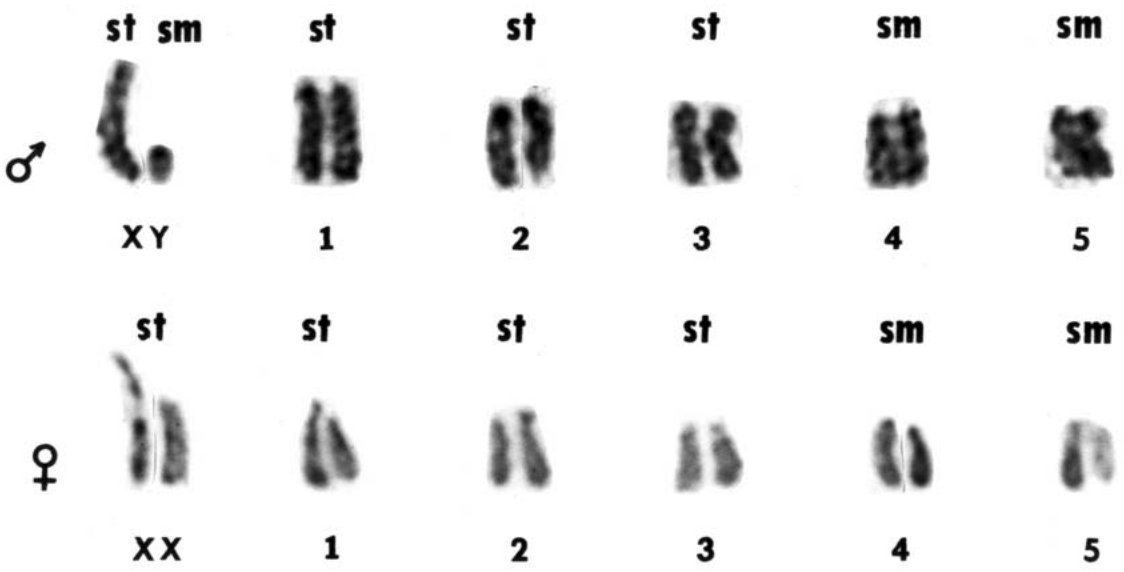

Fig. 1. Karyotype of *Anastrepha suspensa* (Loew); st, subtelocentric chromosome; sm, submetacentric chromosome.

discs are larger than nuclei of other tissues observed. The chromosomes from eye imaginal disc cells were expanded and nicely separated from each other (Table 1), and this is the best tissue for chromosome preparation in the Caribbean fruit fly larvae. In contrast, Radu et al. (1975) found that brain neuroblasts were the most favorable cells for chromosome studies in *Ceratitis capitata*.

Leg imaginal discs are small and poorly defined before the 5th day. In 5-day-old larvae, leg imaginal discs are growing in size, but still small and difficult to prepare for observation of cell division. Some metaphase plates were observed in leg discs from 6- and 7-day-old larvae.

The mitotic karyotype in both sexes of A. *suspensa* comprises 6 pairs of chromosomes (2n = 12), 5 autosomal pairs plus the pair of sex chromosomes (Fig. 1). As in most of the Diptera (White 1973), the male of *A. suspensa* is heterogametic (XY) and the female is homogametic (XX). Based on the centromeric index (Levan et al. 1964) the chromosomes of *A. suspensa* can be grouped into subtelocentric and submetacentric chromosomes. The largest pairs are subtelocentric (pairs 1, 2, 3 and XX), and the shortest pairs are submetacentric (pairs 4, 5, and Y) (Table 2). The relative length of the somatic chromosomes was about the same in all of the pairs (Table 2). One X chromo-

TABLE 2. CENTROMERIC INDEX AND RELATIVE LENGTH OF CHROMOSOMES OF *ANASTREPHA SUSPENSA* LARVAE.

Chromosome number	Centromeric Index* Mean ± SE	Relative length* Mean ± SE	Morphology*
1	25.9 ± 1.8	10.7 ± 0.6	Subtelocentric
2	25.8 ± 1.5	9.5 ± 0.3	Subtelocentric
3	25.7 ± 1.5	8.3 ± 0.3	Subtelocentric
4	23.3 ± 1.3	8.5 ± 0.3	Subtelocentric
5	22.6 ± 1.2	8.0 ± 0.2	Subtelocentric
6	23.8 ± 1.5	7.7 ± 0.1	Subtelocentric
7	27.1 ± 2.0	7.3 ± 0.1	Submetacentric
8	26.9 ± 1.9	7.4 ± 0.2	Submetacentric
9	26.4 ± 1.9	6.9 ± 0.1	Submetacentric
10	25.9 ± 1.7	6.8 ± 0.2	Submetacentric
X1	25.9 ± 2.1	11.3 ± 0.3	Subtelocentric
X2	22.1 ± 2.9	10.7 ± 0.9	Subtelocentric
Y	29.9 ± 3.8	3.5 ± 0.3	Submetacentric

*Calculations and morphology designation based upon 19 metaphase preparations. Relative length is calculated as the (average length of each chromosome divided by the summed length of all chromosomes) × 100. Morphology and centromeric index are based upon work of Levan et al. (1964).

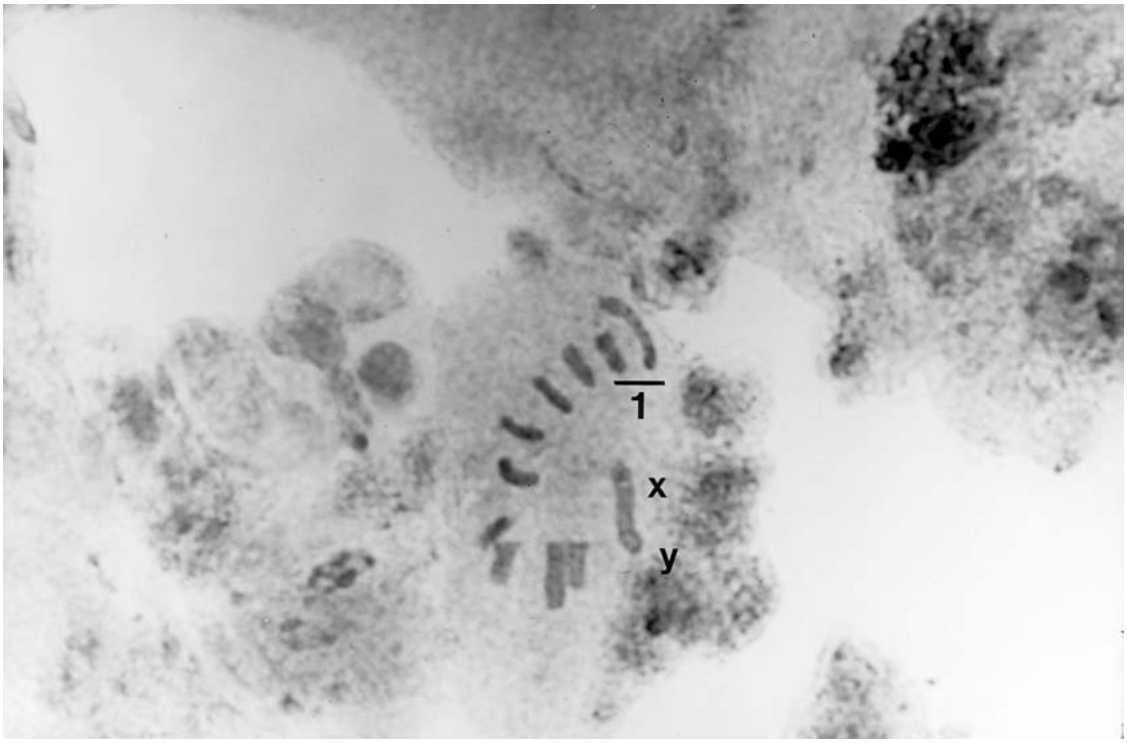

Fig. 2. Metaphase chromosomes from a male brain squash. The long, curved chromosome at the top is a member of pair 1, a pair that sometimes showed heteromorphology. The longer chromosome at the lower right shows the commonly observed short y chromosome associated with the longer x chromosome.

some was the longest, and the Y chromosome the shortest (Table 2). The somatic pairing reported in most dipterans (White 1973; Radu et al. 1975; Southern 1976) was also characteristic in *A. suspensa,* and homologous autosomes usually were associated as somatic bivalents. The Y chromosome was usually joined to the short arm of the X chromosome (Fig. 2). The sex chromosomes in females were often paired, but not joined end to end as the X and Y in the males. Another feature of the male karyotype was the heteromorphism sometimes observed in pairs 1 and 2, where one of the chromosomes appeared to be extended (Fig. 2).

Although all the larval tissues surveyed are suitable for studies of chromosomes, the best tissue for chromosome preparations was the compound eye imaginal discs because the size of the nuclei are larger than the nuclei from other tissues. The squash technique and the cell suspension procedure produced extended and separated chromosomes, but the squash technique was more useful because of the small size of the tissues. For multiple preparations, the squash technique was a relatively simple and faster procedure. Treating tissues with coumarin for 3 minutes helped to relax chromosomes, but prolonged exposure caused shrunken nuclei and very swollen chromosomes. The Caribbean fruit fly has 12 chromosomes, in agreement with 9 other species of *Anastrepha* (Bush 1962), and the Mediterranean fruit fly (Radu et al. 1975). Florida Agricultural Experiment Station Journal Series No. R-09467.

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TWO NEW *ZAMMARA* SPECIES FROM SOUTH AMERICA (HEMIPTERA: CICADOMORPHA: CICADIDAE)

ALLEN F. SANBORN
Barry University, School of Natural and Health Sciences, 11300 N.E. Second Avenue, Miami Shores, FL 33161-6695

ABSTRACT

Two new members of the widespread Neotropical genus *Zammara* Amyot & Serville, *Zammara olivacea* n.sp. from Columbia and *Zammara medialinea* n.sp. from Venezuela are described.

Key Words: new species, taxonomy, cicada, *Zammara*, Columbia, Venezuela.

RESUMEN

Se describe a dos nuevos miembros del género Neotropical *Zammara* Amyot & Seville: *Zammara olivacea* n. sp. de Colombia y *Zammara medialinea* n. sp. de Venezuela.

Translation provided by author.

The genus *Zammara* was erected by Amyot & Serville (1843) to include New World species with expanded lateral pronotal lobes and "cavités sonores" that exposed a large portion of the timbal dorsally. The type species is *Zammara tympanum* [F.] originally described from Brazil (Fabricius 1803). There are currently 16 species assigned to the genus. This cicada group is generally found within the shaded understory of tropical forests (e.g., Young 1977, 1980, personal observation) in which the cryptic wing coloration may account for the infuscated wing patterns typical of *Zammara* species except *Z. luculenta* Distant (1883) and the new species from Venezuela. *Odopoea* Stål, *Miranha* Distant, *Zammaralna* Boulard & Sueur, *Juanaria* Distant, *Borencona* Davis, *Chinaria* Davis, *Orellana* Distant, and *Uhleroides* Distant are other genera of the tribe Zammarini, which share similar morphological characteristics. The two new species here described were found among unidentified material in museum collections. It should be noted that the coloration patterns, especially of the wings, of *Zammara* females may vary from the patterns observed in the males of the same species. The descriptions provided are for males as no female specimens were available for the descriptions. The terminology used to describe the species follows Duffels (1977).

Zammara olivacea, New Species

Type material.—Type male: "Providencia, Colombia, S.A., 17-V-70, sang while being held", deposited in the Insect Research Collection, University of Wisconsin, Madison. Paratype male: "Providencia, Colombia, 3-5-71, J.M. Thompson" deposited in the author's collection.

Etymology. The species is named for its olive green color similar to that of *Z. smaragdina* Walker, 1850 and *Z. smaragdula* Walker, 1858.

Description (Fig. 1).—Head: about as broad as mesonotum; greenish ground color (faded to tawny in the paratype) with ochraceous area between eyes. Ocelli surrounded by black with a wavy fuscous line extending from between the lateral and central ocelli to the supraantennal plates and continued medially across the postclypeus. A thinner irregular mark appears at about one fourth the distance from the ocelli and arches cephalad to the line between the ocelli and supraantennal plate and caudad of where it joins the mark surrounding the lateral ocellus before terminating caudad to the lateral ocellus. This mark consists of two ovoid spots between the lateral ocellus and the antennae. There is a long testaceous spot between the fuscous line and the eye. There are four black spots along the hind part of the head, two mediad of the posterior lateral curvature of the eye and two smaller spots caudad of the terminus of the irregular marks by the lateral ocelli. A thin black line extends posteriorly along the central sulcus and continues laterally as a thin line at the base of the head laterally to the distance of the small spots. A few long hairs extend from the region posterior to the eye. Antennae testaceous except for the distal aspect of the pedicel and the first flagellar segment, which are black. Postclypeus greenish with the lateral surfaces and the junction of the anteclypeus ochraceous. Its dorsal surface is marked with fuscous and a central fuscous stripe. Anteclypeus greenish and ochraceous. Genae greenish with a fuscous line at the junction with the anteclypeus and along a second parallel line at about half the genal length. Rostrum tawny with the tip black, extending to the opercula.

Thorax: pronotum greenish with a central black fascia broadening anteriorly along the base of the head. The mark broadens slightly caudad before the pronotal collar where it terminates.

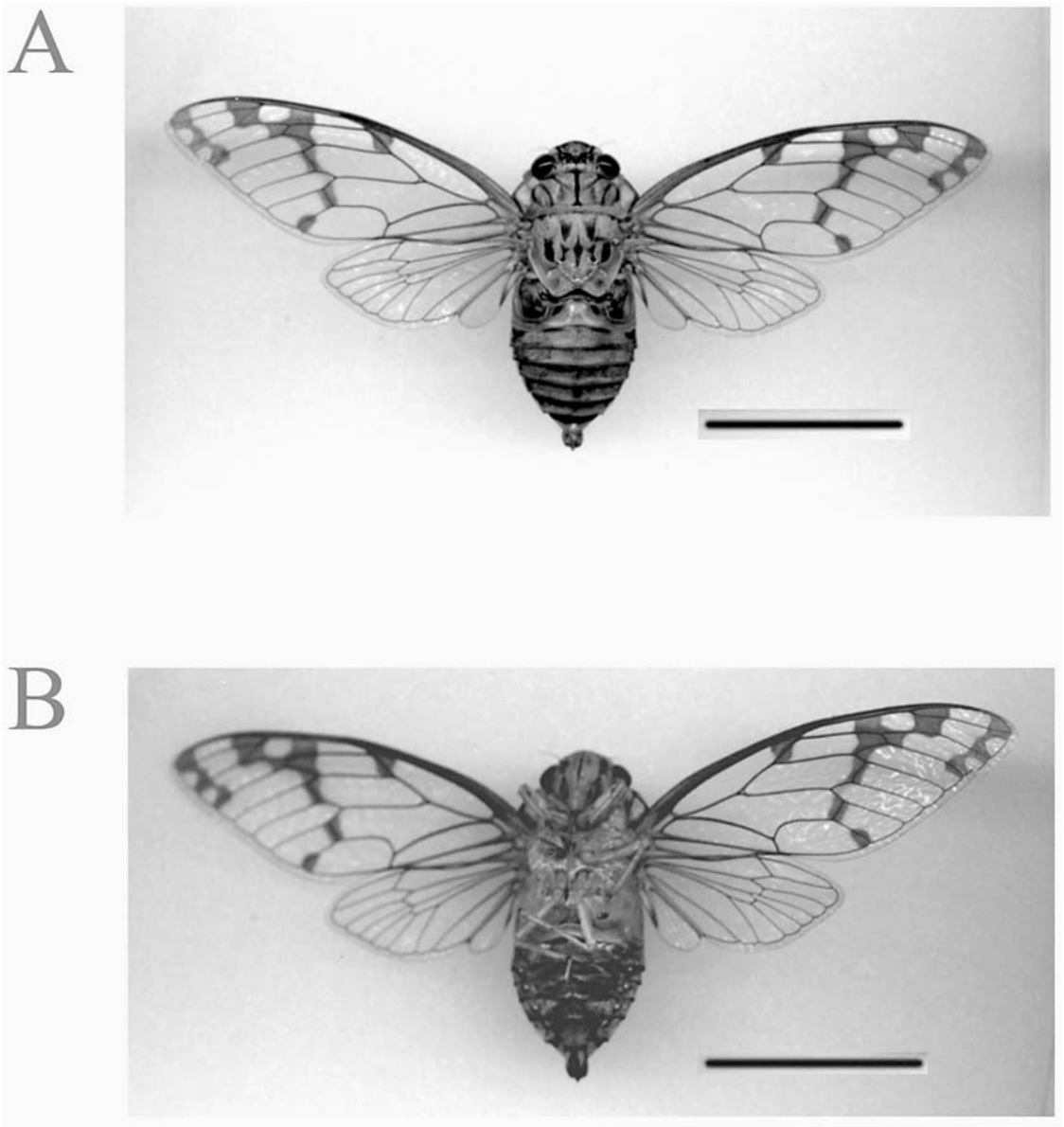

Fig. 1. *Zammara olivacea* n.sp. type male. A. Dorsal view. Bar = 2 cm. B. Ventral view. Bar = 2 cm.

The ambient and posterior oblique fissures are marked with tawny. There is a testaceous spot in the lateral sulcus. Lateral margins of the suprahumeral plates edged in black. Mesonotum with a central median kite-shaped fascia on the posterior half. A pair of discontinuous paramedial fascia which appear to be two separate spots, the first anterior and lateral to the kite-shaped central mark, and which do not reach the pronotum anteriorly; and an irregular mark anterior to the cruciform elevation and lateral to the kite-shaped central mark are present. A thick lateral fascia is found on the posterior half of the mesonotum and extends medially to form a small spot on the anterior arm of the cruciform elevation. There are some irregular marks on the anterior mesonotum extending posteriorly from under the pronotal collar. There are short golden hairs along the lateral and posterior borders of the mesonotum and between the arms of the cruciform elevation. Exposed metanotum and metasternum greenish and tawny. Operculum greenish (darker along posterior and medial borders) with white pruinose wax and a testaceous mark at the base surrounding the meracanthus. Opercula rounded, almost meeting medially.

Wings: fore wings hyaline with eight apical cells; costal area testaceous to just past the node where the coloration becomes fuscous. The basal cell is clouded with green. Crossveins testaceous becoming fuscous in the distal two thirds of the wing. Infuscations at the distal end of the radial cell extending into the proximal portion of ulnar cell 2, a long irregular mark starting in the proximal third of apical cell 1 continuing across the proximal quarter of apical cell 2 and distal portion of the ulnar cell 1, constricting along the vein between apical cell 3 and ulnar cell 1 then expanding across the center of the first ulnar cell to the costal margin and across the proximal portion of apical cell 3 and ulnar cell 2. The mark continues across the proximal portion of apical cell 4 and along the basal veins of the fifth and seventh apical cells. There is a faint mark across the proximal portion of apical cell 6. There are infuscations along the base of the longitudinal veins 1, 2, 3, and 7 (also on the longitudinal veins 5 and 6 in the paratype) continuous to the apex of the tegmina. The marks on longitudinal veins 1, 2, and 3 are connected medially through the apical cells and continue to fill the terminal end of apical cell 1. The spots on longitudinal vein 7 and basal vein 7 are continuous in the paratype. Hind wings with six apical cells; venation testaceous marked with fuscous.

Legs: greenish and ochraceous with the distal tips of the coxa, tibia, tarsi and claws fuscous except the forelegs where the distal part of the femur and the proximal part of the tarsi are also marked with fuscous. Fore femora armed with two distal spines, the proximal one longer and more robust than the other. Hind tibia armed with three fuscous spines along shaft and 13 of these spines around the distal terminus of the segment.

Abdomen: tergites ochraceous, edges of anterior margins of segments 3-8 fuscous, the markings becoming thicker toward segment 8. Medial anterior and lateral anterior fuscous spots next to the timbals on segment 2. Abdominal segments three through seven have a lateral fuscous spot, that is incorporated into the anterior marking on segment 8. Sparse, fine golden hairs cover the central portion of segment 2 and the lateral surfaces of segments 3 and 4. Timbal cover incomplete, exposing the timbal dorsally. The timbal cover folds over itself laterally and posteriorly forming an L-shaped opening. Sternal segments transparent green with a dark green stripe along the midline with some white pruinose wax on the lateral surfaces. Fuscous markings ventrally both at the junction with the metathorax and at the junction of segments 6 and 7. Sternite 8 fuscous except for lateral green bands. Pygofer fuscous with green on lateral surfaces and the dorsal extension very small. Uncus folded at approximate right angle, bulbous laterally at the fold. The terminal medial uncus lobe has an open semicircular shape into which the aedeagus fits.

Measurements (mm): n = 2 males, range given for available specimens. Length of body: 30.4-31.3; length of fore wing: 39.6-41.1; width of fore wing: 13.9; length of head: 4.0-4.1; width of head including eyes: 9.7; width of pronotum including suprahumeral plates: 13.7-14.5; width of mesonotum: 9.4-9.8.

Zammara medialinea, New Species

Type material.—Type male: "VENEZUELA, Aragua Ranch Grande, Rio Res. St. nr Maracay, alt. 3508 ft., 10.15n × 67.36w, 19 Sept 1980" deposited in the collection of the Buffalo Museum of Science. Paratype male: "VENEZUELA, Aragua Ranch Grande, Rio Res. St. nr Maracay, alt. 3508 ft., 10.15n × 67.36w, 24 April 1981" deposited in the author's collection.

Etymology. The species is named for the marks on the head, prothorax and mesonotum which appear to form a stripe along the midline of the dorsal surface of the head and prothorax.

Description (Fig 2).—Head: about as broad as the mesonotum; greenish ground color with an ochraceous band around the eye. A black and fuscous band crosses the anterior part of the head encompassing the median ocellus but not reaching the edge of the supraantennal plates. There is a small caudolateral extension of the band to the eye, a small anterior extension of the band along the midline to the suture with the postclypeus, and a posterior extension of the band encompassing the lateral ocelli except for the caudolateral quarter. This mark continues to narrow posteriorly and terminates anterior to the junction of the prothorax. Some short silver hairs extend from the region posterior to the eye. Antennae fuscous except for the scape and proximal part of the pedicel, which are greenish ochraceous. Postclypeus greenish with some posterior tawny marks which appear to be continuous with the central marking of the head. There is some ochraceous coloration on the transverse postclypeal ridges of the paratype. Anteclypeus greenish but with a central ochraceous mark in the paratype. Genae green. Some white pruinose wax found on the gena, anteclypeus and postclypeus. Rostrum greenish with the tip black, extending to the abdomen.

Thorax: pronotum greenish with a central testaceous fascia broadening anteriorly along the base of the head to about half the distance to the eye. The fascia narrows medially where it terminates anterior of the pronotal collar. Testaceous spot in the anterior portion of the ambient fissure near the junction with the head. An ochraceous mark surrounds the posterior portion of the central fascia and extends into the medial half of the ambient fissure. Pronotal collar green. Mesonotum greenish with a central median testaceous

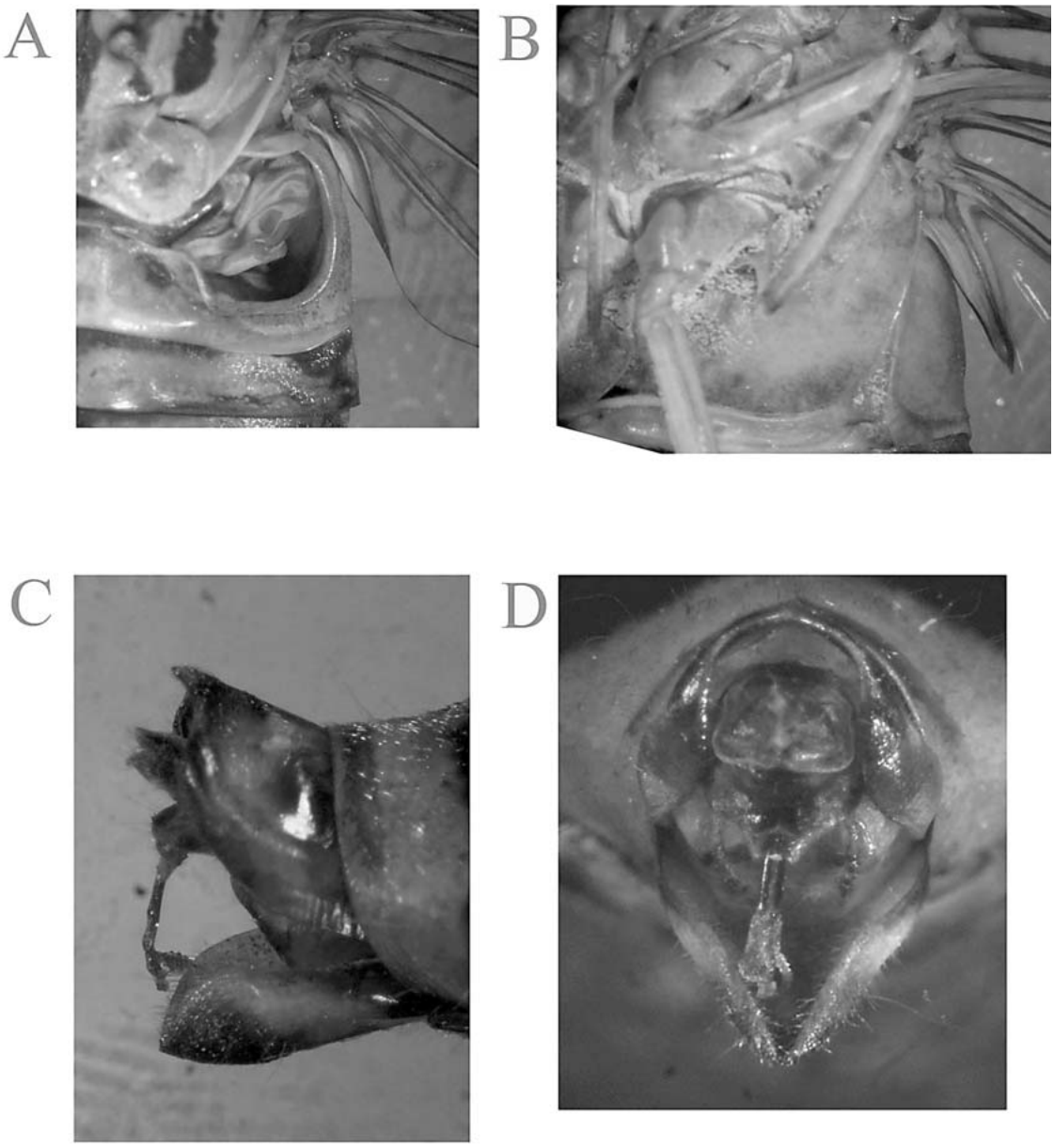

Fig. 2. *Zammara olivacea* n.sp. type male. A. Dorsal opening to the timbal. B. Male operculum. C. Lateral view of the type male genitalia. D. Posterior view of male genitalia.

fascia that expands laterally to the arms of the cruciform elevation. A testaceous spot lateral to the anterior arm of the cruciform elevation. Lateral edges of the wing grooves ochraceous. There are silvery hairs along the posterior borders of the mesonotum and between the arms of the cruciform elevation. Metanotum and metasternum greenish. Operculum and mercanthus greenish flecked with white pruinose wax. Opercula expand laterally before curving toward the midline. Opercula approach one another but do not meet medially.

