DIVERSITY OF SCOLYTINAE (COLEOPTERA: CURCULIONIDAE) ATTRACTED TO AVOCADO, LYCHEE, AND ESSENTIAL OIL LURES

PAUL E. KENDRA[1*], JORGE S. SANCHEZ[1], WAYNE S. MONTGOMERY[1], KATHERINE E. OKINS[2], JEROME NIOGRET[1], JORGE E. PEÑA[3], NANCY D. EPSKY[1] AND ROBERT R. HEATH[1]
[1]USDA-ARS, Subtropical Horticulture Research Station, Miami, FL 33158

[2]Florida Department of Agriculture and Consumer Services, DPI, CAPS, Gainesville, FL 32608

[3]University of Florida, Tropical Research and Education Center, Homestead, FL 33031

*Corresponding author; E-mail: paul.kendra@ars.usda.gov

ABSTRACT

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring insect that vectors laurel wilt, a lethal vascular disease of trees in the Lauraceae, including avocado (*Persea americana*) and native *Persea* species (redbay, swampbay). As part of research to identify host-based attractants for *X. glabratus,* we discovered that a diverse array of non-target ambrosia beetles was attracted to the same substrates as *X. glabratus*. During Sep-Dec 2009, several field tests were conducted in north Florida (in woodlands with advanced stages of laurel wilt) with traps baited with commercial lures of the essential oils, manuka and phoebe, and with freshly-cut wood bolts of avocado (a known host) and lychee (*Litchi chinensis*, a non-host high in the sesquiterpene α-copaene, a putative host attractant). In addition, manuka-baited traps were deployed in avocado groves in south Florida to monitor for potential spread of *X. glabratus.* The combined trapping results indicated that none of these substrates was specific in attraction of *X. glabratus*. Numerous non-target ambrosia beetles were captured, including 17 species representative of 4 tribes within the subfamily Scolytinae. This report provides photo-documentation and data on the species diversity and relative abundance for a group of poorly-studied beetles, the scolytine community in Florida *Persea* habitats.

Key Words: ambrosia beetles, *Persea americana*, *Litchi chinensis*, manuka oil, phoebe oil

RESUMEN

El escarabajo de la ambrosía del laurel rojo (redbay), *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), es un insecto exótico barrenador de madera que transmite la marchitez del laurel, una enfermedad vascular mortal de árboles de la familia Lauraceae, los cuales incluyen el aguacate (*Persea americana*) y las especies nativas del género *Persea* (redbay, swampbay). Como parte de la investigación para identificar las substancias químicas que atraen a *X. glabratus*, fue descubierto que un arsenal diverso de otros escarabajos de la ambrosía que no eran de interés económico fue atraído a las mismas substancias. Entre septiembre y diciembre del 2009, se realizaron varias pruebas en el norte de la Florida (en arboledas con etapas avanzadas de la marchitez del laurel) usando trampas con cebos comerciales de los aceites esenciales manuka y de phoebe, y con madera de aguacate recien cortada (un huésped conocido) y del lychee (*Litchi chinensis*, que no es huésped, pero es alto en el sesquiterpeno α-copaene, una substancia química atractiva). Además, trampas con manuka fueron desplegadas en arboledas de aguacate en el sur de la Florida para supervisar la extensión potenciál del *X. glabratus*. Los resultados de las capturas combinados indicaron que ninguna de estas substancias eran específicas en la atracción del *X. glabratus*. Numerosos escarabajos de la ambrosía fueron capturados, incluyendo 17 especies que representan cuatro tribus dentro de la subfamilia Scolytinae. Este informe proporciona la foto-documentación y datos en la diversidad de la especie y la abundancia relativa para un grupo de escarabajos poco estudiado, la comunidad del Scolytinae en los hábitats de *Persea* de la Florida.

Translation provided by the authors.

The redbay ambrosia beetle, *Xyleborus glabratus* Eichhoff (Coleoptera: Curculionidae: Scolytinae), is an exotic wood-boring insect that vectors laurel wilt, a lethal vascular disease of trees in the Lauraceae (Fraedrich et al. 2008). Haploid males are flightless and remain within the host tree, while diploid females (typically sibling-mated) disperse to colonize new hosts. Unlike most ambrosia beetles, female *X. glabratus* are primary colonizers, capable of attacking healthy

unstressed trees. During gallery excavation, females introduce spores of a symbiotic fungus, *Raffaelea lauricola* T.C. Harr., Fraedrich & Aghayeva (Harrington et al. 2008), carried in mycangial pouches located at the base of the mandibles (Fraedrich et al. 2008). The fungus provides food for both larvae and adults, but it also invades the host vascular system and results in systemic wilt and ultimately tree death. Native to southeastern Asia, *X. glabratus* was first detected in the U.S. in 2002 near Port Wentworth, Georgia (Rabaglia et al. 2006). Since then, the vector-disease complex has spread along the coastal plain into South Carolina and Florida, and has been reported from a single county in Mississippi (USDA-FS 2010). In northern Florida, high mortality has occurred in native *Persea* species, including redbay (*P. borbonia* (L.) Spreng.) and swampbay (*P. palustris* (Raf.) Sarg.), and the rapid southward spread of the pest complex currently threatens commercial groves of avocado (*P. americana* Mill.), a confirmed susceptible host (Mayfield et al. 2008). Florida's avocado production, centered in Miami-Dade County, is worth $13 million annually (USDA-NASS 2010), and replacement costs of all avocado trees (commercial and backyard) in Miami-Dade, Broward, Palm Beach, and Lee Counties have been estimated at $429 million (Evans & Crane 2008).

Due to the serious economic threat posed by *X. glabratus*, there is a critical need for effective attractants to detect, monitor, and control the spread of this invasive pest. Preliminary research provided no evidence of an aggregation pheromone and no strong attraction to its fungal symbiont, to its frass, or to ethanol (a standard attractant for ambrosia beetles); suggesting that host tree volatiles are the primary attractants for dispersing females (Hanula et al. 2008). Additional studies identified manuka and phoebe oils (essential oil extracts from the tea tree, *Leptospermum scoparium* Forst. & Forst., and the Brazilian walnut, *Phoebe porosa* Mez., respectively) as effective baits for field monitoring of *X. glabratus* in South Carolina (Hanula & Sullivan 2008). Based on comparisons of volatile chemicals emitted from chipped redbay wood, manuka oil, and phoebe oil, Hanula & Sullivan (2008) hypothesized that 2 sesquiterpenes, α-copaene and calamenene, were likely the primary host attractants.

While conducting research to evaluate attraction of female *X. glabratus* to wood volatiles and essential oil lures, we discovered that a diverse number of non-target ambrosia beetles (both endemics and exotics established in Florida) were attracted to the same substrates as *X. glabratus*. Several field tests were conducted in north-central Florida (Alachua and Marion Counties) in natural stands of redbay and swampbay with known infestations of *X. glabratus* and visible signs of laurel wilt disease. We used 4-funnel Lindgren traps and/or sticky panels baited with commercially available essential oil lures (manuka and phoebe) or with freshly-cut wood bolts of avocado (a confirmed host) and lychee (*Litchi chinensis* Sonn., a presumed non-host). Lychee is in the family Sapindacaeae; it lacks the typical aromatic laurel volatiles, but it has a high content of α-copaene (Niogret et al. unpublished). During that same period, monitoring traps (Lindgren traps baited with manuka lures) were deployed in avocado groves in south Florida (Miami-Dade County). This report summarizes and illustrates the ambrosia beetles captured over a 4-month period (Sep-Dec 2009) in Florida to (1) provide a tool for action agencies and field scientists to facilitate identification of non-target species captured in *X. glabratus* monitoring traps, (2) document the species diversity and relative abundance for the scolytine community in *Persea* habitats, and (3) identify potential secondary colonizers of *Persea* hosts subsequent to initial attack by *X. glabratus*.

Materials and Methods

Field test 1 was conducted in Citra, Marion County, FL at the University of Florida Agricultural Experiment Station (PSREU). The back edge of the station bordered an upland wooded area dominated by mature live oak (*Quercus virginiana* Mill.) with an understory that included redbay trees symptomatic for laurel wilt. Test 1 was run from 27 Aug-22 Oct 2009 and consisted of 6 treatments: a commercial manuka lure (Synergy Semiochemicals, Burnaby, BC), wood bolts from 3 avocado cultivars representative of the 3 horticultural races ('Simmonds', West Indian race; 'Brooks Late', Guatemalan race; and 'Seedless Mexican', Mexican race), bolts from lychee (cv. 'Hanging Green'), and an unbaited control. Wood bolts were collected from the USDA-ARS germplasm collection at the Subtropical Horticulture Research Station (SHRS), Miami, FL 1 d prior to test deployment. The ends of the bolts were coated with wax to prevent desiccation, and then both ends re-cut when used as baits at the start of the test. All baits were deployed in four-funnel Lindgren traps (BioQuip, Rancho Dominguez, CA) with 300 mL of an aqueous solution of 10% propylene glycol (Low-Tox antifreeze; Prestone, Danbury, CT) added to the collection cup. For the manuka treatment, a single lure was hung from the trap lid by a wire twist tie. For the wood substrates, 2 freshly-cut bolts (5 cm diam × 15 cm length) were suspended with wire from the lid on opposite sides of the trap. Experimental design was a randomized complete block, with 5 replicate blocks arranged in a linear array along the fence at the back of the research station. Within a block, traps were spaced 10 m apart, 1.5 m above the ground, and spacing was 50 m between repli-

cate blocks. Traps were checked every 2 weeks for a total of 8 weeks. At each sampling date, the retention solutions (with insect captures) were collected, a thin layer was sawed from the lower end of each bolt (to "renew" release of wood volatiles), the collection cups were refilled, and trap positions were rotated sequentially within each block to minimize potential positional effects on beetle capture.

Field tests 2 and 3 were conducted in Cross Creek, Alachua County, FL at the Lochloosa Wildlife Conservation Area (St. John's River Water Management District). The study site consisted of mesic flatwoods composed of an overstory of slash pine (*Pinus elliottii* Englem) with a mixed understory that included numerous swampbay trees exhibiting advanced stages of laurel wilt. Test 2 was conducted from 7 Oct-2 Dec 2009 and evaluated the same 6 treatments described above. Test 3 was conducted from 5 Nov-29 Dec (at a site adjacent to test 2) and contained a commercial phoebe lure (Synergy Semiochemicals) in addition to the 6 treatments above. However, with tests 2 and 3 there were differences in trap type and trap layout. The essential oil lures were still deployed in four-funnel Lindgren traps, but the wood bolts were paired and hung vertically with 2 white sticky panels (23 cm × 28 cm, Sentry wing trap bottoms; Great Lakes IPM, Vestaburg, MI) stapled back-to-back at the bottom of the bolts. Sticky panels were further secured with several binder clips around the edges. Tests 2 and 3 followed randomized complete block design, with 5 replicate blocks arranged in a rectangular grid. Each block consisted of a row of traps hung ~2 m high in non-host trees, with a minimum of 10 m spacing between adjacent traps in a row, and with 50 m spacing between rows. Both tests were 8 weeks in duration and checked every 2 weeks. At each check, the retention solutions and sticky panels were collected, a thin layer was sawed from the bottom of each bolt, the solutions/panels were replaced, and the trap positions were rotated sequentially within each row.

In addition to the field tests conducted in north Florida, monitoring/survey traps were deployed in several avocado groves in Miami-Dade County, FL during the fall of 2009. All monitoring traps consisted of four-funnel Lindgren traps baited with manuka lures, which were hung ~2 m above ground within the canopy of avocado trees. Traps were checked at 2-week intervals and sites included the SHRS avocado germplasm collection in Miami and 3 commercial groves in Homestead.

All sample collections (from monitoring traps and field tests) were sorted in the laboratory at SHRS, and scolytine species were counted, photographed, and stored in 70% ethanol. Specimens removed from sticky panels were soaked overnight in histological clearing agent (Histo-clear II; National Diagnostics, Atlanta, GA) prior to storage in alcohol. Beetle identifications were confirmed at FDACS-DPI (Gainesville, FL) by K. E. Okins, and voucher specimens were deposited at both DPI and SHRS.

Results

The combined trapping results from field tests and monitoring traps totaled 659 ambrosia beetles, consisting of 17 species from 4 tribes within the subfamily Scolytinae (Table 1). More than 90% of the captures were from the tribe Xyleborini, and only 1 specimen (*Coccotrypes distinctus* (Motshulsky)) was representative of the tribe Dryocoetini. With the exception of *X. glabratus* (Fig. 1), most beetles were captured in fairly low numbers, with many species represented by only a single capture, despite significant trapping efforts with a variety of host-based attractants. *Xyleborus glabratus* comprised the majority of captures in north Florida, as expected due to its invasive pest status, but the percentages varied by site (15% in Marion County with test 1; 75.4% and 86.2% in Alachua County with tests 2 and 3, respectively). Four other species of *Xyleborus* were captured (Fig. 2), with *X. ferrugineus* (Fabricius) (Fig. 2A) and *X. affinis* (Eichhoff) (Fig. 2B) the 2 most abundant species after *X. glabratus*. There were several other representatives within the tribe Xyleborini (Fig. 3), with *Ambrosiodmus obliquus* (LeConte) (Fig. 3A) a dominant species at both the Alachua site and in the Miami-Dade avocado groves.

The tribe Cryphalini was represented by *Hypothenemus dissimilis* (Zimmerman) and several other *Hypothenemus* species difficult to discern to species level (Fig. 4). *Hypothenemus* beetles were major components at the Marion site and in Miami-Dade County. Within the tribe Corthylini, 5 species were captured, of which 4 are presented in Fig. 5. Two of those ambrosia beetles had distinctive morphological features. Females of *Corthylus papulans* Eichhoff (Fig. 5C) have greatly enlarged terminal antennal segments which bear several long, recurved setae (Fig 5E); males of *C. papulans* lack the characteristic setae. In *Pityoborus comatus* (Zimmerman) (Fig. 5D), females are unique in that the mycangia are located on the pronotum, and consist of a pair of large shallow depressions covered with dense pubescence (Fig. 5F; Furniss et al. 1987).

Discussion

The subfamily Scolytinae contains 2 functionally distinct groups of beetles - bark beetles which feed on phloem from the inner bark of host trees, and ambrosia beetles which cultivate and feed on symbiotic fungi within the xylem layers (Rabaglia 2002). Among the bark beetles, there are major forest pests which have been well studied, includ-

TABLE 1. AMBROSIA BEETLES (CURCULIONIDAE: SCOLYTINAE) CAPTURED IN MARION, ALACHUA, AND MIAMI-DADE COUNTIES, FL FROM SEP-DEC 2009, ARRANGED ACCORDING TO LAWRENCE & NEWTON (1995).

	Marion Co.	Alachua Co.		Miami-Dade Co
	Test 1[a]	Test 2[b]	Test 3[b]	Monitoring[c]
Tribe Dryocoetini				
Coccotrypes distinctus (Motschulsky)			1	
Tribe Xyleborini				
Ambrosiodmus lecontei Hopkins				1
Ambrosiodmus obliquus (LeConte)		6	14	22
Premnobius cavipennis Eichhoff				1
Theoborus ricini (Eggers)				1
Xyleborus affinis (Eichhoff)	2	11	4	
Xyleborus californicus Wood	2	1		
Xyleborus ferrugineus (Fabricius)	3	34	22	1
Xyleborus glabratus Eichhoff	3	193	287	
Xyleborus volvulus (Fabricius)		7	3	
Tribe Cryphalini				
Hypothenemus dissimilis (Zimmerman)	1	1		
Hypothenemus spp.	9	1	1	22
Tribe Corthylini				
Subtribe Corthylina				
Corthylus papulans Eichhoff				1
Monarthrum mali (Fitch)		1		
Subtribe Pityophthorina				
Pityoborus comatus (Zimmerman)			1	
Pseudopityophthorus minutissimus (Zimmerman)				1
Pseudopityophthorus pruinosus (Eichhoff)		1		

[a]8-wk field test in redbay; Lindgren traps baited with wood bolts (avocado, lychee) or manuka oil lures.
[b]8-wk field test in swampbay; Sticky traps baited with wood bolts (avocado, lychee); Lindgren traps baited with manuka/phoebe oil lures.
[c]Monitoring in avocado groves; Lindgren traps baited with manuka oil lures.

ing the southern pine beetle, *Dendroctonus frontalis* Zimmerman (Chellman & Wilkinson 1980), the western pine engraver, *Ips pini* (Say) (Kegley et al. 1997), and several other *Ips* spp. found in the southeastern U.S. (Conner & Wilkinson 1998). In contrast, the ambrosia beetles are generally not of economic importance and consequently have received less attention. They are minute beetles, spend the majority of their life concealed within host trees, and typically attack stressed or dying trees. Despite the large number of described species (e.g., >500 currently recognized *Xyleborus* spp. worldwide, Rabaglia et al. 2006), much is unknown regarding the basic biology, ecology, host range, fungal symbionts, and population dynamics of many endemic ambrosia beetles. Far less is known about exotic invasive species, which are not pests in their native lands but may acquire pest status when introduced into new environments, as is the case with *X. glabratus* in the U.S.

Although our research was focused on identification of attractants specifically for detection and control of *X. glabratus*, information was obtained concurrently on the species diversity and relative abundance for the ambrosia beetles found in native *Persea* habitats in north-central Florida. In south Florida the trapping effort was less intensive, but preliminary data was also obtained for the species composition in avocado groves. This summary report identifies the species of Scolytinae most likely to be encountered while monitoring for *X. glabratus*, and the photo-documentation provides fellow researchers (non-taxonomists) and action agency personnel with a convenient tool for preliminary identification of non-target captures.

Some of the non-target beetles identified herein are species that may potentially function as secondary vectors of the laurel wilt pathogen. Once healthy trees are attacked by *X. glabratus*, the stressed trees are susceptible to further attack by secondary colonizers that can contribute to the rapid mortality seen in laurel hosts. Observations made on dead swampbay trees at the Lochloosa Conservation Area (Kendra et al. unpublished) indicated that multiple wood-boring species attacked those *Persea* trees, as evidenced

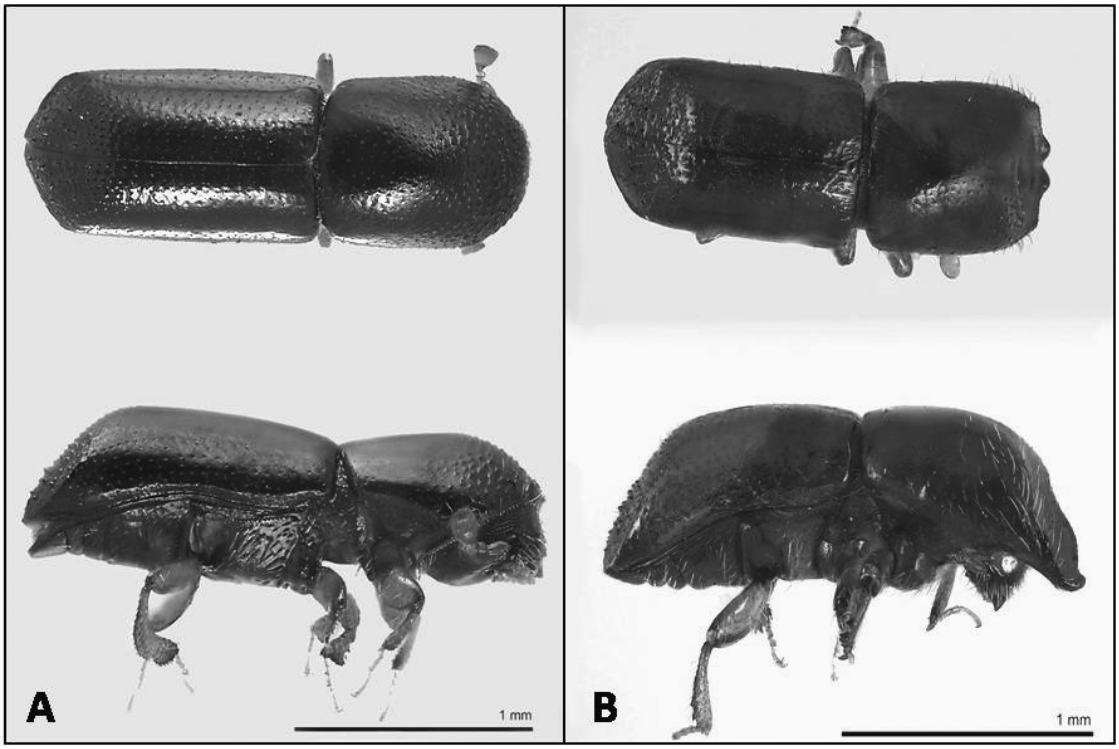

Fig. 1. The redbay ambrosia beetle *Xyleborus glabratus* Eichhoff, vector of a lethal wilt fungus (*Raffaellea lauricola*) causing high mortality of trees in the Lauraceae in the southeastern U.S. A. Female. B. Male. (Note: Males of *X. glabratus* are flightless; this specimen was obtained from host wood, not from a flight trap.)