Wings: fore wings hyaline with eight apical cells; costal area greenish to just past the node where the coloration becomes testaceous. The basal cell is clouded with green. There is a testaceous mark on the vein between the basal and radial cells. Crossveins greenish at base becoming ochraceous and finally testaceous in the distal portion of the wing. Very slight infuscations at the

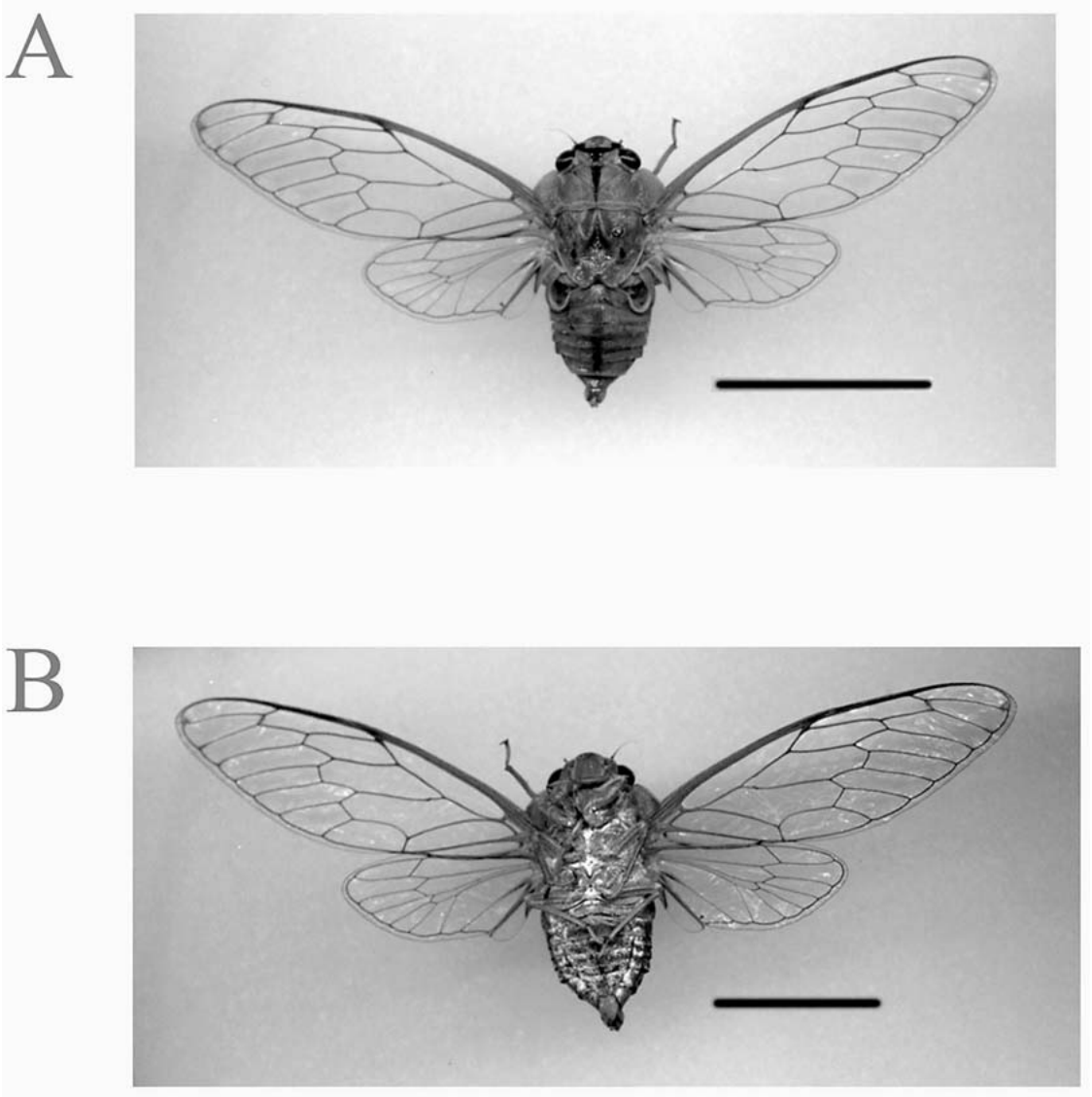

Fig. 3. *Zammara medialinea* n.sp. type male. A. Dorsal view. Bar = 2 cm. B. Ventral view. Bar = 2 cm.

basal vein of the second apical cell and at the distal portion of the longitudinal veins 1, 2, and 3. The infuscations on the first longitudinal veins extends into the apex of the first apical cell. There is a slight bronze tint to the apical cells of the wing. Hind wings with six apical cells; venation ochraceous and testaceous except the cubital vein which is greenish and ochraceous.

Legs: greenish, distal part of the tibia and tarsi ochraceous and claws fuscous. Small testaceous marks on proximal parts of tarsi. Fore femora armed with two greenish distal spines, the proximal one larger, with a testaceous tip, the distal one very small. Hind tibia armed with five testaceous spines with fuscous tips along shaft and 13 of these spines around the distal terminus of the segment.

Abdomen: tergites greenish ochraceous. Small fuscous lateral spots on the anterior portion of segments 7 and 8 partially covered by the anterior segments. Thin medial fuscous band along anterior border of segment 8 not continuous with lateral spots. Sparse silver hairs cover the abdomen, particularly the lateral surfaces and segment 8. Timbal cover incomplete, expanding laterally beyond the edge of the abdomen. Timbal exposed dorsally. The timbal cover folds posteriorly where the coloration is ochraceous. Sternal

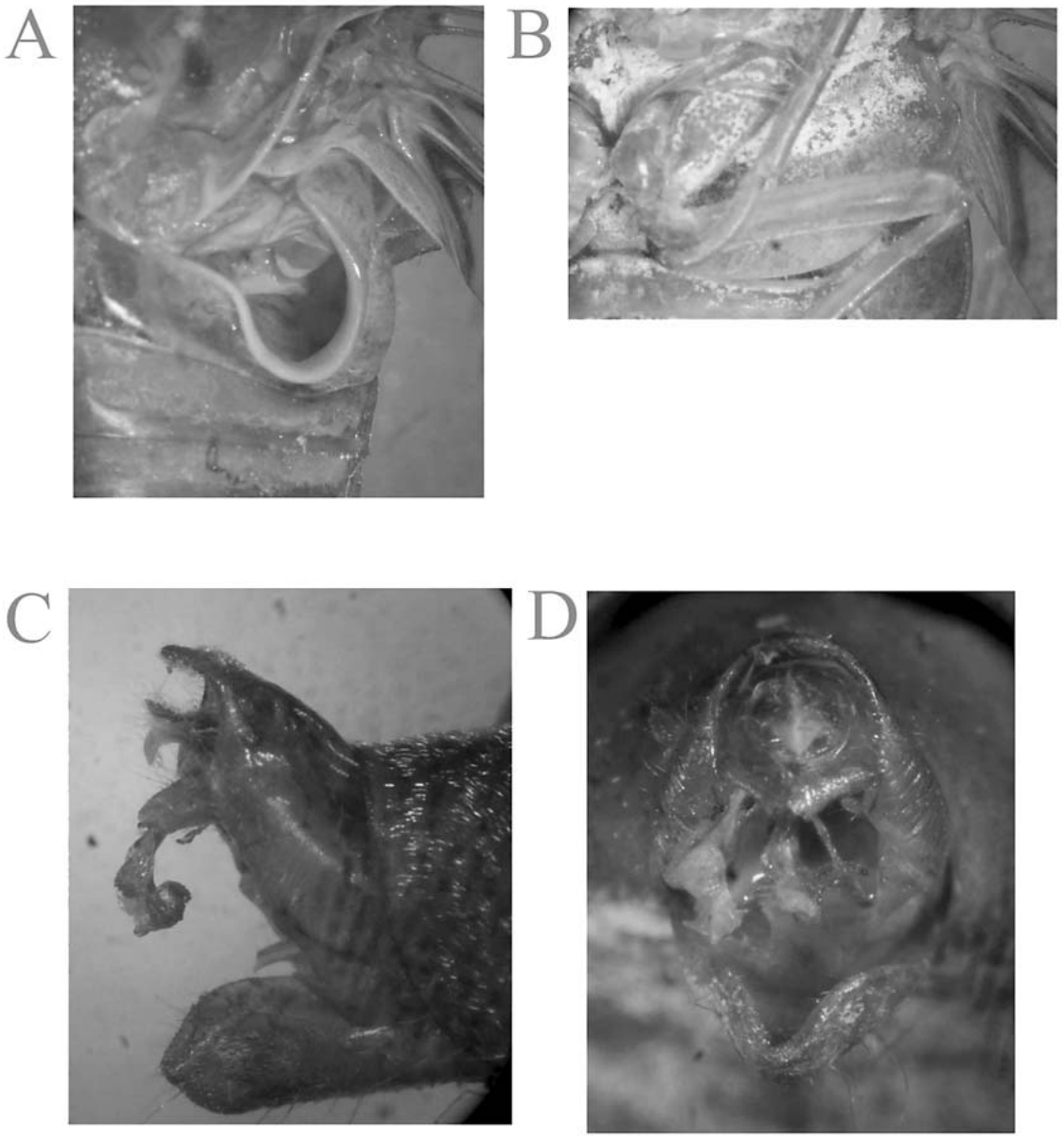

Fig. 4. *Zammara medialinea* n.sp. type male. A. Dorsal opening to the timbal. B. Male operculum. C. Lateral view of the type male genitalia. D. Posterior view of male genitalia.

segments greenish ochraceous with white pruinose wax on the surfaces. Pygofer greenish, ochraceous lateral dorsally with the dorsal extension small. Uncus short. The lateral lobes fold under the medial lobe which is arched distally.

Measurements (mm). N = 2 males, range given for available specimens. Length of body: 26.2; length of fore wing: 36.9-37.7; width of fore wing: 12.1-12.2; length of head: 4.0-4.4; width of head including eyes: 8.9-9.2; width of pronotum including suprahumeral plates: 13.4-13.8; width of mesonotum: 8.8-9.0.

Discussion

The exact locality of the type location for *Zammara olivacea* is unclear. Providencia is the capital and name of an island approximately 233 km off the eastern coast of Nicaragua (13°19'N 81°23'W) that forms part of the Intendency of San Andrés y

Providencia of Colombia (Cohen 1998). This is the location that is identified in most atlases and gazetteers. However, several small towns with the name Providencia can be found on the mainland as well. The origin of the specimens in the University of Wisconsin Collection is uncertain, so assigning a type location is uncertain except for Providencia, Colombia. The recommendations of the International Code of Zoological Nomenclature (1999) concerning a type locality cannot be applied in as much as any Providencia location in Colombia is well within the range of the genus *Zammara*, which extends from Argentina in the south to Mexico in the north (Metcalf 1963).

Zammara olivacea is larger than but morphologically close to *Z. smaradula*. The wing infuscation patterns are similar in the two species but more complete in *Z. olivacea*, particularly the marks that extend across the ulnar cells and longitudinal veins. Body coloration differs in that the prothorax is more highly marked and the markings of the mesothorax and abdomen are much larger than in *Z. smaragdula*. The genitalia differ in that the terminal extension of the medial lobe of the uncus is broader and does not bend over as great an angle as in *Z. smaragdula*. The dorsal opening to the timbal approximates two right angles posteriorly in *Z. olivacea*, while in *Z. smaragdula* the dorsal opening to the timbal is rounded.

Zammara medialinea is the second *Zammara* species not to have obvious infuscations in the tegmina. Although teneral specimens can have faint wing patterns, there are no indications of markings and both specimens appear to be fully pigmented. The faint markings found in *Z. medialinea* make the species more like *Z. luculenta* (known only from the type specimen from an unknown locality) than like any other species of *Zammara*. The new species differs from *Z. luculenta* (Distant 1883) in the markings on the prothorax and mesothorax, the coloration of the abdomen, and the slight bronze tint to the wings.

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DISPERSAL ADAPTATIONS OF IMMATURE STAGES OF THREE SPECIES OF LEAFHOPPER (HEMIPTERA: AUCHENORRYNCHA: CICADELLIDAE)

CHRISTOPHER TIPPING, RUSSELL F. MIZELL III AND PETER C. ANDERSEN
University of Florida, NFREC Quincy, 155 Research Road, Quincy, FL 32351

ABSTRACT

Xylem-feeding leafhoppers have evolved several behavioral and physiological adaptations to utilize xylem fluid of variable composition, including polyphagy and high assimilation efficiency. They also display high vagility coupled with excellent visual acuity. We investigated the spectral frequency responses of the nymphal stages of three leafhopper species: *Homalodisca coagulata*, *H. insolita*, and *Oncometopia nigricans*. Under laboratory conditions, the nymphs of *H. coagulata* and *O. nigricans* discriminated spectra and were highly attracted to hues of yellow, with safety yellow being the most attractive. The nymphs of *H. insolita* were also attracted to yellow hues, but were more attracted to cream yellow. In the laboratory, maximum jumping distances of third instar *H. coagulata*, *H. insolita*, and *O. nigricans* were 68.0, 49.7, and 39.2 cm respectively, when provided a target. The fifth instars of *H. coagulata*, *H. insolita*, and *O. nigricans* had maximum jumping distances of 78.8, 29.2, and 45.5 cm, respectively. Additionally, all nymphal stages of *H. coagulata* dispersed up to 10 meters after three days under field conditions when released into an outdoor grass-covered arena. The neonates of *H. coagulata*, *H. insolita*, and *O. nigricans* survived on average, 83.5, 70.5, and 83.0 h without plant feeding, respectively.

Key Words: leafhoppers, *Homalodisca coagulata*, *H. insolita*, *Oncometopia nigricans*, color perception, nymphal dispersal.

RESUMEN

Los saltahojas que se alimentan del xilema han producido por evolución varias adapciones fisiológicas y de comportamiento para utilizar el liquido de xilema de diferentes composiciones, incluyendo polifagia y una alta eficiencia de asimilación. Ellos demuestran también, en una alta dispersión adjunta a una agudeza visual excelente. Nosotros investigamos las respuestas de la frecuencia espectra de los estados ninfales de tres especies de saltahojas: *Homalodisca coagulata*, *H. insolita*, y *Oncometopia nigricans*. Baja condiciones del laboratorio, las ninfas de *H. coagulata y O. nigricans* discriminaron los espectros y fueron altamente atraidas a los tonos de amarillo, con el amarillo brillante siendo lo mas atractivo. Las ninfas de *H. insolita* también fueron atraidas a los tonos de amarillo, pero fueron mas atraidas al amarillo crema. En el laboratorio, la distancia maxima de salto de los tercer estadios ninfales de *H. coagulata*, *H. insolita*, y *O. nigricans* fueron 68.0, 49.7, y 39.2 cm respectivamente, cuando se proveyó una meta. Los quinto estadios de *H. coagulata*, *H. insolita*, y *O. nigricans* saltaron una distancia maxima de 78.8, 29.2, y 45.5 cm, respectivamente. Además de todas las etapas ninfales de *H. coagulata* se dispersaron hasta 10 metros después de tres dias bajo condiciones de campo cuando fueron liberadas en un área exterior de arena cubierta con pastos. Los recien nacidos de *H. coagulata*, *H. insolita*, y *O. nigricans* sobrevivieron un promedio de 83.5, 70.5, y 83.0 horas sin alimentarse de la planta, respectivamente.

Xylophagous leafhoppers seek host plants nutritionally important for development as well as for reproduction. The ability to disperse, locate, and utilize host plants is essential for leafhoppers because the composition of xylem fluid is dilute and highly variable, not only between host plant species, but also within a single plant over time (Horsfield 1977; Andersen & Brodbeck 1989a). Xylem fluid has the most dilute concentrations of dietary nitrogen and carbon of any plant tissue (Andersen et al. 1989). It is composed of >95% water with the predominate organic compounds consisting of amino and organic acids and small amounts of sugars (Andersen et al. 1989; Clark et al. 1986). Many factors are responsible for the dynamic changes associated with xylem composition including temperature, fertilization, water stress, and plant phenology (Andersen et al. 1995; Andersen & Brodbeck 1989b; Brodbeck et al. 1999).

Adult leafhoppers of the Proconiini are strong flyers. The reported host ranges for many members of this group are quite broad (Turner & Pollard 1959; Adlerz 1980; Brodbeck et al. 1993). Presently, studies relating to dispersal and abundance of leafhoppers are limited to the adult stage. The relative seasonal abundance of adult glassy-winged sharpshooters, *Homalodisca coagulata* (Say), has been well documented on a variety of host plants in its native range in the southeastern US as well as in its introduced range in parts of California (Ball 1979; Mizell & French 1986; Blua et al. 2001).

The attractancy of various colors to insects of several orders including Hemiptera, Thysanoptera, and Diptera has been well documented (Prokopy et al. 1975; Prokopy & Owens 1983; Kirk 1984). Yellow colors have been shown to be especially attractive to the greatest variety of insects (Hoback et al. 1999). However, some species of aphids and thrips are attracted to hues of blue spectra depending on their physiology and relating to their dispersal and host plant selection (Walker 1974; Chang et al. 2000). The use of colored traps to successfully monitor insect pest populations has been recognized as an important tool for many pest management programs (Vaishampayan et al. 1975; Prokopy et al. 1979; Webb et al. 1994).

The glassy-winged sharpshooter is a highly polyphagous leafhopper with a native distribution throughout the southeastern US and northern Mexico (Turner & Pollard 1959). This insect was accidentally introduced into southern California (Gill 1995). *Homalodisca insolita* (Walker) was first found in northern Florida and southern Georgia in 1950 (Pollard et al. 1959). It prefers to feed upon grasses. Its native range includes parts of Texas, Arizona, and Mexico. The black-winged leafhopper, *Oncometopia nigricans* (Walker), is another xylem-feeding leafhopper that is commonly found throughout the southeastern US. Its host plant range is similar to that reported for *H. coagulata* (Turner & Pollard 1959). All three species are competent vectors of strains of *Xylella fastidiosa* (Wells et al. 1987), a Gram negative, xylem limited bacterium that is the causative agent for several important plant diseases including phony peach, oleander leaf scorch, and Pierce's disease of grapevine.

The introduction of *H. coagulata* into California is of great concern for producers of a variety of commodities including grapes, peaches, and almonds as well as ornamental nursery plants such as oleander. Although several strains of *Xylella fastidiosa* have been reported from many plant species native to California, the indigenous vectors such as the green sharpshooter, *Draeculacephala minerva* Ball, and the red-headed sharpshooter, *Carneocephala fulgida* Nottingham, have a relatively narrow host range when compared to *H. coagulata* (Purcell & Frazier 1985). Additionally, *H. coagulata* is routinely intercepted in other regions of California, including the Napa Valley, as a result of the transportation of infested ornamentals from southern California. (Bugspot [webpage] 2003).

Our objectives were to examine characteristics important for nymphal dispersal and survivorship by determining the visual wavelength discrimination by nymphs of *H. coagulata*, *H. insolita*, and *O. nigricans* as well as the absolute jumping distances of the third and fifth instars for each species. Also, the ability of the nymphal stages of *H. coagulata* to disperse under field conditions is described. Finally, the survivorship of starved neonates of the three species was investigated to determine the potential for nymphs to be transported without plants.

Materials and Methods

Insects and Plants

Immature leafhoppers used in all studies were taken from cultures maintained in 1-m^3 screened cages. Each cage was provisioned with a mixture of host plants depending on leafhopper species as well as life history stage. Cages for nymphs of *H. coagulata* contained glabrous soybean, (*Glycine max* (L.) 'D90-9216'), cowpea, (*Vigna unguiculata* (L.) 'California #5'), and basil, (*Ocimum basilicum* L. 'Lemon'). Cages containing adult *H. coagulata* had glabrous soybean, cowpea, and saltbush, (*Baccharis halimifolia* L.). Neonates of *H. coagulata* were removed from the adult cages daily and placed into cages for nymphs. Cages of *H. insolita* contained Texas millet, (*Panicum texicum* Buckl.), and Johnson grass, (*Sorghum halepense* (L.). *Oncometopia nigricans* culture cages were provisioned with cotton, (*Gossypium hirsutum* L. 'Deltapine 88'), *Coleus* sp., okra, (*Hybiscus esculentus* L. 'Clemson spineless'), and wild periwinkle vine, (*Vinca major* L.). All plants used in the colony cages were potted in a 3:1:1 pine bark: sphagnum moss: sand mixture before placement into colony cages. Greenhouse temperatures ranged between 25-32°C with indoor lighting to maintain a 16:8 light/dark photoperiod. Plants were replaced in each cage when they began to show signs of decline which included wilting, discoloration, or reduction in growth. The soil medium for all plants was watered to saturation twice daily.

Visual Response to Color

Wavelength detection and discrimination of the nymphal stages of *Homalodisca coagulata*, *H. insolita*, and *Oncometopia nigricans* were tested within a structure constructed from 0.3 mm plastic sheeting that was 30 cm in height and tubular in shape with a 50 cm radius. The interior of the structure, or arena, was spray painted flat black (Chase Products, Maywood, IL). Clear plastic push pins were inserted along the inner wall 5 cm from the base of the arena at 8 cm intervals. Plastic panels (5 cm^2) were painted safety yellow (Pittsburg Paints, Pittsburgh, PA), cream yellow (Lucite Paints, Pittsburgh, PA), neon blue (General Paint and Manufacturing Company, Cary, IL), and neutral gray (Rustoleum Corporation, Vernon Hills, IL). Colored panels were held in the arena with 1.9-cm binder clips and hung on the clear plastic push pins along the inside of the arena. Panels painted flat black were also placed in the arena to serve as controls. All panels were

coated with Tangle-trap insect coating (The Tanglefoot Company, Grand Rapids, MI).

Leafhoppers collected from colony cages were released in the center of the arena where they could exhibit preference among the colored panels by orienting and leaping on to them. Tests were performed at random times between the hours of 11:00 am and 6:00 pm in a darkened room at 24°C. The arena was illuminated with an overhead fluorescent light source with an intensity of 915 lux measured on the floor in the center with a light meter (Extech Instruments, Waltham, MA). The arena was randomly rotated 45 degrees after each nymphal release and colored panels were randomly repositioned. Mixed nymphal life stages of each of all three species were released together in groups of 15-20 depending on availability. Colored panels were examined for trapped nymphs 1h after release in the arena. After observing the preferred color choices of the nymphs of *H. insolita*, the spectral reflectance of the cream and safety yellow panels as well as leaves and stalks of *S. halepense* was measured with a fiber optic spectrometer (USB-2000, Ocean Optics, Dunedin, FL). Leaves and stalks of *S. halepense* that were used for the reflectance measurements were chosen from healthy plants maintained as described previously.

Outdoor Dispersal Study

An arena was constructed outdoors and consisted of two concentric rings of cardboard tubes. The two rings were situated on a field of Bahiagrass, *Paspalum notatum* Flugge, at the North Florida Research and Education Center (NFREC) in Quincy, Florida. This field was chosen because *P. notatum* is not an acceptable host plant for *H. coagulata*. The field was mowed weekly to an approximate height of 6 cm. Weeds were removed daily from areas inside the arena. The two rings were concentric and were 10 and 20 m in diam. All tests with the 10-m ring were completed before testing with the 20-m ring. The cardboard tubes (30.5 in height and 7.6 cm in diam) were painted safety yellow and coated with Tangletrap. They were placed upright on the ground at one-meter intervals along each of the rings. Small stakes were used to keep the tubes standing. Twenty individuals of each nymphal stage of *H. coagulata* were released in the center of either the 10- or 20-m ring for each test. Three days after each release, the cardboard tubes were checked for captured leafhoppers. Nymphal releases were replicated 3 times for the 10-m ring followed by three separate nymphal releases in the 20-m ring.

Jumping Distance of Nymphs

The jumping distance of 30 third and 30 fifth instars of the three leafhopper species was determined by introducing the nymphs onto a 'platform' placed in a water-filled 10-cm plastic Petri dish. The 'platform' was a 250-ml polyethylene cup 8 cm tall and 7 cm in diameter. The lid on the cup had a small slit to facilitate the introduction of aspirated nymphs. After the appropriate number of nymphs was collected, the cup was placed in the center of the water-filled dish and the lid carefully removed allowing the insects to crawl to the rim of the cup. The dish was centered onto a 2-m^2 corrugated plastic sheet (coreplast) that was coated with a thin layer of Tangle-trap. A single cardboard tube**,** the same dimensions as described previously, was painted safety yellow and was placed at the edge of each of the four corners of the coreplast sheet to provide a target for the nymphs. Ten nymphs of each stage were released between the hours of 4:00 and 6:00 PM in a darkened room with overhead fluorescent illumination and maintained at a temperature of 25°C. After 24 h nymphs were counted and the distance they jumped recorded. Each release was replicated three times.

Neonate Survival Study

Five egg masses of *H. coagulata*, *H. insolita*, and *O. nigricans* were collected on intact entire leaves of *V. unguiculata*, *H. esculentus,* and *G. hirsutum*, respectively, and placed into 10-cm diam plastic Petri dishes. The dishes were filled with approximately 10 ml of (1.2%) water agar (Fisher Chemicals cat. no. BP1423-500). A 5-cm circle was cut out of each dish lid and covered with nylon mesh. Dishes with egg masses were held at 25°C until eclosion. Thirty minutes after eclosion, the leaves were removed and the neonates were observed until death. Preliminary observations of dozens of cohorts of neonates for the three species were useful in determining approximate times of hatching and death. Death was determined when nymphs were not responsive to touch with a small sable hair paintbrush. Time measurements were rounded to the hour.

Statistical Analysis

Data from the visual acuity study were analyzed as one-way analysis of variance by general linear models procedures (GENMOD) (SAS 1990). Data from releases of individuals of each species were analyzed separately by instar. The proportion of each number of individuals of each instar (n = 25 to 73) that was trapped on the colored panels produced test statistics based on a pair-wise comparison with data from the safety yellow panels as the dependent variable. The proportion of the individuals captured by each of the colored panels was then compared by chi-square. Descriptive statistics (means and standard errors) were applied to data from the outdoor dispersal and neonate survival studies.