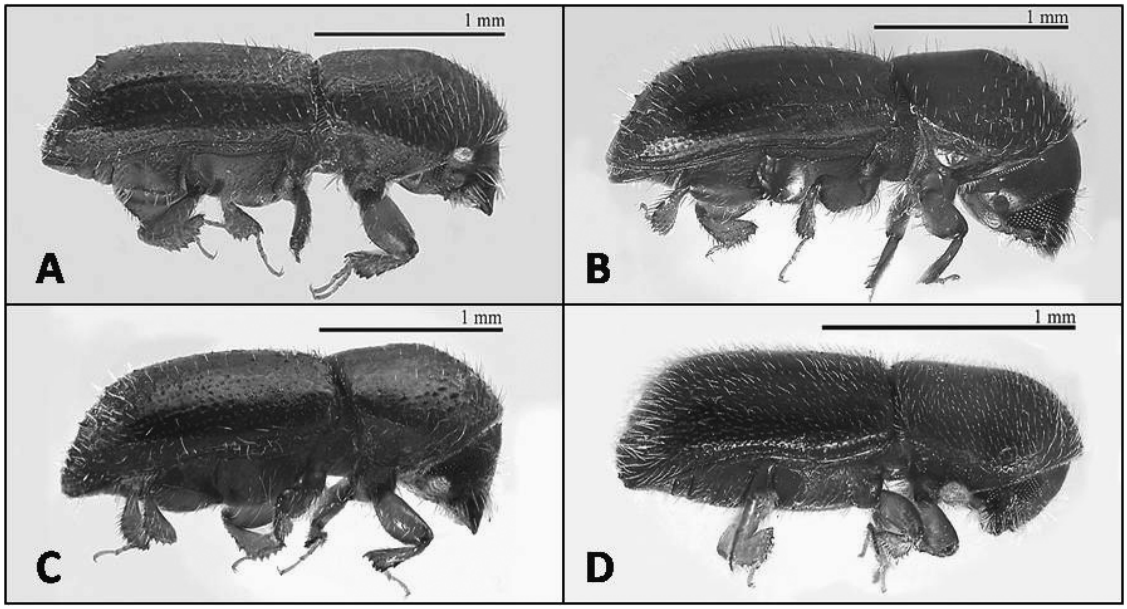

Fig. 2. Four species of *Xyleborus* not of economic importance. A. *X. ferrugineus* (Fabricius). B. *X. affinis* (Eichhoff). C. *X. volvulus* (Fabricius). D. *X. californicus* Wood. (All specimens female.)

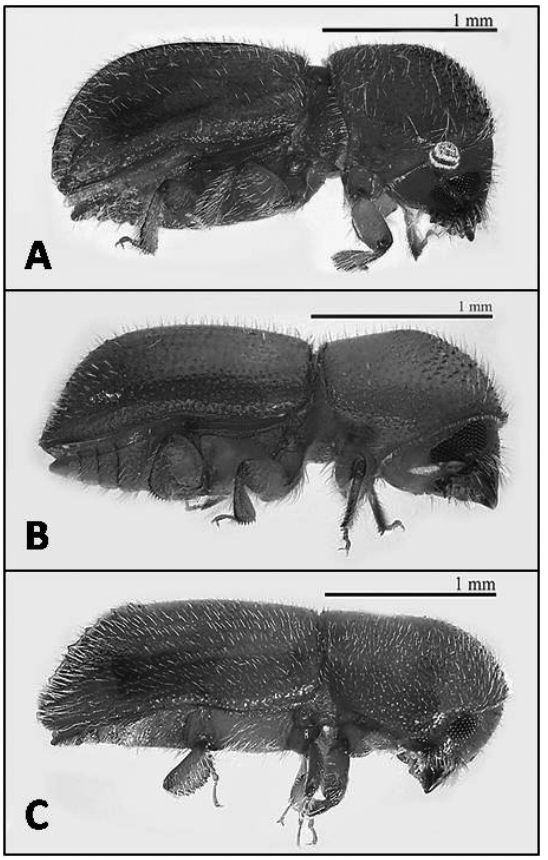

Fig. 3. Ambrosia beetles within the tribe Xyleborini. A. *Ambrosiodmus obliquus* (LeConte). B. *Theoborus ricini* (Eggers). C. *Premnobius cavipennis* Eichhoff. (All specimens female.)

by bore holes of various diameters. These secondary colonizers may potentially pick up *Raffaelea* from the host xylem and transport it to new trees, accelerating the spread of laurel wilt. In north Florida, *X. ferrugineus*, *X. affinis*, and *A. obliquus* were the most abundant species in native *Persea* habitats; in avocado *A. obliquus* and *Hypothenemus* spp. were dominant. In South Carolina, Hanula et al. (2008) found that *Xylosandrus crassiusculus* (Motschulsky) was attracted to wounded redbay trees. Further research should evaluate these additional beetle species to (a) determine if stressed (diseased) trees in the Lauraceae can serve as hosts, and if so, then (b) determine if *Raffaelea* spores can be recovered from the mycangia of ambrosia beetles that developed within *Raffaelea*-infected hosts. In other systems, there is evidence that exchange or "cross contamination" of symbiotic fungi may occur among ambrosia beetle species that occupy a common breeding site (Gebhardt et al. 2004). Alternatively, *Raffaelea* spores may potentially be transported passively by the setae and/or cuticular asperities (protuberances) commonly found on the anterior slope of the female pronotum, as has been demonstrated for *Hypothenemus hampei* (Ferrari) and spores of *Fusarium solani* (Martius) (Morales-Ramos et al. 2000).

The commercial lures currently available for *X. glabratus* are non-specific in attraction, so high numbers of non-target captures are likely to be encountered with the monitoring system (manuka-baited Lindgren traps) employed by the State of Florida. Manuka and phoebe oil lures were originally developed for field monitoring of another (phylogenetically distant) wood-boring beetle, the emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) (Crook et al. 2008). Preliminary research (Kendra et al. unpublished) indicated that these essential oil lures were not only non-specific, but may have limited field life for attraction of *X. glabratus*. With the data set presented here, approximately 30% of the captures were non-target species of Scolytinae. Development of effective strategies for early detection and control (i.e., attract-and-kill systems) of *X. glabratus* is contingent on identification of specific attractants. In the absence of species-specific pheromones or food-based attractants for *X. glabratus* (Hanula et al. 2008), this will be a difficult challenge.

Conclusions

Multiple trapping studies targeting the redbay ambrosia beetle, *Xyleborus glabratus,* effectively generated a survey of the overall scolytine community resident in Florida *Persea* habitats. These ambrosia beetle species that co-occur with *X. glabratus*, are attracted to the same host-based volatile chemicals; and they are the non-target species likely to be encountered in traps set out to monitor for *X. glabratus*. Those species that can function as secondary colonizers of *Persea* hosts should be evaluated as potential secondary vectors for transmission of the laurel wilt pathogen, *Raffaelea lauricola*.

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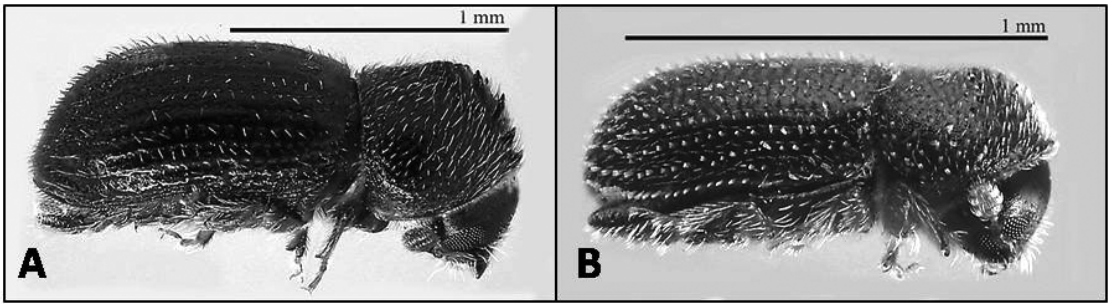

Fig. 4. Ambrosia beetles within the tribe Cryphalini. A. *Hypothenemus dissimilis* (Zimmerman). B. *Hypothenemus* sp. (Both specimens female.)

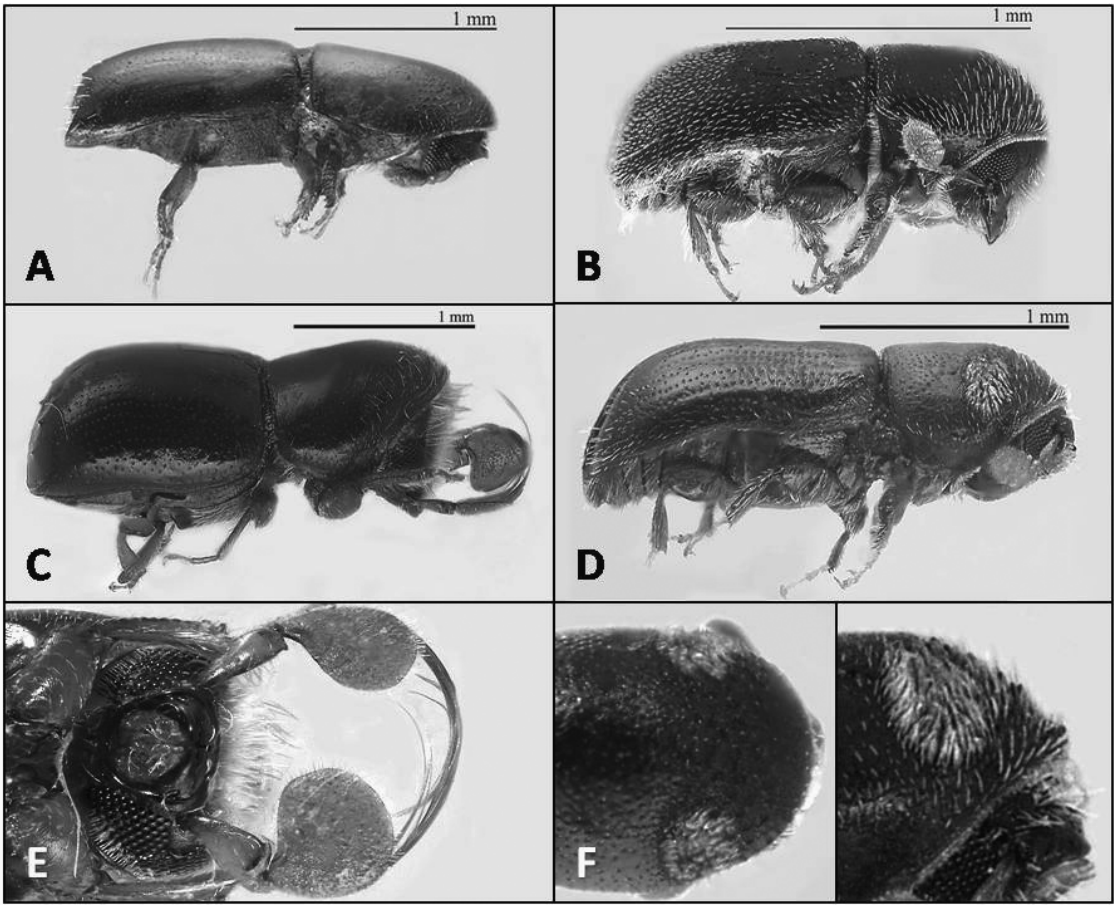

Fig. 5. Ambrosia beetles within the tribe Corthylini. A. *Monarthrum mali* (Fitch). B. *Pseudopityophthorus pruinosus* (Eichhoff). C. *Corthylus papulans* Eichhoff. D. *Pityoborus comatus* (Zimmerman). E. Detail of *C. papulans* (anterior end; ventral view) showing enlarged terminal antennal segments bearing long recurved setae. F. Detail of *P. comatus* (anterior end; dorsal view on left, lateral view on right) showing pronotal mycangia (oval pits covered with dense setae), the storage site for symbiotic fungal spores. (All specimens female.)

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LIGHT ATTRACTION AND SUBSEQUENT COLONIZATION BEHAVIORS OF ALATES AND DEALATES OF THE WEST INDIAN DRYWOOD TERMITE (ISOPTERA: KALOTERMITIDAE)

MARIA TERESA FERREIRA[1,2] AND RUDOLF H. SCHEFFRAHN[1,2]
[1]Fort Lauderdale Research and Education Center, University of Florida,3205 College Avenue, Davie, FL 33314

[2]Azorean Biodiversity Group (CITA-A), Departamento de Ciências Agrárias, Universidade dos Açores, Pico da Urze, Angra do Heroísmo, Portugal

ABSTRACT

Laboratory studies were conducted during the 2007 and 2008 dispersal seasons of the West Indian drywood termite *Cryptotermes brevis* (Walker), a serious urban pest of wooden structures. Attraction to light and subsequent colonization of this species were studied by observing the response of alates to lit and dark chambers. Several intensities of light were tested to determine if light intensity had a role in the alates' attraction to light and subsequent colonization. A bioassay was conducted with semi-shaded wood blocks to quantify negative phototaxis for the dealates. We found that the alates of *C. brevis* preferred flying into lit areas for colonization, and that the number of colonizations was highest in the high light intensity treatments. Negative phototaxis of the dealates was observed because these preferred to colonize in the dark habitat treatments. This information is important when deciding what control methods may be used to prevent *C. brevis* from colonizing wood structures. Traps with a high intensity light to attract *C. brevis* alates and to prevent infestation may be a way to monitor and control this urban pest.

Key Words: *Cryptotermes brevis,* phototaxis, colonization, alates, dealates

RESUMO

Estudos num laboratório foram realizados durante a época de voo de dispersão de 2007 e 2008 para a térmita da madeira seca *Cryptotermes brevis* (Walker), uma praga urbana de estruturas de madeira séria. A atracção à luz e subsequente colonização desta espécie foi estudada, observando a resposta dos alados a câmaras de preferência de luz. Várias intensidades de luz foram testadas para determinar se a intensidade da luz tinha um papel na atracção dos alados pela luz e subsequente colonização. Um ensaio usando blocos de madeira semi-cobertos para quantificar o comportamento fototáctico negativo dos dealados foi conduzido. Nós observámos que os alados de *C. brevis* preferem áreas com maior iluminação para colonizarem, e que o número de colonizações era maior no tratamento com maior intensidade de luz. O comportamento fototáctico negativo dos dealados foi observado porque os dealados preferem colonizar nos tratamentos de habitats escuros. Esta informação é importante quando se tem de decidir que métodos de controlo podem ser usados para prevenir a térmita *C. brevis* de colonizar estruturas de madeira. Usar armadilhas com uma intensidade luminosa elevada para atrair alados de *C. brevis* e prevenir uma infestação poderá ser uma forma de monitorizar e controlar esta praga.

Translation provided by the authors.

The West Indian drywood termite *Cryptotermes brevis* (Walker) (WIDT) is a serious urban pest that causes significant levels of damage to wooden structures. This termite was first described in Jamaica in 1853 and has a tropicopolitan urban distribution except in Asia (Scheffrahn et al. 2008). Like most Kalotermitidae the WIDT nests in its food source, wood, where it spends most of its life cycle. A colony of drywood termites can vary in size from hundreds to a few thousand termites (Nutting 1970), and several colonies can be found inside a single piece of wood. Myles et al. (2007), for example, found as many as 30 colonies of WIDT in a single floor board.

Drywood termites are major pests, accounting for about 20% of the budget spent on termite control in the United States (Su & Scheffrahn 1990). One of the main methods for controlling these pests has been the use of fumigation to eliminate existing colonies. Fumigation, however, does not prevent new infestations, and therefore it is beneficial to combine fumigation with additional preventative control methods. Preventing this species from founding a new colony during the dispersal flight season is an important technique for the control of this pest. This study aims to develop a better understanding of the behavior of the WIDT during the dispersal flight season, the time

when WIDT is most accessible to physical, chemical, or behavioral management efforts.

The life cycle of WIDT includes a dispersal flight where the mature winged forms (alates) leave their previous colony to form new colonies. The dispersal flights are the only occasion when this species is found outside of wood (Kofoid 1934) because it never leaves the nest to forage for new food sources (Korb & Katrantzis 2004). After flying, the alates shed their wings and associate as pairs of female and male dealates. These pairs will crawl on the substrata in search for a suitable place to start a new colony (Snyder 1926; Wilkinson 1962; Nutting 1969; Minnick 1973).

According to Light (1934a) most termites castes are negatively phototactic, but the alates, attracted to light, seek to emerge into openings and fly toward light. However, there are no data correlating colonization sites with lighting conditions. Positive phototaxis of *C. brevis* and congenus alates is followed by negative phototaxis of the dealates (Wilkinson 1962; Minnick1973), although no data have been produced to confirm this observation.

The present study quantified the phototactic colony site selection of *C. brevis* alates and dealates. The first hypothesis tested was that favorable colonization sites in lighted areas are more likely to be selected by alates of *C.brevis*, and that colonization will be higher at higher light intensities. The second hypothesis tested was that dealates search for darker areas on the substrate to colonize.

Materials and Methods

Termites

Experiments were conducted at the University of Florida, Fort Lauderdale Research and Education Center (FLREC), Davie, Florida, in a room partially filled with wood infested by *C. brevis* that originated from several sites around South Florida. The wood was stored in a dark room at ambient temperature (average 25.6ºC) and ambient relative humidity (average 73.5%). The termite alates used in the experiments dispersed naturally from the infested wood. The room remained dark except for the time the experiments were conducted, and the alates were free to fly anywhere in the room. The first experiments took place between Apr and Jul 2007, and the second experiments between Apr and Jun 2008.

Light Intensity Experiments

Twenty four transparent plastic boxes (36 × 23 × 28 cm) served as light preference chambers. The boxes were wrapped in aluminum foil to isolate the light box from adjacent boxes. The boxes were placed with the open side facing the infested wood and a hole was cut in the side of each box in order to fit (Fig. 1) an electrical cord for a set of white Light Emitting Diodes (LED) (HolidayLEDS™ model No TS-70) strung in a series of 5 single light bulbs attached with tape and hung through the hole. The LEDs were all connected to each other in a continuous string of lights mounted in a series and connected to a single power source. Electrical tape was used to position the light bulbs in place as well as prevent light from dispersing through the cut hole, so that the only light source was inside each box. The LED sets were randomly distributed among 12 lit boxes and 12 dark boxes. The lit boxes had a light intensity of approximately 40 lux as measured by a light meter (Extech Instruments model No 403125) and the dark boxes had approximately 0.11 lux (due to some contaminating light from nearby experiments occurring at the same time). A cube of wood (5 × 5 × 5 cm) with 6 drilled holes was placed in the center of each box (Fig. 1). Each 2.3 mm diam hole was 1.5 cm deep, and there was a single hole per face of the block. Four thumb push pins were placed on the underside of the block to allow for enough space (3 mm) for the termite to access the hole on the underside.

The difference in colonization between different light intensities was analyzed with the same 24 boxes previously described. The LED lights were also used but this time there were 4 different set ups for the different light intensities with 6 replicates per light intensity (measured by the light meter): 6 of the boxes were dark with (≈0.11 lux); 6 boxes had 1 LED light bulb with an intensity of approximately 11 lux; 6 boxes had 5 LEDs together (≈40 lux); 6 boxes had 10 LEDs attached with an intensity of approximately 480 lux. The boxes with the different light intensities were randomly distributed. A block of wood (15x2x9 cm) with 24 holes (12 on top and 12 on the bottom, and each 1.5 cm deep and 2.3 mm diam) was placed in the center of each box. After 3 months, the blocks were collected and the number of colonized holes per block was counted. A hole was considered to be colonized when a complete fecal seal (covering of the hole with hardened fecal material from the termites) was present.