RESULTS

Visual Response to Color

The safety yellow-colored sticky panels captured a greater number of the nymphs of *H. coagulata*, regardless of instar, than the other colored panels of the arena. The proportions of nymphs captured on safety yellow was higher when compared to the proportion captured by the second most attractive color, cream yellow, and were 67.1% ($\chi^2 = 22.59$; $df = 99$; $P < 0.0001$), 81.4% ($\chi^2 = 12.29$; $df = 86$; $P < 0.0005$), 69.2% ($\chi^2 = 12.00$; $df = 87$; $P = 0.0005$), 71.4% ($\chi^2 = 2.2$; $df = 72$; $P < 0.0001$), and 81.5% ($\chi^2 = 6.2$; $df = 87$; $P < 0.0001$), for first through fifth instars, respectively (Fig. 1).

Significantly greater numbers of the nymphs of *O. nigricans* were attracted to the panels colored with safety yellow when compared to cream yellow (Fig. 1). The proportion of first through fifth instars trapped on the safety yellow panels was 71.4% ($\chi^2 = 11.81$; $df = 99$; $P < 0.005$), 78.8% ($\chi^2 = 10.22$; $df = 86$; $P < 0.01$), 88.9% ($\chi^2 = 11.40$; $df = 87$; $P < 0.005$), 86.7% ($\chi^2 = 10.22$; $df = 72$; $P < 0.001$), and 96.0% ($\chi^2 = 4115.7$; $df = 87$; $P < 0.0001$), respectively (Fig. 1).

A greater proportion of all nymphal stages of *H. insolita* were attracted to the cream yellow panels than any other color (Fig. 1). The cream yellow panels trapped 40.7, 54.8, 59.5, 51.7, and 63.3% of the first through fifth instars, respectively. However, only the third ($\chi^2 = 8.86$; $df = 87$; $P < 0.005$), and fifth ($\chi^2 = 5.10$; $df = 87$; $P < 0.05$) instar catches were statistically significant when compared to safety yellow.

The instances when the number of nymphs not captured was greater than that of captured nymphs on neon blue, neutral gray, or flat black panels were due to mortality associated with collection and handling or escape (Fig. 1).

Outdoor Dispersal Study

The average number of nymphs trapped on the cardboard tubes in the 10- and 20-m arenas was 6.0, 10.0, 10.4, 5.0, 7.0 and 3.0, 4.0, 3.3, 2.7, and 6.0 for first through fifth instars, respectively (Table 1). The weather during all tests was fair with an average day and evening temperature of 31.0° and 16.6°C, respectively.

Nymphs were observed feeding on seedlings of cut-leafed evening primrose (*Oenothera laciniata* Hill) that escaped detection during the weed check of the arena. These weeds have a fast rate of growth as well as a prostrate growth pattern making their detection difficult. This most likely reduced the number of nymphs captured on the cardboard tubes because they would remain feeding on these plants for several days and not disperse.

Jumping Distance of Nymphs

Nymphs that jumped off of the platform were trapped where they landed on the Tangle-trap-coated coreplast sheet. The longest recorded jump of third instars of *H. coagulata*, *H. insolita*, and *O. nigricans* was 68.0, 46.2, and 39.2 cm, respectively (Fig. 2). The fifth instars of *H. coagulata*, *H. insolita*, and *O. nigricans* had the longest jumps of 78.8, 29.7, and 45.5 cm, respectively (Fig 3). Many nymphs were observed walking down the side of the platform where they would encounter the water and proceed back to the top before jumping towards the yellow cardboard tubes.

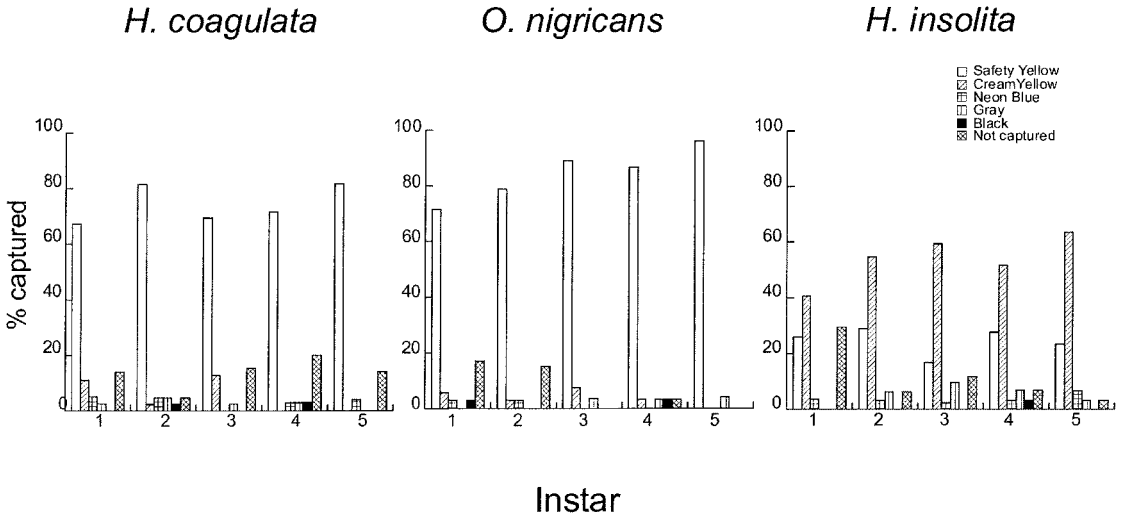

Fig. 1. Attractiveness of various colored panels to nymphal stages of *H. coagulata*, *O. nigricans,* and *H. insolita*.

TABLE 1. NUMBER OF *H. COAGULATA* NYMPHS CAPTURED BY YELLOW STICKY TRAPS IN OUTDOOR ARENAS 10 AND 20 METERS IN DIAMETER.

	Instar[1]				
Arena diameter	1	2	3	4	5
10 meters	6 ± 0.3	10 ± 0.3	10 ± 0.6	5 ± 0.0	7 ± 2.0
20 meters	3 ± 1.0	4 ± 1.0	3.3 ± 1.9	2.7 ± 0.7	6 ± 1.5

[1]Mean and SE, for each instar $n = 20$.

Neonate Survival Study

First instars of *H. coagulata*, *H. insolita*, and *O. nigricans* lived an average of 83.5, 70.5, and 83.0 h, respectively, when placed in the Petri dishes containing agar at 25°C (Table 2). Neonates eclosed from an individual egg mass within 20 min of each other, regardless of the number of eggs in each mass. Additionally, all nymphs from a single egg mass died within a 30-minute period, regardless of species. Nymphs that were held in the agar dishes probed the agar. They were quite mobile and moved around the entire inner surface of the agar dishes.

DISCUSSION

Insects are attracted to different spectra for a variety of reasons including resource finding and dispersal (Prokopy & Owens 1978). Yellow traps are especially attractive to many herbivorous insects including leafhoppers, because this spectrum represents what has been described as 'super normal' green (Staddon 1975). Spectra that represent potential optimal host plants would be expected to be and indeed are, attractive to the nymphs of *H. coagulata*, *H. insolita*, and *O. nigricans*. The adults of all three species are also attracted to yellow traps (Pollard et al. 1959; Ball 1979). Cream yellow was more attractive than safety yellow to the nymphs of *H. insolita* under the conditions of this study. The grassy host plants preferred by *H. insolita* grow in thick clumps with reduced light reaching the base of the stalks. In the field and in culture, nymphs are found only at the base of clumps of *S. halepense*. The spectral reflectance of stalks of *S. halepense* more closely resembles cream yellow than safety yellow (Fig. 4). Conversely, the spectral reflectance of the leaf blades of *S. halepense* is closer to safety yellow. Previous reports (Ball 1979) indicating the attractancy of yellow to the adults of *H. insolita* are not unexpected considering the similarity in the reflectance of leaf blades of *S. halepense* to safety yellow.

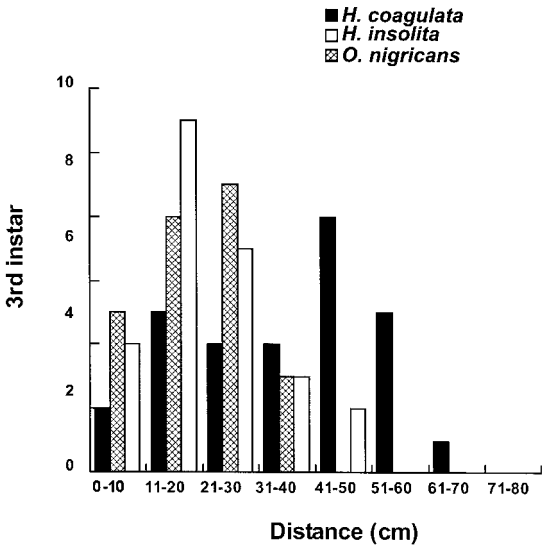

Fig. 2. Lengths of jumps in cm by third instar *H. coagulata* ($n = 29$), *H. insolita* ($n = 27$), and *O. nigricans* ($n = 27$).

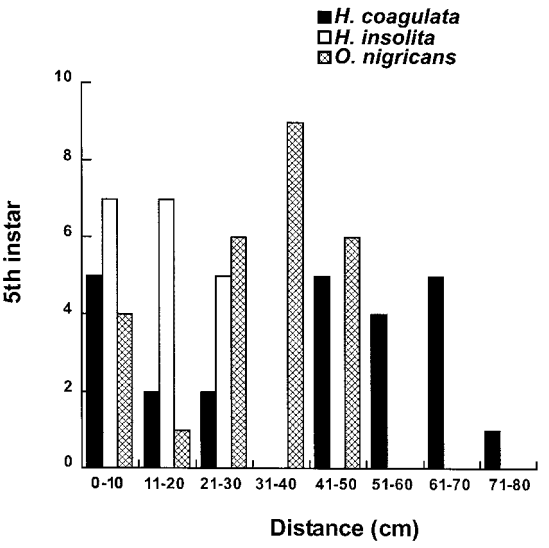

Fig. 3. Lengths of jumps in cm by fifth instar *H. coagulata* ($n = 24$), *H. insolita* ($n = 19$), and *O. nigricans* ($n = 26$).

TABLE 2. MEAN NUMBER OF HOURS NEONATES REMAINING ALIVE AFTER PLACEMENT INTO AGAR-FILLED PETRI DISHES.

	Species[1]		
	H. coagulata	*H. insolita*	*O. nigricans*
Mean survivorship in hours (± SE)	83.5 ± 0.3	70.5 ± 0.4	83.0 ± 0.4
Mean eggs per mass (± SE)	12.0 ± 1.6	19.0 ± 1.1	14.0 ± 1.0
Total *n* of neonates	72	93	74

[1]Data collected from 5 egg masses held at 25°C, >90% RH.

The dispersal ability of adult leafhoppers has been well documented (Taylor 1985). The majority of studies describing the dispersal of leafhoppers have focused primarily on movement patterns of the adults (Medler 1957; Cook 1967; Nestel & Klein 1995; Blackmer et al. 2004). The dispersal ability of leafhopper nymphs has received little attention. The nymphs of leafhoppers are capable of jumping long distances relative to their body size. This tremendous leaping ability is not only important for avoiding potential predators but for dispersing and finding host plants essential for development. We have shown that the nymphal instars of *H. coagulata* are capable of dispersing by walking and or jumping up to 10 meters in three days when placed in a field of Bahia grass. Under laboratory conditions we observed first, third, and fifth instars of *H. coagulata* moving 10 meters in one h on a flat surface.

In the field and in culture, females of *H. coagulata* and *O. nigricans* will readily oviposit on herbaceous and grassy host plants that are not suitable for successful nymphal development. The nutrient requirements of immature *H. coagulata* are different and much more restricted than those of the adults (Brodbeck et al. 1995). The adults prefer to feed on xylem fluid containing proportionally higher concentrations of amides; however, nymphs develop poorly on these diets (Brodbeck et al. 1995). Therefore, visual discrimination of wavelengths by the nymphal stages of leafhoppers is extremely important for their ability to find better quality host plants essential for successful development. Differential survivorship between instars of *H. coagulata* on a variety of hosts suggest the benefits of utilizing different host plants during development due to the changing nutritional requirements for immature stages

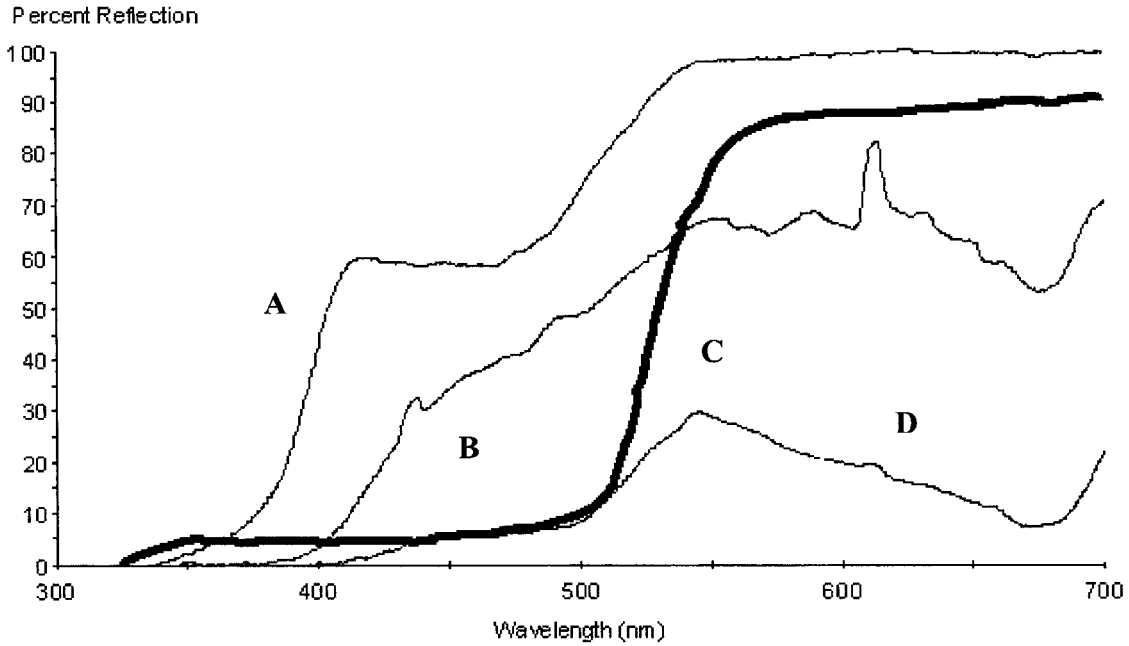

Fig. 4. Spectral reflectance of cream yellow, safety yellow, and *S. halepense* stalk and leaf blade. A, cream yellow; B, *S. halepense* stalk; C, safety yellow; D, *S. halepense* leaf blade.

and adults (Brodbeck et al. 1995; Brodbeck et al. 2004). Although egg masses of *H. coagulata* and *O. nigricans* can occasionally be found on *S. halepense* and *P. texicum*, Turner and Pollard (1959) reported the eggs masses of *H. insolita* were found almost exclusively on these two grasses as well as crabgrass, *Digitaria sanguinalis* (L.). Our field observations agree with previous reports. Additionally, in northern Florida, we also have found southern sandbur (*Cenchrus echinatus* L.) as another plant utilized by *H. insolita* as an oviposition host.

Under normal environmental conditions, newly eclosed neonates of all three species cluster together on the surface of the leaf near the egg mass for 2 to 3 h until their cuticle hardens. Nymphs were also observed to feed and become visibly larger in size during this time. After the initial period of clustering, nymphs would then disperse to the smaller stems of the host plant.

Excellent visual discrimination of wavelengths coupled with the ability to disperse and utilize a variety of host plants allow the nymphal stages of *H. coagulata* to successfully complete development within a dynamic nutritional environment of changing xylem fluid compositions. As the nutritional quality of host plants changes due to phenology and environmental conditions, instars of *H. coagulata* have the ability to disperse and seek better hosts. Future strategies related to the management of *H. coagulata* in California should consider the dispersal capabilities of the nymphal stages. In many sections of southern California, grape vineyards are located adjacent or near to citrus groves. *H. coagulata* has been shown to oviposit heavily on several varieties of citrus including lemon and orange (Al-Wahaibi & Morse 2000). Dispersal studies involving movement patterns of nymphs from citrus to adjacent or nearby vineyards have not been explored, but there is an obvious potential for such a phenomenon.

The survivorship of the neonates for up to three days without food represents an additional consideration for the accidental shipment of *H. coagulata*. Trucks, trains, or shipping containers that were used to transport plants infested with egg masses could potentially contain viable neonates. Under ideal conditions, neonate nymphs could survive transport for several days without feeding. Whether they would then be capable of feeding and becoming established remains to be investigated.

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A NEW SPECIES OF *NEMOMYDAS* AND A NEW RECORD FOR *BALIOMYDAS GRACILIS* (DIPTERA: MYDIDAE) FROM HISPANIOLA

BORIS C. KONDRATIEFF[1] AND DANIEL E. PEREZ-GELABERT[2]
[1]Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, CO 80523

[2]Research Associate, Department of Entomology, National Museum of Natural History, Smithsonian Institution Washington, DC 20560-0169, USA

ABSTRACT

A new species of mydas fly, *Nemomydas dominicanus* is described from two males collected from the Dominican Republic, the first record of this genus from Hispaniola. A new record for *Baliomydas cubanus* (Curran) is noted for the Dominican Republic.

Key Words: Diptera, Mydidae, *Nemomydas*, new species, Dominican Republic.

RESUMEN

Se describe una nueva especie de Mydidae, *Nemomydas dominicanus*, en base a dos machos colectados en Republica Dominicana, el primer registro de este genero para la Hispaniola. Se señala el nuevo registro de *Baliomydas cubanus* (Curran) para la Republica Dominicana.

Translation provided by authors.

A recent survey of orthopteroids and associated insects throughout the Dominican Republic has provided the opportunity to collect large numbers of robber flies (Asilidae), which are being studied in collaboration with Dr. A. Scarbrough (Towson University). Among these flies, the junior author collected four specimens of mydas flies belonging to two species. These represent the second and third known species of this family known from the Caribbean island of Hispaniola.

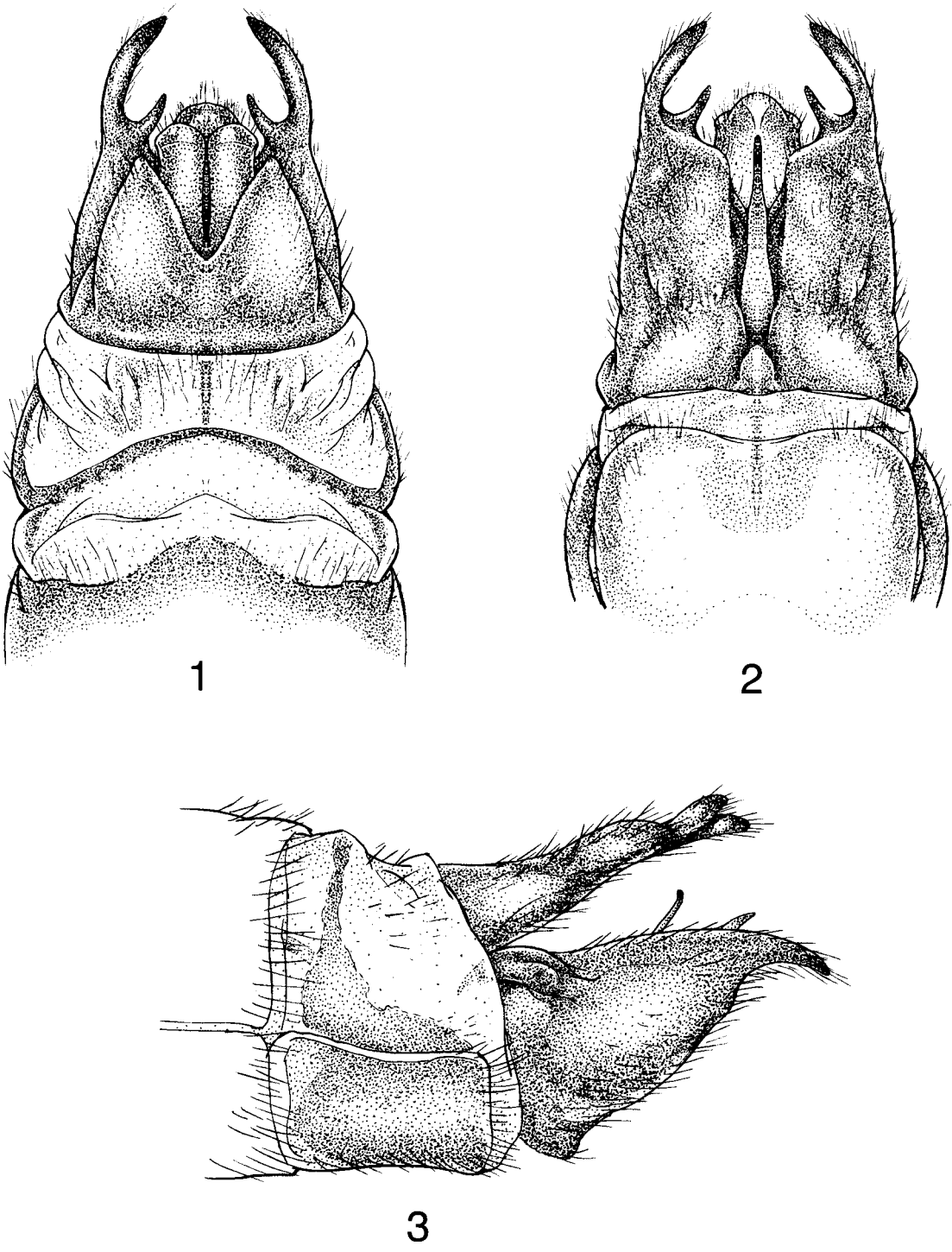
Mydidae have a worldwide distribution. Six subfamilies and 23 genera of these flies are known from the New World (Papavero & Artigas 1990). The only Mydidae previously recorded from the Greater Antilles are *Baliomydas cubanus* (Curran), *B. gracilis* (Macquart), and *B. tricolor* (Wiedemann) from Cuba and *Ceriomydas darlingtoni* Papavero and Wilcox from the Dominican Republic. A male and female of *B. gracilis*, previously known only from Cuba, were recently collected from the Dominican Republic.

Two of the above specimens represent a new species of *Nemomydas* Curran, a genus that includes 20 species distributed in the United States, Mexico, Central America, Taiwan and Japan (Nagatomi & Tawaki 1985; Kondratieff & Welch 1990; Papavero & Artigas 1990; Welch & Kondratieff 1990; Welch & Kondratieff 1991; Welch & Kondratieff 1994; Fitzgerald & Kondratieff 1998) and with perhaps an undescribed species from Cuba (Alayo & Garcés 1990). These authors provide a generalized illustration of a representative of the genus. No material from Cuba was available to the authors.

Nemomydas dominicanus n. sp.
Figs. 1-3.

Male—Length 15.1 mm. Head black, orbital margin of compound eyes gray pollinose, antennae 3.5 mm long; mystax anteriorly with black hairs, laterally and posteriorly with white hairs; antennae bases with black hairs; occiput with black hairs, inner edge of orbital margin with patch of white hairs; antennae black with tinge of gray pollinose apically; proboscis long, 1.5× as long as subcranial cavity, black and brown. Scutum black, generally shiny, with pair of submedian thin white pollinose stripes slightly converging posteriorly, a pair of wide pollinose stripes, posteriorly directed forward as a wedge; short black hairs dorsally, longer white hairs laterally and posteriorly; scutellum black with a tinge of gray pollinose; postnotum black with lateral gray pollinose areas; katatergite with long white hairs. Wings hyaline, venation typical for genus, longitudinal veins brown, M_1 ending on costa, first posterior cell open. Halter brown. Fore- and midfemur brown, black dorsally, hindfemur brown basally, black distally, tibia and tarsi of fore-, mid-, and hindlegs brownish black, all hairs and bristles black. Abdominal tergites shiny black, posterior margins of tergites 1-7 yellow, bulla brown, setae black appressed, tergites 1-2 with long white hairs. Terminalia black, ventral digitate process of gonocoxite large, tapered at apex (Figs. 1-3). Aedeagus tube-like distally, swollen basally (Figs. 1-3).

Female—Unknown.



Figs. 1-3. *Nemomydas dominicanus*, n. sp. Male terminalia. 1. dorsal, 2. ventral, 3. lateral.

Material examined: Holotype ♂: Dominican Republic: RD-097 La Malena de Boca Chica, Santo Domingo Prov., nr. sea level, 18°25.461'N 69°33.408'W, 21-III-2003, D. Perez, B. Hierro, S. Medrano (day). Paratype ♂: Dominican Republic, RD-226, 2 km SE Montecristi, Montecristi Prov.,

44 m, dry forest, 19°50.127'N 71°37.252'W, 17-IV-2004, D. Perez, B. Hierro. Both the holotype and paratype deposited at the National Museum of Natural History (NMNH), Smithsonian Institution, Washington, D.C.

Etymology—In reference to the Dominican Republic.

Habitat—Both localities of *N. dominicanus* are coastal scrub forest. At Boca Chica the forest is of a mixed deciduous type growing on a limestone substrate, with trees growing in between soil pockets. At Montecristi located in the northwestern corner of the country, the habitat is xeric with sandy soil. The tall bunch grass *Leptochloosis virgata* (Poaceae) is one of the dominant plant species of this low forest. The great distance between these localities hints of a wider distribution for this species on the island.

Remarks—The male of *N. dominicanus* is similar to three other species that have a combination of black abdominal tergites with yellow transverse posterior margins and tube-like aedeagus. These include *N. loreni* Welch and Kondratieff (Costa Rica, Welch & Kondratieff 1991), *N. melanopogon* Steyskal (Florida, Welch & Kondratieff 1994) and *N. venosus* (Loew) (Colorado, Kansas west to Arizona and Mexico, Kondratieff & Welch 1990). The brown wings, long proboscis, 2.6× as long as the subcranial cavity of *N. loreni* (hyaline wings, proboscis, 1.5× as long as the subcranial cavity in *N. dominicanus*), the short proboscis, 0.2× as long as the subcranial cavity in *N. melanopogon* (long proboscis 1.5× as long as the subcranial cavity in *N. dominicanus*), and the large thumblike ventral process of the gonocoxite, completely white mystax in *N. venosus* (tapered ventral process (Figs. 1-3), mixed white and black mystax in *N. dominicanus*) will allow separation of the species. The male terminalia in lateral view resembles *N. fronki* Kondratieff and Welch, but the completely black coloration of *N. fronki* easily distinguishes it from *N. dominicanus*.

The record for *B. gracilis* is given below.

DOMINICAN REPUBLIC: RD-053 Matadero, 11 km N entrance to Honduras, 10 km W Baní, Peravia Prov., 28-VII-2002, 18°24.367'N 70°25.703'W, 1,600 ft., D. Perez, R. Bastardo, 1 ♂, 1 ♀. Deposited at Museo Nacional de Historia Natural, Santo Domingo (MHND).

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A NEW *ANAGRUS* (HYMENOPTERA: MYMARIDAE) FROM ARGENTINA, AN EGG PARASITOID OF *DELPHACODES SITAREA* (HEMIPTERA: ARCHAEORRHYNCHA: DELPHACIDAE)

SERGUEI V. TRIAPITSYN[1] AND EDUARDO G. VIRLA[2]
[1]Entomology Research Museum, Department of Entomology, University of California, Riverside, CA 92521, USA

[2]CONICET, PROIMI—Biotecnología (Biological Control Division)
Av. Belgrano y Pje. Caseros (4000), San Miguel de Tucumán, Tucumán, Argentina

In a review of the Argentine species of the fairyfly genus *Anagrus* Haliday (Hymenoptera: Mymaridae), Triapitsyn (2000) keyed, diagnosed, and illustrated an unnamed species (called *A.* sp. E) from Buenos Aires, Misiones, Neuquén, and Santiago del Estero Provinces of Argentina. It was not described as new then because all the specimens were improperly slide-mounted and had no host association data. Recently, the junior author reared a large series of individuals of *Anagrus* near San Miguel de Tucumán from eggs of the planthopper *Delphacodes sitarea* Remes Lenicov and Tesón (Hemiptera: Archaeorrhyncha: Delphacidae) laid in pasture or lawn grass, *Stenotaphrum secundatum* (Walter) Kuntze (Poaceae). These specimens were sent for identification to the senior author, who found them to be conspecific with *A.* sp. E of Triapitsyn (2000). Availability of fresh, reared specimens of this species has enabled us to describe it herein as *A.* (*Anagrus*) *miriamae* sp. nov.

Terms for morphological features are those of Gibson (1997). Measurements are given in micrometers (μm) as length or, where appropriate, as length/width. Abbreviations (codens) for depositories of specimens are as follows: CNCI, Canadian National Collection of Insects, Ottawa, Ontario, Canada; IEFA, E. Chiappini collection, Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Piacenza, Italy; IMLA, Fundación e Instituto Miguel Lillo, San Miguel de Tucumán, Tucumán, Argentina; MLPA, Museo de La Plata, La Plata, Argentina; UCRC, Entomology Research Museum, University of California, Riverside, California, USA. An abbreviation used in the text is: F = funicle (in females) or flagellar (in males) antennal segment.

Anagrus (*Anagrus*) *miriamae* S. Triapitsyn and Virla, **sp. nov.**
(Figs. 1, 2)

Anagrus (*Anagrus*) sp. E; Triapitsyn, 2000: 220-221.

Types. Holotype female [IMLA] on slide, labeled: 1. "ARGENTINA: Tucumán, Las Talitas, ix.1999, E. Virla. Ex. eggs of *Delphacodes sitarea*. Mounted by V. Berezovskiy 2000 Canada balsam"; 2. "*Anagrus* (*Anagrus*) *miriamae* S. Triapitsyn & Virla HOLOTYPE ♀". Paratypes: 25 females and 3 males on cards or points [CNCI, IEFA, IMLA, MLPA, UCRC] and 2 females and 2 males on slides [IMLA, UCRC], all same data as the holotype. Other material from Argentina, stored in MLPA (Triapitsyn 2000), is not included in the type series because those slide-mounted specimens are in poor condition.

Description (holotype and paratypes, $n = 3$).

Female: Color. Body and appendages more or less uniformly light brown to brown except scape, pedicel, and F1 pale; eye and ocelli pink. Head a little wider than mesosoma. Antenna (Fig. 1) much shorter than body. Scape 2.9-3.0 × as long as wide; pedicel much longer than F1; all funicle segments longer than wide, F1 shortest and F2 longest, F3-F6 subequal in length, longitudinal sensilla on F3 (1), F4 (1), F5 (usually 2, as in holotype, but sometimes 1), F6 (2); clava about 3.5 ×

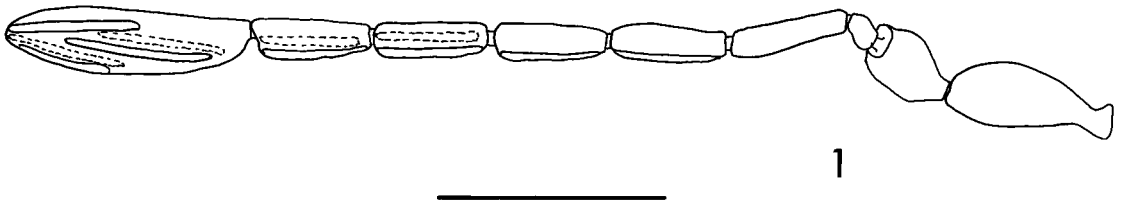

Figure 1. *Anagrus* (*Anagrus*) *miriamae* S. Triapitsyn and Virla, **sp. nov.**, antenna, female (holotype). Scale bar = 0.1 mm.

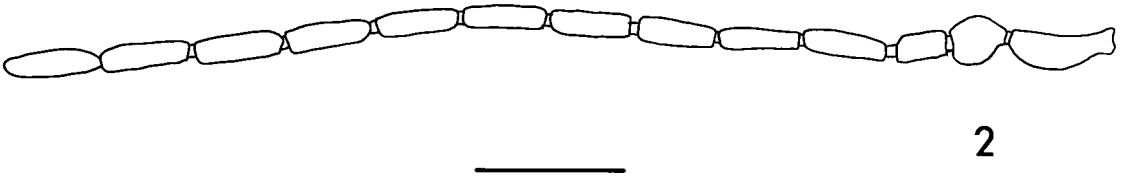

Figure 2. *Anagrus* (*Anagrus*) *miriamae* S. Triapitsyn and Virla, **sp. nov.**, antenna, male (paratype). Scale bar = 0.1 mm.

as long as wide (in lateral view), with 5 longitudinal sensilla, 3 of them subapical.

Mesosoma: Mesoscutum with a pair of adnotaular setae. Forewing narrow (see Fig. 18, p. 220 in Triapitsyn 2000), 8.4-9.3 × as long as wide; distal macrochaeta 1.5-2.0 × length of proximal macrochaeta; longest marginal cilia about 3 × maximal width of blade; disc hyaline, with several rows of microtrichia beyond venation not leaving any distinct bare area apically, only 1 such row behind and immediately beyond stigmal vein. Hind wing about 25 × as long as wide; disc hyaline, with microtrichia only along margins; longest marginal cilia 7-8 × maximal width of blade.

Metasoma: Gaster longer than mesosoma. Ovipositor occupying about 4/5 length of gaster, not reaching or barely reaching tip of mesophragma anteriorly and slightly exserted beyond apex of gaster posteriorly (exserted part of ovipositor 1/10 to 1/7 of its total length); ovipositor length/foretibia length ratio 2.5-2.9:1. External plate of ovipositor with 3 setae.

Measurements (holotype): Body (length, taken before slide-mounting): 644; head (length, taken before slide-mounting): 122; mesosoma: 227; metasoma: 336; ovipositor: 315. Antenna: scape: 76; pedicel: 39; F1: 20; F2: 57; F3: 52; F4: 51; F5: 50; F6: 52; clava: 104. Forewing: 545/61; longest marginal cilia: 188. Hind wing: 509/20; longest marginal cilia: 149. Legs (given as femur, tibia, tarsus): fore: 127, 118, 166; middle: 112, 161, 161; hind: 121, 185, 173.

Male: Similar to female except for normally sexually dimorphic characters such as antenna (Fig. 2) and genitalia (Fig. 3); the latter are typical in shape and structure for the *incarnatus* group species as discussed by Chiappini & Mazzoni (2000). Coloration of body a little darker than in female (dusky).

Diagnosis

The new taxon, which belongs to the *incarnatus* species group of the nominate subgenus of *Anagrus* as defined by Chiappini et al. (1996), appears to be most closely related to the widespread New World species *A. flaveolus* Waterhouse, a well-known egg parasitoid of several economically important planthoppers (Triapitsyn 1997, 2002), and also to the common Palaearctic species *A. ni-*

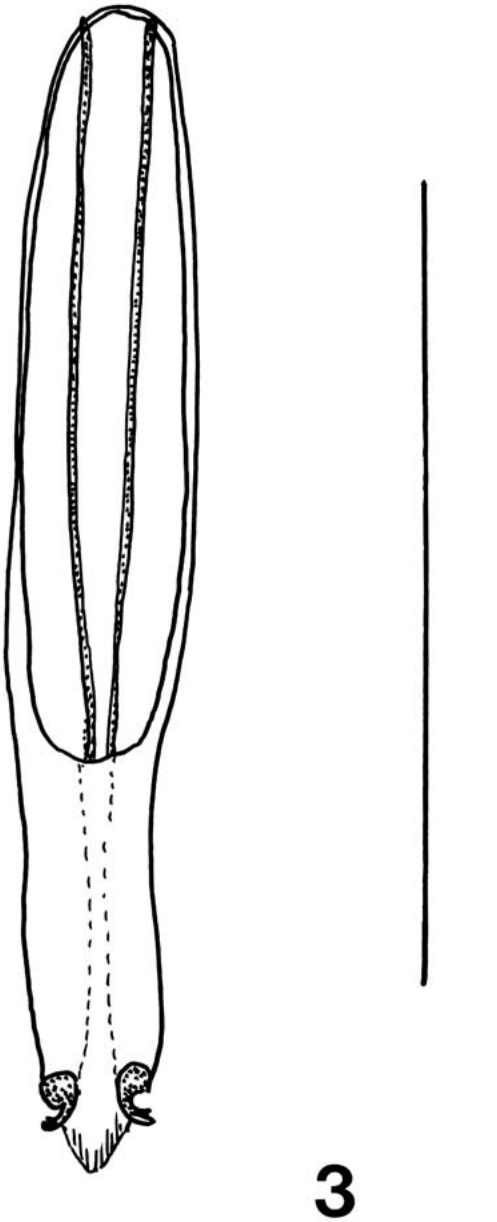

Fig. 3. *Anagrus* (*Anagrus*) *miriamae* S. Triapitsyn and Virla, **sp. nov.**, genitalia, male (paratype). Scale bar = 0.1 mm.

griceps (Smits van Burgst). It differs from *A. flaveolus* in having a longitudinal sensillum on F3 and usually two, not one, longitudinal sensilla on F5 of the female antenna. The forewing of *A. miriamae* sp. nov. differs from the forewing of *A. nigriceps* in having just one, not two, rows of microtrichia on the blade behind and immediately beyond the stigmal vein. Females of the new species can be recognized with the key by Triapitsyn (2000).

Etymology

This species is named in honor of Miriam Virla, the junior author's wife, who generously helped with field work.

Distribution

Known from the type locality in Tucumán as well as from several other provinces in Argentina (Triapitsyn 2000). The host planthopper is widely distributed in Argentina where it was found in low densities mostly on grasses and also on corn, wheat, sorghum and other commercial crops (Remes Lenicov & Tesón 1979; Remes Lenicov & Virla 1993).

We thank Vladimir V. Berezovskiy (UCRC) for help with specimen preparation and line drawings.

Summary

A new species of the mymarid wasp genus *Anagrus* Haliday is described from the Province of Tucumán, Argentina. The type series of *A.* (*Anagrus*) *miriamae* S. Triapitsyn and Virla sp. nov. was reared from eggs of the planthopper *Delphacodes sitarea* Remes Lenicov and Tesón on a common pasture and lawn grass, *Stenotaphrum secundatum* (Walter) Kuntze. Both the parasitoid and its host are widely distributed in Argentina.

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SOIL MACROINVERTEBRATES ALONG A SUCCESSIONAL GRADIENT IN CENTRAL FLORIDA

JAN FROUZ[1,2] AND ARSHAD ALI[1]
[1]Mid-Florida Research and Education Center and Department of Entomology and Nematology, IFAS University of Florida, 2725 Binion Road, Apopka, FL 32703-8504

[2]Institute of Soil Biology, Academy of Sciences of the Czech Republic Na Sádkách 7, České Budějovice, CZ-37005, Czech Republic

Soil macrofauna are a diverse group of biota that play important roles in many ecosystem processes. Soil macrofauna succession has been the subject of several studies in the temperate zone (Strüve-Kusenberg 1981; Tajovsky 1990; Pižl 1992; Frouz 1997). However, few data are available concerning succession of soil macrofauna in tropical and subtropical areas of the world (Silva Del Pozo & Blandon 1991a,b), including subtropical Florida, USA. The present study was undertaken to quantify changes in soil macrofauna during secondary succession in north-central Florida.

The study was conducted at the University of Florida's Natural Area Teaching Laboratory (NATL) at Gainesville, Florida (29°41'N, 82°19'W). This undeveloped area located at the southwest corner of the university campus covers 19 ha and is maintained to demonstrate local natural habitats, primarily three Florida upland ecosystems, including upland hammock, upland pine, and old-field. Five plots of different successional stage were selected for the study. These included two earlier stage plats, disturbed 2 and 7 years earlier by disking as a part of the old-field succession project of NATL. The other three plots are considered to have been subjected to clear-cutting combined with burning and mechanical disturbance, i.e., with a history typical of forest lands in this region. By comparing aerial photographs of this area taken every 10 years since 1949, we determined the appearance of different succession stages. From this and examination of the trees in the plots, we estimated that the last major disturbances in the three plots took place 20-30 years ago, 60-90 years ago, and more than 150 years ago, respectively. The last two sites appear on the oldest aerial photography as young forest about 10-30 years old (former site) or as an old growth hardwood (latter site). The study plots area extended over 1.2 ha for 2- and 7-years-old plots, 0.3 ha for 20-30-year-old plot, and ca. 3-4 ha for the two oldest plots. The first three plots were quite close to each other, whereas the last two plots bordering with each other were relatively distant.

The plot most recently disturbed (2 years) supported a dense cover of weeds, with soil covered by a discontinuous thin layer of litter. The second plot, disturbed 7 years earlier, was dominated by blackberries (*Rubus cuneifolius* Pursh.) and dog fennel (*Eupatorium capillifolium* [Lam.] Small). In this plot the soil surface was covered by a continuous 3-5 cm thick layer of shrub and herb litter. The third plot, not disturbed for 20-30 years, was dominated by Loblolly pine (*Pinus taeda* L.), with soil covered by a 5-7 cm thick layer of pine litter. The fourth plot, not disturbed for 60-90 years, was covered by 1-2 cm thick layer of litter and was dominated by hardwoods, including sweetgum (*Liquidambar styraciflua* L.), oaks (*Quercus hemisphaerica* Bartr. ex Willd and *Quercus nigra* L.), plus hop hornbeam (*Ostrya virginiana* (Mill.) K. Koch) and *P. taeda*. The last sampling plot located in an area undisturbed for > 150 years was very similar to the fourth plot in terms of vegetation and litter cover.

All plots were sampled in February, September, and October 2001, with a circular corer 11 cm in diameter. In each plot, soil samples were collected from three locations. Individual sampling locations were 20-70 m apart from each other. Except for the oldest plot, the distance between two most distant sampling locations selected on the same plot was typically greater than the distance between any two closest sampling locations existing on different plots. At each plot location, three 16-cm-deep core samples were collected. Each core sample was divided into two depth classes: 0-8 cm (top) and 9-16 cm (bottom). Thus, a total of nine cores for each depth class were taken from each plot. The top and the bottom core samples at each location were separately combined. Only apparently sandy soil in each plot was sampled, whereas clay soil patches were avoided, as were apparent soil depressions or elevations in a plot. The collected samples were extracted for 7 days in the laboratory for soil macrofauna with the Tullgren-type extraction apparatus. Extracted material from each sample was fixed in 2% formaldehyde, transferred to 80% ethanol and sorted under various magnifications of a dissection microscope to separate morphologically distinguishable morphospecies (Beattie & Oliver 1994). Morphospecies represent here morphologically distinguishable forms, which are assumed to represent separate species but which cannot be adequately determined. Morphospecies were determined to lowest practical level and these data were used for grouping into trophic groups. Morphospecies were grouped into higher taxa, dried at 40°C for 24 h, and weighed to determine

dry biomass (OHAUS AS120, Florham Park, NJ, accuracy 0.1 mg). If some higher taxa consisted of several trophic groups, these were grouped and weighed separately. The statistical package, SPSS 10.0 (SPSS, 1999) was used for ANOVA and *t*-tests; nine replicates per plot were used unless mentioned otherwise.

A total of 71 soil macrofaunal morphospecies was recorded during the study, with 23-38 morphospecies identified from individual sites (Table 1). Mean number of morphospecies occurring per sample in individual plots, total number of morphospecies per plot as well as Shannon-Weiner diversity index increased with time since last disturbance. These findings agree with those of Loranger et al. (1998) who reported that the species diversity increased with successional age of plots in the Martinique (Caribbean).

Density of soil total macrofauna ranged from 512 to 962 individuals/m^2 (Table 1). These values are comparable with those given for soil under a mixed forest in France (Geoffroy et al. 1981), or a mountain forest in Ecuador (Silva del Poso & Blandon 1991a,b). However, substantially higher densities (than in our study) of soil macrofauna have been reported for some wet tropical areas (Decaens et al. 1994, Loranger et al. 1998, Höfer et al. 2001). In our study, omnivores represented mainly by Formicidae were the most abundant among soil macrofauna at the investigated sites (Table 1). In the 2-year post disturbance plot, Hymenoptera, larval Coleoptera, larval Diptera, and adult Coleoptera were the most abundant. In other studies, high abundance of soil insects with flying adults, especially Diptera larvae, in initial succession stages has been reported by Strüve-Kusenberg (1981), and Frouz (1997); such an abundance has been attributed to high migration potential of the insects (Strüve-Kusenberg 1981; Frouz 1997). Omnivores and the highest proportion of phytophagous organisms, such as insect larvae, were recorded in the earliest stage. With post-disturbance time increase, the proportion of herbivores decreased and the number of saprophagous organisms increased (Table 1). Diplopoda (Julidae) were abundant among saprophagous groups in the 7- and 20-30-years post-disturbance plots. Isoptera were abundant in the 60-90-year post-disturbance hardwood plot and Diplopoda (mostly Polyxenidae) were abundant in the oldest site. The relatively lower number of saprophagous groups, in comparison with their reports from temperate zone studies (Axelon et al. 1984) may be attributable to the occurrence of oligochaetes in low densities. In sandy soils, oligochaete densities are usually low because of the lower water holding capacity as well as lower organic matter content of such soils (Hendrix et al. 1992; Kalisz & Powell 2002 a,b).

The proportion of macrofauna occurring in the deeper (9-16 cm) soil layer was greatest in the 7-year post-disturbance plot and generally decreased in older plots, with only slight increase in the oldest hardwood plot. Vertical distribution of macrofauna differed seasonally. More invertebrates were recorded in the upper soil layer during September ($P < 0.05$, paired *t*-test, all plots). However, no significant differences were noted between invertebrate densities in the two soil layers collected in February and October 2001. Temporal differences were most pronounced in the 7-year post-disturbance plot, where 55 and 60% of all soil macrofauna were recorded in the deeper layer in February and October, respectively, but only 23% in September. In contrast, the proportion of macrofauna in the deeper soil layer of the 60-90-year post-disturbance hardwood remained fairly constant, and ranged between 8-12% during the observation period. We postulate that vertical distribution in our plots reflects changes in shelter against soil desiccation during succession. As the vegetation and litter layer develop during succession, the topsoil layer is protected against desiccation, thus decreasing the necessity of downward faunal migration during the dry season. The sandy soil may be more conducive to this process because it can desiccate easily, and also because burrowing in sandy soil is likely easier than in clay soil. Contrary to this observation, Silva Del Pozo & Blandon (1991a) observed an increase in proportion of macroinvertebrates inhabiting deeper soil layers during succession.

Total macrofaunal biomass varied from 1.87 to 3.71 g/m^2 (Table 2). Similar values were recorded in a Kentucky forest (Kalisz & Powel 2000a) and in a rain forest in the Amazon (Höfer et al. 2001). However, Decaens at al. (1994) recorded substantially higher biomass of soil fauna during the rainy season in tropical West Africa. In the 2-year post-disturbance plot, Coleoptera (mostly Tenebrionidae) formed the highest proportion of total biomass, while in the 7- and 20-30-year post-disturbance plots, Diplopoda (Julidae) dominated. Coleoptera formed the largest proportion of total biomass in the 60-90-year post-disturbance plot, whereas oligochaetes dominated the total biomass in the oldest plot. In our study, phytophagous macroinvertebrates dominated total biomass during early succession, while the proportion of saprophagous macrofauna increased with post-disturbance age (Table 2).

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Summary

Density, biomass, and community structure of soil macroinvertebrates were studied in five types of plots developing by secondary succession at 2,

TABLE 1. DENSITY AND DIVERSITY OF SOIL MACROINVERTEBRATES IN UP TO 16- CM DEPTH IN FIVE SUCCESSIONAL OLD-FIELD PLOTS AT THE NATURAL AREA TEACHING LABORATORY, UNIVERSITY OF FLORIDA, GAINESVILLE, FL (FEBRUARY-OCTOBER 2001).

	Successional Stage (post-disturbance years)				
	2	7	20-30	60-90	>150
Taxa	Mean ± SE (individuals/m^2)				
Annelida					
Lumbricidae	0 ± 0	19 ± 15	0 ± 0	8 ± 7	16 ± 11
Arthropoda					
Crustacea					
Isopoda	4 ± 4	0 ± 0	43 ± 25	47 ± 27	4 ± 4
Amphipoda	4 ± 4	0 ± 0	8 ± 4	0 ± 0	0 ± 0
Chelicerata					
Arachnida	8 ± 5a	12 ± 11 a	19 ± 10 ab	31 ± 10 b	16 ± 6 ab
Myriapoda					
Symphyla	0 ± 0	4 ± 4	12 ± 5	12 ± 8	0 ± 0
Chilopoda	0 ± 0	8 ± 7	4 ± 4	4 ± 4	16 ± 8
Diplopoda	12 ± 11 a	276 ± 163 b	241 ± 68 ab	23 ± 8 a	66 ± 21 ab
Hexapoda					
Diplura	4 ± 4	0 ± 0	27 ± 15	31 ± 15	12 ± 8
Insecta					
Thysanoptera	0 ± 0	0 ± 0	4 ± 4	8 ± 7	4 ± 4
Hemiptera					
Auchenorrhyncha	0 ± 0 a	0 ± 0 a	4 ± 4 ab	16 ± 8 b	0 ± 0a
Sternorrhyncha	0 ± 0	0 ± 0	0 ± 0	8 ± 7	12 ± 11
Heteroptera	43 ± 32	54 ± 27	0 ± 0	16 ± 8	27 ± 12
Isoptera	0 ± 0	39 ± 37	43 ± 36	101 ± 95	0 ± 0
Blattodea	0 ± 0	0 ± 0	4 ± 4	0 ± 0	16 ± 11
Dermaptera	0 ± 0	4 ± 4	4 ± 4	0 ± 0	0 ± 0
Hymenoptera	257 ± 143	393 ± 88	397 ± 157	408 ± 189	198 ± 88
Coleoptera—adult	47 ± 19	47 ± 26	47 ± 23	89 ± 43	70 ± 26
Coleoptera—larvae	78 ± 26	82 ± 42	43 ±15	27 ± 9	47 ± 10
Lepidoptera	51 ± 40	12 ± 8	0 ± 0	4 ± 4	0 ± 0
Diptera—larvae	74 ± 43 a	12 ± 8 b	8 ± 5 b	8 ± 5 b	8 ± 5b
Total macrofauna	582 ± 144	962 ± 185	904 ± 160	841 ± 230	512 ± 90
% in 9-16 cm[1]	36.2 ab	47.8 b	23.4 ab	10.4 a	19.5 ab
Phytophagous (%)[2]	30	14	7	10	18
Predator (%)	13	6	7	9	12
Saprophagous (%)	13	39	40	33	31
Other (%)	44	41	46	48	39
Species no. total[3]	23	31	26	38	38
Exclusive species no.[4]	7	4	2	2	8
Species no. mean[5]	4.5	5	5.9	6.5	6.7
Shannon-Weiner Diversity	3.62	3.72	3.55	3.81	4.18

Values in the same row followed by the same letter are not significantly different (ANOVA, Tukey's test, $P<0.05$), and values in the same row without any letters are not significantly different.

[1]Mean percent of macroinvertebrates collected from 9-16 cm depth of the total macroinvertebrates occurring in 0-16 cm soil depth.

[2]Percentage of total density.

[3]Total number of morphospecies recorded in a plot.

[4]Total number of morphospecies recorded exclusively in a plot (absent on other plots studied).

[5]Number of morphospecies per one sample.

7, 20-30, 60-90 and >150 years after last major disturbance. Formicidae, Diplopoda, and larval Diptera constituted the highest density, while Coleoptera, Isoptera, and Oligochaeta were among the most important groups in terms of total biomass. The highest numbers of morphospecies

TABLE 2. DRY BIOMASS OF SOIL MACROINVERTEBRATES IN UP TO 16-CM-DEPTH IN FIVE SUCCESSIONAL OLD-FIELD PLOTS AT THE NATURAL AREA TEACHING LABORATORY, UNIVERSITY OF FLORIDA, GAINESVILLE, FL (FEBRUARY-OCTOBER, 2001). GROUPS WHICH DID NOT REACH BIOMASS 0.5 MG/M^2 (E.G., ALL AUCHENORRHYNCHA) ARE NOT INCLUDED EVEN IF MENTIONED IN THE PLOTS (SEE TABLE 1).

	Successional Stage (post-disturbance years)				
	2	7	20-30	60-90	>150
Taxa	Mean ± SE (mg/m^2)				
Annelida					
Lumbricidae	0 ± 0	51 ± 12	0 ± 0	18 ± 4	741 ± 108
Arthropoda					
Crustacea					
Isopoda	23 ± 6	0 ± 0	12 ± 3	295 ± 44	49 ± 11
Amphipoda	2 ± 1	0 ± 0	2 ± 1	0 ± 0	0 ± 0
Chelicerata					
Arachnida	14 ± 3	23 ± 5	4 ± 1	173 ± 21	1 ± 1
Myriapoda					
Symphyla	0 ± 0	1 ± 1	4 ± 1	4 ± 1	0 ± 0
Chilopoda	0 ± 0	2 ± 1	6 ± 1	126 ± 26	26 ± 6
Diplopoda	210 ± 50	1057 ± 57	3163 ± 552	105 ± 14	298 ± 69
Hexapoda					
Diplura	1 ± 1	0 ± 0	6 ± 1	6 ± 1	5 ± 1
Insecta					
Hemiptera					
Sternorrhyncha	0 ± 0	0 ± 0	0 ± 0	11 ± 2	13 ± 3
Heteroptera	93 ± 15	237 ± 33	0 ± 0	18 ± 4	467 ± 33
Isoptera	0 ± 0	35 ± 29	56 ± 46	168 ± 8	0 ± 0
Blattodea	0 ± 0	0 ± 0	12 ± 4	0 ± 0	53 ± 40
Dermaptera	0 ± 0	1 ± 1	1 ± 1	0 ± 0	0 ± 0
Hymenoptera	83 ± 40	112 ± 74	200 ± 19	76 ± 29	53 ± 10
Coleoptera	1593 ± 664	244 ± 102	245 ± 137	1047 ± 295	307 ± 134
Lepidoptera	88 ± 72	26 ± 21	0 ± 0	36 ± 30	0 ± 0
Diptera	16 ± 13	76 ± 28	1 ± 1	1 ± 1	117 ± 92
Total macrofauna	2123 ± 155	1865 ± 60	3712 ± 585	2084 ± 132	2130 ± 150
Phytophagous (%)[1]	59	21	4	24	31
Predator (%)	18	8	3	36	8
Saprophagous (%)	19	65	88	35	59
Other (%)	4	6	5	5	2

[1]Percentage of total biomass.

were recorded in the oldest plots. The proportion of invertebrates found in deeper soil (9-16 cm) generally decreased with successional age. This may be attributed to more pronounced downward migration of soil macrofauna during drier periods of the year in earlier succession plots where the soil could be more sensitive to desiccation. This is Florida Agricultural Experiment Station Journal Series No. R-09606.