Negative Phototaxis Experiment

The negative phototaxis of the dealates was studied with a white PVC pipe (51 cm × 7 cm inside diam) wrapped with electrical tape and closed on one end. A 102 × 2 × 5 cm board with 40 holes, each 2.3-mm diam and drilled 2.5 cm apart, was placed inside the pipe. The outermost holes were 1 cm from the edge of the board. Of the 40 holes, 20 were always exposed to light (regular fluorescent indoor lighting) and 20 were exposed to decreasing levels of light toward the closed end of the PVC pipe (Fig. 2). The board was visually

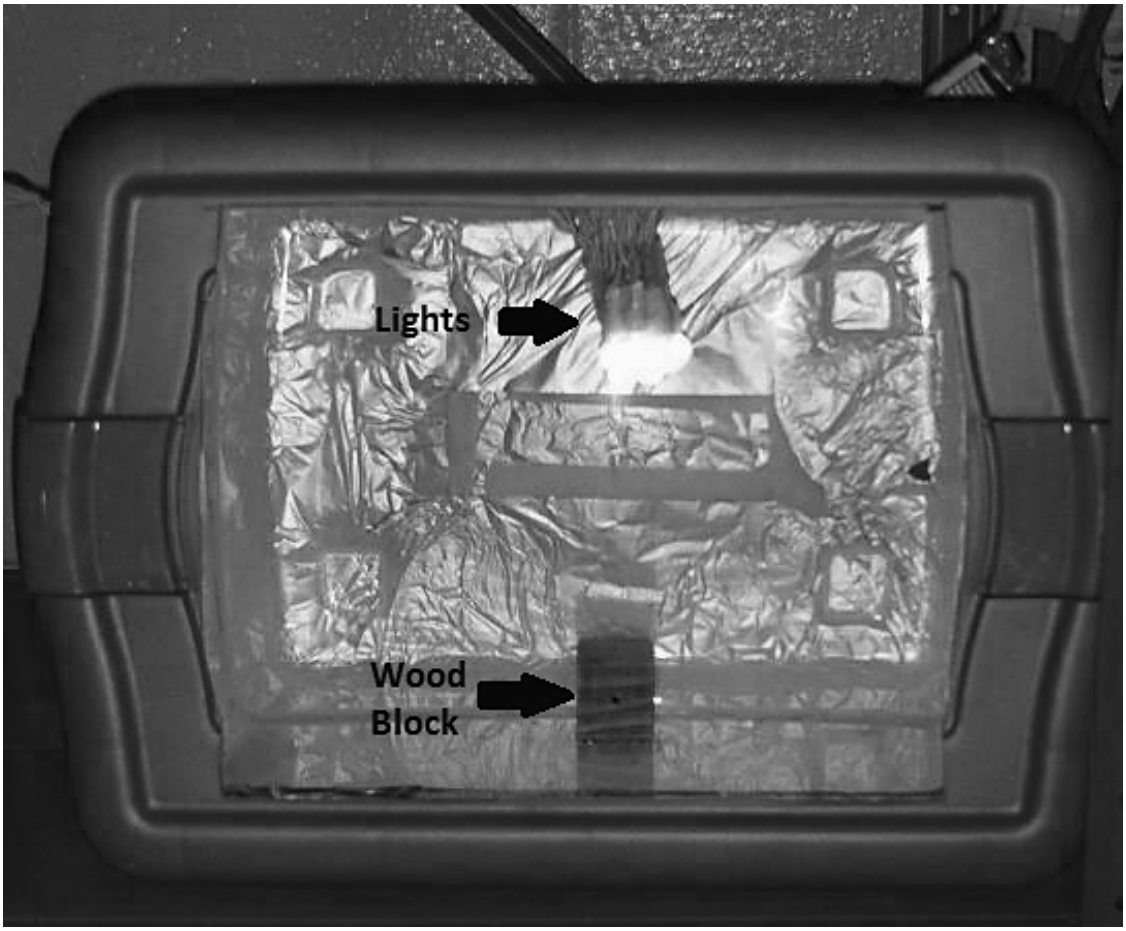

Fig. 1. Arena for light intensity experiments. Aluminum foil isolated the box and the LED lights were placed above the block of wood.

divided into 10-cm sections (excluding 1 cm at each end). Each section had 4 holes available for colonization. A board with the same dimensions and number of holes was used as a control and was completely exposed to the same light intensity. The 10-cm sections were lettered from A to J with sections A, B, C, D, and E inside the PVC pipe and sections F, G, H, I, and J outside (Table 1). This experimental protocol was replicated 4 times. Light measurements were made inside and outside the PVC pipe (Table 1). After dispersal flight season was over the number of colonized holes was counted for each 10-cm section based on the previously described colonization criteria.

Statistical Analysis

The data for the lit versus dark boxes were analyzed by a non-parametric Wilcoxon Matched Pairs test (SAS Institute 2003) to test whether the numbers of colonizations in the dark and lit areas were different. Colonization differences between the light intensities were tested with Student's *t*-test (SAS Institute 2003).

A Chi-squared test for independence (SAS Institute 2003) was used to test whether the distribution of colonizations was dependent on the light intensity. A Student's *t*-test for dependent samples was used to determine whether the differences between the numbers of colonizations in each 10-cm section were significant, (SAS Institute 2003).

Results

Light Intensity Experiments

Lit vs dark boxes. A total of 43 holes were colonized. There were significantly (t-test = 4.01E-05, $P < 0.0001$) more holes colonized in the lighted boxes (2.8 ± 0.3, mean ± SEM) than in the dark areas (0.8 ± 0.2 (mean ± SEM)).

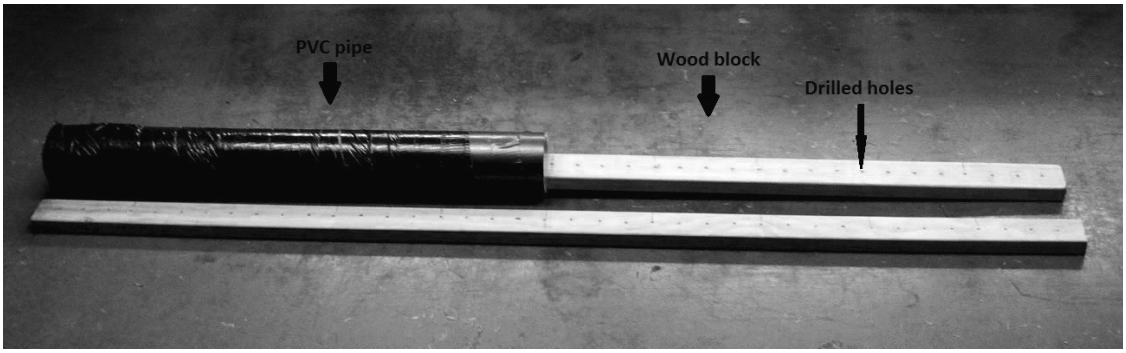

Fig. 2. Arena for negative phototaxis experiment. PVC pipe wrapped in black tape covered half of the wood block. Controls had no PVC pipe cover.

Light intensity. A total of 76 holes were colonized in the light intensity experiment. A higher number of colonizations was recorded in high light intensity boxes. There were significant (*t*-test, $P < 0.05$) differences in colonizations among the various light intensities (Fig. 3).

Negative Phototaxis Experiment

A total of 175 holes were colonized during the negative phototaxis experiment. There were no significant differences in number of colonizations between the 10-cm sections ($P \geq 0.29$) in the control boards. The distribution of number of colonizations was independent of the section where the colonizations occurred (Chi-squared = 5.9541, $P = 0.75$; n.s.) with no section of the control board preferred over any other section.

In the boards placed in the PVC pipes colonization distribution was not independent of the section where it occurred (Chi-squared = 33.3939, $P = 0.001$) with a significantly higher number of colonizations occurring in the dark sections (Fig. 3). Sections A, B, C, and D inside the PVC pipe had a significantly higher number of colonizations than sections G, H, I, and J outside the PVC pipe ($P < 0.05$). Section E (inside the PVC pipe) was not significantly different ($P \geq 0.16$) from sections G-J (outside the PVC pipe); and section F (outside the PVC pipe) was not significantly different ($P \geq 0.08$) from sections A-D (inside the PVC pipe) (Fig. 4).

Discussion

The results of the light versus dark experiment confirmed the hypothesis that colonization of the alates occur more frequently in lit areas. The termite *C. brevis* in South Florida flies mainly between 1:00 and 2:00 AM (unpublished data) when it is very dark. These results showed that colonization sites located in areas that are lit during the night may be more susceptible to infestation by *C. brevis* during dispersal flights. The presence of artificial lights may cause a change of behavior for some species of animals (Longcore & Rich 2004), but artificial lights may be beneficial to structure infesting termites like *C. brevis.* The attraction of these termites to artificial lights puts structures that have a continuous nighttime light source at higher risk of being infested than structures near by that are not lit.

The light intensity experiment showed that increasing light intensities increased the number of termite colonizations (Fig. 3). Minnick (1973) reported differences in the wavelength of light preferred by *C. brevis,* and Guerreiro et al. (2007) reported differences in color preference for the alates. Neither of them reported results based on light intensity. The fact that we found that 11 lux of light intensity was significantly different from 480 lux shows that the increase in light intensity caused an increase in colonization of the wood blocks by the termites. *Cryptotermes brevis* has

TABLE 1. DISTANCES OF 10-CM WOOD SECTIONS FROM THE CLOSED END OF 51-CM PVC PIPE AND THE LIGHT INTENSITY AT THE CENTER OF EACH SECTION.

	Inside of PVC pipe					Outside of PVC pipe				
Section	A	B	C	D	E	A	B	C	D	E
Distance from closed end of PVC pipe (cm)	10	20	30	40	50	60	70	80	90	100
Light Intensity (lux)	0.01	0.04	0.08	0.20	0.70	600	600	600	600	600

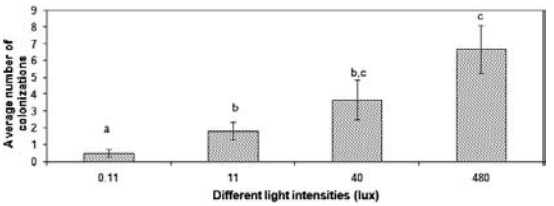

Fig. 3. Average number of holes colonized by *C. brevis* per light intensity (n = 43). Different letters represent significant differences at $P < 0.05$.

been observed to have as many as 2 founded colonies in a total of 22 nuptial chambers (Scheffrahn et al. 2001) which means that there is a 9% success rate of colonization. This shows that investing in preventing *C. brevis* alates from founding colonies is important because their success rate is high enough to make prevention methods necessary. The attraction to light by alates can be used to create light traps as a form of preventing infestations and re-infestations by *C. brevis* alates and as a means of partial control of this species. Such light traps inside structures that are already infested may minimize the spread of the infestation, and the results of this study showed that more intense the light used the more alates will be attracted; thereby making the use of light a possible alternative to use of chemicals for prevention. Further studies with different light wavelengths can help improve light traps as an alternative method to prevent the founding of colonies by *C. brevis*.

Previous experiments with *C. havilandi* (Sjöstedt) (Wilkinson 1962) and *C. brevis* (Minnick 1973) showed negative phototaxis in dealates of these species. After landing, the dealates search and colonize dark areas. Due to the nature of wood structures, it could be argued that this behavior is not really negative phototaxis but that because the cracks and holes are usually hidden and in dark areas the dealates end up colonizing there. If so, the behavior would be dependent not on light intensity but on the locations of a good places to colonize. However, the present study showed that negative phototaxis of dealates did occur. Independent distributions of colonizations occurred in the controls, but independent colonizations did not occur in the semi-shaded blocks; this confirmed the negative phototaxis hypothesis. The lighter segment of the wood block inserted in the PVC pipe had significantly less colonizations than the darker segment. However, the numbers of colonizations in section F (immediately outside the PVC pipe) were not significantly different from the numbers in the darker areas inside the PVC pipe.

Colonization of section F might have occurred because the termites colonizing that area had searched for colonizing sites in the dark area inside the PVC pipe where earlier colonizers had already taken all suitable sites, i.e., a site saturation. Also they may have landed near the PVC pipe cueing in on the darker area nearby and colonizing the sites near that dark area. However, further studies on this are needed to understand why the section closest to the PVC pipe on the light side was significantly more colonized than the section immediately inside the dark PVC pipe; where it would be expected considering the negative phototaxis behavior. One way to approach this might be to use a higher density of colonizing holes or fewer termites, so that the number of holes is not a limiting factor.

In termites, both the alates and dealates have well developed compound eyes (Light 1934b) and their behavior during flight season has shown that they do respond to light while in flight (positive phototaxis) and to have negative phototaxis after landing. Further studies on the behavior of *C. brevis* during the flight season can help improve different methods to prevent colonization and subsequent infestation by this species.

This study has shown that alates fly to and colonize more in higher light intensity areas, while the dealates have an opposite behavior colonizing more in darker areas. This knowledge is useful to improve light traps as a control method against colony foundation from *C. brevis*.

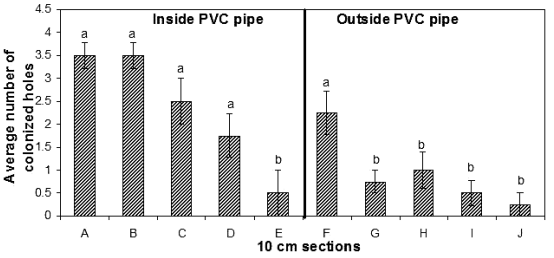

Fig. 4. Average number of *C. brevis* colonized holes per section (n = 175). Sections A, B, C, D, and E were inside the PVC pipe and sections F, G, H, I, and J were outside the PVC pipe. Bars with the same letter were not significantly different at $P < 0.05$.

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INFLUENCE OF METHOPRENE AND DIETARY PROTEIN ON MALE *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE) LIPID AND PROTEIN CONTENT

RUI PEREIRA[1,2], JOHN SIVINSKI[2], JEFFREY P. SHAPIRO[2] AND PETER E. A. TEAL[2]
[1]Entomology and Nematology Department, University of Florida, Gainesville, Florida

[2]Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida

ABSTRACT

Because both the application of a juvenile hormone analog, methoprene, and the addition of protein to the adult diet increased the sexual success of male Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), it was hypothesized that both might also impact male nutritional status. Total content of lipid and of protein in *A. suspensa* males were measured to discover if there was an effect of these treatments alone or in combination on the content of each of these subtstances. In the first 24 hours following adult emergence, 6 different treatments were applied (all possible combinations of methoprene in acetone solution or acetone alone, and protein-diet enrichment). Adult weight was determined for all treatments at 5, 10, 15, 20, 25, 30 and 35 d post-emergence. Dietary protein had a positive effect on the weight and total lipid and protein contents during the first 35 d of adult male life. There were minimal negative impacts from methoprene applications. Even though males were more active sexually, there was no significant change in weight or protein content during the study period. However, total lipid content decreased with age. The usefulness of methoprene to enhance the sexual performance of mass-reared tephritids destined for sterile release appears to outweigh any physiological costs/limitations that such treatment might confer.

Key Words: adult age, adult weight, Caribbean fruit fly, hydrolyzed yeast, juvenile hormone, sexual maturation

RESUMEN

Debido a que tanto la aplicación de un análogo de la hormona juvenil, metopreno, y la adición de proteínas a la dieta del adulto aumento el éxito sexual del macho de la mosca de la fruta de Caribe, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), se planteó la hipótesis de que ambos también podrían afectar el estatus nutricional masculino. Se midió el contenido total de lípidos y de proteínas en los machos de *A. suspensa* para descubrir si había un efecto de estos tratamientos solos o en combinación sobre el contenido de cada una de estas sustancias. En las primeras 24 horas después de la emergencia de adultos, se aplicaron 6 diferentes tratamientos (todas las combinaciones posibles de metopreno en solución de acetona en solución o solo acetona y con el enriquecimiento de proteínas en la dieta). Se determinó el peso adulto para todos los tratamientos a los 5, 10, 15, 20, 25, 30 y 35 días después de la emergencia. Las proteínas dietéticas tuvo un efecto positivo sobre el peso y el total de lípidos y proteínas durante los primeros 35 días de vida de los machos adultos. Hubo un mínimo de impactos negativos de las aplicaciones de metopreno. A pesar de que los machos eran más activos sexualmente, no hubo ningún cambio significativo en el peso o el contenido de proteína durante el período de estudio. Sin embargo, el contenido de lípidos totales disminuyeron con la edad. La utilidad de metopreno para mejorar el desempeño sexual de moscas tefrítidas criadas en masa destinadas para programas que liberan los machos estériles parece superar los costos fisiológicos y las limitaciones que dicho tratamiento puede conferir.

Topical application of the juvenile hormone analog, methoprene, on the dorsal surface of adult male Caribbean fruit flies, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), increases male sexual success (Pereira et al. 2009), apparently because it increases the production of male sex pheromone. In addition it accelerates sexual maturation by several days (Teal & Gomez-Simuta 2002). The addition of protein to the adult diet has a similar effect on sexual performance, but the underlying cause(s) has yet to be investigated experimentally. When methoprene and protein are combined there is an additive increase in male sexual performance, and males are ~ 4 times more likely to mate than males not exposed to methoprene nor given access to protein (Pereira et al. 2010).

Presumably, increased pheromone production occurring at an earlier age, as well as accelerated sexual activity, is energetically demanding and

may affect the balance of the metabolic compounds (Teal et al. 2000). One hypothesis frequently mentioned for the relatively long, sometimes more than 2 weeks, pre-reproductive period found in many adult frugivorous Tephritidae is that the time is used to acquire resources needed for reproduction (Sivinski et al. 2000). Decreases in resource foraging time due to accelerated sexual maturation and increases in body nutrients resource expenditure might be expected to result in substantive changes in a fruit fly's nutritional status that could affect longevity and long-term sexual performance. We further supposed that these expenses could be particularly difficult to incur in the absence of a protein enriched adult diet.

As a result, we hypothesized that the nutritional effects of methoprene and diet, both alone and in combination, might have an important effect on sexual performance of male flies reared for sterile insect technique (SIT) programs. It is from this perspective that we address the following questions: (1) What influence does methoprene treatment have on male *A. suspensa* weight and lipid/protein nutrient stores over a period of 35 d; ~ 33% of males survive to this age under laboratory conditions (Sivinski 1993); and (2) do protein enriched and protein deprived diets affect male weight and lipid/protein content, and is there an interaction between diet and methoprene treatment?

Considering the potential importance of adult diet to SIT, relatively little nutritional work has been done with *A. suspensa*. The rate and the temporal patterns of consumption of carbohydrates, proteins, and amino acids by adults (Sharp & Chambers 1984; Landolt & Davis-Hernandez 1993), as well as the role of food availability and quality on male pheromone production have been studied to some extent (Epsky & Heath 1993; Teal et al. 2000; Teal & Gomez-Simuta 2002). A positive influence of sucrose on male pheromone calling (Landolt & Sivinski 1992) and survival (Teal et al. 2004) has been reported. However, no work has been done specifically on the nutritional impact of protein incorporation into adult diets. This is the first study of male tephritid nutritional balance challenged by artificially elevated "juvenile hormone" titers to improve sexual performance.

Material and Methods

Insects

The Caribbean fruit flies used in this study were obtained from laboratory colony at the Center for Medical, Agricultural and Veterinary Entomology (CMAVE) USDA-ARS, at Gainesville, FL. At the time of the study, the colony was 3 years old and had been produced according to the conventional mass rearing protocols (FDACS 1995). Pupae were collected from the colony and sorted by size in a pupal sorting machine (FAO/IAEA/USDA 2003). Pupal size was homogenized to reduce male size and weight variability; large males have been shown to have a sexual advantage over smaller males (Burk & Webb 1983; Burk 1984; Webb et al. 1984; Sivinski & Dodson 1992; Sivinski 1993). Males used for this experiment were from pupal size class of 10.9 0.71 mg (n = 30) in weight. This is considered a mid-size pupal weight for field collected *A. suspensa* males in infested guava fruits (Hendrichs 1986). Throughout the experiment flies were maintained in a laboratory room with a photoperiod of 13L:11D (light from 0700 to 2000 h), a light intensity of 550 50 lux, a temperature of 25 1°C and a relative humidity of 55 5%.

Diet and Hormonal Treatments

Following emergence, males were subjected to 1 of the following 6 diet and hormonal treatments:

- M^+P^+: topical methoprene in acetone; access to sugar and hydrolyzed yeast
- M^+P^-: topical methoprene in acetone; access to sugar
- M^-P^+: topical acetone; access to sugar and hydrolyzed yeast
- M^-P^-: topical acetone; access to sugar
- P^+: no topical application; access to sugar and hydrolyzed yeast
- P^-: no topical application; access to sugar.

Methoprene (5 µg in 1 µL acetone) was applied topically within the first 24h after emergence. Controls consisted of application of 1 µL acetone only (M^-) or no topical application (P^+ and P^-). In order to conduct the topical application, males were immobilized in a net bag, and the solution was applied through the mesh on the dorsal surface of the thorax from a micro-pipette. No anaesthesia was used to immobilize the flies. Precautions were taken to avoid cross contaminations among experimental subjects. Male flies exposed to the different treatments were maintained in screen cages (30 cm by 30 cm by 30 cm), with a maximum male density of 200 flies/cage. Flies were allowed free access to food (according to above treatments) and water. In protein-deprived treatments (P^-) flies were only provided with sugar. Protein was provided to the flies in the form of hydrolyzed yeast mixed with sugar (1:3 parts, respectively). This mixture is considered a high quality diet for *Anastrepha* species (Jácome et al. 1995; Aluja et al. 2001).

Experimental cages were maintained for up to 35 d. For weight and chemical analysis, male flies

were sampled at the following ages: 5, 10, 15, 20, 25, 30, and 35 d of adult age. For each age and treatment, 5 flies were randomly sampled and stored at -84ºC until used for analysis. In order to obtain the base line information after emergence, 5 newly emerged (without access to any food or water) and untreated flies were collected as well. Because lipid content in *Ceratitis capitata* (Wied.) has been found to vary according to the time of the day, due to the different activities in which males were engaged (Warburg & Yuval 1997), we sampled males at the same time each day (16:30 h, immediately before the beginning of the calling period). Flies were weighed individually prior to homogenization for lipid and protein determination.

Quantification of Lipids and Proteins

Individual male flies were homogenized in a solution of PBS buffer at pH 7.25 (8.77 g of 0.15 M NaCl and 7.1 g of 50 mM Na_2HPO_4 in 1 L of water). The homogenate was then brought up to 4.0 mL with PBS. Lipids were extracted from the homogenate by adding 40 mg of Na_2SO_4 to half of the initial volume, and 3.75 mL of chloroform: methanol (1:2) (Bligh & Dyer 1959) was used to separate polar and non-polar constituents of the homogenate. An additional 1.25 mL of chloroform was added to the homogenate and vortexed for 4 min at 4,000 rpm. The non-polar chloroform phase was collected. The remaining solution was re-extracted with chloroform (1.875 mL), vortexed and collected. Chloroform was evaporated in a Speed Vac device (Thermo Savant, San Jose, CA).

Lipid contents were determined by the vanillin reagent method (Van Handel 1985; Warburg & Yuval 1996), with triolein being used as a standard. Quantification of lipids was done by reacting 10 µL of sample with 190 µL of vanillin reagent. Lipid content was determined colorimetrically at 530 nm in a spectrophotometer (Bio-tek Instruments, Winooski, VT).

Protein determination was done according the Pierce BCA protein assay (Pierce, Rockford, IL). One mL of the polar fraction of the homogenate was centrifuged for 1 min at 14,000 rpm. Half the volume was mixed with 100 µL of sodium deoxychoate reagent (0.15 w/v) and 100 µL of 72% (w/v) tricloroacetic acid (TCA) to precipitate the proteins. After incubation at room temperature for 10 min and centrifugation for 10 min at 14,000 rpm the supernatant was discarded. The precipitate was dissolved and reacted with 50 µL of 5% (w/v) sodium dodecyl sulfate (SDS) and 1 mL of Pierce micro BCA™ protein assay reagent (Pierce, 1999). After incubation in a water bath at 37ºC for 30 min, proteins in samples and standards were determined colorimetrically at 562 nm in a spectrophotometer (Bio-Tek Instruments, Winooski, VT).

Statistical Analyses

Data were analyzed by two-way analysis of variance (ANOVA) to detect the interactions between age and treatment for the parameters studied, independently (weight, lipid content, and protein content). These analyses were followed by an ANOVA to detect differences between means in the treatments. Tukey's test was used to separate means (Ott & Longnecker 2001). Statistical analyses were performed with R software (version 2.1.0, www.r-project.org).

Results

Male weight

Average adult weight varied between 5.8 mg and 11.6 mg (Fig. 1). There was no interaction between treatment and adult age ($F_{35,192} = 1.16$, $P = 0.256$), and no effect of age ($F_{7,192} = 1.59$, $P = 0.140$; Table 1) on adult weight. There was, however, a significant effect of treatment ($F_{5,192} = 24.46$, $P < 0.05$). Protein-fed males generally had significantly higher fresh weights than sugar fed males (Fig. 1, Table 2).

Lipid content

Significant effects of treatment ($F_{5,192} = 131.37$, $P < 0.001$), adult age ($F_{7,192} = 83.14$, $P < 0.001$), and the interaction of adult age and treatment ($F_{35,192} = 6.34$, $P < 0.001$) were found. Male lipid content per treatment per age (Fig. 2) differed both among ages and for different treatments (Table 1) and among treatments for different ages (Table 2). In protein-deprived males, lipid contents dropped at 5 d after emergence, while protein-fed males maintained stable lipid levels during the first 10 d of adult life (Fig. 2). Afterwards, lipids dropped to lower levels. Methoprene treatment did not affect lipid levels in either protein-fed or protein-deprived male flies.

Protein Content

Significant effects of treatment ($F_{5,192} = 44.63$, $P < 0.001$), adult age ($F_{7,192} = 15.00$, $P < 0.05$), and the interaction of adult age and treatment ($F_{35,192} = 4.77$, $P < 0.001$) were found. Significant differences in protein content among the different ages were found within each treatment except for treatment M^-P^+ (Fig. 3, Table 1), and among treatments at all ages (Table 2). Protein-fed males maintained higher protein levels than protein-deprived males (Fig. 3). In protein-fed males, protein content steadily increased through time, while in protein-deprived males, protein levels declined. Methoprene did not affect the level of protein in either protein-fed or protein-deprived males.

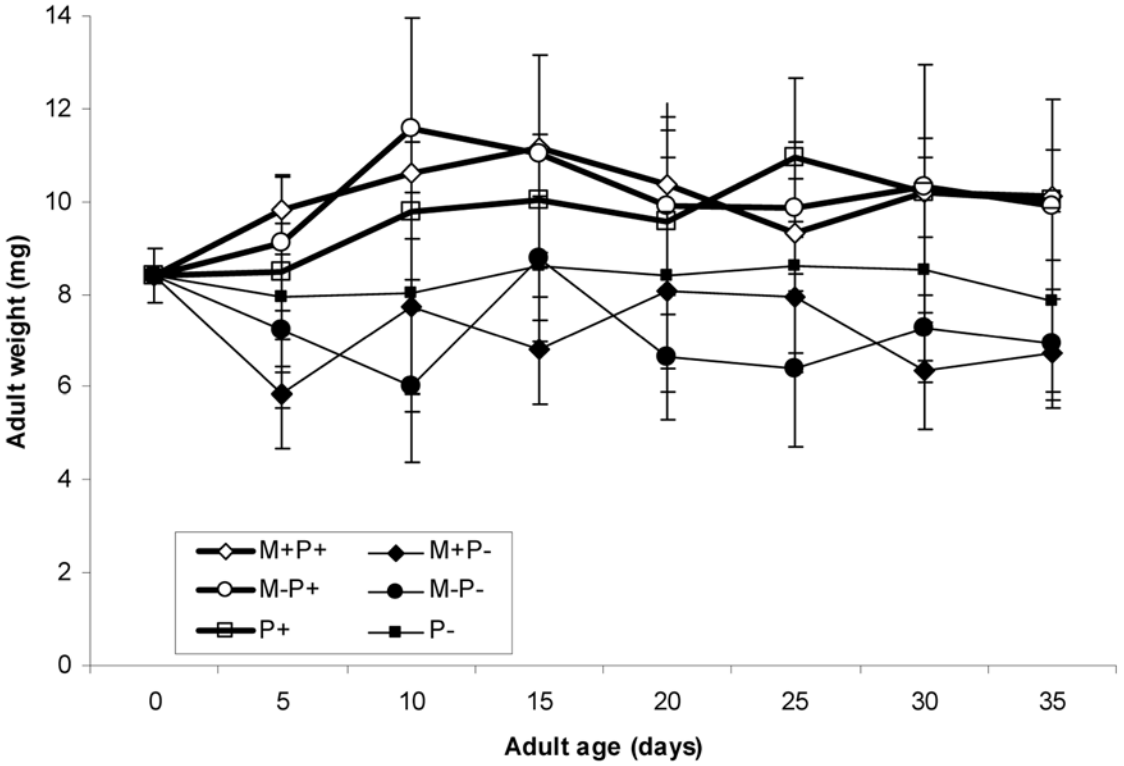

Fig 1. Mean (±SD) adult weight (n = 5) of male Caribbean fruit flies at different adult ages among the 6 treatments featuring methoprene (M) and protein (P) and their various combinations.

Discussion

In male *A. suspensa* there was a clear effect of a protein-enriched diet on weight, total lipid, and total protein content over the first 35 d of adult life. In contrast, there was no effect of methoprene or acetone application on the studied parameters. Regardless of diet type, weight and total protein content were relatively stable during adult life. In contrast, total lipid content steadily decreased with age. This decline began later, however, in flies fed a protein-enriched diet (10 d after emergence).

In all the treatments, consumption of protein resulted in insects able to regulate their weight, protein levels, and lipid content at higher level than insects without a protein food source. This is broadly consistent with what has been observed in Tephritidae in general and other *Anastrepha* spp. in particular (Aluja et al. 2001). In nature, adult tephritids feed on a variety of carbohydrates and proteins derived from fruit juices, honeydew, and bird feces (Hendrichs et al. 1991; Warburg & Yuval 1997; Yuval & Hendrichs 2000). Protein enhances reproductive performance in *C.*

Table 1. Analysis of variance (ANOVA) for male Caribbean fruit fly weight, total lipids, and total proteins among different ages in 6 different treatments featuring methoprene (M) and protein (P) and their various combinations (NS, non significant differences, $P > 0.05$; * $0.01 < P < 0.05$; *** $P < 0.001$).

Treatments	Male weight	Total lipids	Total proteins
M+P+	$F_{7,32} = 1.1559$ (ns)	$F_{7,32} = 7.5932$ ***	$F_{7,32} = 3.2711$ *
M+P-	$F_{7,32} = 1.9367$ (ns)	$F_{7,32} = 32.76$ ***	$F_{7,32} = 8.6007$ ***
M-P+	$F_{7,32} = 1.8041$ (ns)	$F_{7,32} = 11.124$ ***	$F_{7,32} = 2.0186$ (ns)
M-P-	$F_{7,32} = 2.0277$ (ns)	$F_{7,32} = 35.906$ ***	$F_{7,32} = 7.7853$ ***
P+	$F_{7,32} = 1.0071$ (ns)	$F_{7,32} = 12.504$ ***	$F_{7,32} = 2.9685$ *
P-	$F_{7,32} = 0.1291$ (ns)	$F_{7,32} = 20.805$ ***	$F_{7,32} = 4.8732$ ***

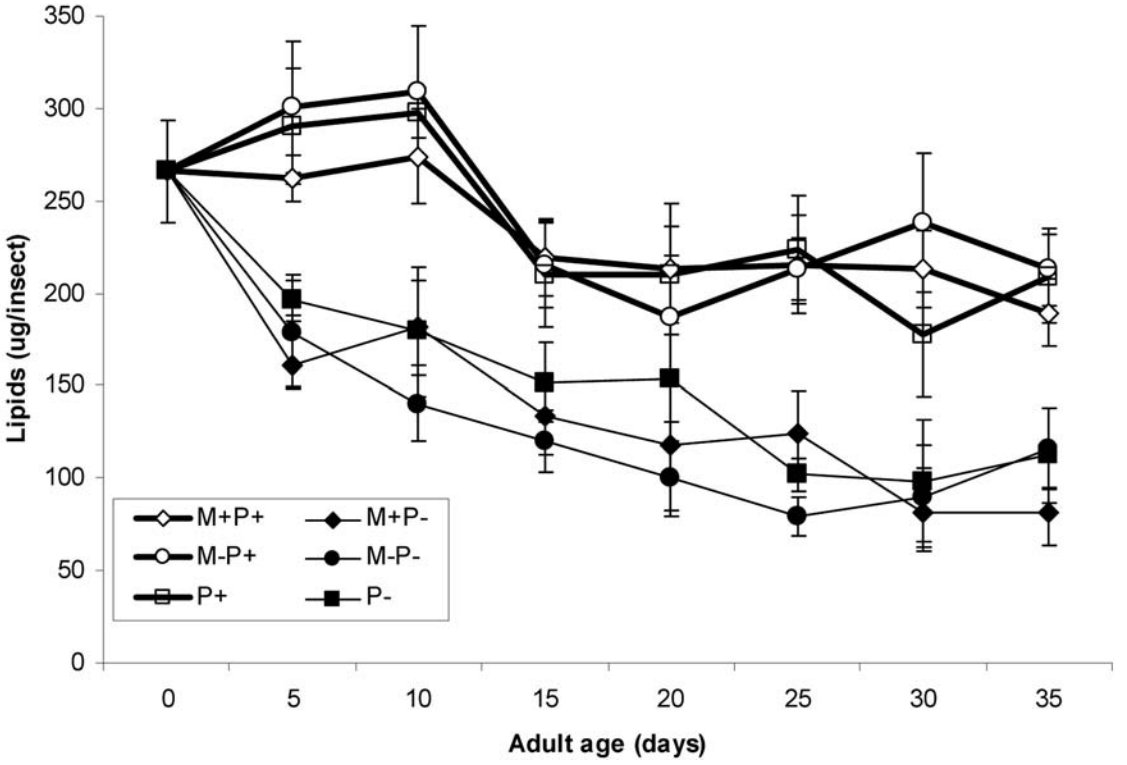

Fig. 2. Mean (±SD) total lipid content (n = 5) of male Caribbean fruit flies at different adult ages among the 6 treatments featuring methoprene (M) and protein (P) and their various combinations.

capitata (Warburg & Yuval 1997; Kaspi et al. 2000; Shelly & Kennelly 2002; Shelly et al. 2002; Yuval et al. 2002), and protein-fed males start to call earlier in life (Papadopoulos et al. 1998). Protein-fed males are more competitive in terms of post copulatory sexual selection as well (Taylor & Yuval 1999). In *Bactrocera dorsalis* (Hendel), incorporation of protein into adult diet significantly increases survival and mating success (Shelly el al. 2005). Among *Anastrepha* species, Aluja et al. (2001) evaluated the effects of different adult nutrients, including protein and sugar, on male sexual performance in adults of 4 species, (*A. ludens* (Loew), *A. obliqua* (Macquart), *A. serpentina* (Wied.), *A. striata* Schiner). Overall, protein-fed males were more sexually successful than protein-deprived, except for *A. ludens* where no differences were found. Neither did male diet influence *A. ludens* female reproductive potential following trophalaxis (Mangan 2003). However, in a more recent study protein did improve *A. ludens* sexual performance (Aluja et al. 2008).

Thus, perhaps not surprisingly, protein-enhanced diets typically, but not always, enhance

TABLE 2. ANALYSIS OF VARIANCE (ANOVA) FOR MALE CARIBBEAN FRUIT FLY WEIGHT, TOTAL LIPIDS, AND TOTAL PROTEINS AMONG TREATMENTS FOR DIFFERENT AGES FEATURING JUVENILE HORMONE (JH) AND PROTEIN (P) AND THEIR VARIOUS COMBINATIONS (* $0.01 < P < 0.05$; ** $0.001 < P < 0.01$; *** $P < 0.001$).

Adult age (days)	Male weight	Total lipids	Total proteins
5	$F_{5,24} = 4.546$ **	$F_{5,24} = 26.285$ ***	$F_{5,24} = 3.5202$ *
10	$F_{5,24} = 4.566$ **	$F_{5,24} = 28.881$ ***	$F_{5,24} = 26.629$ ***
15	$F_{5,24} = 5.521$ **	$F_{5,24} = 12.548$ ***	$F_{5,24} = 10.277$ ***
20	$F_{5,24} = 2.624$ *	$F_{5,24} = 13.873$ ***	$F_{5,24} = 8.9948$ ***
25	$F_{5,24} = 4.370$ **	$F_{5,24} = 50.275$ ***	$F_{5,24} = 4.2223$ **
30	$F_{5,24} = 4.429$ **	$F_{5,24} = 21.339$ ***	$F_{5,24} = 8.9493$ ***
35	$F_{5,24} = 5.120$ **	$F_{5,24} = 36.197$ ***	$F_{5,24} = 9.5048$ ***

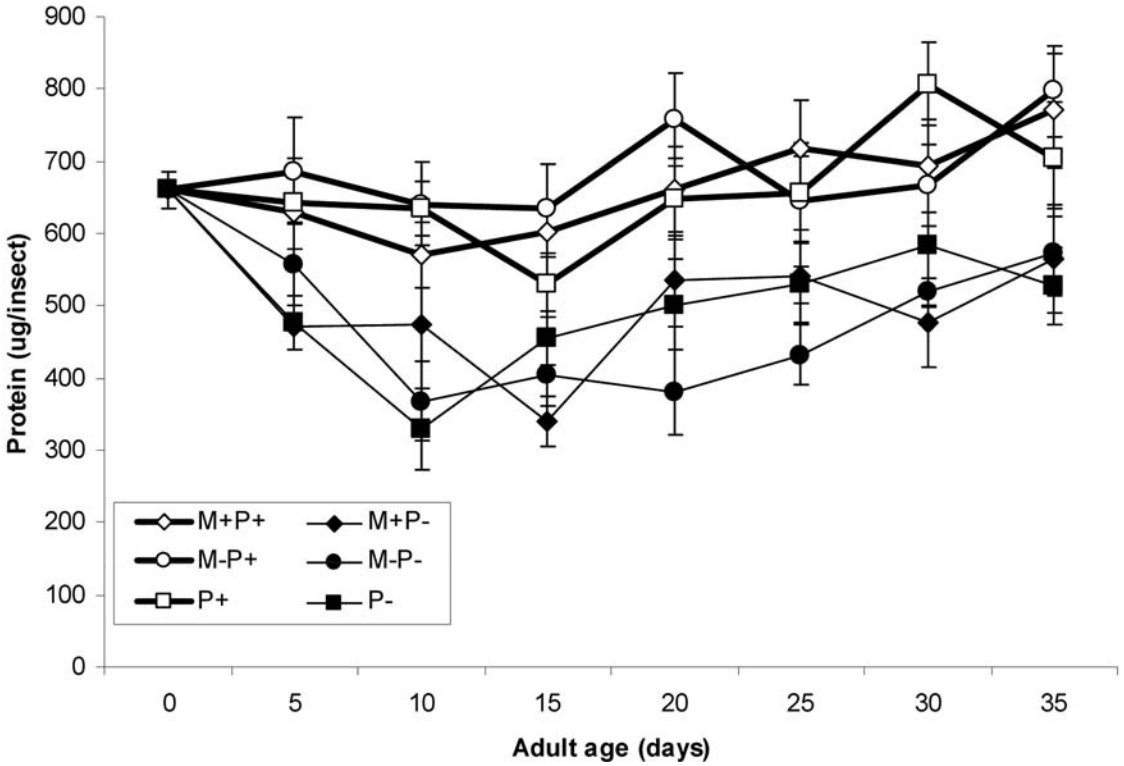

Fig. 3. Mean (±SD) total protein content (n = 5) of male Caribbean fruit flies at different adult ages among the 6 treatments featuring methoprene (M) and protein (P) and their various combinations.

sexual success. However, there are perhaps revealing differences in physiology and foraging tactics for food and mates among males of different species with different diets and activities. For instance, unlike *A. suspensa*, protein-fed *C. capitata* males have lower lipid content than those that are protein-deprived (Kaspi et al. 2000). In addition, while lekking males are heavier and contain significantly more protein and sugar than resting males, they do not contain more lipids (Yuval et al. 1998). Lipids (fatty acids, phospholipids, and sterols) have a nutritional role distinct from carbohydrates and proteins. Yuval et al. (1994) described lipids metaphorically as an energetic trust fund, whereas carbohydrates are comparable to a readily accessible cash account. Perhaps the difference between *A. suspensa* and *C. capitata* in their use of protein for lipogenesis reflects a difference in energy use patterns, with *A. suspensa* putting more reserves in "long-term" accounts for future use. This in turn might reflect more predictably encountered food sources for *C. capitata* or a lower daily chance of mortality for *A. suspensa* that leads in turn to "planning" for the future.

Many kinds of fatty acids and phospholipids are synthesized by insects, but all insects require sterols in their diet (Chapman 1998). Reduction of total lipid content with age in *A. suspensa* can be the result of somatic activities, since lipids represent stored energy, even if some restoration of lipid reserves occurs by lipogenesis (Warburg & Yuval 1996). In male *A. suspensa*, at least in the first 10 days of adult life of protein-fed males, there is a slight increase in lipid content. The same phenomenon occurs in *C. capitata* (Warburg & Yuval 1996) and *A. serpentina* (Jácome et al. 1995). Total lipid content declined following male *A. suspensa* sexual maturation. Sharp decreases indicate that males started to utilize their metabolic reserves, and this seems likely to correspond to the energetic requirements of any number of sexual and agonistic behaviors and processes (e.g., Sivinski et al. 2000). One of these potential expenditures that can be indirectly examined with the present data is pheromone production.

Nestel et al. (1986) suggested that lipid reserves in male *C. capitata* may play an important role in the regulation and production of sex pheromone. Nestel et al. (2005) found a decrease in lipid body content after sexual maturation (as the present data reveal for *A. suspensa*), but later on the content displayed a harmonic pattern where total lipid content increased and decreased at a periodicity of 10 days. In *A. suspensa*, male pheromone production increases when methoprene is

applied (Teal et al. 2000). However, we found that application of methoprene did not affect lipid content of flies maintained on different diet treatments.

The differences in total protein content between protein-fed and protein-deprived males may be influenced by ingested protein in the gut. However, the gradual increase in total protein content over time in both protein-deprived (after 10 d as adult) and protein-fed males is both difficult to explain and inconsistent with artificial-diet protein alone accounting for the difference. Tephritids are known to feed on animal excrement (Prokopy et al. 1993; Epsky et al. 1997), and perhaps the consumption of bacteria from their own feces or bacteria growing on dead flies or on food sources, inadvertently provided them with a protein source. Regardless of the origin of this additional protein, the difference in protein contents on the different diets suggests it was not sufficient to completely satisfy nutritional requirements.

The findings of this study have implications for SIT programs. Among the most important is that while the addition of methoprene has male sexual advantages it appears to have no immediate nutritional detriments. Thus it is a relatively "cost-free" means of improving the performance of mass-reared and released flies. The incorporation of dietary protein has a positive effect on adult weight and lipid and protein content all of which are plausibly related to performance as well (Yuval et al. 1998). Due to these effects, the incorporation of protein in adult diet for SIT programs is also recommended.

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CLARIFICATION OF THE TAXONOMIC STATUS OF *CUCUJUS CLAVIPES* WITH DESCRIPTIONS OF THE LARVAE OF *C. C. CLAVIPES* AND *C. C. PUNICEUS* (COLEOPTERA: CUCUJIDAE)

JONGEUN LEE[1] AND MICHAEL C. THOMAS[2]
[1]Department of Biological Science, College of Natural Sciences, Andong National University, Andong, 760749 Korea
E-mail: jelee@andong.ac.kr

[2]Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Florida 326147100, USA
E-mail: michael.thomas@freshfromflorida.com

ABSTRACT

The larvae of *Cucujus c. clavipes* Fabricius and *C. c. puniceus* Mannerheim are fully described and illustrated in detail for the first time. Based on larval and adult morphology the present recognition of two subspecies is maintained.