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IRRADIATION DISINFESTATION OF DIAPREPES ROOT WEEVIL (COLEOPTERA: CURCULIONIDAE) AND PAPAYA FRUIT FLY (DIPTERA: TEPHRITIDAE)

WALTER P. GOULD[1] AND GUY J. HALLMAN[1,2]
[1]USDA-APHIS-PPQ, 4700 River Road, Unit 147, Riverdale, MD 20737

[2]Subtropical Agricultural Research Center, USDA-ARS, 2413 E. Highway 83, Weslaco, TX 78596

Ionizing irradiation has become a viable disinfestation technique to overcome biological barriers to export of agricultural commodities produced in Florida. During the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), outbreaks of 1997-98, some boxes of mangoes were irradiated at a minimum absorbed dose of 225 Gy so they could be moved out of quarantined areas. Since 1999, about 80 metric tons per year of guavas have been irradiated with 150 Gy against Caribbean fruit fly, *Anastrepha suspensa* (Loew), and shipped to Texas and California. Beginning in 2000, an increasing amount of 'boniato' sweetpotatoes (now >200 metric tons annually) has been irradiated with 165 Gy against sweetpotato weevil, *Cylas formicarius elegantulus* (Summers) and shipped to California (Hallman 2001a). Greater potential lies ahead; in August of 2003, California accepted irradiation at 150 Gy as a quarantine treatment for any fruit from Florida where Caribbean fruit fly is the only quarantined insect. That dose may be reduced in the future; Gould & von Windeguth (1991) found that 50 Gy would suffice.

To be able to use this promising treatment on a wider variety of commodities, doses for other quarantined insects in Florida are needed. The root weevil *Diaprepes abbreviatus* (L.) has been in Florida since at least 1964 (Woodruff 1968) and is associated with about 270 plant species, including sugarcane, citrus, and many ornamentals (Simpson et al. 1996). Although it would not be advisable to use irradiation disinfestation on plant propagative materials, such as nursery stock, because it would be detrimental to their growth, some agricultural commodities, such as sugarcane pieces and root crops, may be infested with *D. abbreviatus* and, thus, be amenable to radiodisinfestation. Invariably, radiotolerance increases positively with insect development (Hallman 2001b). Although adult *D. abbreviatus* are not normally found in host commodities that may be disinfested via irradiation, it is possible, and a quarantine treatment must be effective against the most radiotolerant stage that may be present in a shipped commodity regardless of its frequency of occurrence.

Papaya is the only commodity that the papaya fruit fly, *Toxotrypana curvicauda* Gerstaecker, normally infests. The fly is found in Florida (introduced about 1905), Central and South America, and the Caribbean. As such, shipment of papayas from these areas to other areas where papayas are grown is prohibited. No disinfestation treatment is available. Irradiation at 150 Gy has been proposed as a generic treatment for all tephritid fruit flies infesting all fruits (Hallman & Loaharanu 2002). The measure of efficacy of irradiation disinfestation against tephritids is prevention of emergence of adults capable of flight when late third instars are irradiated inside fruit. Papaya fruit fly is considerably larger and otherwise morphologically distinct from all other tephritids studied with irradiation. It would lend more confidence to the proposal of a generic dose of 150 Gy if that dose could be shown to control papaya fruit fly.

The objectives of this research were to investigate a dose that could disinfest commodities of *D. abbreviatus* and determine if 150 Gy is sufficient to prevent adult emergence from irradiated third instar papaya fruit fly.

Diaprepes abbreviatus. Adults were field collected near Homestead, FL, and separated according to sex. Twenty females were irradiated (Gammacell model 220, Atomic Energy of Canada, Ltd., dose rate about 50 Gy/min) in 0.5-liter plastic containers at the following doses: 0 (control), 10, 20, 30, 40, or 50 Gy. Each female was placed separately without males in the containers with sugar water for food and small bundles of waxed paper in which to oviposit. There were 11 replicates. Egg numbers were counted for 6 days, and the eggs held to observe eclosion rates. Eclosion data were subjected to regression (Prism 4.0, GraphPad Software, San Diego, CA) and probit analysis (SAS 1986).

The mean number (±SEM) of egg masses and eggs laid per replicate of 20 females during the 6-day period was 24.3 (±0.68) and 1,354 (±50), respectively, and the slopes were not significantly non-zero in the dose range studied (for number of egg masses, F statistic for test of non-zero slope was 0.38; $df = 1, 64$; $P = 0.54$. For number of eggs, $F = 0.19$; $df = 1, 64$; $P = 0.67$). The mean number (±SEM) of females per replicate that oviposited during the 6-day period was 14.0 (±0.19), and the slope was not significantly non-zero ($F = 0.50$; $df = 1, 64$; $P = 0.48$). Egg hatch was significantly affected by dose and fit the normal probability density function (n = 88,372; slope + SE = 0.093 + 0.0029; ED(effective dose)$_{99}$ = 20.0 Gy; 95% CL = 19.5-20.6 Gy; $\chi^2 = 0.32$).

Irradiation up to 50 Gy did not affect the number of eggs nor masses laid or the proportion of females ovipositing. It did reduce eclosion, with one egg of 15,730 hatching at 40 Gy and none of 13,835 hatching at 50 Gy. Therefore, *D. abbreviatus* is quite radiosusceptible. Other adult curculionids are prevented from reproducing at doses between 80-165 Gy (Hallman 2001b). Because the adults were not observed until they died and 30% of the females did not oviposit during the 6-day observation period, it is probable that the dose to confidently prevent reproduction by irradiated *D. abbreviatus* adults is somewhat >50 Gy. Plum curculio, *Conotrachelus nenuphar* (Herbst) (Coleoptera: Curculionidae), adults lived up to 25 days after they were irradiated at a dose (80 Gy) that prevented reproduction; after 25 days only 13% of non-irradiated controls had died (Hallman 2003). In any case, this research shows that commodity disinfestation of *D. abbreviatus* could probably be accomplished by low dose irradiation, which is tolerated by a great many commodities.

Papaya fruit fly. Over the course of 3 replicates, 1,180 papayas (560 kg) naturally infested with papaya fruit fly were harvested at the U.S. Dept. Agr., Agric. Research Service Subtropical Horticulture Research Station, Miami, Florida, and divided into 2 groups of 590 each. One group was irradiated at 150 Gy, and the other was held as a control. Most of the papaya fruit flies inside the papayas were third instars. After treatment, the papayas were placed on perforated plastic seedling trays at 24°C, which allowed for emerging larvae to fall into sand in fiberglass bins under the trays. Larvae and puparia were sifted from the sand with flour sifters, collected, and counted. After larvae stopped emerging from the fruit, the remains of the papayas were examined for any additional larvae and puparia. Puparia were held for at least a month to observe adult emergence.

A total of 1,640 larvae emerged from the irradiated papayas, 1,131 (69%) pupariated, but no adults emerged, not even partially. A total of 2,098 larvae emerged from the control papayas, 1,918 pupariated (91%), and 1,093 (52%) emerged as adults. Fifty five percent of the adults were females. Although the numbers studied fall far short of the numbers usually required to confirm a quarantine treatment against fruit flies (about 30,000), this research demonstrates that papaya fruit fly is not considerably more radiotolerant than other tephritids and lends support to a generic dose of 150 Gy for all tephritids.

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Summary

Irradiation disinfestation is being used in Florida and elsewhere to overcome biological barriers to trade. More potential exists. This research examined radiosusceptibility of two pests in Florida, the root weevil *Diaprepes abbreviatus* and the papaya fruit fly, *Toxotrypana curvicauda*. The root weevil was found to be quite susceptible to radiosterility; 50 Gy prevented eclosion of eggs laid by irradiated adults. A dose of 150 Gy prevented adult emergence from mostly third instar papaya fruit flies naturally infesting papaya fruit; lower doses were not tried.

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EFFECT OF CALCIUM SILICATE ON FEEDING AND DEVELOPMENT OF TROPICAL SOD WEBWORMS (LEPIDOPTERA: PYRALIDAE)

ANA PAULA KORNDORFER, RON CHERRY AND RUSSELL NAGATA
University of Florida, IFAS, Everglades Research and Education Center
3200 E. Palm Beach Road, Belle Glade, FL 33430

The tropical sod webworm (TSW), *Herpetogramma phaeopteralis* Guenee, has a wide tropical distribution and is considered to be one of the most destructive turfgrass pests in Florida. This webworm is common throughout most of Florida, although it probably does not survive the winter in the northern part of the state. TSW larvae cause damage to many turfgrass species by feeding on grass foliage. Damage appears as patches that become yellowish, then brown. Turf adjacent to flower beds and shrubs usually shows the first sign of damage, since adults rest in such foliage and moths lay more eggs in nearby turf (Kerr 1955).

Silicon (Si) is the second most common element on earth, but it is not considered to be an essential element for plant growth (Arnon & Stout 1939). However, there is a growing body of evidence that Si can enhance plant resistance to insect pests. The solid silica that is associated with the plant cell walls may constitute a mechanical barrier to penetration of the mandibles of insects (Jones & Handreck 1967). Applied Si and higher available soil Si have improved the resistance of rice to several economically important rice insect pests (Savant et al. 1997). Recently, Carvalho et al. (1999) reported that Si reduced the feeding and reproduction of greenbug (*Schizaphis graminum* Rond.), a sorghum herbivore. Also, Saigusa et al. (1999) reported reduced insect feeding in turf treated with calcium silicate. The objective of this study was to determine if calcium silicate applications to turf plants increase silicon in the plants and affect feeding and development of TSW.

Tests were conducted from August to December 2003. In the first test, five species of turfgrasses were used. Each species received two treatments: one with calcium silicate slag (Calcium Silicate Corporation, Inc., Lake Harbor, FL) at 10 MT/ha of slag mixed into a soil mix and one treatment without slag. The rate of 10 MT/ha of calcium silicate slag was selected based on previous research on pest control in rice and sugarcane (Seebold 1998; Savant et al. 1999) to ensure a high concentration of Si within plant tissue. The turfgrasses used were Tifdwarf Bermudagrass (*Cynodon dactylon* (L.) Pers. × *Cynodon transvaalensis* Burtt-Pavy), SeaIsle Seashore paspalum (*Paspalum vagintium* Swartz), NUF-76 St. Augustinegrass (*Stenotaphrum secudatum* (Walt) Kuntze), Meyers Zoysiagrass (*Zoysia japonica* Steud) and Centennial Centipedegrass (*Eremochloa ophiuroides* (Munro) Hack). All plants were grown in 225-cm^3 pots with 1:1 volume sand and Farfard #2 soil mix (Conrad Fatard, Agawam, MA). One fifth instar TWS (range = 4 to 8 mg) was weighed and placed on each host plant and the plant set inside a one liter polypropylene container and covered with mesh cloth. After four days larvae were recovered and weighed to determine weight gain. During the experiment, larvae and plants were maintained in an insectary at 28°C and 12-h photoperiod. Five host plants × the two treatments were set up at the same time to be one replication and ten replications were conducted.

In the second test, the same turf grass species and treatments were used as in the first test. Two moist filter papers were placed on the bottom of a 9-cm diameter petri dish. Excised leaves from one potted host plant were placed into a dish and a newly hatched TSW larvae (neonate) was placed upon the excised leaves. Neonates were not weighed because they are very small and fragile averaging less than 0.001 g per larva. Leaves were added "ad libitum" as needed. Petri dishes were stored in the previously mentioned insectary. Because the larvae are sensitive to dehydration, petri dishes were placed in plastic bags to maintain high humidity. Larvae were weighed after 7 days and returned to petri dishes until adults had emerged. Petri dishes were examined daily to insure fresh leaves and to note pupation and adult emergence date. Kerr (1955) reported that TSW took about 7 days from pupation to adult emergence at 26°C. We waited 14 days at 28°C before considering pupae dead. Five host plants × the two treatments were again set up at the same time to be one replication and 15 replications were conducted. Leaves from seven plants of each treatment in each of the five host plant species were randomly selected to determine the concentration of Si in the leaves. Silicon analysis of plant tissue was made according to methods for autoclave-induced digestion and colorimetric determination of Si as described by Elliott & Snyder (1991).

Our experimental design in both tests was a 5 × 2 factorial experiment with five levels of plant factor and two levels of treatment (= plus or minus slag) factor. Hence, a two way Analysis of Variance by the GLM procedure (SAS 1996) was used to analyze our data.

In the first test with potted plants, plant species and treatment were not significant factors ($P > 0.05$) in larval initial weights and there was no interaction ($P > 0.05$) between the two factors (Table 1). F values were 1.3 (4 df) for plant species,

TABLE 1. GROWTH OF TROPICAL SOD WEBWORMS ON DIFFERENT HOST PLANTS TREATED WITH CALCIUM SILICATE SLAG.

Plant	Slag	Mean ± SD[1]		
		Initial wt (mg)	Final wt (mg)	% Growth
Bermudagrass	–	5.7 ± 0.8	44.7 ± 10.6	784
	+	5.4 ± 1.3	42.2 ± 12.9	781
Centipedegrass	–	6.0 ± 1.1	30.8 ± 14.2	513
	+	5.8 ± 1.6	24.9 ± 6.2	429
Seashore paspalum	–	5.5 ± 1.5	34.8 ± 10.3	632
	+	5.5 ± 1.5	37.5 ± 15.4	681
St. Augustinegrass	–	5.6 ± 1.3	27.1 ± 11.9	483
	+	5.8 ± 0.8	29.9 ± 11.9	515
Zoysiagrass	–	5.0 ± 1.0	17.2 ± 6.2	344
	+	5.6 ± 1.3	15.9 ± 6.5	283

[1]A two way Analysis of Variance with the GLM procedure (SAS 1996) was used to analyze the data. See text for discussion of results.

0.1 (1 *df*) for treatment, and 0.9 (4 *df*) for plant-treatment interaction. The lack of statistical significance was expected since larval weights were selected initially to be similar in all treatments. After four days, plant species was a factor ($P < 0.0001, F = 15.2, 4\ df$) in larval weights and treatment was not ($P > 0.05. F = 0.3, 1\ df$). No interaction ($P > 0.05, F = 0.7, 4\ df$) was observed. Percentage survival of larvae after four days in all 10 groups averaged 94.0 ± 7.0% (SD), with some larvae surviving in all 10 groups.

In the second test with excised leaves, plant species was again a factor in larval weights ($P < 0.0001, F = 11.8, 4\ df$), days to pupation ($P < 0.0001, F = 17.0, 4\ df$), and days to emergence ($P < 0.0001, F = 15.6, 4\ df$) (Table 2). Treatment was not a factor in larval weights ($P > 0.05, F = 2.1, 1\ df$), days to pupation ($P > 0.05, F = 3.9, 1\ df$), or days to emergence ($P > 0.05, F = 0.5, 1\ df$). No interaction was observed in larval weights ($P > 0.05, F = 0.5, 4\ df$), days to pupation, ($P > 0.05, F = 0.1, 4\ df$) or days to emergence ($P > 0.05, F = 1.5, 4\ df$). Percentage survival of neonate larvae to adult emergence in all 10 groups averaged 66.0 ± 18.2% (SD), with some larvae surviving in all 10 groups. Leaf tissue analysis showed that treatment was a factor ($P < 0.0001, F = 35.5, 1\ df$) in % Si in leaf tissue. This is corroborated by noting that all five plant species had more Si in leaves after slag applications than controls with no slag application. Plant species was also a factor ($P < 0.05, F = 2.7, 4\ df$) in % Si in leaves for reasons not fully understood. No interaction ($P > 0.05, F = 1.0, 4\ df$) was observed.

As noted earlier, increased Si in plant tissue has been shown to reduce insect feeding damage. However, our data consistently showed that using two different testing methods (whole plant and

TABLE 2. GROWTH AND DEVELOPMENT OF TROPICAL SOD WEBWORMS IN DIFFERENT HOST PLANTS TREATED WITH CALCIUM SILICATE SLAG AND PERCENTAGE OF SILICON IN LEAVES OF HOST PLANTS.

Plant	Slag	Mean ± SD[1]			
		Larval wt[2]	Days to Pupation	Days to emergence	Si (%)[3]
Bermudagrass	–	33.2 ± 13.2	10.5 ± 0.7	16.5 ± 0.5	0.7 ± 0.2
	+	29.4 ± 10.0	10.7 ± 0.9	16.6 ± 1.3	1.4 ± 0.5
Centipedegrass	–	23.0 ± 13.0	12.7 ± 1.8	18.7 ± 1.7	0.8 ± 0.3
	+	16.9 ± 11.7	13.2 ± 2.8	20.0 ± 2.5	1.4 ± 0.6
Seashore paspalum	–	39.4 ± 15.8	10.1 ± 0.9	15.5 ± 1.0	0.7 ± 0.2
	+	37.0 ± 16.5	10.8 ± 0.9	16.3 ± 1.2	0.9 ± 0.2
St. Augustinegrass	–	30.0 ± 15.3	11.0 ± 0.9	17.3 ± 1.2	0.5 ± 0.07
	+	22.3 ± 20.3	11.4 ± 1.6	17.2 ± 2.0	0.9 ± 0.3
Zoysiagrass	–	12.8 ± 9.6	12.7 ± 1.4	19.0 ± 1.3	0.5 ± 0.2
	+	15.3 ± 12.1	13.3 ± 1.7	18.0 ± 1.3	1.2 ± 0.6

[1]A two way Analysis of Variance by the GLM procedure (SAS 1996) was used to analyze the data.
[2]Weight (mg) of neonates held seven days at 28°C.
[3]Si (%) in dry weight of plant tissue.

excised leaves) on five different species of turf grasses, increased Si in plant tissue from calcium silicate applications did not affect growth and development of TSW. Reasons for the lack of response of TSW to Si in leaves is not known. However, the insect-plant-silicon response is complex and not always predictable. For example, Peterson et al. (1988) showed that high levels of silica decreased digestibility in *Spodoptera eridania* (Cramer) and promoted increased consumption rates. However, larval growth rates were not different from the control at the highest level of silica (20% dry weight). Also, it has been shown that certain plant genotypes are more efficient than others in their accumulation of Si, thus making them more resistant (Savant 1997). Our study, like other studies, shows application of elements to plants may affect insects in different and not always predictable ways.

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Summary

The objective of this study was to determine if the calcium silicate applications to turf plants increase silicon concentration within the plants and affect feeding and development of tropical sod webworms. Five species of host plants were used with each species receiving two treatments: one with calcium silicate slag (10 MT/ha) and one without. Higher concentrations of silicon found in slag treated plants did not affect tropical sod webworm feeding or development.

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NEW INVASIVE SPECIES OF MEALYBUGS, *PALMICULTOR LUMPURENSIS* AND *CHAETOCOCCUS BAMBUSAE* (HEMIPTERA: COCCOIDEA: PSEUDOCOCCIDAE), ON BAMBOO IN FLORIDA

GREG HODGES[1] AND AMANDA HODGES[2]
[1]Taxonomic Entomologist, Florida Department of Agriculture and Consumer Services
Division of Plant Industry, Gainesville, FL 32614

[2]University of Florida, Entomology & Nematology Department, P.O. Box 110620, Gainesville, FL 32611-0620

Homeowners, theme parks, botanical gardens, and water gardens utilize bamboo as an ornamental planting in the Southeastern United States. Nurserymen and collectors acquire bamboo cuttings from various regions, including the Orient, which can be infested with exotic pest insects. Unfortunately, immature and adult pests beneath nodal regions and sheaths, and on the roots of a bamboo plant may be difficult for plant inspectors to detect. Two bamboo mealybugs *Palmicultor lumpurensis* (Takahashi) (Hemiptera: Pseudococcidae) and *Chaetococcus bambusae* (Maskell) which feed underneath leaf sheaths on the bamboo stalks recently invaded Florida.

While over 30 species of mealybugs are known to occur on bamboo worldwide, few species have been reported in the United States. The most commonly reported is the noxious bamboo mealybug, *Antonina pretiosa* Ferris, an established invasive species that is considered a minor pest of bamboo with aesthetics being affected more so than actual plant damage (Miller et al. 2002). The adults are legless, generally located at the nodal regions of various bamboo species and fairly easy to detect due to the presence of sooty mold and long, white, tapering wax filaments emerging from nodal regions. *Chaetococcus bambusae* (Fig. 1) is an obscure mealybug that first was introduced in Florida during 1956 and subsequently eradicated. However, a small population was able to establish at Coral Gables, Florida in 1998. Adults of this mealybug are legless and generally found beneath the sheaths of their bamboo hosts. The infestation at Coral Gables went unnoticed until the mealybugs were exposed by removal of older leaf sheaths by grounds workers. This population did not induce significant economic damage to stands of bamboo at the infestation site. Subsequent populations were found in Miami and Orange County, Florida.

In 2002, *P. lumpurensis* was introduced in the United States at Lake Buena Vista, Florida (Hodges 2002). Bamboo (*Bambusa oldhammi* Munro) at the site of infestation displayed signs of obvious mealybug contamination with large quantities of white wax on new bamboo shoots and beneath the leaf sheaths. Subsequent surveys revealed additional populations on stands of *B. olehammi* and an *Arundinaria* sp. in Orange County and Seminole County. Level of infestation ranged from slight to severe and is probably indicative of the amount of time the mealybugs had been present within the individual stands. Unlike the noxious bamboo mealybug and *C. bambusae*, this mealybug does cause considerable damage to the host plant. New bamboo shoots are aborted from heavy populations of this mealybug. Severe infestations potentially could kill stands of bamboo.

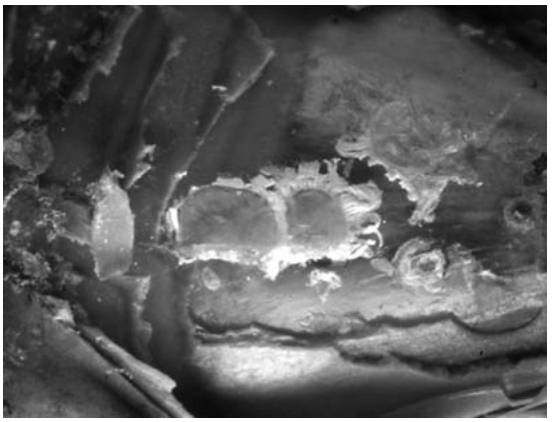

Fig. 1. *Chaetococcus bambusae* adults on bamboo.

The biology of *P. lumpurensis* is poorly known. Only a brief taxonomic and host description of *P. lumpurensis* was documented by Takahashi (1950) and Ben Dov (1994), respectively. The adults and immatures of this mealybug are grayish-pink, lack lateral wax filaments and are covered by a fine, white mealy wax (Fig. 2). This mealybug superficially resembles both the pink hibiscus mealybug (*Maconellicoccus hirsutus* (Green)) and the sugarcane mealybug (*Saccharicoccus sacchari* Cockerell). Although the pink hibiscus mealybug does not occur on bamboo, the sugarcane mealybug occasionally has been reported on bamboo within Florida. Adult females of *Palmicultor lumpurensis* mounted on slides are distinguished from these species by having 14-17 pairs of cerarii in contrast to 4-6 pairs in pink hibiscus mealybug and 1 pair in sugarcane mealybug.

SUMMARY

Palmicultor lumpurensis and *C. bambusae* have become established in Florida. The potential

Fig. 2. *Palmicultor lumpurensis* adults on bamboo.

economic impact of these invasive species for Florida's bamboo is not yet known. Monitoring of populations from each of these invasive species will be important for the native bamboo species, *Arundinaria gigantea* Walter, and for ornamental bamboo stands.

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IMPACT OF SEED PREDATORS ON THE HERB *BAPTISIA LANCEOLATA* (FABALES: FABACEAE)

SCOTT HORN AND JAMES L. HANULA
USDA Forest Service, 320 Green Street, Athens, GA 30602

The reproductive success of plants is a complex interaction among beneficial organisms such as pollinators, and destructive ones such as defoliators or seed predators that eat plant tissue. Many insects that consume reproductive tissue destroy much of a plant's reproductive output (Breedlove & Ehrlich 1968; Janzen 1971; Evans et al. 1989). In particular, the predation of seeds serves as a major selective force affecting plant abundance, distribution, and evolution (Harper et al. 1970; Moore 1978; Duggan 1985). Seed predation (destruction prior to dispersal) often accounts for a large portion of a plant population's mortality (Janzen 1969; Louda 1978; Norambuena & Piper 2000).

Lance-leaf wild indigo, *Baptisia lanceolata* (Walter) Elliott (Fabaceae), is a member of a large group of plants containing several alkaloids (Cranmer & Turner 1967) that deter some herbivores (Frost 1945). In particular, *Baptisia* spp. contain alkaloids called quinolizidines that are toxic to herbivores (Gibbons et al. 1990). *Baptisia lanceolata* occurs sporadically on sandhills, in open woods, and along roadsides in Alabama, Georgia, South Carolina, and northern Florida. The plant is considered rare in South Carolina because it is found in only two counties (Knox & Sharitz 1990). Because this species is of special concern to the state of South Carolina, research on its life history is needed to identify factors affecting populations and to aid efforts to maintain and increase *B. lanceolata* populations.

Leguminous seeds are a concentrated source of nutrition (Brashier 2000). In a nutrient-poor habitat, these seeds are important resources for many of the animal species residing there. Several insect predators are known to feed on *Baptisia* seeds. One such insect is *Apion rostrum* Say (Coleoptera: Curculionidae), a weevil that feeds on seeds of several wild indigo species. Females lay eggs in developing seed pods where the larvae eat the seeds. Haddock & Chaplin (1982) found that *A. rostrum* consumed as much as 5% of the yearly seed crop of *B. leucantha* in the tallgrass prairies of Missouri. Peterson (1989) examined the relationship between these same two species in an Illinois tallgrass prairie and found that limiting seed predator access to plants with a sticky barrier increased seeds per pod and pods per plant. Similarly, a study on two *Haplopappus* spp. (Asteraceae) in California showed that insecticides were effective in reducing seed predation (Louda 1982).