Key Words: taxonomy, *Cucujus*, larva, North America

RESUMEN

Por primera vez se describen e ilustran las larvas de *Cucujus c. clavipes* Fabricius y *C. c. puniceus* Mannerheim. Basándose en la morfología larval, se acepta el reconocimiento de las dos subespecies.

Translation provided by the authors.

Cucujus clavipes Fabricius (1781) was described from "America boreali." *Cucujus puniceus* Mannerheim (1843) was described from "insula Sitkha", now Baranof Island in southeastern Alaska and the site of the modern city of Sitka. Both descriptions are of adults only, are based on the adult stage and are brief and relatively uninformative. Of *C. clavipes*, Fabricius wrote: "*ruber, thorace fuscato, femoribus clavatis rufis*" (red, thorax dark, femora clavate, red); of *C. puniceus*, Mannerheim wrote: "*elongatus, depressus, laete sanguineus, antennis nigrofuscis, pectore abdomineque rufoferrugineis, thorax subrotundato, lateribus leviter denticulato, supra obsolete bisulcato*" (Elongate, depressed, rich red, antennae nigro-fuscus, abdomen rufo-ferrugineous; thorax rounded, laterally weakly denticulate, above obsoletely bisulcate).

LeConte (1854, 1861, 1863) consistently treated *C. puniceus* as a valid species. Casey (1884) reduced it to a variety of *C. clavipes* and said of it: "The body is more elongated, and usually of a brighter color. The first joint of the antennae is usually of a dark testaceous, while in *clavipes* it is black. The antennae are slightly longer, and the neck slightly narrower in *puniceus*." Leng (1920) treated *C. puniceus* as either a variety or subspecies of *C. clavipes* [In the Leng Catalogue, a lettered taxon following a numbered species name could be ". . . variety, subspecies, race, etc." (Leng 1920: v)] and Hetschko (1930) followed Casey in treating it as a variety of *C. clavipes*. Schaeffer (1931) described *Cucujus clavipes subnitens* as a variety from Arizona and Utah. Thomas (1993) in a list of Nearctic Cucujidae treated *C. puniceus* as a subspecies of *C. clavipes* and Schaeffer's taxon as a variety as previously described.

In an effort to resolve the status of *Cucujus clavipes* we examined adults and larvae from both eastern and western North America.

Larvae

Larvae of Japanese *Cucujus coccinatus* Lewis were described and illustrated by Hayashi (1980, 1986) and the larva of *C. mniszechi* Grouvelle was described by Lee and Sato (2007).

Larvae of *C. clavipes* Fabricius were briefly and partially illustrated (head and mandible) by Bøving and Craighead (1931) and Klausnitzer (2001). Peterson (1951) provided extensive illustrations of *C. clavipes* but provided only a brief description. In neither case was the origin of the specimen illustrated provided. Lawrence (1991) re-used Peterson's illustrations and added scanning electron micrographs of mouthparts of a specimen from California. The larva of both North American subspecies of *C. clavipes* Fabricius are fully illustrated and described for the first time in the present paper. The larva of *C. clavipes* is similar to *C. mniszechi* (Lee and Sato 2007), but can be distinguished by absence of a distinct epicranial stem and presence of a sharp prostheca. In *C. mniszechi* the epicranial stem is present and the prostheca is blunt.

Larvae of *C. clavipes* are reported to be predaceous (Smith and Sears 1982) or facultatively predaceous (Lawrence 1991). Their extreme cold tolerance, which increases with increasing latitude, has been extensively studied (Sformo et al. 2010, and references therein).

Materials and Methods

The larvae were preserved in 70% ethyl alcohol, cleared in 10% KOH solution for 1 hour, rinsed in water, and dissected under a stereoscopic microscope (Leica® MS5). Slide mounting procedures were carried out according to LeSage (1984), and the larval terminology follows Lawrence (1991). Specimens were measured with an ocular micrometer and the measurements were transferred to graph paper. The illustrations were then sketched in pencil, the sketches inked, and assembled into plates, which were optically scanned and cleaned up in a graphics editor. Specimens examined are deposited in the Florida State Collection of Arthropods (FSCA) and the University of Alberta E. H. Strickland Entomological Museum (UASM).

Descriptions

Cucujus clavipes clavipes Fabricius, 1781 (Fig. 1 AJ)

Diagnosis**:** See this section under *C. c. puniceus*.

Material examined**:** 37 total from: INDIANA**:** Morgan Co.: Martinsville (10); Tippecanoe Co. (1); OHIO**:** Champaign Co. (1); Columbiana Co. (1); WISCONSIN**:** Calumet Co.: Forest Junction (1); Ingham Co.: Dansville State Game Area (1); Shawano Co.: Shawano (16); Shawnee Co.: Tilleda (6) (all deposited in the FSCA).

Description: Late instar (Fig. 1A). Body 22.0 - 26.0 mm long, elongate, subparallel, strongly dorsoventrally flattened with strongly forked median process at abdominal apex (Fig. 1A). Head and abdominal segment 8 moderately sclerotized, yellowishbrown to brown, tergite of abdominal segment 9 strongly sclerotized and brown.

Head (Fig. 1B): prognathous, strongly transverse and dorsoventrally flattened. Lateral margin rounded. Median endocarina absent; epicranial stem present but very short; frontal sutures lyriform, strongly curved; bases contiguous. Stemmata well-developed, 6 on each side of head (Peterson (1951) reported 5 on each side; we count 6 but 1 is small and difficult to see). Frontoclypeal suture absent. Fronotoclypeal region with 3 long setae anterior to angles of frontal arms, 1 pair anterior to the apex of the frontal arms on each side of the head, 1 pair medially between the frontal arms, and 1 pair at the apex of the frontoclypeal region near the clypeolabral suture. Clypeolabral suture complete. Labrum (Fig. 1G) free, with 3 pairs of setae and anterior border fimbriate. Epipharynx glabrous medially, with 5 anterior setae on each side. Antennae 3segmented, ratio of lengths of antennomeres 1, 2, and 3 about 1.0: 1.2: 1.0. Mandibles (Fig. 1H) heavily sclerotized, symmetrical, apices bidentate with a smaller subapical tooth; with 2 dorsolateral mandibular setae; prostheca acuminate, spinelike, with a broad base; mola with numerous setae medially and penicillus posteriorly (The scanning electron micrographs in Lawrence (1991: 464, figs. 34.528, c-f) show a conspicuous patch of microtrichia on both the dorsal and ventral surfaces of the mandible near the base; these are virtually invisible in liquid and are not illustrated here). Maxilla (Fig. 1E) with cardo triangular, divided by an internal ridge, basal portion trapezoidal, 1 moderately elongate seta near latero-basal margin; stipes elongate; mala falciform with 5 apical spines and a medial brush composed of several thick setae; maxillary palpus 3segmented, segment 1 asetose, segment 2 with 2 setae, segment 3 with 4 minute apical setae. Labium (Fig. 1F) with conspicuous mentum and prementum; mentum about as long as wide, with 2 pairs of setae and prementum with 1 pair of setae and 1 pair of sensilla; ligula rounded anteriorly, 1 pair of setae and microtrichia anteriorly; labial palpi 2-segmented and widely separated at base.

Thorax: Meso and metathorax tergites, and abdominal tergites and ventrites 18 each with 1 transverse ridge near anterior margin, ridge on ventral surface of abdominal segment 1 lightly sclerotized. Prothorax subquadrate, transverse, 0.5 times as long as wide, sides slightly curved, dorsal surface smooth; prosternal surface smooth, 3 setae (1 elongate) at anterolateral angles and 2 short setae at posterolateral angles; prosternum trapezoidal, sides oblique, posterior margin straight, pair of medial setae present posterior to posterior margin of presternum. Meso- and metathorax transverse, both 0.5 times as long as wide, sides curved, dorsal surface of both tergites smooth with 3 short setae at anterolateral angles and 2 short setae at posterolateral angles; both sterna without well-defined subdivisions, each smooth with a pair of discal setae near anterior margin; spiracular sclerite projecting strongly from lateral margin, spiracles (Fig. 1C) annular and angled posterolaterally. Legs (Fig. 1D) moderately long, 5segmented; claw falciform, large.

Abdomen: Segments 17 transverse, tergite surface smooth with 2 setae anterior to spiracles and 2 setae posterior to spiracles; ventrite surface with 3 setae, 2 anteriorly and 1 posteriorly. Segment 8 slightly enlarged, tergite (Fig. 1I) with a stout spicule at each posterolateral margin, posterolateral angles with 4 long and 4 short setae, 3 pairs of short setae anteromedially; sternite (Fig. 1J) with 7 pairs of setae and with large stout pro-

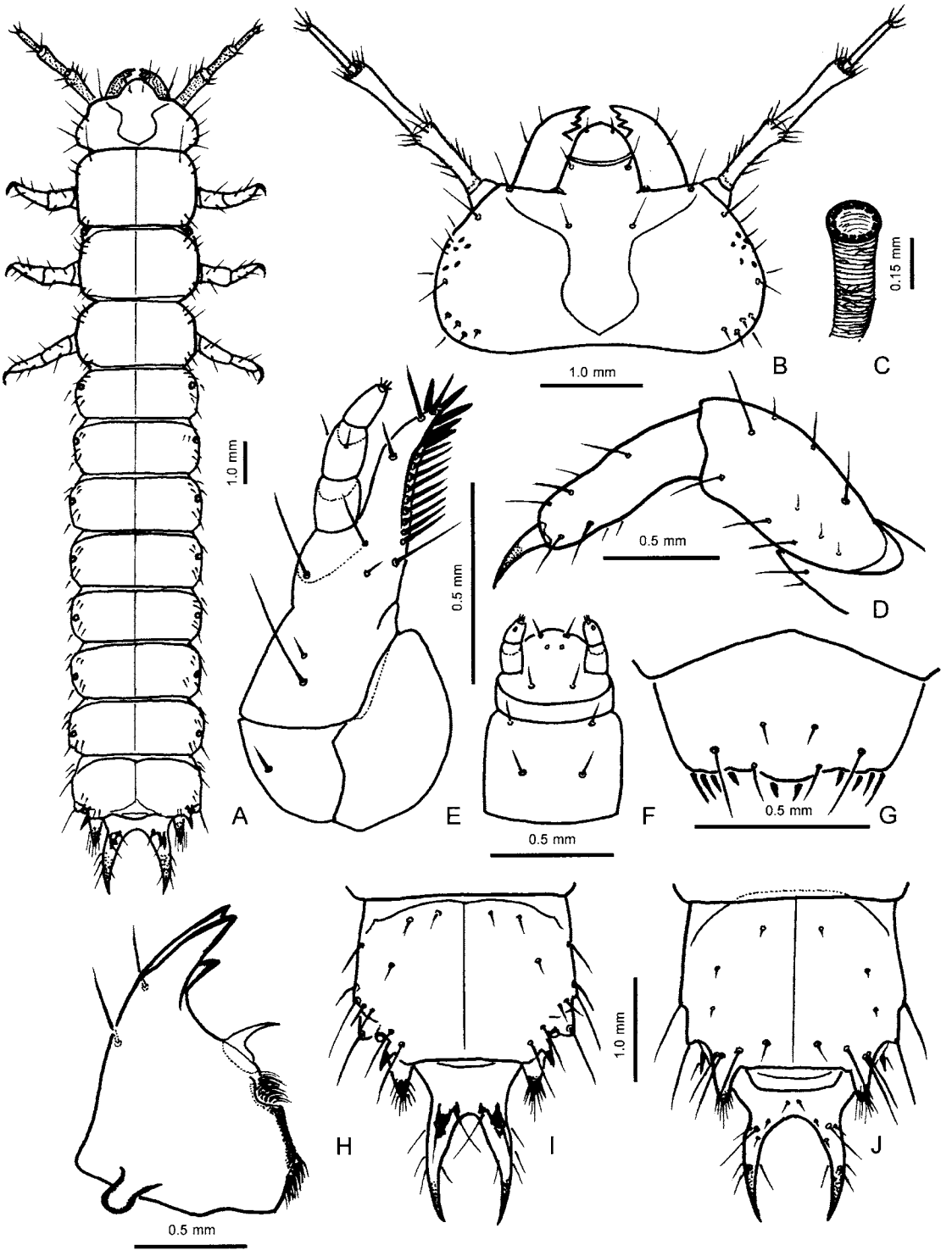

Fig. 1. Larva of *Cucujus c. clavipes*. A, habitus, dorsal view; B, head, dorsal view; C, A7 spiracle, D, prothoracic leg; E, left maxilla, dorsal view; F, labium, ventral view; G, labrum, dorsal view; H, left mandible, dorsal view; I, abdominal segments 89, dorsal view; J, same, ventral view.

cess posteriorly with many minute setae apically. Tergum 9 with a basally forked process, directed dorsad; base of process with a pair of short, apically forked processes, 1 short seta at apex of forked process; anterior margin with laterally curved processes projecting from tergum 8; ventrite 9 reduced and concealed from above.

Cucujus clavipes puniceus Mannerheim
(Fig. 2 AJ)

Diagnosis. The larva of this species is very similar to that of *Cucujus c. clavipes*, but can be distinguished by the ratio of the 8th abdominal segment length vs length of the forked process (4:3 in *C. c. puniceus*; 1:1 in *C. c. Clavipes*), and the ratio of the 8th abdominal segment width vs the width of forked process (measured at tips) (5:3 in *C. c. puniceus*; 3:2 in *C. c. clavipes*).

Material examined: 7 total, from: CANADA: ALBERTA: George Lake (2, UASM); USA: CALIFORNIA: El Dorado Co.: Blodgett Forest (1, FSCA); Tulare Co.: Sequoia National Park, Stoney Cr. Picnic Area (2, FSCA);UTAH: Cache Co.: Logan Valley (2, FSCA)

Description: Late instar larva (Fig. 2A). Body 21.0-24.0 mm long, elongate, subparallel, strongly dorsoventrally flattened with forked median process at abdominal apex (Fig. 2A). Head and abdominal segment 8 moderately sclerotized, brown, tergum 9 strongly sclerotized and dark brown.

Head (Fig. 2B): prognathous, strongly transverse and dorsoventrally flattened. Lateral margin rounded. Hind corners of epicranium slightly produced posteriorly. Median endocarina and epicranial stem very short; frontal sutures lyriform, strongly curved; bases contiguous. Stemmata well-developed, 6 present on each side of head. Frontoclypeal suture absent. Fronotoclypeal region with 3 long setae anterior to angles of frontal arms, 1 pair anterior to the apex of the frontal arms on each side of the head, 1 pair medially between the frontal arms, and 1 pair at the apex of the frontoclypeal region near the clypeolabral suture. Clypeolabral suture complete. Labrum free (Fig. 2G), with 5 pairs of setae. Epipharynx medially glabrous, 6 anterior setae on each side. Antennae 3segmented, ratio of lengths of antennomeres 1, 2, and 3 about 1.0: 1.4: 1.0. Mandibles (Fig. 2H) heavily sclerotized, symmetrical, apices bidentate with a smaller subapical tooth; with 2 dorsolateral mandibular setae present; prostheca acuminate, spinelike, with a broad base; mola with numerous setae medially and posteriorly. Maxilla (Fig. 2E) with cardo, divided by an internal ridge, basal portion trapezoidal, with 1 moderately elongate seta near basal margin; stipes elongate; mala falciform, mala falciform with 5 apical spines and a medial brush composed of several thick setae; maxillary palpus 3segmented, segment 1 asetose, segment 2 with 3 setae, segment 3 with 1 seta and 4 minute apical setae. Labium (Fig. 2F) with conspicuous mentum and prementum; mentum about as long as wide, with 3 pairs of setae, prementum with 3 pairs of setae; ligula transverse, with anterior microtrichia; labial palpi 2 segmented.

Thorax: Meso and metathorax tergites, and abdominal tergites and ventrites 18 each with 1 transverse ridge near anterior margin, ridge on ventral surface of abdominal segment 1 smaller lightly sclerotized. Prothorax subquadrate, transverse, 0.5 times as long as wide, sides curved, dorsal surface smooth; prosternal surface smooth, 3 setae (1 elongate) at anterolateral angles and 2 short setae at posterolateral angles; prosternum trapezoidal, sides oblique, posterior margin straight, a pair of medial setae present posterior to posterior margin of presternum. Meso- and metathorax transverse, both 0.5 times as long as wide, sides curved, surface of both tergites smooth with 3 short seta at anterolateral angles and 2 short setae at posterolateral angles; both sterna without well- defined subdivisions, each smooth with a pair of discal setae near anterior margin; spiracular sclerite projecting strongly from lateral margin, spiracles (Fig. 2C) annular and angled posterolaterally. Legs (Fig. 2D) moderately long, 5segmented; claw falciform, with 2 setae.

Abdomen: Segments 17 transverse, tergite surface smooth with 2 setae anterior to spiracles and 2 setae posterior to spiracles; ventrite surface with 3 setae, 2 anteriorly and 1 posteriorly. Segment 8 enlarged, tergite (Fig. 2I) with a stout spicule at each posterolateral margin, posterolateral angles with 8 short setae, 3 pairs of short setae anteromedially, 2 pairs of short setae posteromedially. Ventrite (Fig. 2J) with 9 pairs of setae and large stout process posteriorly with numerous minute setae apically. Tergite 9 with a basally forked process, directed dorsad, as wide as long; base of process with a pair of short, apically forked processes, 1 short seta at apex of forked process; anterior margin with lateral curved processes projecting from tergite 8; sternite 9 reduced and concealed from above.

Adults

Given the differences discovered in the larvae of the 2 subspecies, we examined adults to determine if there were corresponding adult differences. We examined 120 adult specimens of *C. c. clavipes* in the FSCA from the following states and provinces: CANADA: Ontario; USA: Colorado, Illinois, Indiana, Iowa, Kansas, Maine, Maryland, Massachusetts, Michigan, Mississippi, Missouri, New York, New Jersey, North Carolina, Ohio, Pennsylvania, Virginia, Wisconsin. We examined 46 adult specimens of *C. c. puniceus* in the

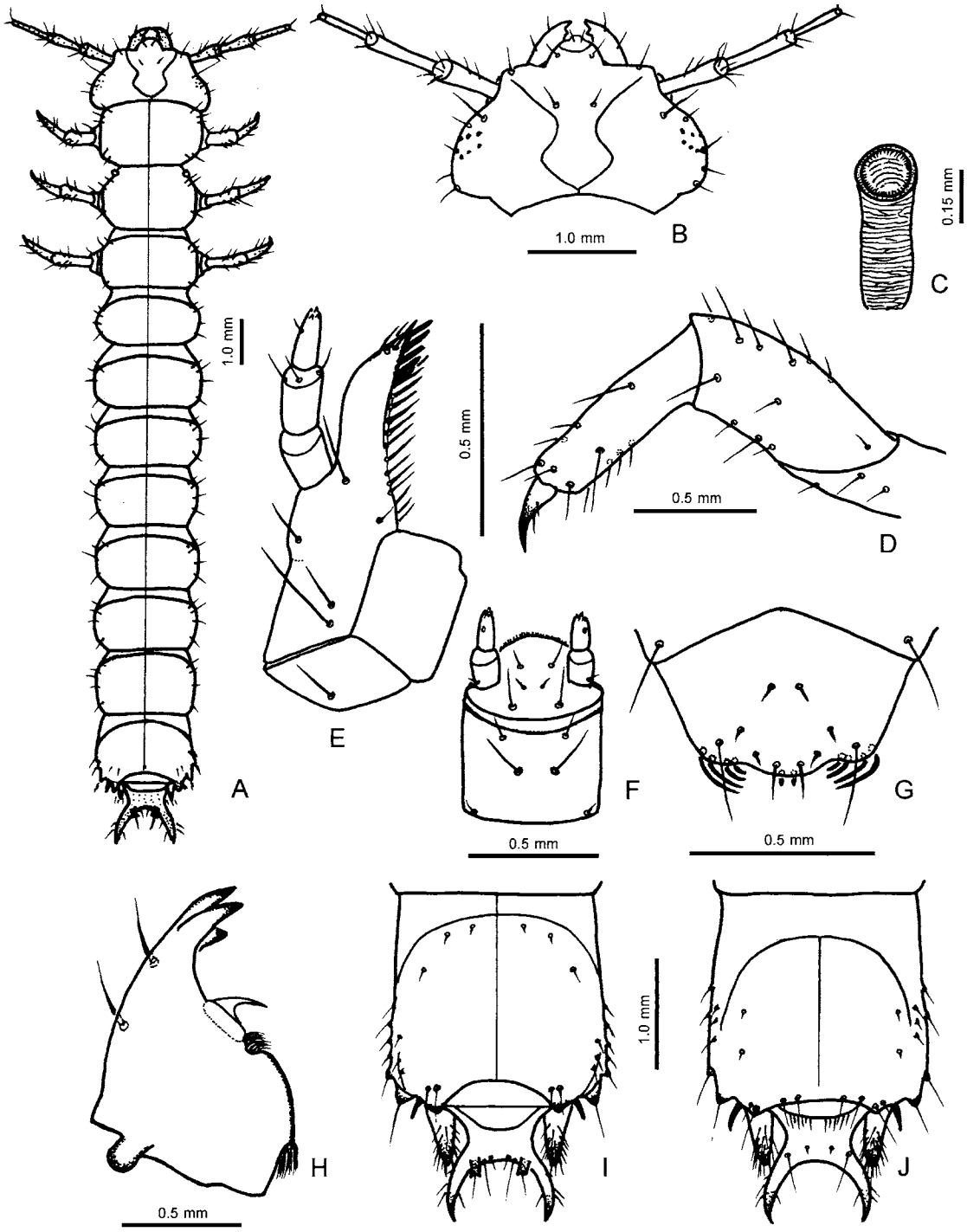

Fig. 2. Larva of *Cucujus c. puniceus*. A, habitus, dorsal view; B, head, dorsal view; C, A7 spiracle, D, prothoracic leg; E, left maxilla, dorsal view; F, labium, ventral view; G, labrum, dorsal view; H, left mandible, dorsal view; I, abdominal segments 89, dorsal view; J, same, ventral view.