Little is known about the life history and factors influencing survival of *B. lanceolata.* In preliminary examinations, *B. lanceolata* seed pods in South Carolina were commonly infested by *A. rostrum*. Therefore, the objectives of this study were to determine the extent of *A. rostrum* seed predation on *B. lanceolata* and the efficacy of an insecticide to limit damage to *B. lanceolata* seed production. Insecticides could provide managers with an additional tool to use in efforts to increase local populations.

The study was conducted during the spring and summer of 2003 at the Savannah River Site (SRS) near Aiken, South Carolina. The SRS is owned and operated by the Department of Energy (DOE), and the land is managed as a National Environmental Research Park. *Baptisia lanceolata* is only found at a few scattered localities in the southwestern corner of the SRS where it is mainly associated with pine forests (Knox & Sharitz 1990). This species blooms from April to May, with seed pods being present from June to November. We were unsure when seed predators began oviposition on plants in our study area so we started insecticide applications early to increase the likelihood of protecting seeds. We selected 37 plants as untreated controls and 33 plants that were treated with insecticide at two locations on the SRS. Plants were sprayed with permethrin (1% AI) until runoff. The first application occurred on 29 April at the time several of the plants started to bloom. Plants were treated three more times (9 May, 21 May, and 18 June) throughout the blooming and fruiting period. Seed pods were collected on 17 July and examined for number of seeds produced and the presence of insect seed predators. A *t*-test was used to compare treated and control plants statistically.

Plants treated with insecticide experienced greater reproductive output (Table 1). The number of damaged seeds per plant was significantly lower on insecticide-treated plants ($P = 0.046$) resulting in an increase of 5 seeds/plant. In addition, percent success (no. pods formed / no. original flowers) was significantly higher on treated plants ($P = 0.010$) probably due to reduced weevil attacks in the early stages of pod development. Studies have suggested that *A. rostrum* may be responsible for premature pod abortion (Peterson 1989; Peterson & Sleboda 1994; Peterson et al. 1998). Thus, it appears insecticides effectively lowered initial damage by ovipositing females. Insecticide applications resulted in a net increase of 15 undamaged seeds per plant. Because treated and control plants had similar numbers of seeds per pod and seeds per plant, the increased seed yield resulted from reduced propagule damage.

TABLE 1. MEAN ± SE OF SELECTED FLOWER AND SEED YIELD PARAMETERS FROM CONTROL AND INSECTICIDE TREATED *BAPTISIA LANCEOLATA* PLANTS.

	Control (n = 37)	Treated (n = 33)
Blooms/plant	43.0 ± 5.68	36.7 ± 5.76
Pods/plant	7.78 ± 1.82	10.6 ± 2.23
% success (no.pods/no.flowers)*	16.5 ± 3.03	29.1 ± 4.08
Seeds/plant (damaged + viable)	42.7 ± 8.09	52.9 ± 9.88
Damaged Seeds/plant*	7.10 ± 2.07	2.20 ± 1.11
% of seeds damaged	14.0 ± 2.81	4.30 ± 1.62
A. rostrum/plant	0.49 ± 0.15	0.79 ± 0.41
Tortricid larvae/plant*	0.49 ± 0.15	0.00 ± 0.00

*denotes a significant difference ($P < 0.05$).

Peterson & Sleboda (1994) stated that *A. rostrum* was the only known predator impacting *Baptisia* spp. in their study area in Illinois. Likewise, *A. rostrum* was the only seed predator encountered at SRS during preliminary seed counts in 2002. However, when pods were collected for analysis during summer 2003, *A. rostrum* appeared to be less common than the year before and another seed predator was found.

The latter was an unidentified caterpillar in the family Tortricidae found on almost half of the plants sampled. Previous authors found tortricid larvae to be prevalent on plants in Missouri (Haddock & Chaplin 1982) and Kansas (Evans et al. 1989). It is unclear whether this caterpillar was overlooked the previous year or was not present. Unlike *A. rostrum*, the caterpillar seemed to consume almost all of the seeds within a pod. Infested pods contained frass and silk similar to that observed by Haddock & Chaplin (1982). Although we were able to collect several specimens, many larvae apparently exited the pods before 17 July. We found no evidence of pupation in the pods. Our insecticide treatments were especially effective in reducing tortricid numbers. None of the 18 larvae collected from seed pods were from treated plants.

The insecticide effectively protected seeds from tortricid larvae, but weevils and their damage were still evident, despite four applications. Although the treatments only reduced damaged seeds from 7.1 to 2.2, it is likely that they would have increased yields more if weevil populations were higher. For example, Peterson (1989) found a sticky barrier increased yields by 0.4 and 2.6 seeds per pod in 1985 and 1988, respectively. Even though our treatments resulted in an increase of only 15 viable seeds per plant, this would be an input of 600 additional seeds in a 40-plant population. More research is needed to determine if increasing seed yield within a plant population is sufficient to increase plant numbers, or whether other factors after seed dispersal limit population densities. Insecticide applications were able to limit insect seed predator damage, but the impacts on nontarget insects is less clear. Six butterfly species occur in South Carolina that feed on wild indigo (Scott 1986) and all six may occur at the Savannah River Site. These include *Colias eurytheme* (Lepidoptera: Pieridae), *Colias philodice* (Lepidoptera: Pieridae), *Callophrys irus* (Lepidoptera: Lycaenidae), *Everes comyntas* (Lepidoptera: Lycaenidae), *Achalarus lyciades* (Lepidoptera: Hesperiidae), and *Erynnis baptisiae* (Lepidoptera: Hesperiidae). These species are closely associated with wild indigo and may require it for survival, so it may be appropriate to survey plants targeted for insecticide application to ensure that larvae are not present. However, this may not be necessary since most of these butterfly species can survive on any plants in the genus *Baptisia*. Areas containing plants of other more common *Baptisia* species such as *B. alba* or *B. australis* are likely to have sufficient host plants to lessen the impacts of insecticide application to *B. lanceolata* on butterfly populations. In addition to insecticide applications, other methods such as fire, fertilization, or planting may be useful for increasing local populations of *B. lanceolata*.

Future work should investigate how seed predation is affected by biotic and abiotic factors and how it changes over time; how to time sprays better so less insecticide is needed; and whether other insecticides may be better at controlling certain seed predators. Insecticides may be a simple, inexpensive, and effective means of increasing seed yields of other rare plants.

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SUMMARY

Preventive insecticide applications were used to determine the impact of insects on *Baptisia*

lanceolata seed yield. Treated plants had an increased rate of success of flowers developing into seed pods and lower damage. Two insects, *Apion rostrum* and an unidentified tortricid, were responsible for seed damage. Seed yields were increased by 15 seeds per plant. More work is needed to determine if increased seed yields result in increased plant population densities.

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DISTRIBUTION OF THE ASIAN CITRUS PSYLLID, *DIAPHORINA CITRI* KUWAYAMA (RHYNCHOTA: PSYLLIDAE) IN THE CARIBBEAN BASIN

SUSAN E. HALBERT[1] AND CARMELO A. NÚÑEZ[2]
[1]Florida Department of Agriculture and Consumer Services, Division of Plant Industry
P.O. Box 147100, Gainesville, FL 32614-7100

[2]Sección de Entomología, Museo Nacional de Historia Natural, Plaza de la Cultura "Juan Pablo Duarte"
Calle César Nicolás Penson, Santo Domingo, D.N., Dominican Republic

Asian citrus psyllid, *Diaphorina citri* Kuwayama 1907 (Rhynchota: Psyllidae), was described from Taiwan and is native to Asia. It has been known in the Western Hemisphere for several decades in Brazil (Hodkinson & White 1981; Lima 1942). During the past decade, its range has expanded into northern South America and the Caribbean. The focus of this paper is to document the distribution of *D. citri* in the Caribbean Basin.

Burckhardt & Martinez (1989) reported *D. citri* intercepted in France on citrus plants from Honduras and "reconditioné aux Etats-Unis avant d'être importé en France." This report has been difficult to verify. There is no doubt that the intercepted insects were *D. citri*, but the source of the shipment is questionable. The circumstances under which these infested plants spent time being rehabilitated in the United States are not stated. Citrus plants from foreign sources are not allowed in the USA except under strict quarantine conditions (Title 7 (Agriculture), Chapter III, Part 319.19, Code of Federal Regulations). Additionally, *D. citri* was not present in the USA in 1989, so the infestation did not come from the USA. Visiting scientists Ronald Cave (Department of Entomology, University of Florida, pers. comm. 2003) and James Baker (Department of Entomology, North Carolina State University, pers. comm. 2003) spent many months in Honduras in the 1990s and failed to find *D. citri*.

In 1997, specimens of *D. citri* were found on citrus in Corrientes, Argentina, by Sara Cáceres (Florida State Collection of Arthropods (FSCA) accession # E1997-3427). Evidently, the infestation was minor, because the Argentina Department of Agriculture was unaware of the presence of *D. citri* in Argentina in 1998. This low infestation level suggests that *D. citri* had been there prior to 1997 long enough for populations to subside to low levels controlled by local natural enemies.

In 1998, *D. citri* was found in Guadeloupe (Étienne et al. 1998) and in south Florida (FSCA# E1998-1751) (Halbert 1998; Halbert et al. 2003). Since then, movement throughout the Caribbean has been rapid.

In 1999, *D. citri* was found on Abaco Island and Grand Bahama Island, Bahamas, on both *Citrus* spp. and on *Murraya paniculata* (L.) Jack. (FSCA# E1999-1975, 1976, Robert C. Bullock and Robert R. Pelosi, University of Florida). Two years later, high numbers of *D. citri* were intercepted on citrus fruit from Abaco, Bahamas sent for processing in Ft. Pierce, FL (FSCA# E2001-747, 850, 978, 1135, and 2049, Kenneth L. Hibbard and James J. Walukiewicz, Florida Department of Agriculture and Consumer Services, Division of Plant Industry (DPI) inspectors). These repeated interceptions of *D. citri* indicate beyond doubt that *D. citri* can move on fresh, unprocessed citrus fruit.

Infestations were found in West Bay, Grand Cayman, Cayman Islands in June 2000 (FSCA# E2000-2102, Joan Steer and Sasha Frederick). In 2001, *D. citri* was intercepted in passenger baggage on leaves of *Murraya koenigii* (L.) Sprengel from St. Thomas, U.S. Virgin Islands, probably indicating a population of *D. citri* on that island (FSCA# E2001-696). An infestation was found in Jamaica for the first time in Bodles, St. Catherine on 18 January 2003 (FSCA# E2003-259, Sharon McDonald).

Diaphorina citri was first found in the Dominican Republic in September 2001, but because of its wide distribution in the country, we believe its introduction occurred at least one year before its detection. The specimens from the Dominican Republic are housed at the Museum of Natural History, Santo Domingo, Dominican Republic. All collections are by C.A. Núñez unless otherwise noted. They are labeled as follows: 17 adults on *Murraya paniculata*—Distrito Nacional, Santo Domingo, Ciudad de los Millones, 16-IX-2001; 9 adults on *Citrus sinensis* (L.) Osbeck, Plaza de la Cultura "Juan Pablo Duarte," 20-IX-2001; 6 adults, 1 nymph on *Murraya paniculata*, Ensanche Miraflores, 21-IX-2001; 3 adults on *Citrus limon* (L.) Burm. f., 2 adults on *Citrus reticulata* Blanco, 6 adults on *Citrus limetta* Risso, 5 adults on *Citrus sinensis*, 7 adults on *Citrus maxima* (Burm.) Merr., Urbanización Las Praderas, 22-IX-2001; 7 adults, 3 nymphs on *Citrus aurantium* L., Ensanche Quisqueya, 23-IX-2001; 11 adults on *Murraya paniculata*, 3 adults on *Citrus limon*, 3 adults on *Citrus sinensis*, Province of Santo Domingo, Santo Domingo Oeste, Engombe 26-VII-2002; 6 adults, 9 nymphs on *Murraya paniculata*, Zona Industrial, Herrera, 14-XI-2002; 8 adults, 10 nymphs on *Murraya paniculata*, Santo Domingo Este, Ensanche Ozama, 8-VIII-2002; 7 adults on *Citrus sinensis*, San Cristobal, General Leger Ave., 30-IX-2001; 9 adults on *Murraya paniculata*, Monte Plata, Parque Central, 23-VII-

2002; 6 adults on *Citrus limetta*, 5 adults on *Citrus maxima*, Yamasá, Palmita de los Botados, 23-VII-2002; 13 adults on *Murraya paniculata*, Independencia, Jimaní (near the border of Haiti), 8-X-2002, C. A. Núñez and H. Takizawa.

Diaphorina citri was found in Cuba in 2001. Several specimens from Cuba, also housed at the Museum of Natural History, Santo Domingo, Dominican Republic, are labeled as follows: La Habana city, Playa, on *Citrus sinensis*, 24-VI-2001, Y. Hernández; Guanabo, on *Citrus limon*, 12-IX-2002, L. L. Vázquez; Granma, Bayamo, on *Citrus sinensis*, 10-VIII-2001, L. L. Vázquez.

We have specimens from Puerto Rico collected on 3 December 2002 at Isabela (FSCA# E2003-1439, Ana Escribano). Prior to that collection *D. citri* was reported (no specimens collected) on the coast of Isabela and the mountains of Adjuntas in Puerto Rico in June 2001; both *Citrus* and *Murraya* were infested (Philip Stansly & Robert Rouse, Southwest Florida Research and Extension Center, University of Florida, pers. comm., 2003).

Diaphorina citri was reported for the first time in Venezuela in 1999 in the Peninsula de Paraguaná, State of Falcón (Cermeli et al. 2000). Hosts were reported as *Citrus aurantifolia* (Christm.), *Citrus reticulata*, *Citrus latifolia* Tan., and *Murraya paniculata*.

French et al. (2001) reported *D. citri* in Weslaco, Texas, in September 2001 (FSCA# E2001-3720, J. Victor French). So far, there are no records from other U.S. citrus growing areas of Alabama, Mississippi, Louisiana, Arizona, or California.

There is a report of *D. citri* intercepted from Belize on *Citrus* in baggage at Houston, TX in October 2002 in the USDA/APHIS/PPQ interception database. We do not have any specimens of *D. citri* from Belize.

Finally, we have specimens, of *D. citri* from Mexico. Roy Morris (Bayer Corp., pers. comm., 2003) reported *D. citri* in Cancún, Mexico in April 2002 but collected no specimens. He collected specimens when he returned to the same location in November 2003 (FSCA# E2003-6158). There is an interception report in the USDA/APHIS/PPQ interception database of *D. citri* on *Citrus reticulata* fruit from Mexico in Texas in April 1996.

In Asia, *D. citri* primarily causes damage to citrus as a result of transmission of the pathogens that cause huanglongbing (黄龙病, citrus greening disease). In July 2004, as this paper was going to press, citrus greening disease was reported in Brazil by Fundecitrus. This is the first credible report of the disease in the Western Hemisphere.

Summary

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Rhynchota: Psyllidae), originally is from Asia but has been known in the Western Hemisphere for several decades. In 1998, it was discovered for the first time in the Caribbean Basin both in Guadeloupe and in Florida. Since then, it has spread widely among islands and adjacent mainland countries, including the Bahamas, the Cayman Islands, Jamaica, Dominican Republic, Cuba, Puerto Rico, Venezuela, Mexico, and Texas (USA). Additionally, there were interceptions of *D. citri* from St. Thomas and Belize. In Asia, *D. citri* primarily causes damage to citrus as a result of transmission of the pathogens that cause citrus greening disease. In July 2004, as this paper was going to press, citrus greening disease was reported in Brazil by Fundecitrus. This is the first credible report of the disease in the Western Hemisphere.

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AN EXTENSIVE SURVEY OF *BEMISIA TABACI* (HOMOPTERA: ALEYRODIDAE) IN AGRICULTURAL ECOSYSTEMS IN FLORIDA

C. L. McKenzie[1], Pamela K. Anderson[2] and Natalia Villarreal[3]
[1]USDA-ARS, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945

[2]International Potato Center, Apartado 1558, Lima, Peru

[3]International Center for Tropical Agriculture, A.A. 6713, Cali, Colombia

Bemisia tabaci (Gennadius) is a cryptic whitefly species, owing to lack of morphological characters that permit recognition of behavioral and/or genetic variants (Mound 1963; Mohanty & Basu 1986; Gill 1992; Gawel & Bartlett 1993; Bedford et al. 1994) and, consequently, has been considered a species complex (Brown et al. 1995; Rosell et al. 1997; Frohlich et al. 1999) or a complex species (Perring et al. 1993; Bellows et al. 1994). The *B. tabaci* complex is abundant in cultivated and uncultivated subtropical and tropical habitats worldwide and is unusual among whiteflies in that it is polyphagous on herbaceous plant species, whereas most whitefly species are monophagous on woody species (Mound 1963; Lopez-Avila 1986; Martin 1987). Among the few whitefly species that colonize herbaceous hosts, *B. tabaci* is unique in that its host range is highly variable across the species. Host range phenotypes vary from restricted to highly polyphagous, illustrating the key character that led to the recognition of 'host races' for *B. tabaci* (Bird 1957).

Host races are one variant of biotype (Diehl & Bush 1984) of *B. tabaci*, now referred to as 'biological types', that exhibit variation in geographical distribution, host range, fecundity, dispersal behavior, insecticide resistance, natural enemy complexes, and endosymbiont complement (Rowland et al. 1991; Costa et al. 1993a, b; Bedford et al. 1994; Costa et al. 1995; Kirk et al. 2000). Some of these factors may influence biotype evolution and possibly the formation of 'new' biotypes.

The first recorded whitefly outbreak occurred on tobacco in Greece in 1889 and led to the description of the whitefly as *B. tabaci* (Genn.) (Gennadius 1889). This insect was reported in Florida within the same decade (Mound 1963; Hamon & Salguero 1987), but was not considered a pest in the state until 1986 when large populations infested poinsettia (Price 1987). The appearance of a previously undescribed tomato irregular ripening disorder and a squash silverleaf disorder were associated with the introduction of this exotic biotype in Florida (Schuster et al. 1990, 1991). The spread of geminiviruses in beans and tomatoes (Blair et al. 1995; Polston & Anderson 1997) was also associated with the introduction of this new *B. tabaci* biotype. The new biotype (a.k.a. *B. argentifolii* Bellows & Perring, the silverleaf whitefly) was designated B to separate it from the original A biotype. The objective of this work was to conduct an extensive survey of *B. tabaci* populations in Florida agricultural ecosystems to determine if the B biotype had excluded non-B biotypes.

During the 2000-2001 growing seasons, an extensive survey was conducted in 13 locations, representing 8 different counties and corresponding principal vegetable producing areas in Florida (FDACS/FASS 1998), including 15 economically important agricultural crops and eight weed hosts found in proximity to the crop fields (Table 1). The same crops were surveyed across locations, when possible, and many counties were sampled multiple times. Adult whiteflies were collected at each location by vacuuming host plants with a Makita Cordless Cleaner (Model 4073D; Anjo, Aichi, Japan) outfitted with size 12-dram plastic collection vials (BioQuip Products, Gardena, CA). The plastic bottom of the collection vials was cut out, screened and placed directly into the hole made for vacuum attachments for easy sampling of host plants. At each location, leaves from crop and weed plants were collected to obtain whitefly nymphs. Adult whiteflies were counted and sexed, nymphs were removed from host leaves, and all whitefly samples were stored in 95% ethanol for molecular analysis. At the International Center for Tropical Agriculture (CIAT), in Cali, Colombia, whitefly species were verified as *B. tabaci* by classical morphological characteristics of the 4th nymphal instars and adult whiteflies were biotyped by RAPD/PCR analysis following methods adapted from De Barro and Driver (1997). CIAT has maintained whitefly colonies of the A biotype on multiple hosts since the mid-1980s. The B biotype of *B. tabaci* was detected and characterized in Colombia by Quintero et al. (1998), and colonies of this biotype have been maintained at CIAT since that time. Thus, CIAT provided the positive controls for this study. Whitefly data (mean number of nymphs, male, female and sex ratios (female: male) were analyzed by the General Linear Models (GLM) procedure where hosts were sampled more than once, and differences among hosts were determined by Ryan-Einot-Gabriel-Welsch multiple-range test (REGWQ) at $\alpha = 0.05$ (SAS Institute 2000).

TABLE 1. *BEMISIA TABACI* SURVEY SITES AND HOST PLANTS IN FLORIDA AGRICULTURAL ECOSYSTEMS, MARCH 2000-MAY 2001.

Crop/weed host common name[1]	Scientific name[1]	County (# of collection sites)
Tomato	*Lycopersicon esculentum* Mill.	St. Lucie (2); Palm Beach (1); Collier (1); Seminole (1); Gadsden (1)
Cucumber	*Cucumis sativus* L.	Palm Beach (1); Seminole (1); Gadsden (1); Suwannee (1)
Cabbage	*Brassica oleracea* L. var.*capitata* L	Dade (1); Palm Beach (2)
Broccoli	*Brassica oleracea* L. *botrytis* L.	Palm Beach (1)
Kale	*Brassica oleracea* L. var. *acephala* DC	Palm Beach (1)
Eggplant	*Solanum melongena* L.	Dade (1); Palm Beach (2); Seminole (1)
Crook-neck squash (butternut squash)	*Cucurbita moschata* (Duchesne) Duchesne ex Poir.	Dade (1)
Summer squash (zucchini squash)	*Cucurbita pepo* L.	Dade (1); Palm Beach (4); Gadsden (2); Manatee (1)
Cantaloupe	*Cucumis melo* L. var. *cantalupensis* Naudin	Collier (1); Gadsden (1); Manatee (1)
Upland cotton	*Gossypium hirsutum* L.	Gadsden (1); Suwannee (1)
Peanut	*Arachis hypogaea* L.	Gadsden (1); Suwannee (1)
Bean	*Phaseolus vulgaris* L.	Dade (1); Palm Beach (1)
Black-eyed pea (southern pea)	*Vigna unguiculata* (L.) Walp	Palm Beach (2); Gadsden (1)
Soybean	*Glysine max* (L.) Merr.	Gadsden (1)
Watermelon	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Gadsden (1)
Swiss-chard	*Beta vulgaris* L. subsp. *cicla* (L.) W. Koch	Palm Beach (1)
Sow-thistle	*Sonchus oleraceus* L.	St. Lucie (1)
American black nightshade	*Solanum americanum* Mill.	Gadsden (1)
Wild morning-glory	*Convolvulus arvensis* L.	Gadsden (1)
Lance-leaf ground-cherry	*Physalis angulata* L.	Gadsden (1)
Wild poinsettia	*Euphorbia heterophylla* L.	Gadsden (1)
Bristly star-bur	*Acanthospermum hispidum* DC.	Gadsden (1)
Wild radish	*Raphanus raphanistrum* L.	Gadsden (1)

[1]Common and scientific names according to Brako et al. 1995.

TABLE 2. MEAN NUMBER ± SE OF *BEMISIA TABACI* NYMPHS, FEMALES, AND MALES, AND SEX RATIO COLLECTED FROM SELECTED HOST PLANTS IN FLORIDA.

Host	n[1]	Nymph	Female	Male	Sex ratio (female: male)
Tomato	6	90.5 ± 19.3 a	138.3 ± 22.5 ab	50.7 ± 14.7 a	0.73 ± 0.05 a
Cucumber	4	72.8 ± 38.1 a	210.0 ± 69.9 a	71.0 ± 23.1 a	0.71 ± 0.11 a
Brassica[2]	5	136.2 ± 28.5 a	163.8 ± 35.3 ab	41.0 ± 9.0 a	0.80 ± 0.01 a
Eggplant	4	107.3 ± 44.4 a	47.5 ± 18.2 ab	11.3 ± 4.3 a	0.80 ± 0.05 a
Squash[3]	9	30.0 ± 28.5 a	86.3 ± 20.1 ab	9.7 ± 2.6 a	0.91 ± 0.01 a
Cantaloupe	3	70.0 ± 36.2 a	65.3 ± 40.7 ab	16.7 ± 13.7 a	0.84 ± 0.04 a
Cotton	2	101.5 ± 15.5 a	184.5 ± 27.5 ab	48.5 ± 13.5 a	0.80 ± 0.02 a
Peanut	2	54.0 ± 4.0 a	38.0 ± 14.0 ab	22.0 ± 15.0 a	0.68 ± 0.09 a
Bean	2	21.0 ± 9.0 a	29.0 ± 2.0 ab	12.0 ± 9.0 a	0.75 ± 0.15 a
Pea	3	35.3 ± 18.1 a	17.7 ± 2.3 b	11.3 ± 3.9 a	0.63 ± 0.06 a

Means within columns followed by the same lowercase letter are not different ($\alpha = 0.05$, REGWQ).

[1] n = the number of sites (replication) whitefly collections were made for a given host; hosts sampled only once were not included in this analysis.

[2] All *Brassica oleracea* varieties were combined for analysis.

[3] All *Cucurbita* spp. were combined for analysis.

A total of 9,963 nymph and adult *B. tabaci* were collected in 13 locations across Florida (nymph = 3,364; female = 5,061; male = 1,538). Sex ratios (female: male) were not different among host plants (F = 2.23; df = 9,21; P = <0.0629) and ranged from 0.63 on pea to 0.91 on *Cucurbita* spp. (Table 2).

RADP/PCR analysis of statewide whitefly samples using Primer set H16 (Fig. 1) indicated that only the B biotype of *B. tabaci* was present. Gels for all locations and hosts were identical to Fig. 1 (data not shown). Primer set H9 was used (data not shown) to confirm the biotype B origin of each whitefly sample. Band pattern variation in lanes 14 (Fig. 1a) and 12 (Fig. 1b) represent natural within biotype variation related to the RAPD method used as described by De Barro and Driver (1997). Minor band variability within the 600 to 900 bp region demonstrates some polymorphism between different B biotype individuals. However, the real area of interest is the region between 300 and 600 bp which demonstrates a consistently reproducible pattern (520, 500, and 344 bp) that is unique to the B biotype (De Barro and Driver 1998). These bands are not present in the case of other biotypes studied.