FSCA from the following states and provinces: CANADA: Alberta, British Columbia; USA: Alaska, California, Idaho, Oregon.

As noted in previous literature, *C. c. clavipes* has a black scape, while *C. c. puniceus* has a red scape. However, specimens of *C. c. puniceus* from Alaska have black scapes. We had formed the impression that individuals from the western U.S. were on average more elongate than those from the eastern part of the country. Measurements of series from both populations revealed considerable overlap in body proportions, with specimens of the *C. c. puniceus* slightly more elongate, ranging in size from 12.5mm to 16.6mm, while specimens of *C. c. clavipes* ranged in size from 9.5mm to 14.6mm.

Lee and Sato (2007) found taxonomically useful genitalic differences among Asian species of *Cucujus*. Male genitalia from specimens of *C. clavipes* from all parts of its distribution were examined and found to be indistinguishable.

Conclusions

Despite the larval differences, the lack of consistent and significant morphological differences in the adults suggests that at this point given the state of our knowledge, the present treatment of these 2 populations as subspecies of the same species is valid. Research into molecular differences may prove useful in understanding the limits of both taxa.

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TEPHRITOID FLIES (DIPTERA, TEPHRITOIDEA) AND THEIR PLANT HOSTS FROM THE STATE OF SANTA CATARINA IN SOUTHERN BRAZIL

FLÁVIO R. M. GARCIA[1] AND ALLEN L. NORRBOM[2]
[1]Federal University of Pelotas, Institute of Biology, Department of Zoology and Genetics
Laboratory of Insect Ecology, 96010-900 P.O. Box: 354, Pelotas, RS, Brazil
E-mail: flavio.garcia@pq.cnpq.br

[2]Systematic Entomology Laboratory, c/o National Museum of Natural History, MRC-168,
P.O. Box 37012, Washington, DC 20013-7012, USA
E-mail: allen.norrbom@ars.usda.gov

ABSTRACT

A total of 12,540 ripe fruits belonging to 46 species in 25 plant families were sampled from either the trees or the ground in 6 municipalities in the state of Santa Catarina, Brazil between 2002 and 2006 to determine which fruit fly species developed on various host plants. Each fruit was weighed and placed into a plastic flask filled with sterilized sand 7 cm deep, and the opening of the flask was covered with sheer fabric. The flasks were kept under controlled conditions (25 ± 3°C, 70 ± 10% RH and 12h photophase). After 7 d, the pupae were sifted from the sand and transferred to Petri dishes lined with filter paper. Twenty-one species of Tephritoidea were recovered consisting of 13 species of Tephritidae, 6 of Lonchaeidae, and 2 of Ulidiidae. We present new host records for some species of fruit flies.

Key Words: Tephritidae, Lonchaeidae, Ulidiidae, fruit pests, new host records

RESUMEN

Este trabajo dirigido a la evaluación de las especies de moscas de la fruta y sus plantas hospederas en el estado de Santa Catarina Brasil. Un total de 12.540 frutos maduros que pertenecen a 46 especies y 25 familias de arboles o del suelo en seis municipios del estado de Santa Catarina, Brasil entre 2002 y 2006 fueran muestradas. Cada fruto fue pesado y se coloca en un frasco de plástico cubierto con Voil, con 7 cm de arena esterilizada. Los frascos fueron mantenidos en condiciones controladas (25 ± 3°C, UR 70 ± 10% y 12h de photophase). Después de siete días, la arena se tamiza y la pupas fueron transferidas a placas de petri con papel filtro como sustrato. Veintiún especies de Tephritoidea fueron recuperados - 13 especies de Tephritidae, seis especies de Lonchaeidae, y dos de Ulidiidae. Se presentan los registros de para algunas especies de fruta o moscas.

Translation provided by the authors.

Approximately 70 species of Tephritidae are considered important pests of fruit production worldwide. The majority of the species of economic importance belong to 5 genera: *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus*, and *Rhagoletis* (Garcia 2009). The genus *Neosilba* of the family Lonchaeidae (McAlpine & Steyskal 1982) includes 16 described species (Strikis & Prado 2005), some of which cause severe damage to certain species of fruit crops in the American tropics.

Field surveys of fruit flies (Tephritoidea) and their host plants and parasitoids are essential for understanding the bioecology of the economically important genera and species in this superfamily (Bateman 1972). The creation of the common market, Mercosul, involving Brazil, Argentina, Paraguay and Uruguay, has elevated the importance of such studies because knowledge of these pest species, their hosts and natural enemies is key to containing their destructive effects as trade in fruits between these countries expands. In Brazil, most of the pest tephritids belong to the genus *Anastrepha,* but host plants are known for only 44% of the species (Zucchi 2007).

Santa Catarina has the most host plant records, 81, for species of Tephritidae among the Brazilian states (Garcia 2011). However, only 46 plant species belonging to 18 families are recorded in the state as hosts for fruit flies in the genus *Anastrepha* (Nora et al. 2000).

This work reports new information from a survey of fruit fly species and their host plants in the state of Santa Catarina, Brazil.

MATERIALS AND METHODS

Fruit Sampling

Between 2002 and 2006, a total of 12,540 ripe fruits from 46 plant species belonging to 25 fami-

lies were sampled. Fruits were picked from the plants, or freshly fallen fruits were gathered from the ground below them. Sampling occurred in 6 municipalities of Santa Catarina, Brazil: Anchieta (26º 53'S and 53º 33'W), Chapecó (27º 06'S and 53º 16'W), Cunha Porã (26º 07'S and 53ºW 16'), Palmitos (27º 06'S and 53º 16W'), São Carlos (27º 07' S and 53º 00' W), and Xanxerê (26º 87' S and 52ºW' 40). Each fruit was weighed and placed into a plastic flask containing 7 cm of sterilized sand, and the opening of the flask was covered with sheer fabric. The flasks were kept under controlled conditions (25 ± 3°C, 70 ± 10% RH and 12h photophase). After 7 d, the sand was sifted and the pupae transferred to Petri dishes with filter paper as substrate.

Identification of fruit flies and host plants

Characters of the females, primarily of the aculeus, and body and wing markings, were considered in identifying species of *Anastrepha* (Zucchi 2000) identified by Garcia and Zucchi. *Ceratitis capitata* (Wiedemann) is the only species of *Ceratitis* in Brazil and was easily recognized by the description by Zucchi (2000). Lonchaeidae were identified by Dr. Pedro Strikis, and other Tephritidae and *Notogramma cimiciforme* Loew (Ulidiidae) were identified by Norrbom. The host plant species were identified by the botanists Dr. Sérgio Augusto de Loreto Bordignon, Dr. Rosiane Berenice Denardin, and Lúcia Salengue. Some voucher specimens of fruit flies and host plants were deposited at the Zoobotanic Museum of the University of Chapecó.

Data Analysis

The infestation indexes were calculated in 2 ways: (1) by dividing the total number of puparia obtained by the number of fruits in the sample (puparia/fruit); or (2) by dividing the total number of puparia by the total mass (kg) of fruits in the sample (puparia/kg). The host plants of *Anastrepha* obtained in this work were compared to the lists of hosts assembled by Norrbom (2004) and Zucchi (2007, 2008) with the aim of providing new host records for Brazil.

Results and Discussion

Twenty-one species of Tephritoidea were recovered: 13 species of Tephritidae, 6 of Lonchaeidae, and 2 of Ulidiidae (= Otitidae) (Table 1). The species, *Parastenopa guttata* Aczél and *P. montana* Aczél, are new records of fruit flies for the state of Santa Catarina, and the total number of known species of Tephritidae from the state is now 81 (Garcia 2011). The development of flies from the fruit of yerba maté, *Ilex paraguariensis* A. St. Hil., is reported for the first time. Two species of the genus *Parastenopa*, *P. guttata* and *P. montana*, were reared. The only *Parastenopa* species previously known to attack this plant were reared from stems or from leaf galls of the Paraguay tea psyllid, *Gyropsylla spegazziniana* Lizer & Trelles (Hemiptera, Psyllidae) (Blanchard 1929; psyllid as *Metaphalara spegazziniana*), although the North American *P. limata* (Coquillett) breeds in the fruit of several *Ilex* species (Benjamin 1934; Phillips 1946). Araticum, *Annona rugulosa* (Schltdl.) H. Rainer (Annonaceae), *Inga sellowiana* Benth. (Fabaceae), and the iguana hackberry, *C. iguanaea* (Jacq.) Sarg. (Ulmaceae) are recorded for the first time as host plants of *Anastrepha fraterculus* (Wiedemann). Rio Grande cherry, *Eugenia involucrata* DC., is recorded for the first time as a host plant of *Anastrepha obliqua* (Macquart); and sete-capas, *Campomanesia guazumifolia* (Cambess.) O. Berg. (Myrtaceae), is recorded as a host plant of *Anastrepha sororcula* Zucchi. Strawberry guava, *P. cattleianum* Sabine (Myrtaceae), is recorded for the first time as host plant of both *A. obliqua* and *A. sororcula* in Brazil. Previously strawberry guava had been reported as a host of *A. obliqua* in Guatemala (Eskafi & Cunningham, 1987).

The greatest infestations based on the number of puparia per fruit were found in pumpkin, *Cucurbita pepo* L. (6.59), followed by pineapple guava, *Acca sellowiana* (O. Berg) Burret (6.23), and common guava, *Psidium guajava* L. (6.16). Regarding the parameter puparia/kg, the greatest infestations occurred in strawberry guava, *P. cattleianum* (422), followed by pineapple guava, *P. cattleianum* (278), yerba maté, *I. paraguariensis* A. St. Hil. (260), and wild cherry, *P. avium* (L.) L. (232). Considering both parameters, pineapple guava, *P. cattleianum*, was the species most infested by fruit flies.

The highest number of plant hosts was recorded for *A. fraterculus* (20 plant species from 8 families) (Table 1); predominantly fig, *Ficus carica* L. (Moraceae) (75.0% of the total of samples collected were infested); guavirova, *Campomanesia xanthocarpa* O. Berg, (60.7%); guaviju, *Myrcianthes pungens* (O. Berg) D. Legrand (57.1%); Surinam cherry, *Eugenia uniflora* L. (55.3%); wild cherry, *P. avium* (L.) L. (Rosacae) (52.0%); pineapple guava, *P. cattleianum* (51.7%); common guava, *P. cattleianum* (51.4%); guava (48.0%), *Campomanesia guazumifolia* (45.4%) (Myrtaceae); and carambola, *Averrhoa carambola* L. (Oxalidaceae), (35.3%).

Nine new host plants of *A. fraterculus* were recorded in Brazil: araticum, *A. rugulosa* (Annonaceae); *Inga sellowiana* (Fabaceae); common fig, *F. carica* (Moraceae); pineapple guava, *P. cattleianum* (Myrtaceae); jaboticaba, *Myrciaria cauliflora* (Mart.) O. Berg (Myrtaceae); *Campomanesia guazumifolia* (Myrtaceae); wild cherry, *P. avium* (Rosaceae); bergamot orange, *Citrus reticulata*

TABLE 1. PLANTS SAMPLED WITH THEIR RESPECTIVE ORIGIN (O), FRUIT WEIGHT (FW), NUMBER OF FRUITS SAMPLED (N), NUMBER OF PUPAE (P), AVERAGE NUMBER OF PUPAE PER FRUIT (P/N), AND AVERAGE NUMBER OF PUPAE PER KG (P/KG). N = NATIVE AND E = EXOTIC. NUMBER IN PARENTHESES FOLLOWING FLY SPECIES NAMES = NUMBER OF SPECIMENS REARED.

Plant Species	O	FW (kg)	# fruits *n*	#pupae P	P/n ± SE	P/kg ± SE	Tephritidae	Lonchaeidae & Ulidiidae
Annonaceae								
Araticum, *Annona rugulosa*	N	4.64	102	33	0.32 ± 0.1	7.10 ± 2,4	*A. fraterculus* (3)	*Neosilba zadolicha* (18)
Aquifoliaceae								
Erva-mate, *Ilex paraguariensis*	N	1.00	2465	259	0.11 ± 0.1	259.70 ± 20.5	*Parastenopa* spp.(254)	
Cactaceae								
Pereskia aculeata	N	0.37	50	45	0.90 ± 0.2	121.62 ± 10.4	*A. barbiellinii* (19)	
Cucurbitaceae								
Abóbora, *Cucurbita pepo*	E	139.77	68	448	6.59 ± 2.1	3.21 ± 1,8	*A. grandis* (310)	*Dasiops* sp. (8)
Chuchu, *Sechium edule*	E	8.94	120	46	0.38 ± 0.1	5.15 ± 3.2		*Euxesta* sp.(12) *Neosilba padroi* (40) *Euxesta* sp. (22) *Lonchaea* sp. (12) *Neosilba padroi* (10)
Melancia, *Citrullus lanatus*	E	58.30	14	2	0.14 ± 0.1	0.03 ± 0.1	*A. grandis* (2)	
Melão, *Cucumis melo*	E	10.80	13	12	0.92 ± 0.3	1.11 ± 0.7		*Neosilba padroi* (9)
Pepino, *Cucumis sativus*	E	8.22	43	11	0.26 ± 0.1	1.34 ± 0.8		*Euxesta* sp. (7)
Ebenaceae								
Caqui, *Diospyros kaki*	E	9.47	126	367	2.91 ± 1.1	38.74 ± 12.0	*A. fraterculus* (11) *C. capitata* (293)	
Euphorbiaceae								
Mandioca, *Manihot esculenta*	N	0.52	210	2	0.01 ± 0.0	3.83 ± 1.3	*A. montei* (2)	
Fabaceae								
Ingá, *Inga sellowiana*	N	1.75	246	49	0.20 ± 0.2	27.97 ± 6.2	*A. fraterculus* (5) *C. capitata* (4)	*Lonchaea* sp. (12) *Neosilba* sp. (19)
Moraceae								
Figo, *Ficus carica*	E	1.22	52	22	0.42 ± 0.2	18.10 ± 8.3	*A. fraterculus* (16)	
Myrtaceae								
Araçá, *Psidium cattleianum*	N	5.67	670	2393	3.57 ± 1.3	421.99 ± 25.1	*A. fraterculus* (1220) *C. capitata* (10)	*Neosilba zadolicha* (5) *Neosilba padroi* (7) *Neosilba* sp. (6)

TABLE 1. (CONTINUED) PLANTS SAMPLED WITH THEIR RESPECTIVE ORIGIN (O), FRUIT WEIGHT (FW), NUMBER OF FRUITS SAMPLED (N), NUMBER OF PUPAE (P), AVERAGE NUMBER OF PUPAE PER FRUIT (P/N), AND AVERAGE NUMBER OF PUPAE PER KG (P/KG). N = NATIVE AND E = EXOTIC. NUMBER IN PARENTHESES FOLLOWING FLY SPECIES NAMES = NUMBER OF SPECIMENS REARED.

Plant Species	O	FW (kg)	# fruits *n*	#pupae P	P/n ± SE	P/kg ± SE	Tephritidae	Lonchaeidae & Ulidiidae
Cereja, *Eugenia involucrata*	N	2.85	516	155	0.30 ± 0.1	54.47 ± 13.0	*A. fraterculus* (79) *C. capitata* (15)	*Neosilba padroi* (6)
Goiaba, *Psidium guajava*	N	12.47	236	1454	6.16 ± 2.4	116.64 ± 10.9	*A. fraterculus* (697) *A. obliqua* (14) *A. sororcula* (7) *C. capitata* (13)	*Neosilba padroi* (29)
Goiaba-do-campo, *Acca sellowiana*	N	1.79	80	498	6.23 ± 3.2	277.80 ± 23.2	*A. fraterculus* (254)	
Guaviju, *Myrcianthes pungens*	N	0.25	52	21	0.40 ± 0.1	84.31 ± 13.3	*A. fraterculus* (12)	
Guavirova, *Campomanesia xanthocarpa*	N	2.61	717	53	0.07 ± 0.0	20.27 ± 6.8	*A. fraterculus* (32)	
Jabuticaba, *Myrciaria cauliflora*	N	0.16	25	3	0.12 ± 0.1	18.75 ± 7.7	*A. fraterculus* (3)	
Pitanga, *Eugenia uniflora*	N	4.49	1699	406	0.24 ± 0.1	90.37 ± 15.6	*A. fraterculus* (223)	*Neosilba padroi* (12)
Sete-capotes, *Campomanesia guazumifolia*	N	4.51	398	799	2.01 ± 1.0	177.08 ± 23.1	*A. fraterculus* (360) *A. obliqua* (4) *A. sororcula* (5)	*Neosilba padroi* (5)
Uvaia, *Eugenia pyriformis*	N	1.60	334	148	0.44 ± 0.2	92.48 ± 17.9	*A. fraterculus* (51) *C. capitata* (43)	*Neosilba padroi* (3)
Oxalidaceae								
Carambola, *Averrhoa carambola*	E	3.31	65	25	0.38 ± 0.1	7.56 ± 3.12	*A. fraterculus* (9) *A. obliqua* (2)	*Neosilba padroi* (12)
Passifloraceae								
Maracujá, *Passiflora edulis*	N	26.58	298	628	2.11 ± 0.5	23.63 ± 15.7	*A. dissimilis* (9) *A. pseudoparallela* (363) *C. capitata* (12)	*Lonchaea* sp. (185) *Neosilba padroi* (29)
Rosaceae								
Ameixa, *Prunus domestica*	E	5.24	148	267	1.80 ± 1.2	50.94 ± 10.8	*A. fraterculus* (148)	*Neosilba* sp. (14)
Cereja-do-mato, *Prunus avium*	E	0.40	36	94	2.61 ± 1.1	232.45 ± 27.9	*A. fraterculus* (47)	
Nêspera, *Eriobotrya japonica*	E	12.79	1263	1285	1.02 ± 0.7	100.44 ± 30.1	*A. fraterculus* (218) *C. capitata* (816)	
Pera, *Pyrus communis*	E	9.85	96	52	0.54 ± 0.2	5.28 ± 2.6	*A. fraterculus* (33)	
Pêssego, *Prunus persica*	E	27.32	652	1151	1.77 ± 0.9	42.13 ± 18.1	*A. fraterculus* (372) *C. capitata* (322)	*Lonchaea* sp. (14) *Neosilba zadolicha* (43) *Neosilba* sp. (41)
Rutaceae								
Bergamota, *Citrus reticulata*	E	8.67	138	44	0.32 ± 0.1	5.07 ± 3.3	*A. fraterculus* (12)	*Neosilba padroi* (12)

TABLE 1. (CONTINUED) PLANTS SAMPLED WITH THEIR RESPECTIVE ORIGIN (O), FRUIT WEIGHT (FW), NUMBER OF FRUITS SAMPLED (N), NUMBER OF PUPAE (P), AVERAGE NUMBER OF PUPAE PER FRUIT (P/N), AND AVERAGE NUMBER OF PUPAE PER KG (P/KG). N = NATIVE AND E = EXOTIC. NUMBER IN PARENTHESES FOLLOWING FLY SPECIES NAMES = NUMBER OF SPECIMENS REARED.

Plant Species	O	FW (kg)	# fruits *n*	#pupae P	P/n ± SE	P/kg ± SE	Tephritidae	Lonchaeidae & Ulidiidae
Laranja, *Citrus sinensis*	E	17.20	176	105	0.60 ± 0.2	6.10 ± 4.0		*Notogramma cimiciforme* (9) *Neosilba padroi* (69) *Notogramma cimiciforme* (29)
Sapindaceae								
Camboatá-vermelho, *Cupania vernalis*	N	5.80	63	2	0.03 ± 0.0	0.34 ± 0.2		*Neosilba padroi* (2)
Sapotaceae								
Aguaí, *Chrysophyllum gonocarpum*	N	0.24	87	9	0.10 ± 0.1	37.50 ± 12.3	*A. elegans* (7)	
Solanaceae								
Joá, *Solanum sisimbrifolium*	N	0.22	32	13	0.41 ± 0.2	59.09 ± 29.5		*Neosilba padroi* (12)
Tomate, *Lycopersicum esculentum*	E	6.57	193	107	0.55 ± 0.3	16.29 ± 8.2		*Neosilba padroi* (52)
Ulmaceae								
Esporão-de-galo, *Celtis iguanaea*	N	911.66	807	608	0.75 ± 0.3	0.67 ± 0.2	*A. fraterculus* (3) *R. pastranai* (577)	*Neosilba padroi*(3)

Blanco (Rutaceae); and iguana hackberry, *C. iguanaea* (Jacq.) Sarg. (Ulmaceae).

Pereskia aculeata Mill., also known as Orapro-nobis or Barbados gooseberry, was found to be a host plant for *Anastrepha barbiellinii* Lima; and *Campomanesia guazumifolia* (Myrtaceae) was recorded for the first time as a host plant for both *A. obliqua* and *A. sororcula*.

Native plant species served as hosts of 12 fruit fly species from 4 genera of Tephritidae, whereas exotic plant species served as hosts of only 4 species from 2 genera. *Ceratitis capitata* developed in 9 plant species from 5 families, with the following order of predominance: khaki, *Diospyros kaki* Thunb. (Ebenaceae) (93.1% of the fruits sampled were infested); medlar, *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) (63.5%); uvaia, *Eugenia pyriformis* Cambess. (Myrtaceae) (29.2%); and peach, *P. persica* (L.) Batsch (28.1%). Some fruit fly species occurred exclusively in 1 plant species: *Anastrepha barbiellinii* in ora-pro-nobis, *Pereskia aculeata*; *Anastrepha grandis* (Macquart) only in pumpkin, *C. pepo*; *Rhagoletotrypeta pastranai* Aczél only in esporão-de-galo, *Celtis iguaneae* (Jacq.) Sarg.; *Anastrepha dissimilis* Stone and *A. pseudoparallela* (Loew) only in *Passiflora edulis* Sims; *Anastrepha montei* Lima only in cassava, *Manihot esculenta* Crantz; and *Parastenopa guttata* and *P. montana* only in yerba maté, *I. paraguariensis* St. Hil.

Lonchaeid flies were recorded from 22 host plant species from 9 families of which 12 were native and 10 exotic. Araújo & Zucchi (2002) have also described the indiscriminate infestation of native and exotic fruits by Lonchaeidae. *Neosilba padroi*, a species described recently by Strikis & Lerena (2009), had the highest number of host species (7 native, 15 exotic) belonging to 8 families; the lance fly, *Lonchaea* sp., had 4 host species (2 native and 2 exotic) in 4 families; *Neosilba zadolicha* McAlpine & Steyskal had 3 host species (1 native and 2 exotic) in 3 families; and *Dasiops* sp. occurred only in *C. pepo* (exotic). *Neosilba zadolicha* occurred in araticum, *A. rugulosa* (Annonaceae), araçá, *P. cattleianum* (Myrtaceae), and peach, *P. persica* (Rosaceae). *Spondias* spp. (Anacardiaceae) (Santos et al. 2004) and medlar, *Eriobotrya japonica* (Strikis & Prado 2009) also may serve as hosts of *N. zadolicha*.

The Ulidiidae occurred only in 5 exotic species of Rutaceae and Cucurbitaceae; *Euxesta* sp. occurred only in Cucurbitaceae and *N. cimiciforme* Loew only in Rutacaeae. *Euxesta* sp. occurred on 3 plant species, with predominance in chayote, *Sechium edule* (Jacq.) Sw., (48.9%). *N. cimiciforme* occurred only in bergamot orange, *C. reticulata* Blanco, and orange, *Citrus sinensis* (L.) Osbeck. This species has a wide geographic range in the New World and is a scavenger recorded from a wide variety of plants (Steyskal 1963). Unlike our results, Uchôa-Fernandes et al. (2003) and Aguiar-Menezes et al. (2004) obtained specimens of *N. cimiciforme* in passion fruit (*Passiflora* sp.), with occurrences also in tangerine, *C. reticulata*, and orange, *C. sinensis*. Such differences may be due to the interpopulation differences or seasonal availability of host plants in different regions (Selivon 2000).

Pumpkin was infested by 4 species of flies belonging to 3 families. Guava, passion fruit, and peach were infested by 5 species each, and these fruits were found to support infestations only of species of Tephritidae and Lonchaeidae.

Under the conditions in which this research was conducted, we conclude that a wide diversity of fruit-bearing plant species in the state of Santa Catarina was attacked by 22 species of tephritoid flies. The most predominant fly was *A. fraterculus*, and *P. cattleianum* was the host species most frequently infested by these flies.

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SUSCEPTIBILITY OF GENERA AND CULTIVARS OF TURFGRASS TO SOUTHERN CHINCH BUG *BLISSUS INSULARIS* (HEMIPTERA: BLISSIDAE)

JAMES A. REINERT, AMBIKA CHANDRA AND M. C. ENGELKE
Texas AgriLife Research & Extension Center, Texas A&M System, 17360 Coit Road, Dallas, TX 75252-6599 USA
E-mail: j-reinert@tamu.edu

ABSTRACT

The southern chinch bug (*Blissus insularis* Barber) is the most damaging insect pest of St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze), across the southern U.S.A. Susceptibility to the southern chinch bug and reproductive potential of the bugs on 24 cultivars from 7 genera in 8 turfgrasses were evaluated under greenhouse conditions. *Stenotaphrum secundatum* ('Raleigh', 'Texas Common', and 'Captiva') cultivars were the most susceptible among all the turfgrass genera and each produced populations ≥97.5 bugs per 15-cm diameter plant within the 11-week test period from Jul to Sep 2008. Substantial populations also developed on zoysiagrass (*Zoysia* spp.) ('Emerald', 'Empire', 'Palisades', and 'Zorro') cultivars and on '609' buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.). Low population development was recorded on cultivars of bermudagrass (*Cynodon* spp.), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), seashore paspalum (*Paspalum vaginatum* Swartz), bahiagrass (*Paspalum notatum* Flugge), and tall fescue (*Festuca arundinacea* Schreb.).

Key Words: turfgrass pests, host plant resistance, host range, pest management, host plants

RESUMEN

La chinche sureña del pasto (*Blissus insularis* Barber) es el insecto mas dañino para St. Augustingrass (*Stenotaphrum secundatum* Walt. Kuntze), en el sur de U.S.A. La susceptibilidad de materiales y el potencial reproductivo del insecto fueron evaluados en 24 cultivares pertenecientes a siete géneros de pasto para césped en condiciones de invernadero. Los cultivares de *S. secundatum* ('Raleigh', 'Texas Common', y 'Captiva') fueron los mas susceptibles con poblaciones de ≥97.5 chinches por planta en maceta de 15 cm de diámetro, en 11 semanas de evaluacion de julio a septiembre 2008. Considerables niveles de poblaciones también fueron registrados en zoysiagrass (*Zoysia* spp.) (cultivares 'Emerald', 'Empire', 'Palisades', y 'Zorro'), así como en el cultivar '609' de buffalograss (*Buchloe dactyloides* (Nutt.) Engelm.). Bajos niveles de desarrollo se registraron en cultivares de bermudagrass (*Cynodon* spp.), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), seashore paspalum (*Paspalum vaginatum* Swartz), bahiagrass (*Paspalum notatum* Flugge), y tall fescue (*Festuca arundinacea* Schreb.).

The authors provided the translation by Carlos Campos

The southern chinch bug (SCB) (*Blissus insularis* Barber) (Hemiptera: Blissidae) is the most damaging insect pest of St. Augustinegrass (*Stenotaphrum secundatum* Walt. Kuntze), across the southern U.S.A., Bermuda, Mexico, and throughout the Caribbean Archipelago (Henry & Froeschner 1988; Sweet 2000). In the U.S.A. it is found from South Carolina to Florida, westward to Oklahoma and along the Gulf Coast to Texas and in California, Hawaii, Puerto Rico, and Guam (Reinert et al. 1995; Mortorell 1976; Vittum et al. 1999).

This pest begins to damage St. Augustine lawns as early as Mar in parts of Southern Florida and Texas and first instars have been found during all 12 months in Southern Florida (Reinert, unpublished data). Damage begins as small patches of dead grass early in the season, with entire lawns killed as the summer progresses. During heavy infestations, large populations will progress from one lawn to another as they move from one city block to the next (Reinert & Kerr 1973). According to Painter (1928) *B. leucopterous* L. damages grasses by "removal of the synergic food-bearing solutions which flow to the roots by way of the phloem; the stopping up of the sieve tubes, and perhaps also the removal of water from the xylem, together with the stoppage of the tracheids." It is believed that this same process takes place when SCB feeds at the node and the crown area of *Stenotaphrum*, which mimics the effects of a toxin being injected into the plant. SCB infestations soon turn the grass yellow, brown, and it eventually dies within a few days. Both nymphs and adults feed in aggregates in localized areas early in the season, with these areas coalescing

into large dead areas or entire lawns as the season progresses (Reinert et al. 1995).

Stenotaphrum is cultivated extensively in subtropical and tropical climates around the world (Busey 2003; Sauer 1972). It is used widely across the southern U.S.A. as a turfgrass in urban landscapes, including residential and commercial lawns, parks, some sports complexes, and as a pasture grass (Busey 2003; Sauer 1972). *Stenotaphrum* has long been considered the primary host of the SCB (Reinert & Kerr 1973; Reinert et al. 1995; Vittum et al. 1999). The SCB has been identified on 9 other grass hosts (Cherry & Nagata 1997; Slater 1976).

Resistant cultivars 'Floratam', 'Floralawn', 'FX-10', and 'Captiva' have been developed and deployed to help manage this pest (Busey 1993; Cherry & Nagata 1997; Dudeck et al. 1986; Horn, et al. 1973; Reinert & Dudeck 1974). Recently, populations of SCB have been identified that have overcome the resistance in each of these cultivars (Busey & Center 1987; Cherry & Nagata 1997; Reinert 2008).

This study was established to characterize the reproductive potential and development of the SCB on 24 cultivars of turfgrass from 7 genera in 8 turfgrasses used across the Southern U.S.A.

Materials and Methods

This study was conducted under greenhouse conditions during Jul-Sep 2008 at the Texas AgriLife Research and Extension Center at Dallas, TX, U.S.A. A total of 24 cultivars (Table 1) including St. Augustinegrass, (5) zoysiagrass (*Zoysia* spp.) (5), bermudagrass (*Cynodon* spp.) (5), buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.) (2), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) (1), seashore paspalum (*Paspalum vaginatum* Swartz) (2), bahiagrass (*Paspalum notatum* Flugge) (2), and tall fescue (*Festuca arundinacea* Schreb.) (2) were evaluated for their susceptibility to SCB infestation and development.

Plugs of grass grown either in the field or greenhouse were divided and planted into 18-cell trays and allowed to grow to cover the whole cell. Cells measured 7.5 × 7.5 cm and 4 cm deep. Plants were fertilized bi-monthly during establishment with Miracle-Gro All Purpose fertilizer (24-8-16 + B (200 ppm), Cu (700 ppm), Fe (1500 ppm), Mn (500 ppm), Mo (5 ppm), Zn (600 ppm)) (Scotts, 14111 Scottslawn Road, Marysville, Ohio) at ~8.25 kg of N ha^{-1} $month^{-1}$. Once sufficient growth was achieved to provide near complete coverage of the entire cell (ca. 14 weeks), plugs from 4 cells of each cultivar were repotted into 15-cm diam plastic pots and allowed to establish for 2 weeks. Each pot was filled with soil within 2.5 cm of the top. Potted plants were then fitted with a cylindrical plastic cage (a modification of Starks & Burton 1977) to exclude extraneous insects and to confine the SCB. Cages were made of Lexan® 8010 Film (0.2 mm thickness) (General Electric Plastics, 4600 AC Bergen op Zoom, The Netherlands) and measured 32.5 cm tall and 2.5 cm in diam and were vented on opposite sides with two, 8-cm diameter ventilation holes to allow air circulation within the cage. Ventilation holes and the top end of the cage were covered with Voile 118" Decorator Fabric in White # 235-004-81 (Hancock Fabrics, Plano, Texas, hancockfabrics.com) cut 15 mm larger than the holes and secured with glue.

On 6-8 Jul 2008, 10 adults (5 male and 5 female) were introduced into each cage. Before bugs were introduced, each pot was filled to the top with fine topdressing sand. When each cage was inserted over the plant in the pot the area between the cage and the wall of the pot was backfilled with additional sand to form an escape-proof barrier to the confined insects.

Pots were maintained in the greenhouse in a randomized complete block design with 4 replicates and held on full size aluminum sheet pans that were 45 cm × 65 cm 18 gauge (WINCO Industries Co., Lodi, New Jersey). Potted plants were provided sub-surface irrigation by filling the pans with 1.5-2.0 cm of water as needed to avoid wilting of the test grasses. Watering was done every 3-4 d. After the pots were allowed to soak-up water for about 2 h, the excess water was drained to avoid causing deterioration of the root system. Cages had to be opened about every 2 weeks so the grass could be clipped, since there was not enough room for the continued plant growth. The clipping process was done over one of the aluminum sheet pans so that any SCB adults or nymphs that were removed with the clippings or that tried to escape could be collected with a hand aspirator and returned to the grass when the cage was put back in the pot.

SCB for this experiment were collected by vacuum sampling the bugs from a residential lawn of *S. secundatum* in the Houston, Texas area. A modification of the procedure for vacuuming (Nagata & Cherry 2007; and personal communication) was used. An Echo Shred 'N' Vac® model ES-210 (Echo Inc., Lake Zurich, Illinois) leaf blower/vacuum was modified by cutting-off the distal 15-cm end of the vacuum tube. This unit has an 87.5 cm long intake tube (11.25 cm diam) and produces 225.31 km/h (140 mph) of vacuum. A 20-cm long piece of French drain pipe (10.3 cm outside diameter) that fit loosely within the intake tube was shimmed to fit the inside diam of the vacuum tube by wrapping it with duct tape, close to each end, so it would fit snugly inside to reattach the 2 pieces of the intake vacuum tube. When the 2 pieces of tube were re-joined, a 20-cm diameter piece of polyester Tricot interlocking netting (mesh size ca. 9.6 × 8 per cm, 24 × 20 per inch) cut

TABLE 1. RATE OF REPRODUCTION AND DEVELOPMENT OF SOUTHERN CHINCH BUG ON CULTIVARS AND GENERA OF TURFGRASS.

Genera of grasses Cultivars	Nymphs 1st [a, b]	 5th[a, b]	 Total nymphs[a, b] (N)	Adults[a, b] (A)	Total[a, b] (N + A)
Stenotaphrum secundatum (St Augustinegrass)					
Raleigh	34.3 a*	45.8 a*	163.0 a*	17.8 a*	180.8 a* A**
TX Common	25.3 a	30.0 b	117.5 ab	4.3 b	121.8 b A
Captiva	30.7 a	18.5 b	93.3 b	4.3 bc	97.6 b A
FX-10	1.0 bc	0.0 c	1.3 d	0.0 d	1.3 d B
Floratam	0.8 bc	0.3 c	1.1 d	0.0 d	1.1 d B
Zoysia spp. (Zoysiagrass)					
Palisades	5.8 b	0.5 c	16.5 c	2.5 bcd	19.0 c A
Emerald	1.3 bc	1.0 c	8.8 cd	1.0 cd	9.8 cd AB
Zorro	1.3 bc	2.3 c	8.0 cd	0.0 d	8.0 cd AB
Empire	0.8 bc	0.8 c	4.3 cd	1.3 bcd	5.6 cd AB
Cavalier	0.0 c	0.0 c	0.3 d	0.8 d	1.1 d B
Buchloë dactyloides (Buffalograss)					
609	3.7 bc	0.3 c	7.5 cd	2.5 bcd	10.0 cd ns
Prairie	0.8 bc	0.0 c	1.0 cd	0.8 d	1.8 d
Festuca arundinacea (Tall Fescue)					
Rebel	2.0 bc	1.0 c	5.0 cd	1.0 cd	6.0 cd ns
Paladin	2.8 bc	0.0 c	3.3 cd	0.8 d	4.1 cd
Cynodon spp. (Bermudagrass)					
Tifton 10	1.3 bc	0.5 c	2.8 cd	0.3 d	3.1 cd ns
Tifway	0.0 c	1.3 c	1.3 cd	0.0 d	1.3 d
Texturf 10	0.3 c	0.0 c	0.5 d	0.0 d	0.5 d
TifSport	0.0 c	0.0 c	0.0 d	0.0 d	0.0 d
Common	0.0 c	0.0 c	0.0 d	0.0 d	0.0 d
Paspalum notatum (Bahiagrass)					
Argintine	1.8 bc	0.0 c	2.0 cd	0.0 d	2.0 d ns
Pensacola	0.0 c	0.0 c	0.0 d	0.0 d	0.0 d
Paspalum vaginatum (Seashore Paspalum)					
Seadwarf	0.3 c	0.0 c	1.5 cd	0.5 d	2.0 cd ns
AZ-1	0.0 c	0.0 c	0.0 d	0.0 d	0.0 d
Eremochloa ophiuroides (Centipedegrass)					
Tifblaire	0.0 c	0.3 c	0.3 d	0.3 d	0.6 d

[a]Mean number of 1st, 5th instars, total nymphs, adults, and total population on each turfgrass cultivar after an 11-week development period.

[b]Data in each column was transformed as $\sqrt{(n + 0.001)}$ for analysis; untransformed means are reported.

*Means in a column followed by the same lower case letter are not significantly different by Fishers protected LSD ($P = 0.05$) (Analysis among all turf groups).

**Means in the total column for each grass followed by the same upper case letter are not significantly different by Fishers protected LSD ($P = 0.05$) or by Student's t-test. (Analysis within a turf group only).

from the material of a BioQuip® superior aerial net (Cat. No. 7215NA, BioQuip® Products, Rancho Dominguez, CA), material was inserted at the outer end of the French drain insert to form a 15-cm deep collecting basin to catch insects that were dislodged as the grass was vacuumed.

The potted plants were maintained in the greenhouse until the week of 22 Sep 2008, when the total number of bugs produced on each plant was assayed. Plants in the experiment (1 replicate at a time) were individually submerged in 18.9-liter plastic buckets of water (the plant was weighted with a stone so it would stay submerged) and all SCB nymphs and adults that floated to the surface within 30 min were removed, identified by instars, and counted. After a plant had been submerged for 5 min and again at 15 min, the canopy of the plant was agitated by

hand to dislodge any bugs that had failed to let loose and float to the surface. This procedure was a modification of the flotation method that has been used widely to accurately assay field populations of SCB for chemical efficacy tests (Reinert 1974, 1982).

Data Analysis and Statistics

Data for the number of SCB for each growth stage for each cultivar were analyzed by Analysis of Variance (ANOVA) (PROC GLM) for a randomized complete block design with 4 replications to test for differences in the number of progeny that had developed on each cultivar. Data for cultivars were also grouped by species of grass (*Zoysia* and *Cynodon* each contained 2 species, but were analyzed by genus only) and analyzed to determine suitability among the 8 types of turfgrass tested. The transformations $\sqrt{(n + 0.001)}$ was used on each data set to achieve normality and homogeneity of variance before analysis (Kuehl 2000) but untransformed means are presented. Means were compared at the 5% level of significance with Fisher's least-significant difference (LSD) multiple range test. For the total population column, means were also compared within each grass genera by Student's *t*-test (SAS Institute 2009).

Results and Discussion

This method of caging the SCB on potted grass plants in a no-choice experiment worked well to assay the reproductive and developmental potential on each cultivar. Caging was necessary to confine the bugs on the grasses, but the main problem with this type and size of cage was that cages were not large enough to accommodate the growth potential of several of the grasses, and they had to be opened during the experiment to clip and remove leaf material from many of the cultivars. The modified Echo® blower/vacuum also worked well for collecting large numbers of SCB specimens for this type of study.

Stenotaphrum (St. Augustinegrass)

Stenotaphrum, as expected, served as the best host among the 8 turfgrass groups. The highest population was produced on 'Raleigh' with all 5 instars and adults present in substantial numbers for a mean of 163.0 nymphs, 17.8 adults, and a total population of 180.8 bugs per plant after the 11-week test period (Table 1). 'Texas Common' was the second best host (117.5 nymphs, 121.8 total), followed by 'Captiva' with 93.3 nymphs and 97.5 total bugs produced on it. Analysis conducted across all 8 groups of turfgrass showed that Raleigh produced significantly more SCB than either Texas Common or Captiva, but all 3 cultivars serve as good reproductive hosts and all 3 mean populations far exceeded the accepted damage threshold level of 20 to 30 bugs per 0.1 m^2 (Reinert 1972; Buss & Unruh 2006). The highest individual SCB population on any of the replicate plants of Raleigh, Texas Common, and Captiva was 311, 252, and 155, respectively. Neither 'FX-10' nor 'Floratam' served as an acceptable host with this population of SCB and they yielded only an average of 1.3 and 1.1 total bugs, respectively. Moreover, all of the bugs on these 2 cultivars had developed on only 1 replicate plant. Additionally, they were all first instars, except for 1 third instar on 1 of the FX-10 replicate plants and 1 fifth instar on 1 Floratam replicate plant. Analysis conducted only on the 5 cultivars of *Stenotaphrum* showed that population levels on FX-10 and Floratam were not significantly different, and they were significantly lower than those on the 3 susceptible cultivars (Raleigh, Texas Common, and Captiva).

In a related study in a lab no-choice experiment with the same population of SCB, adult survival was high on Raleigh, Texas Common, and Captiva (72-78%), but survival on Floratam and FX-10 was only 48 and 58%, respectively, after 7 d of confinement (Reinert unpublished data). However, when Floratam and FX-10 were first released (Horn et al. 1973; Busey 1993), both cultivars consistently provided >80% antibiosis within 7 d for populations of SCB adults that were collected from lawns in Florida (Reinert & Dudeck 1974; Reinert 1978). More recently, however, Cherry & Nagata (1997) showed that oviposition of eggs was high and survival on Floratam, Seville, Bitterblue, and FX-10 cultivars was 88.6 to 75.6% for populations of SCB collected from Florida lawns.