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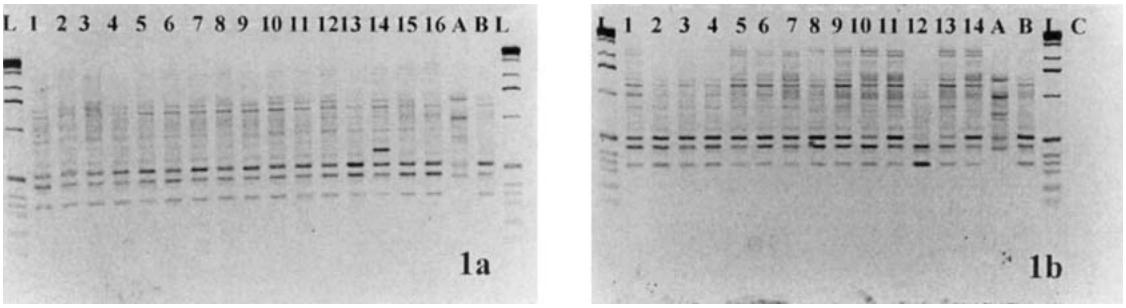

Fig. 1. Electrophoretic profiles of amplified DNA obtained for *Bemisia tabaci* samples collected from agricultural ecosystems in Florida with RAPD-PCR analysis. L, molecular weight marker Gibco-BRL 1-Kb; A, + control for *Bemisia tabaci* biotype A; B, + control for *Bemisia tabaci* biotype B; C, (-) water control with H16 primers. Gel 1a, lanes 1-10 sampled from common bean, *Phaseolus vulgaris*, Kendall Farms, Homestead, FL; Gel 1a Lanes 11-16 and gel 1b lanes 1-4, sampled from tomato, *Lycospersicon esculentum* Mill., U. S. Horticultural Research Laboratory, Fort Pierce, FL; Gel 1b, lanes 5-14 sampled from tomato, *L. esculentum* Mill., Neil's U-Pick Farm, Fort Pierce, FL.

Florida, Tropical Research and Education Center, Homestead) for assistance in surveying their respective research facilities.

Summary

An extensive survey of *B. tabaci* populations in 15 economically important crops and 8 weed species in Florida was carried out from March 2000 through May 2001. Sex ratios did not significantly differ among host plants. Biotype analysis by RADP/PCR indicated the presence of only the B biotype of *B. tabaci* in all collections. These data suggest that in Florida the B biotype of *B. tabaci* has excluded the native non-B biotypes in agricultural ecosystems.

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MONARCH BUTTERFLY LARVAE (LEPIDOPTERA: NYMPHALIDAE) WITH 3 TUBERCLE PAIRS IN SOUTH FLORIDA

BETHANY FARREY AND ANDREW K. DAVIS[1]
Department of Environmental Studies, Emory University, 400 Dowman Dr. Atlanta, GA 30322

Monarch butterflies (*Danaus plexippus*) in southern Florida differ from the larger migratory population in eastern North America in that they are a continuously breeding, non-migratory population (Brower 1995). There they persist year-round perhaps because of the warm climate in South Florida, coupled with the year-round occurrence of several species of milkweeds, the host plants of monarchs. Individuals in South Florida are smaller than those in the eastern population (Arango Velez 1996), and are heavily infected with the protozoan parasite, *Ophryocystis elektroscirrha* (Altizer et al. 2000). In southern Florida, two other danaine species occur which have similar but distinctive coloration from monarchs: the queen (*Danaus gilippus*) and soldier (*Danaus eresimus*). The ranges of monarchs and queens overlap completely in Florida, with both species present throughout the state, although monarchs occur more frequently in winter (Brower 1961; Opler 1998). Soldiers occur only in the southernmost portion of the state (Opler 1985). All three species feed on milkweed as larvae.

The morphology of monarch larvae is distinctive, with black, white and yellow striping, 11 segments, and two pairs of tubercles on the dorsal side of segments 2 and 11 (Ackery & Vane-Wright 1984; Scott 1986). These tubercles are sometimes called tentacles (Oberhauser & Kuda 1997) or filaments (Kitching 1985), and their function is unknown in the Danaidae (Kitching 1985). While tubercles are present on segments 2 and 11 in all Danaidae, queens and soldiers also each have an extra pair of tubercles on segment 5 (which is also abdominal segment 1; Ackery & Vane-Wright 1984). Here we report observations of wild monarch larvae in southern Florida with tubercles on segment 5, similar to the queens and soldiers.

On January 5, 2004, in a residential neighborhood in Miami Lakes, FL, we observed approximately 200 monarch larvae (all five instars were represented) feeding on *Asclepias curassavica* in a backyard flower garden. This is not unusual for this backyard, as 3-5 adult monarchs were frequently seen nectaring and ovipositing throughout the year here (B. Farrey, unpublished data). However on this day we also observed 11 larvae (5.5% of total larvae observed) that had a third set of tubercles on the dorsal side of their 5th segment (Fig. 1). Two days later all larvae containing an additional set of tentacles were transported to our lab at Emory University.

The 11 larvae were placed in two separate plastic rearing containers, fed clippings from potted *Asclepias incarnata*, and reared to adulthood. Of the 11 larvae, only 4 individuals survived to adulthood, but we suspect that the individuals that did not survive were infected with the protozoan parasite, *Ophryocistis elektroshirrha*. We observed the four surviving larvae when they prepared to form their chrysalis (hanging in the 'J' position). At this time, the third tubercle pair was still visible, but was reduced (Fig. 2A). Once the chrysalis had formed, they each appeared as normal monarch pupae, with the light green color and gold spots characteristic of this species (Fig. 2A). We subsequently weighed each pupa and recorded weights of 1.01 g, 1.17 g, 1.01 g, and 1.09 g. This is slightly lower than the mass of captive-reared offspring from adults collected at this same site in June 2003 (mean = 1.31 g, SD = 0.11 g, n = 53; A. K. Davis & B. Farrey, unpublished data). After each individual had eclosed and finished expanding its wings, we examined the wing morphology of each and could find no obvious aberrations to the normal monarch wing characteristics (Fig. 2B).

Larvae with three sets of tubercles on segments 2, 5, and 11 are common in many species of Danaidae, including the queens and soldiers found in Florida. We are aware of no other published report of this form in monarch larvae. We offer that this could be due to a mutation or expression of a recessive trait due to inbreeding; or perhaps a rare hybridization event between monarchs and another danaid species.

We thank N. Vitone for help rearing larvae. Sonia Altizer provided helpful comments on the manuscript.

SUMMARY

We report monarch butterfly larvae in South Florida with a third set of dorsal tubercles on their 5th segment. We reared these individuals to adulthood and observed no other physical differences between these and 'normal' type monarchs.

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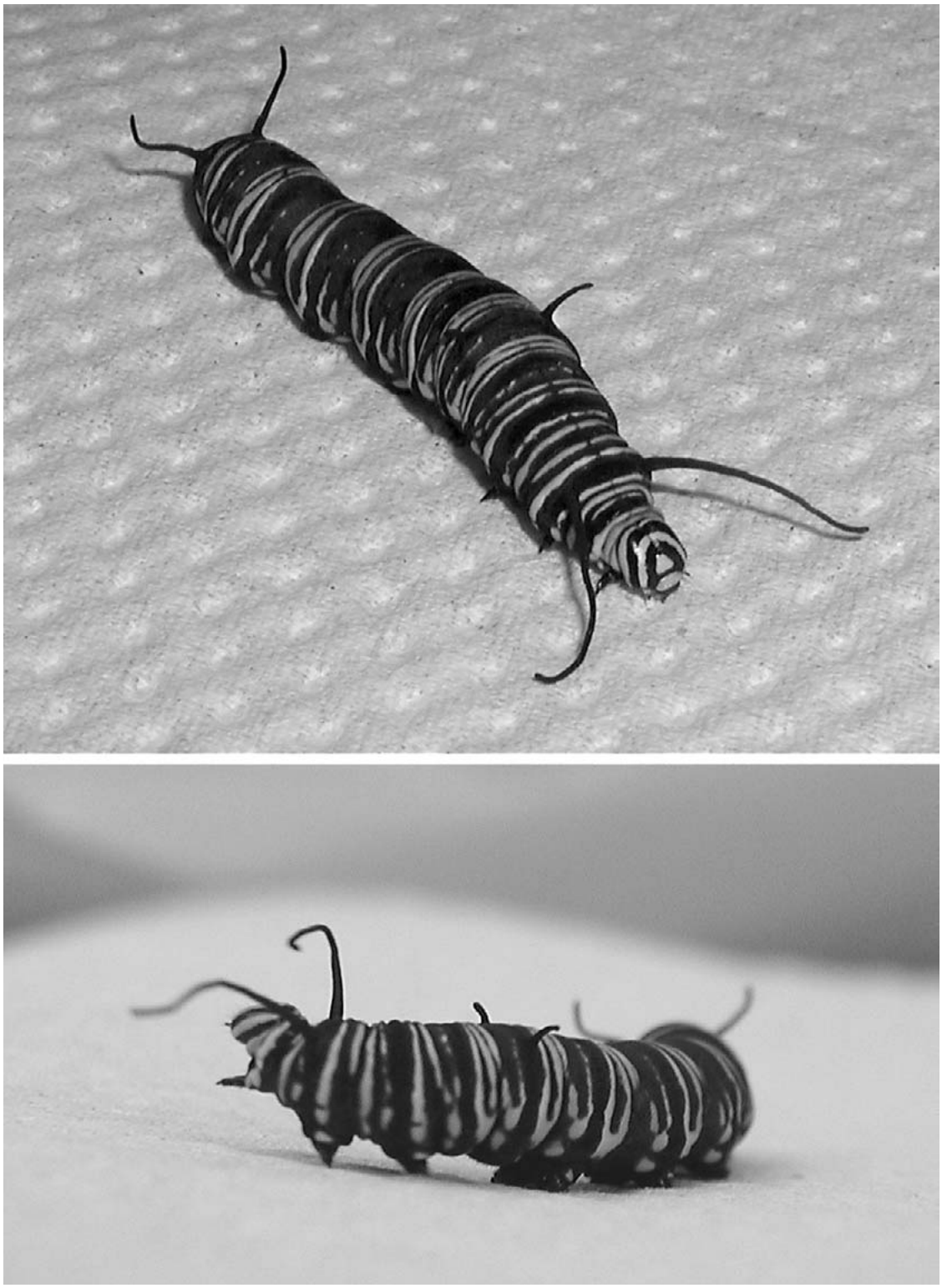

Fig. 1. Two of 11 monarch butterfly larvae with three tubercle pairs found in South Florida. Photos taken by A. K. Davis on Jan. 12, 2004.

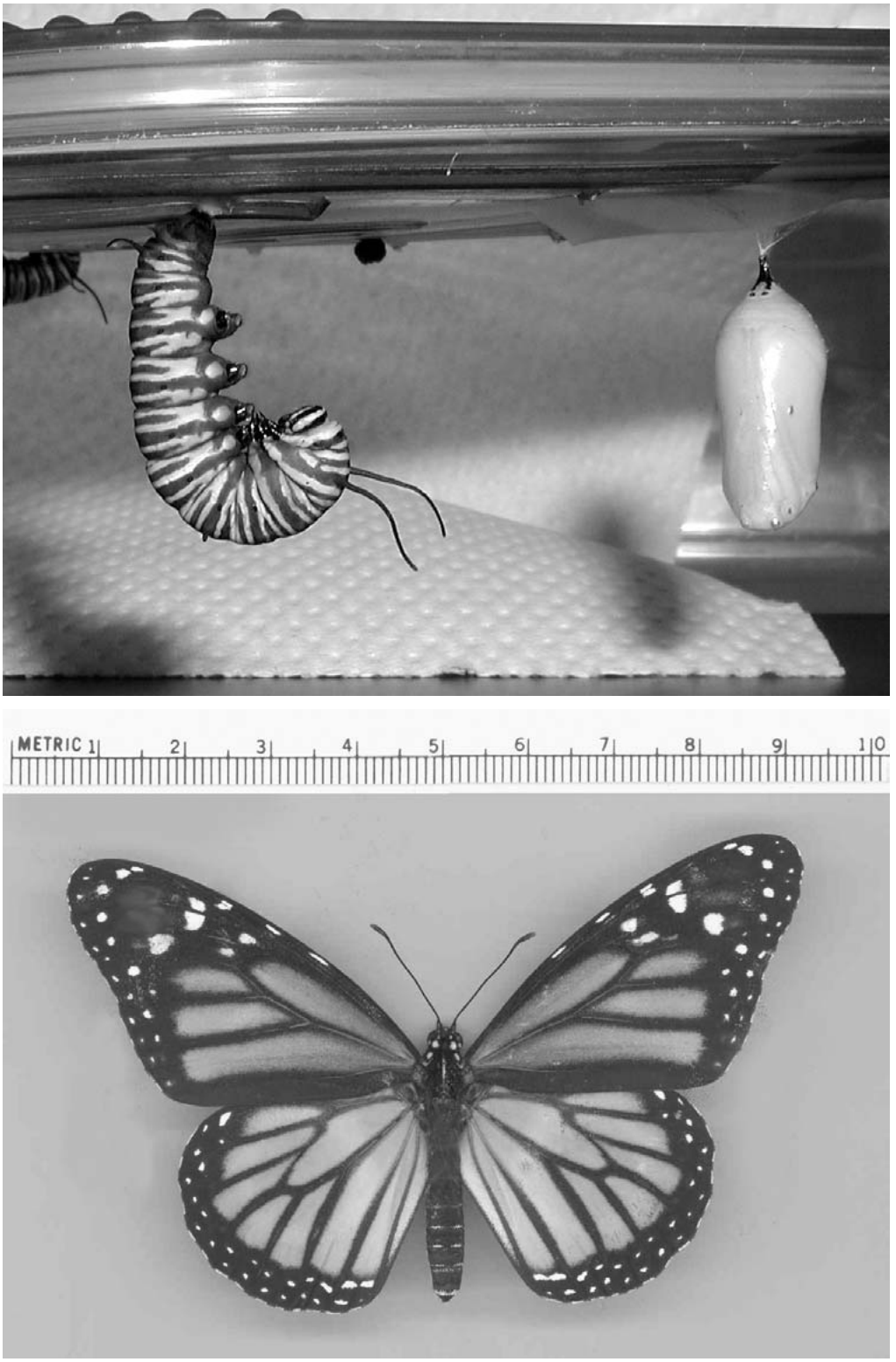

Fig 2. A. The "J" stage and chrysalis of the larvae with three tubercle pairs (note the small third tubercle remnant). B. Adult morphology of one of the 11 aberrant individuals, which was visually indistinguishable from the 'normal' type of individual reared from the same site.

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BATTLING WEST NILE VIRUS AND OTHER MOSQUITO-BORNE DISEASES: A TRIBUTE TO DR. MAURICE W. PROVOST

JOHN D. EDMAN
Center for Vectorborne Diseases, Department of Entomology, University of California, Davis
One Shields Ave., Davis, CA 95616-8584

This pioneer lecture honors the scientific career of Dr. Maurice W. Provost. Dr. Provost's distinguished career with the Florida State Board (later Division) of Health was devoted to freeing Floridians from the scourge of mosquitoes and sand flies, and preventing mosquito-borne diseases such as malaria and St. Louis encephalitis (SLE). The unprecedented invasion of West Nile virus (WNV) into North America in 1999 and its transcontinental spread from the Northeast over the past four years has added a new mosquito-borne disease to the Florida landscape. WNV is a close relative of SLE virus and has a similar maintenance and amplification cycle involving wild birds and *Culex* mosquitoes. SLE virus invaded Florida in 1959-63, just prior to when I joined Dr. Provost, then director of the Florida Medical Entomology Laboratory (FMEL) in Vero Beach. Now, 40 years later, history has been repeated, only this time the invader is an African virus. WNV moved southward into Florida in 2001 bringing human and equine illness and death to many wild birds. It is unfortunate that we no longer have the benefit of Dr. Provost's keen insight in planning our defense as we did when SLE invaded. However, he left us clear directions of how we should respond to this newest vector borne disease challenge.

Dr. Provost was unpretentious and so I will henceforth refer to him, simply as Maury. Despite his distinguished appearance and sophisticated tastes, Maury was at heart a naturalist who encouraged informality. He donned a coat and tie for the annual staff picture on the front steps of the FMEL (Fig. 1) and for the annual Christmas party (Fig. 2) but otherwise he dressed casually in loose, brightly colored shirts.

Fated with a strong genetic predisposition for coronary heart disease, Maury witnessed the untimely death from heart failure of his parents and all his brothers and sisters, except for one younger brother. He too experienced early symptoms of heart disease and as a biologist Maury understood inheritance and statistics. He realized his own longevity might be abbreviated despite his long daily walks, sensible life style, and

Fig. 1. Dr. Provost (front, center) with Senior Staff at the Florida Medical Entomology Laboratory in 1967.

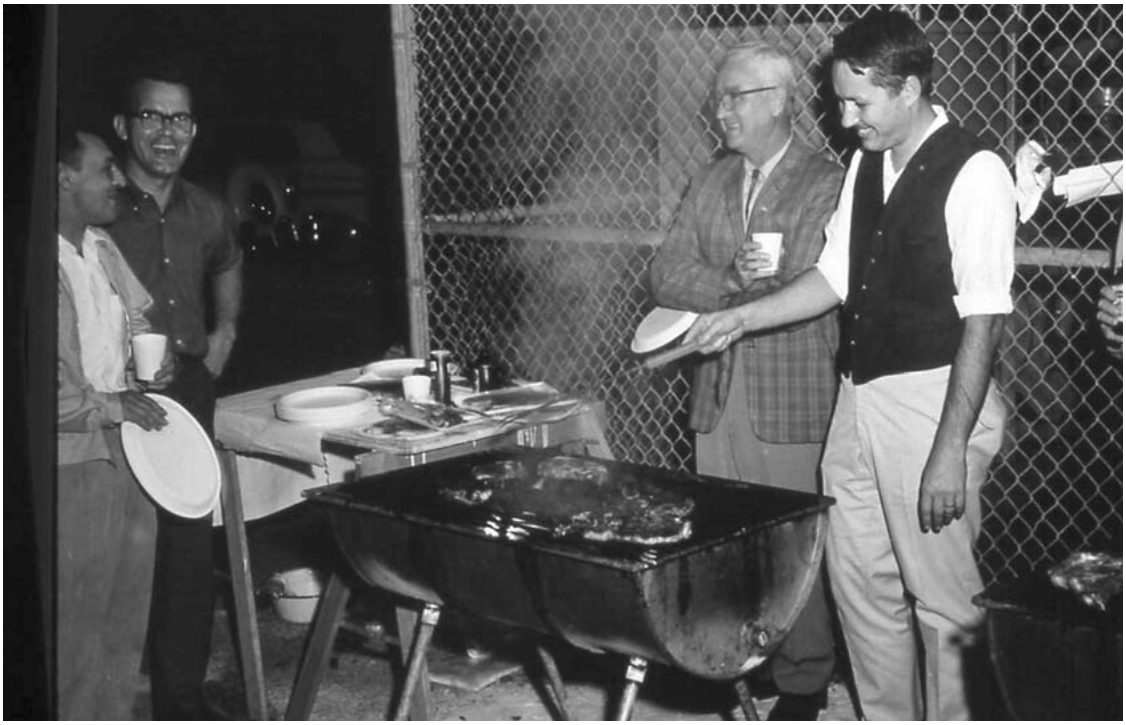

Fig. 2. Dr. Provost talking to the cook, Dr. Arden Lea, and hungry staff, Les Bourinot and Larry Webber, at the annual Florida Medical Entomology Laboratory Christmas Party in about 1962.

positive attitude. This gave focus and a quiet sense of urgency to his life. He was one of famous cardiologist Denton Cooley's early coronary bypass patients, and this hope-filled trip to Texas undoubtedly bought Maury some extra time. He used it unselfishly. Despite all that he contributed and left us there was still much more that he wanted to give to his profession and to the preservation of the wild side of Florida for which he cared so deeply. Those who knew him and worked with him realized that Maury represented someone special. This made his death at just 63 all the more tragic. Some reparation can be found in recalling some of the positive things that I personally learned from Maury, lessons that displayed the depth of his wisdom and demonstrate how slow we as a nation and public health community have been to take them to heart and turn them into positive action.

Born to French Canadian emigrants, Maury grew up hiking the mountains and wading the clear, cold streams of rural New England. Early in life, he developed the deep love of nature that so permeated his interests and values as an adult. His quiet, often stoic approach to controversial environmental issues belied the visceral feelings that were his constant motivator. He was a devoted environmentalist before the term was coined and popularized. Maury was a pragmatist who knew that marching and flag waving were not the best way to recruit those who harbored less passionate feelings toward mother nature than he did—and there were many who didn't during the "drain and develop era" following WW II. Maury, the consummate gentleman, found such displays of activism to be way outside of his comfort zone.

Maury had a special fondness for waterfowl and wetlands, and all their various inhabitants, except perhaps their mosquitoes, sand flies, and black flies. He must have donated considerable blood to these vexers of nature on those summer sojourns through the humid boreal terrain of New England. However, they did not stimulate an early calling to medical entomology. That was something Maury happened into without the medical entomology background and training one might expect. Although initially a student of vertebrate ecology, he ended up becoming a leader and innovative force in the early development of mosquito research and control efforts in Florida. World War II, malaria and chance all played a role in taking him in this fortunate direction. Perhaps it was this unique background that allowed him to step back and take a fresh and broader view of mosquito biology and control possibilities.

Maury's education included undergraduate studies at St. Anselm College in New Hampshire and graduate work at the University of California, Berkeley, and Iowa State University, plus

considerable practical experience. The nesting ecology of waterfowl associated with Iowa's many cattail-ringed potholes was the topic of Maury's Ph.D. dissertation. This came after many summers of fish and waterfowl surveys for the New Hampshire Dept. of Fish and Game while a student at Saint Anselm College and following graduation. He talked fondly about the year he spent in graduate school at U.C. Berkeley before returning to New Hampshire to complete his Master's in Vertebrate Zoology on his native turf. He then began doctoral training at Iowa State in 1940 but was interrupted by service to his country (1942-45) with the U.S. Public Health Service assigned to the 'Malaria Control in War Areas' program in Florida. This assignment provided Maury with his first public health challenge, controlling malaria vectors. Paris Green and swamp drainage were the methods of choice, and seeing the environmental affects of both convinced Maury that there must be better ways to do battle with mosquitoes. When the war ended he stayed on for a year as State Entomologist before returning to Iowa to finish his doctoral studies. These Florida experiences and connections led Maury to return to accept a position in the Bureau of Entomology of the Florida State Board of Health, heading up research on mosquito biology and control.

Malaria was no longer a significant problem in Florida, but land and economic development along the coastline, while expanding, were being inhibited by the hordes of mosquitoes that laid claim to Florida's pristine salt marshes and mangrove swamps. This seemed like an almost insurmountable task and tested the strength of Maury's vision and leadership. Salt marsh mosquitoes were thought to disperse great distances, but no one knew how far they moved from their estuarine sources and, therefore, how far control efforts needed to be extended to protect the growing populace. Living in old lighthouse quarters on the tip of Sanibel Island (no bridge in those days), Maury and his small team of thick-skinned scientists, including Jim Haeger and Bill Bidlingmayer and their devoted wives, began to study the migratory behavior of *Aedes taeniorhynchus*. This was indeed a challenge and one that continued to occupy the rest of Maury's scientific career. Maury encountered a Danish scientist, Erik Nielsen, who was studying migration of the common salt marsh butterfly in Florida, and this interaction stimulated and broadened Maury's thinking about mosquito migration and dispersal. It led to a brief but productive research collaboration between the two. Maury realized that addressing the flood-water mosquito problems associate with Florida's many salt marshes, pastures, glades, and irrigated citrus would require a major research commitment, and so he launched plans for the creation of a research laboratory in Vero Beach. Mosquitoes were so thick it was easy to generate local support for any control effort, and Maury had the backing of his politically astute Bureau Chief, John Mulrennan Sr.

Land was donated by Indian River County, and a special state appropriation funded construction of the initial facilities that opened in 1957 (Fig. 3). Maury knew it would take a long-term commitment and more resources and patience than he could expect from a non-research oriented health agency. Thus, he launched his strategy of applying for NIH grants to support basic research and collaboration with local mosquito control districts for the conduct of more short-term applied research. This strategy was highly successful, and research funding and staff grew rapidly. The lab had four Sections: Ethology, Ecology, and Physiology to conduct basic research, and a Control section to address operational issues of more immediate concern. When the Control section was surreptitiously removed to form a separate laboratory at Panama City in 1964, it disrupted Maury's master plan for a balance between basic and applied research. He quietly accepted the political reality and replaced the Control Section with a Biochemistry Section, an entomological field just coming into its own. He also consolidated the small chironomid midge substation at Winter Haven and used the associated positions and resources to develop a stronger salt marsh management effort within the Ecology section. The ecology research group, which included Maury and the mosquito control district directors in Indian River and Brevard counties, developed and fine-tuned salt marsh impoundments to prevent mosquito egg deposition. This control strategy effectively freed large areas of Florida's East Coast from the traditional hordes of salt marsh mosquitoes. Importantly to Maury, it did so without the extensive use of persistent insecticides.

Along with the expanding research capability at Vero Beach came the first major epidemic of St. Louis encephalitis (SLE) in Florida, near Tampa and St. Petersburg (1959-63). This provided the 3rd major mosquito research challenge in Maury's career, after malaria and salt marsh mosquito control. The SLE epidemic led to creation of the Encephalitis Research Laboratory in Tampa. Maury forged close ties with this new laboratory and enlarged the research focus in Vero Beach to include studies on birds and *Culex nigripalpus*. This mosquito was quickly shown to be the major vector transmitting SLE virus in Florida, both among birds and to dead-end human hosts.

I arrived at the FMEL during this period and began studying the blood-feeding behavior of Florida's mosquitoes. While waiting for the funds from Maury's new NIH grant, I became involved in several ongoing research projects and, despite my youth and naiveté about the subtropics, I quickly grew to appreciate how the Provost philosophy had permeated the way research was ap-

Fig. 3. The Florida Medical Entomology Laboratory at Vero Beach, Florida, as dedicated in 1957.

proached at the laboratory. When I interviewed with Maury, he explained that research ideas at the FMEL were usually generated from field observations, then brought into the laboratory for experimentation and clarification under controlled conditions, and finally taken back to the field and verified again whenever possible. He also explained that most U.S. university researchers did not have the benefit of Florida's long subtropical field season or the opportunity to engage in large, multi-year field studies utilizing the pooled talents of many different scientists all focusing on the same insect. Maury was a proponent of team research long before current biological complexity and rapid technological change forced this realization on the entire research community. He felt that the key to understanding the biology of insects was to be able to bridge field and laboratory based science, an approach that is now gaining many new proponents in medical entomology. For example, genetically modified mosquitoes can be created through novel technology but their population dynamics must be understood before they can be used for mosquito control.

I was personally involved in research at Vero Beach that exemplified Maury's approach. In his many field experiments on the movement of salt marsh mosquitoes, *Ae. taeniorhynychus*, Maury and colleagues observed that dense adult populations emerged synchronously in the salt marsh during the day and engaged in a mass exodus at first twilight. Much swarming and mating behavior preceded their departure, perhaps because they would never find each other again following distant migration. At the same time, Arden Lea and colleagues at the FMEL were engaged in laboratory experiments on the endocrine control of behavior, including mating. They noted that *Aedes aegypti* and other mosquitoes, including a colony of *Ae. taeniorhynchus*, would mate soon after emergence but successful sperm transfer did not take place unless females were 30-40 hr old. This raised doubts about whether the *Ae. taeniorhynchus* observed mating prior to their exodus from the marsh were actually being inseminated. A large field experiment with newly-emerged, dye-marked *Ae. taeniorhynchus* was subsequently conducted on an inland. Indeed, observations alone can sometimes be misleading, for no fertilized females <30 hrs old were recovered in the field.

The advent of West Nile virus (WNV) in the U.S. has revealed how little is still known about many of our most important vector species. The current epidemic also exposed a general deterioration of the public health infrastructure in most states. When WNV arrived, New York City no longer had a mosquito control program, anyone who could identify mosquitoes, or equipment for surveillance and operational mosquito control. An emergency response was mounted with the assistance of the Centers for Disease Control and Pre-

vention (CDC), neighboring states, and private contractors. New York City was forced to use 30-year-old control technology that had not been properly evaluated for effectiveness in a disease control emergency. New, innovative technologies are needed for detecting pathogens, predicting risk, and controlling vectors. Until we better understand mosquito vectors and the cycles of diseases they transmit, we will continue to be vulnerable to natural, accidental, and intentionally caused epidemics. The ongoing WNV epidemic in the U.S., with over 4,000 cases and nearly 300 deaths in 2002, provides a strong argument for a dramatic increase in the kind of research advocated and conducted by Maury Provost.

The large body of research on salt marsh *Aedes* generated by Maury and the staff at the FMEL was published after his death in a monograph dedicated to him (Nayar 1985). Maury contributed a summary of the life history of *Cx. nigripalpis* in a monograph on St. Louis encephalitis published by the Florida State Board of Health (Provost 1969). Much has been added to our knowledge about mosquitoes since Maury's pioneering work, but he was responsible for providing the basis for conducting meaningful research on mosquito biology.

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PATTERSON, G. 2004. The Mosquito Wars. A History of Mosquito Control in Florida. University Press of Florida; Gainesville, xviii + 263 pp. ISBN 0-8130-2720-9. Hardback. $55.00.

Florida was among the last of the states to be populated widely by humans. Some people deemed it uninhabitable because of its insufferable mosquito populations.

Here is explained how focused mosquito control in Florida solved the problems of the major mosquito-borne diseases malaria (transmitted by *Anopheles* mosquitoes), yellow fever, and dengue (transmitted by *Aedes aegypti* only) by the mid 20th century. This was done under the auspices of Florida's Board of Health, later Department of Health [DH]. The pest problem caused by the teeming populations of saltmarsh mosquitoes (*Ochlerotatus taeniorhynchus* and *O. sollicitans*) took even greater effort, lasting for decades and still ongoing. While saltmarsh mosquitoes were being battled, many new residents decided that Florida's coasts were the place to live, and they now expected a mosquito-free environment. It is alleged that mosquito control made possible the USA's space program at Cape Canaveral, adjacent to saltmarshes. Mosquito control districts (for which taxes were paid) were established in many Florida counties. Their charge was more to control pest mosquitoes (pest control) than to control mosquito-borne diseases (health), but the boundaries were not clear.

If you think that all of this was beneficial, then think again. The only realistic way to control saltmarsh mosquitoes (which do not transmit diseases to humans and are 'merely' horrendous pests) was to apply chemicals, or to change the character of the marshes by filling, impounding and flooding, or draining them. Those marshes are the cradle of Florida's offshore fisheries, and are immensely productive. The conflict between those who would maintain the marshes in their natural state, and those who wanted mosquito control at all costs led to a multi-way political fight. Many coastal residents wanted mosquito control at any cost, whatever it took, and they had political clout because they were numerous. Some mosquito control districts (southwest coast) would give it to them by applying chemicals—but of course they ran afoul of the fishing industry and environmentalists, and then of Florida's DNR (Department of Natural Resources, later incorporated into Florida's DEP, Department of Environment Protection). Other mosquito control districts (east coast) would give it to them at first by canalling to drain the marshes, and later by impounding sections of them and flooding the impoundments to prevent saltmarsh mosquito oviposition. This, concept, too, ran afoul of Florida's DNR which would rather have the natural function of the saltmarshes fully restored. Florida's Game and Freshwater Fish Commission, contrarily, viewed flooding of impounded marshes as beneficial, because it promoted populations of ducks, other forms of "wildlife" being of little interest to it. Developers would rather drain the marshes and build condominiums and other housing on them, no matter that this would trash the east coast method of controlling saltmarsh mosquitoes, make chemical control the only way of protecting the human population from these biting pests, and destroy the offshore fishing industry. Developers, too, have a political lobby. Oh, what a fight!

In the late 1950s, additional diseases, transmitted by *Culex* mosquitoes began to arrive in Florida. They were St. Louis encephalitis, eastern, western and Venezuelan encephalitides. People, horses and birds, died from these diseases. The diseases would flare up here and there, and be difficult to study because they were not constantly present. Funding for disease-carrying mosquito control was increased at major flareups, and died between them when urgency was not apparent to politicians. These new diseases were not transmitted by *Aedes*, *Anopheles*, or *Ochlerotatus* mosquitoes, and did not live in saltmarshes, requiring other kinds of control methods. Much more recently, West Nile virus arrived, also transmitted mainly by *Culex* mosquitoes. The attitude of a large segment of the public, especially along the coasts, is that chemical pesticides must be applied to kill mosquitoes (any mosquitoes, because the general public does not understand the differences). However, a growing segment of the public is aghast at profligate use of chemical pesticides which they know kill non-target organisms and threaten human health. Again, the problem is political.

Politics determined the level of mosquito control. In 1964-1969, a U.S. Public Health Service campaign (in conjunction with other campaigns in the Americas) was concocted to eradicate *Aedes aegypti* (vector of yellow fever and dengue) from the USA. Florida became a battleground although it no longer had either of these diseases. The campaign in Florida failed through poor planning and execution and caused ill-will through 'invasion' of private property.

Politics caused absorption of the Florida Department of Health [DH] into the Florida Department of Health and Rehabititative Services [DHRS]. This caused great dissent between medicine and rehabilitative services. Mosquito control and research, the child of DH, became virtually orphaned. Along with politics came the major human participants and their views about mosquito control, which were rapidly politicized. Jack Rogers, eventually 'state entomologist' on behalf of DHRS, thought chemicals were the ultimate answer to mosquito control despite anything that Rachel Carson wrote. Wayne Miller, director of the

Lee County Mosquito Control District, also believed in chemicals (although he supported some applied research), and countered mosquitoes by buying more planes and helicopters, and spraying more chemicals, until his airforce exceeded that of most Central American countries. Jackie Salmela and John Beidler (Brevard County and Indian River County mosquito control directors respectively) believed that impounding and flooding sections of saltmarsh should be the main way to control saltmarsh mosquitoes. Herb Kale, future president of the Florida Audubon Society, demonstrated that purple martins, beloved of the fringe element with quackish cures for mosquito control, did nothing worthwhile to control mosquitoes. John Mulrennan Sr. fought for funding for mosquito control (and research) and set the scene for what happened next. John Mulrennan Jr., a retired naval man, somehow inherited his father's position as director of the DHRS Office of Entomology and imposed a new director on a breakaway laboratory (next paragraph). Elton Gissendanner, administrator of Florida's Department of Natural Resources (later absorbed into Florida's Department of Environmental Protection) was horrified by the cavalier attitudes of some of the foregoing, and wanted to stop mosquito control. However, Florida's laws on pesticide use related to agricultural chemicals, not those used for mosquito control. Ultimately, the Office of Entomology was transferred from DHRS to FDACS (Florida Department of Agriculture and Consumer Services) where pesticide use is regulated.

The hero in this story is Maurice Provost, an environmentalist who was involved in the early control of *Anopheles* mosquitoes to control malaria. He was the founding director of the DH [later DHRS] Entomological Research Center [ERC, later Florida Medical Entomology Laboratory, FMEL] in Vero Beach. This laboratory was dedicated to basic (and applied) research into mosquitoes and some other biting flies. He sponsored research into mosquito ecology, behavior, and physiology and he ran headlong into Jack Rogers (promoted to his superior instead of his inferior by DHRS). Under Rogers, the use of chemical pesticides should prevail, and advocacy research should be the *modus operandi* of FMEL. Provost would have none of it. Provost believed that impoundments in saltmarshes could be managed to maintain their productivity for offshore life **and** to control mosquitoes, with use of chemicals against adult mosquitoes only on the occasions when control of the immature stages in the marshes failed. Provost was assailed now from the highest levels of DHRS with prompting from Rogers. The result was the cutting of the FMEL budget to the bone or worse by DHRS, and an embargo on its Federal research grants. DNR did nothing to support Provost. Dale Patchett, Florida state representative in Vero Beach, suggested a legislative move to transfer FMEL administratively from DHRS to the Institute of Food and Agricultural Research [IFAS] of the University of Florida.

One Saturday morning in 1977, Maurice Provost called a meeting of his senior researchers and explained to them what was afoot. He asked for a vote of confidence to transfer FMEL to IFAS. He explained that this would be considered mutiny against DHRS and might well lead to repercussions. His call for a vote yielded a universal **yes**. The move to transfer FMEL to the University of Florida failed in the first bill presented by Dale Patchett. There followed a 2-year virtual 'war' with the DHRS administration that opposed the transfer. During that war, Maurice was the first casualty: he died of a heart attack in 1977, on the Sunday after Thanksgiving. Two researchers, Bill Bidlingmayer and George O'Meara, valiantly stepped in successively as interim directors. After that, affairs went further downhill.

This book is a tremendous read. It gets deeply into the contorted politics of mosquito control, Florida's environment, and the personalities involved. I recommend it highly.

I was involved in this story because I was one of Maurice Provost's senior researchers who voted **yes** that Saturday morning in 1977. A colleague discovered that two of our peers were "ratting" behind the backs of the others to kept the DHRS administration informed; this poisoned collegial relationships for years, and they remained poisoned long after the bill was passed and transfer was accomplished. This book does not delve deeply into the events of the mutiny, the repercussions, and the aftermath. Perhaps these things are still too raw in the memories of the living participants. Instead, the book presents much information about fascinating events and personalities and politics in the early decades of the 20th century, which otherwise might have been forgotten, and these are its strength.

J. H. Frank
Entomology & Nematology Dept.
University of Florida
Gainesville, FL 32611-0630

EMDEN, H. F. VAN, AND M. W. SERVICE. 1994. Pest and Vector Control. Cambridge Univ. Press, Cambridge, U.K. xii + 349 pp. ISBN 0-521-01083-7. Paperback. $50.00. ISBN 0-521-81195. Hardback. $120.00.

Both authors have previously written well-accepted books. HvE has published textbooks on agricultural pest control, of which this one is a descendant. MWS has published on medical entomology and mosquito ecology. Now, they have been persuaded by a publisher to co-author a book on all aspects of control of all pest arthropods. The integration is seamless. The pests in question include agricultural and horticultural pests, stored products pests, household pests, pests of veterinary importance, and pests of public health and medical importance. The authors do not specifically state the target audience, but I believe it would be useful as a textbook for undergraduate students. The book also is a good read for anyone who wants to know the basics.

The text is not loaded down with references to the sources of information. In fact, the end of the book has only three pages of References. This makes it an unsuitable reference book. The trade-off is that it is easy to read. There are 13 chapters. The first two (Man and insects; The causes of pest and vectored disease outbreaks) serve as a general background, including the ways in which human activities have caused problems with pest arthropods. The next three chapters (Insecticides and their formulation; Application of insecticides; Problems with insecticides) concern chemical pesticides. Then follow seven chapters about alternative control methods (Environmental/cultural control; Biological control; Insect pathogens; Genetic control; Pheromones; Plant and host resistance; Other control measures and related topics). Last follows a very thoughtful chapter (Pest and vector management) in which the authors assemble and discuss the concepts they presented in the previous chapters. There is an appendix listing chemical pesticides, pheromones and repellents, and a few microbial insecticides. My characterization of the last chapter in no way implies that the previous chapters were devoid of thoughtfulness and discussion.

The idea of juxtaposing in one book all types of arthropod targets of control has, I believe, led to many interesting contrasts. Those who have enjoyed reading the book as an introduction and want more depth will probably want to read more specialist texts, for example on toxicology, integrated pest management, and host plant resistance.

How many agriculturists realize, for example, that agricultural methods followed in wetland rice cultivation and pig-farming have led directly to vastly increased mosquito populations and disease transmission to humans and pigs? How many realize that many chemical pesticides (albeit with different trade names), also are used against mosquitoes, and that frequent use of such chemicals against agricultural pests may help to develop resistance to those chemicals by mosquitoes (or vice versa)? How many public health agencies pay attention to the possibility that their use of chemicals against mosquitoes may kill honey bees, reduce pollination of crops, and lead to lower yields? How many agriculturists still do not realize that their use of chemical pesticides against pests may have devastating effects on insect natural enemies which, left alone, might be able to provide a high level of control of the pests in question? How many agriculturists using modern farming methods simply do not want to be bothered with the complexity of scouting for population levels of pests and natural enemies, and would rather apply a chemical pesticide on some schedule to "guarantee" (at least for the moment, and at unwarranted cost) that they will be free of a particular pest problem. From there, we get into pest dynamics, computers, and mathematical models.

Chapter 7, on biological control, in contrast to the other chapters, has several curious statements and errors. On page 154, a statement "The Tachinidae have been far less exploited for biological control; ectoparasitism is the norm" suggests that ectoparasitism is the norm in Tachinidae. I do not know of any ectoparasitoid tachinid. On p. 167, a fly imported into California from Australia as a parasitoid of *Icerya purchasi* Maskell (Margarodidae) is erroneously attributed to Tachinidae. In fact, this fly was *Cryptochetum iceryae* (Williston) (Cryptochetidae).

The topic of one of the discussions is the need by adult insect parasitoids for nectar. The authors write about "repeated failures" of a biological control program headed by George Wolcott in Puerto Rico due to the "absence of suitable flowers for the adult hunting wasp *Larra americana* that were being released to prey on mole crickets (a species of *Gryllotalpa*) in cereal fields." Errors are that the currently correct name of the wasp is *Larra bicolor* (F.), that it is a parasitoid rather than a predator, that its target was *Scapteriscus didactylus* (Latreille), not "a species of *Gryllotalpa*" (in another tribe or even subfamily of mole crickets), which damaged many crops (sugarcane, coffee, vegetable seedlings, pastures, and turf) (Frank & Parkman 1999). In fact, Wolcott's program was proclaimed a partial success by Cruz & Segarra (1992). The truth behind the story is that there exists in Puerto Rico a highly suitable nectar source for the wasp, a native rubiaceous plant called "botón blanco" (*Spermacoce verticillata* L.), but it was and is destroyed as a weed by farmers, grazed by cattle, and exists abundantly only in sandy uncultivated areas, so that its abundance is far from optimal to promote *L. bicolor* popula-

tions (Wolcott 1941). Management of populations of the plant could lead to enhanced populations of the wasp if agriculturists were to treat it as a beneficial wildflower instead of a weed.

The discussion about nectar sources goes on to state that George Wolcott introduced *Cordia* (species not specified) into Mauritius as a nectar source (for what, is not stated) and that this plant became a weed and then itself a target of a successful biological control program. In fact, *Cordia macrostachya* (Jacquin) Roemer and Schultes (Boraginaceae) arrived as a contaminant of sugarcane imported from Guyana about 1890, had become a localized weed in sugarcane fields by 1912, and was dispersed by birds (Goeden 1977). Later, it was for a while propagated as a nectar source for imported tiphiid and scoliid wasps that attacked larvae of a non-native scarab beetle pest of sugarcane (Goeden 1977). Later still, a successful biological control program was undertaken against *C. macrostachya* by importation of a chryomelid beetle and a eurytomid wasp (Wiehe 1960; Goeden 1977). George Wolcott was born in 1889 and did not introduce *C. macrostachya* into Mauritius.

The information on p. 156 about entomopathogenic nematodes is not quite right. The species there called *Neoaplectana carpocapsae* has for over a decade been called *Steinernema carpocapsae*. The trade name Nemasys is claimed to apply to *Steinernema feltiae*, stated to be "particularly useful in controlling a number of soil pests (e.g., vine weevil, *Orthorhinus klugi*)." However, The trade name Nemasys is used, with qualifications, for two nematodes, *Steinernema feltiae* and *Heterorhabditis megidis*. In the U.K, 'Nemasys leather jacket killer' (for control of pest tipulid larvae) and in the U.S.A. Nemasys (for control of sciarid fungus gnats) are *S. feltiae*. In the U.K., 'Nemasys chafer grub killer' and 'Nemasys vine weevil killer' as well in the U.S.A. as 'Nemasys H' (for control of black vine weevil, *Otiorhynchus sulcatus*) are *H. megidis*. In the U.S.A. only is sold Nematac S (*Steinernema scapterisci*, for control of *Scapteriscus* mole crickets), and in the U.K. only is sold Nemaslug (*Phasmarhabditis hermaphrodita*, for control of slugs). These are products of just one company, other species of beneficial nematodes being produced by other companies, so the products and uses are much broader than suggested.

Outside chapter 7, the erroneous use of the verb "to predate" as meaning to prey on, when its correct meaning is "to antedate" (p. 96 and p. 126) caught my attention. The few erroneous examples in this paragraph and the four above are trivial compared to the wealth of correct and appropriate examples interwoven in this most readable text. I would buy this book.

J.H. Frank
Entomology & Nematology Dept.
University of Florida
Gainesville, FL 32611-0630

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PRICE, P. W. 2002. Macroevolutionary Theory on Macroecological Patterns. Cambridge Univ. Press; New York, NY. x + 291 pp. ISBN 0-521-52037-1. Paperback. $30.00. ISBN 0-521-81712-9. Hardback. $85.00.

The familiar metaphor of the "evolutionary play on the ecological stage" is turned askew in this provocative book by Peter Price who stresses the importance of the emergent properties of adaptations that in turn influence the abundance and distribution of plants and animals. At the center of his argument is the "Phylogenetic Constraints Hypothesis", such a constraint being ". . . a critical plesiomorphic character, or set of characters, common to a major taxon, that limits the major adaptive developments in a lineage and thus the ecological options for the taxon." The emphasis here is on macro phenomena, "higher" taxa and "major" suites of adaptation, which leads to unusually powerful comparative experiments with large samples of multiple species.

The best-developed example of his approach is his consideration of population dynamics in sawflies. The observation he addresses is straightforward: Why do some herbivorous sawfly species periodically "explode" and become defoliating pests, while others are rather uncommon species with relatively stable populations? Price argues that these differences in dynamics are emergent effects of adaptations for locating larval feeding sites. In the case of rarer species (Tenthredinidae), females have competed for access to optimal oviposition sites, this has lead to specialization, low fecundity, and because such sites are rare, ultimately low population numbers. In diprionid species where females are less discriminating and much the responsibility for feeding site choice is left to larvae there has been selection for larval mobility, high fecundity, and generalized host preferences. Sub-optimal feeding sites can be relatively abundant and under the right conditions generalist sawfly numbers can skyrocket. Thus divergent oviposition adaptations in the two families, and their resulting phylogenetic constraints, ultimately underlie different degrees of population stability and potential abundance.

I have always enjoyed the work of Peter Price, who has struck me as a "theoretical naturalist", someone whose inspiration comes from loving observations made in the field. This book is no exception, and the somewhat daunting title should not discourage other insect natural historians from picking it up. There is a good deal of interesting insect lore and a lot of stimulating thought on topics ranging from the importance of parasitoids to population regulation to the intellectual condition of contemporary Ecology. I had a macro-good time with the book.

John Sivinski
USDA-ARS
Center for Medical, Agricultural
and Veterinary Entomology
1700 SW 23rd Drive
P.O. Box 14565
Gainesville, FL 32604

ARNETT, R. H., M. C. THOMAS, P. E. SKELLEY, AND J. H. FRANK (Eds.). 2002. American Beetles. Vol. 2. Polyphaga: Scarabaeoidea through Curculionoidea. CRC Press, Boca Raton, Florida. 861 pp. ISBN 0-8493-0954-9. Softback. $125.

This is a magnificent volume that completes the 2-volume series. The first volume dealt with 22 families, the more primitive of the Coleoptera, including Carabidae. This volume deals with 109 families. It is a far thing from the original book by Ross Arnett, The Beetles of the United States, which was published in a single volume in 1962. I own a copy of the 1968 edition of it, which has 1112 pages and is in small format. The quality of that book and its illustrations are nowhere near comparable to the present volumes, which do credit to CRC Press. A very complete bibliography ends each of the chapters.

Sixty-eight "contributors" collaborated in this volume, and the result is excellent. Robert Woodruff, unfortunately, did not contribute to the account of Scarabaeidae, but the account of Passalidae is due to the pen of my friend Jack Schuster, one of the foremost specialists on this group. The Lampyridae, to cite only a few of the accounts, were treated by James Lloyd, the specialist on their nocturnal lights; the Endomychidae and Erotylidae, the latter remarkably illustrated, were revised by Paul Skelley, the Curculionidae were revised by Robert Anderson, and there are so many others that I would have liked to mention. All these families are revised with brio by the best specialists on the American fauna. Ross Arnett died too soon to treat the family dear to his heart, the Oedemeridae, which he was never able to revise completely because he was always occupied with production of some new book. It was he who first had the idea to produce this re-edition with his friend Michael Thomas. He worked hard at it until a terrible illness laid him low in just a few months. He was followed in less than two years by his wife and faithful collaborator, Mary, who lived to see the re-edition of American Insects and the first volume of American Beetles. She showed them to me in 2001.

Thanks to God and John Kingsolver, the Bruchidae were considered an independent family, and were not amalgamated within Chrysomelidae. These latter were fragmented into three families (Megalopodididae and Orsodacnidae are set apart), an arrangement with which not everyone concurs. The subfamily Synetinae are not the tribe Synetini, and have nothing in common except a certain superficial convergence with the subfamily Eumolpinae. At all events, and this is what matters most, the Chrysomelidae were treated with brio by Shawn Clark, Edward G. Riley, R. Wills Flowers, and Arthur J. Gilbert. The genus *Cadiz*, whose larva has never been described, is placed as *incertae sedis*, in the vicinity of *Timarcha*. It will be good if the author of this generic name one day provides us with a description of the adult, the larva, and the biology of this mysterious Californian insect, perhaps with a subfamily of its own to accommodate it. A little molecular biology would help to show the correct situation. It lives in sandy habitats and feeds on Boraginaceae. Under the name Hispinae are lumped the cassidines and hispines; the alticines and galerucines are also lumped, under the name Galerucinae. This is only a question of interpretation, because before his recent sacking, Sicien H. Chen had already proposed similar changes, of which nobody took notice. It is all a question of interpretation, and I will not belabor the point. Like Michael Schmitt, I consider Jacoby's old classification, albeit slightly modernized, to be the best now and best thought-out.

Let's congratulate the authors, the collaborators, and finally the editors for having produced this magnificent work. These two volumes are indispensable for those who wish to study the beetle fauna of North America and northern Mexico, in other words the Nearctic fauna. This fauna has elements in common with that of Asia and especially of Europe, the connections with the neighboring continents having existed in various epochs. The faunas of the Antilles and of Central America are not far removed either, and this book will serve as a basis for their study. A production of this quality will perhaps be necessary for France, bringing together various volumes of the series Faune de France which, unfortunately, are far from complete for the Coleoptera.

Pierre Jolivet
67 Boulevard Soult
B-75012, Paris, France

COHEN, ALLEN CARSON. 2004. Insect Diets: Science and Technology. CRC Press, Boca Raton, vii + 324 pp. ISBN 0-8493-1577-8. Hardback. $129.95

This is a well-written book that will be useful to both experienced and novice workers with insect diets. It is not a "How to" book, not a description of diet formulations; the author's intent is to explain the basic nutritional needs of insects and the processing of diets. There are 15 chapters, 8 appendices, 13 pages of references, and an index. Four-page Chapter 2 gives some of the history of insect diet development and useful definitions encountered in the literature on insect diets. Appendix II near the back of the book is a listing of historical landmarks in insect diet development and could well have been included in Chapter 2, instead of as an appendix. One of the distractions I found in the book is the repetitive nature and disjunct distribution of related information in several different chapters. For example, Chapter 3 is a very good exposition of the nutritional requirements of insects, the chemistry of major nutrients, and explanation of how various dietary components supply nutritional needs, but additional nutritional information occurs in Chapters 4, 5, 8, and 9. Case studies in successful diet development for the screwworm fly and tarnished plant bug make good reading in Chapter 4. Chapter 5 describes the role of water in insect diets, pH effects, antioxidants, and some physical effects such as heating, cold or cool storage of components and diets. The author continues in this chapter with chemistry and possible interactions of various dietary components of diets that can occur during mixing and formulation. In Chapter 7 Cohen describes insect feeding biology and behavior, and the mouthparts and aspects of gut structure and digestive processes in selected insects. Chapter 10, entitled "How to develop artificial diets" is an excellent outline for new workers in diets, as well as some good advice for more experienced researchers. Chapter 12 describes some of the equipment needed to scale-up diet work and processing necessary for mass rearing. Chapter 13 deals with microbial problems in diet work, and has good advice for preventing mold and microbial contamination of diet components as well as formulated diets. On page 240 there is an excellent table of antimicrobial agents used in insect diet work, with additional information and references about the antimicrobials. Chapter 14 covers safety practices and good insectary practice. Finally, in Chapter 15, Cohen discusses some of his ideas for future development and use of insect diets. A useful glossary of diet terms is in Appendix I. Other appendices supply useful information on mineral and vitamin mixes used in insect diets, quality control procedures, and advice on measuring dietary components. There are many references to the literature on insect diets, and an index at the end of the book. Overall, I recommend the book to those involved with insect diet development or management of rearing facilities in which synthetic or semisynthetic diets will be used.

James L. Nation
Dept. of Entomology & Nematology
University of Florida
Gainesville, FL 32611-0620

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ERRATUM

BARANOWSKI, R. M. AND JULIETA BRAMBILA. *Caymanis*, a New Genus of Antillocorini from the Cayman Islands (Hemiptera: Lygaeidae). Florida Entomol. 84(1): 117-118.

The following sentence should be substituted for the sentence beginning 12 lines from the end of the page.

"Species of *Cligenes* also have the trichobothria anterior to the spiracle, but in *Caymanis* they are slightly dorsoventrad to each other rather than linear, the latter a highly derived condition according to Slater (1980); furthermore, *Cligenes* differs from *Caymanis* by the presence of a prosternal groove. Holotype deposited in the Florida State Collection of Arthropods."

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