Zoysia (Zoysiagrass)

Among the 5 *Zoysia* cultivars, 'Palisades' served as the best host for SCB with the developing population consisting of all 5 instars and adults. A mean of 16.5 nymphs and 19.0 total bugs had developed on this cultivar during the 11-week test period. When the *Zoysia* cultivars were analyzed either among the total cultivars or separately for the genus, the same statistical separations were recorded (Table 1). Palisades produced a significantly higher number of SCB than 'Cavalier' (mean total of only 1 SCB), but the population on Palisades was not significantly higher than either 'Emerald', 'Zorro', or 'Empire'.

Although *Zoysia* is not normally considered a primary host of the SCB (Reinert et al. 1995), this study shows certain cultivars, Palisades along with Emerald, Zorro, and Empire can serve as acceptable reproductive hosts with mean development of ≥5.5 total bugs during this study. This would be an equivalent of 31 bugs per 0.1 m^2, which is within the considered threshold of damage on *Stenotaphrum*. Three of the replicate

plants of Palisades had total populations >23 SCB. Also, 1 replicate plant each of Zorro, Emerald, and Empire had total populations of 27, 26, and 14 SCB, respectively. Population development on Cavalier in the present study with *B. insularis* was the lowest among the *Zoysia* cultivars tested with an average of 1.1 bugs per replicate plant.

Studies with a related *Blissus* species, the western chinch bug (*B. occiduus* Barber*)*, showed that *Zoysia* and particularly the cultivars 'Zenith', 'Meyer', and 'Crowne', serve as acceptable hosts for that species as well (Eickhoff et al. 2006, 2007). Populations of *B. occiduus* preferred both *Buchloë* and *Zoysia.* Cavalier along with Emerald and Zorro produced the lowest number of *B. occiduus* in their greenhouse study and these cultivars were listed as moderately resistant (Eickhoff et al. 2007). Cavalier expresses good resistance to both species of *Blissus.*

Buchloë (Buffalograss)

For the 2 cultivars of *Buchloë*, only '609" served as a good host for SCB with 7.5 nymphs and a total of 10.0 bugs per plant (Table 1). This would be equivalent to 56.5 bugs 0.1 m^2 which is within the threshold of damage on *Stenotaphrum*. Although there was a large difference between the mean number of SCB that developed on the 2 cultivars, there was no significant difference due to a large amount of variance among the replicates.

Other Turfgrass Genera

Surprisingly, both cultivars of *Festuca*, 'Rebel', and 'Paladin', did support low development of SCB with total mean numbers of 6 and 4.1 bugs per replicate plant, respectively (Table 1). The development on the 5 *Cynodon* cultivars was very low. Poor development of SCB has also been reported on *Cynodon* (no cultivar identified) by Cherry & Nagata (1997). Slater (1976) in his study of host relationships of Blissinae described *Cynodon* as a breeding host for the SCB. However, Kelsheimer & Kerr (1957) reported *Cynodon* to be rarely attacked by the SCB. One of the authors has received numerous reports of chinch bug feeding on and damage to *Cynodon* in Florida, Texas, and in island nations throughout the Caribbean, but most likely these populations and their damage were caused by another *Blissus* species. Cynodon has been reported as a host of *B. leucopterus leucopterus* Say (Lynch et al. 1987).

The 2 cultivars of *P. notatum*, 2 cultivars of *P. vaginatum*, and 1 cultivar of *Eremochloa* did not support much SCB development (<2.0 bugs per plant) in this study. Also, only 1 adult developed on 1 of the *Eremochloa* cv. 'Tifblaire' replicate plants. Kelsheimer & Kerr (1957) reported *Eremochloa* to be an occasional host for the SCB. Additionally, Kerr (1966) reported that SCB will attack other lawn grasses (*P. notatum*, *Cynodon*, *Eremochloa*, and *Zoysia*) but mostly it is a problem on *Stenotaphrum.* Other common hosts include crabgrass (*Digitaria* spp.), torpedograss (*Panicum repens* L.), and pangolagrass (*Digitaria eriantha* Steud) (Slater & Baranowski 1990).

This study confirms the high suitability of *Stenotaphrum* as a developmental host for the SCB. We also show that 4 cultivars of *Zoysia* and the cultivar 609 *Buchloë* serve as good breeding hosts and may have potential for damage by SCB.

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TOMARUS SUBTROPICUS (COLEOPTERA: SCARABAEIDAE) LARVAL FEEDING HABITS

OLGA S. KOSTROMYTSKA[1] AND EILEEN A. BUSS[2]
[1]Entomology Department, Rutgers, The State University of New Jersey, 93 Lipman Drive, New Brunswick, NJ 08901

[2]Entomology and Nematology Department, University of Florida, P.O. Box 110620, Gainesville, FL 32611

ABSTRACT

The importance of soil organic matter for *Tomarus subtropicus* Blatchley larval development and survival, the amount of damage larvae could cause on turfgrasses, and potential larval host range were investigated in greenhouse experiments. First instars were reared individually in seedling trays containing sand or peat, with or without St. Augustinegrass. Survival, developmental stage, and final weight were recorded 1 month after introduction. First instars died in the pots with peat but no grass, so it appears that grass roots were critical for larval growth and development. Soil organic matter did not significantly affect grub weight gain and development, but more root loss occurred with grass grown in sand. In host range tests (2005 and 2006), first and third instars were reared on 6 species of warm season grasses and ryegrass. Grub weight gain, development, survival and grass root reduction were determined 2 months after introduction. Larval survival ranged from 62-93% if grubs were reared on warm season grasses to only 40% if reared on ryegrass. Grubs reared on warm season grasses gained weight and successfully developed into third instars, indicating that all of the tested warm season turfgrasses were suitable for larval *T. subtropicus* growth and development. Grub feeding caused significant root reduction of all grasses in our study, which ranged from 36 to 87% and differed among grass species. As result, quality ratings and clipping yields decreased for most of the turfgrasses after 5 weeks of infestation, but bahiagrass and seashore paspalum were less affected by *T. subtropicus* root feeding, compared to the other grass species.

Key Words: sugarcane grub, host range, bermudagrass, sugarcane, St. Augustinegrass, ryegrass, root reduction, soil organic matter, grub weight gain and development

RESUMEN

La importancia de materia orgánica en el suelo para el desarrollo y sobrevivencia de larvas de *Tomarus subtropicus* Blatchley, la cantidad de daño que las larvas puedan causar en el césped y el rango potencial de hospederos por las larvas fueron investigados en experimentos realizados en invernaderos. Se criaron los primeros instares individualmente en bandejas usadas para plantillas con arena o turba y con o sin el césped San Augustin. El sobrevivencia, el estadio de desarrollo y el peso final fueron anotados 1 mes después de la introducción. Los primeros instares murieron en las macetas con turbo y sin grama, esto parece indicar que las raices de grama son básicas para el crecimiento y desarrollo de las larvas. La materia orgánica del suelo no afecto el aumento en el peso y el desarrollo de las larvas, pero hubo una mayor pérdida de las raices de la grama sembrada en arena. En pruebas del rango de los hospederos (2005 y 2006), se criaron los primeros y terceros instares sobre 6 especies de grama de la estación cálida y sobre centeno. Se determinaron el aumento en el peso de las larvas, el desarrollo, la sobrevivencia reducción en las raices de la grama 2 meses despues de la introducción. El sobrevivencia de las larvas fue entre 62-93% en las larvas criadas sobre grama de la estación cálida y solo 40% en larvas criadas sobre centeno. Las larvas criadas sobre grama de la estación cálida aumentaron en peso y se desarrollaron exitosamente en instares de tercer estadio, que indica que todas las clases de grama de la estación cálida probadas fueron apropiadas para el crecimiento y desarrollo de larvas de *T. subtropicus.* En nuestro estudio, la alimentación de las larvas causó una reducción significativa de las raices de 36 a 87% y varían entre las especies de grama. Como un resultado, el indice de la calidad y el rendimiento de las cortadas de grama diminuyó para la mayoría de las clases de grama después de 5 semanas de infestación, pero el césped Bahia y el paspalum costero fueron menos afectados por la alimentación de *T. subtropicus* sobre las raices, comparados con las otras especies de grama.

Tomarus subtropicus Blatchley is a destructive turfgrass pest along Florida's Gulf and Atlantic Coasts, but is also distributed along coastal Alabama, Georgia, South Carolina, and North Caro-

lina (Cartwright 1959). As with other grub species, *T. subtropicus* grubs directly damage turf by their root-feeding and tunneling behaviors (Ritcher 1966; Tashiro 1987; Braman & Pendley 1993). This pest is univoltine, with eggs present from late Jun to early Aug, first instars from Jul to Aug, second instars from Aug to Sep, and mostly third instars from Oct to Feb in Florida (Kostromytska & Buss 2008). *Tomarus subtropicus* attacks the roots of sugarcane (*Saccharum* spp.) (Gordon & Anderson 1981), St. Augustinegrass (*Stentaphrum secundatum* (Walt. Kuntze)) (Kostromytska & Buss 2008), and bermudagrass (*Cynodon dactylon* (L.)) (Summers 1974; Reinert 1979; Prewitt & Summers 1981), resulting in crop yield loss and large patches of dead turfgrass. Its potential to feed on and injure other warm season turfgrasses has not been assessed. *Tomarus subtropicus* grubs also feed on plant roots in ornamental plant beds, and cause plant dieback (E. Buss, personal observation).

White grub feeding habits vary depending on the species. Some species (e.g., *Cotinis nitida* L.) obtain nutrients from soils high in organic matter (Brandhorst-Hubbard et al. 2001), while others need live plant roots (e.g., *Popillia japonica* Newman, *Rhizotrogus majalis* (Razumowsky), *Cyclocephala* spp., *Phyllophaga* spp.). Some scarab species consume soil organic matter as first instars, then switch to live roots in later instars (Litsinger et al. 2002). The larval feeding habits of *T. subtropicus* have not been clearly described. *Tomarus subtropicus* females oviposit and their offspring develop in soil with a high organic matter content (e.g., muck soil in sugarcane fields) (Cherry & Coale 1994), but they also survive and develop in sandy soils with low organic matter in residential environments (Kostromytska & Buss 2008).

Because the urban landscape is a complex system with many plant species, understanding the feeding preference of key pests helps to explain pest distribution, abundance, and damage related to feeding, and can affect the management strategies used against them. We conducted no-choice tests to assess the effect of soil organic matter on *T. subtropicus* survival and development, and to determine which other warm season turfgrasses could be hosts for *T. subtropicus* grubs.

Materials and Methods

Effect of 2 Soil Types on First Instars

A no-choice test with a nutrient-poor soil (sand) and an organic soil (Black Velvet peat (Black Gold Compost Co., Oxford, Florida)) was conducted from Jul to Aug 2005 to evaluate *T. subtropicus* larval growth and survival. The roots of 'Palmetto' St. Augustinegrass plugs were washed, and grass plugs were replanted into the seedling tray cells (8 × 8 × 8 cm) with either sand or peat (48 cells for each growing media). Another 24 cells were filled with peat, but no grass. Grass was maintained in the greenhouse for 2 weeks before the experiment. Cells were arranged in a randomized complete block design.

Adult *T. subtropicus* were collected from infested St. Augustinegrass lawns in Punta Gorda (Charlotte County) and Fort Myers (Lee County), Florida, and held in the laboratory to obtain eggs and young larvae (Kostromytska 2007). Grubs (2-6 d old; mean weight: 0.028 ± 0.002 g) were randomly assigned to the following treatments: peat only, peat and grass, or sand and grass (24 replicates or cells for each). Cells of grass planted in sand or peat (24 cells of each) were uninfested controls. Individual first instars were placed in a depression (2.5 cm deep, 0.7 cm in diameter) made in the center of each cell and covered with soil. Each cell was provided 30 mL of water daily. After 1 month, cells were visually inspected, and surviving grubs were weighed. Grass roots were washed with a #10 sieve, oven-dried in paper bags for 48 h at 55°C, and root dry weights were recorded. Data were analyzed by an ANCOVA (SAS Institute 2004) with soil type as a factor, post-treatment grub weight as a dependent variable and initial grub weight as a covariate. A two-way ANOVA was also conducted with soil type and grub presence as factors and dry root weight as a dependent variable. Tukey's HSD test was conducted for mean separation.

Survival and Growth of Third Instar *T. subtropicus* Reared on Different Turfgrass Species

Six warm season and 1 cool season turfgrasses were evaluated as possible hosts for *T. subtropicus* grubs. Tested grasses included Palmetto St. Augustinegrass, 'Tifway' bermudagrass (*C. dactylon* × *transvaalensis* Burtt-Davy), 'Empire' zoysiagrass (*Z. japonica* Steud.), common centipedegrass (*Erimochloa ophiuroides* (Munro) Hack), 'Pensacola' bahiagrass (*Paspalum notatum* Flugge), 'Sea Dwarf' seashore paspalum (*Paspalum vaginatum* Swartz), and 'Gulf' annual ryegrass (*Lolium multiflorum* Lam.). Thirty plugs (15 cm diameter) of each warm season grass species were obtained from the University of Florida Plant Science Unit in Citra (Marion County), Florida, in Aug 2005. Soil was washed off the roots and the grass plugs were planted with FaFard mix #2 (Conrad Fafard, Inc., Agawam, Massachusetts) in plastic pots (15 cm diameter). Annual ryegrass was seeded at a rate of 0.05 kg/m^2. Grass was watered daily and fertilized monthly with 24.4 kg of N per ha during 2 months of establishment. Pots were arranged in a randomized complete block design in the greenhouse.

Initial grub weights were obtained, then 1 recently molted (<7 d old) third instar was put in a shallow depression on the soil of each of 15 pots

for each turfgrass species. Grubs that failed to dig into the soil within 10 min were replaced. Larval survival, weight, and weight gain were determined after 8 weeks. Daylight was supplemented with lights to provide a photoperiod of 16:8 h (L:D) and the average ambient greenhouse temperature was ~23.4°C. Pots were watered with 150 mL of tap water every other day. Each turfgrass species was maintained at its recommended height (Turgeon 2002): 1.3 cm for seashore paspalum, 2.5 cm for bermudagrass, 5.1 cm for centipedegrass and zoysiagrass, and 7.6 cm for annual ryegrass, St. Augustinegrass and bahiagrass. To assess the amount of feeding damage, grass clippings were collected weekly and fresh weights were taken within 2 h. Clippings were then oven-dried for 48 h at 55°C, and weighed. After 8 weeks of infestation, grass roots were cut within 2 mm of the plant crown, washed with a #20 sieve, oven-dried for 48 h (55°C), and dry root weights were recorded.

Tomarus subtropicus Neonate Survival, Growth and Development on 6 Warm Season Grasses

The warm season turfgrass species noted in the previous section were tested as potential hosts for *T. subtropicus* larvae in a greenhouse experiment in 2006. Grass plugs were planted into 10-cm plastic pots with native soil (94% sand, 4% clay, 2% silt and 1.6% organic matter), and allowed 2 months to establish. First instars (1-3 d old) were weighed immediately before being individually placed in a hole (8 mm diameter, 5 cm deep) in the soil of each pot, and were covered with soil. Grass was watered daily as needed and fertilized weekly (0.6 kg of N per ha, Miracle Gro® Scotts Miracle-Gro Products Inc., Marysville, OH). Four replicates per grass species were arranged and each replicate included 6 infested and 6 uninfested control pots. Grubs were removed from the pots after 8 weeks, and larval survival, weight, and head capsule width were determined. Grass clippings were collected and processed weekly, as previously described. Grass color and density were visually assessed on a scale from 1 (yellow, sparse) to 9 (dark green, very dense) and total grass quality was calculated by averaging the two scores. After the grubs were removed, grass roots were cut to within 2 mm of the plant crown, washed with a #20 sieve, and placed in paper bags. The remaining plant parts were collectively placed into paper bags, and oven-dried for 48 h (55°C). Dry root weight and dry total plant yield were recorded.

Statistical Analysis

The correlation between initial and final grub weights was tested before analysis. If the 2 variables were significantly correlated, the ANCOVA GLM procedure (SAS Institute 2004) was used to analyze the effect of turf species on grub final weight with a correction for initial weight for all experiments. Percent of root reduction was calculated as averaged root weights of controls minus root weights of infested plants and divided by averaged weights in controls. Analysis of variance (GLM procedure, SAS Institute 2004) was used to determine the effect of turf species on the percentage of grub survival and development, and effect of grub presence on dry root weight. Proportion data were arcsine square root transformed before analysis. Clipping weights and grass quality ratings were analyzed by a repeated measure analysis (SAS Institute 2004) with time as a repeated within-group factor and grub presence as a between-group factor. Means were separated by Tukey's HSD.

Results and Discussion

Effect of 2 Soil Types on First Instars

Tomarus subtropicus grubs fed on live St. Augustinegrass roots, regardless of soil type, in this test. Two grubs (8%) that were reared on peat without grass survived, but they remained first instars, and all other grubs in this treatment died. All grubs provided with St. Augustinegrass roots, regardless of soil type, were second instars when the test was evaluated. Grub survival to the second instar when reared on peat with grass was 83%, and 75% when reared on sand with grass. *Tomarus subtropicus* body weights were statistically similar when grubs were reared on grass grown in peat (0.87 ± 0.07 g) or grass grown in sand (0.74 ± 0.08 g).

Grub feeding significantly reduced St. Augustinegrass dry root weight compared to uninfested cells, regardless of soil type ($F = 156.66$; $df = 1, 85$; $P < 0.0001$). Uninfested pots had statistically similar dry St. Augustinegrass root weights (1.24 ± 0.06 g in peat; 1.17 ± 0.06 g in sand). The final dry root weight of infested grass grown in sand (0.31 ± 0.05 g) was significantly lower than in infested grass grown in peat (0.59 ± 0.05 g) ($F = 4.59$; $df = 1, 85$; $P = 0.03$), indicating that more root herbivory may have occurred in the pots with sand.

Peat, like muck soil, is an organic soil, consisting of poorly decomposed animal and plant remnants (Brown 2009) with organic matter content ranging from 40 to 80% (Andriesse 1988; Litaor et al. 2005; Kechavarzi et al. 2010) which can provide nutrients (e.g., carbon, nitrogen, sulfur, and phosphorous) to plants and soil fauna (Andriesse 1988; Killham 1994). Although the nutrient content of the soils or turfgrass were not measured in our test (fertilization and irrigation were consistent across all treatments), it is possible that the peat provided additional nutrients to the grass, which could lead to either more efficient grub

feeding or increased compensatory plant growth (Radcliffe 1970; Brown and Gauge 1990; Steinger & Müller-Schärer 1992; Sparling et al. 2006). In addition, grubs may, while feeding on grass roots, acquire additional nutrients when ingesting soil with greater organic matter content (Seastedt 1985; Brown & Gange 1990), and thus cause less root damage. The tendency for insects to consume more of a nutritionally inferior food to overcome the lack of needed nutrients has been documented for many taxa (King 1977; Yang & Joern 1994; Obermaier & Zwolfer 1999; Berner et al. 2005).

Survival and Growth of Third Instar *T. subtropicus* Reared on Different Turfgrass Species

Third instar initial weights (1.97 ± 0.08 g) were statistically similar among treatments (F = 0.71; df = 6, 76; P = 0.64). However, initial and final grub weights were significantly correlated (Pearson's r = 0.37, P = 0.001) and were included as covariates in the analysis. Final grub weight differed statistically among grasses (Table 1). Third instar weights when reared on ryegrass (1.96 ± 0.2 g) and bermudagrass (2.1 ± 0.2 g) were significantly lower than grub weights on any of the other grasses tested (F = 8.51; df = 6, 76; P < 0.0001). However, 66.7% of the grubs survived on bermudagrass and only 40% survived on ryegrass. Analysis of grass dry root weight with and without grubs indicated that there was a significant root reduction in all pots with warm season grasses, but not in the pots with ryegrass (Fig. 1). These data suggest that ryegrass was a poor larval host for *T. subtropicus*. Annual ryegrass was the only C_3 grass included in the test. C_3 grasses typically are more beneficial nutritionally to herbivores than C_4 grasses (Barbehenn & Bernays 1992), however physical properties could be a key factor for herbivore host selection (Scheirs et al. 2001). Suitability of annual ryegrass as a host for *T. subtropicus* larvae may be affected by the physical structure of the grass root system. Numerous ryegrass fine roots created a dense mesh through the entire pot, which may have reduced grub movement. At evaluation, live grubs in ryegrass pots were in soil chambers that were located near pot walls and not more than 5 cm deep. In contrast, the other grasses had thick main roots from which roots branched closer to the pot walls and bottom, and grubs were often found in the center of the pots at different depths.

Grass leaf growth changed differently among grass species over time (summarized statistics are in Table 2). For St. Augustinegrass, the main effect of grub presence and the interaction of grub feeding with time were significant. On

TABLE 1. LARVAL *T. SUBTROPICUS* SURVIVAL AND GROWTH ON DIFFERENT GRASS SPECIES AND ASSOCIATED ROOT REDUCTION IN 2005 AND 2006.

Grass species	% Grub survival	Initial grub weight (g ± SEM)[1]	Final grub weight (g ± SEM)[2]	Proportional weight gain[3]	Root reduction (% ± SEM)[4]
2005					
Bahiagrass	93.3 ± 6.7	1.8 ± 0.2a	3.05 ± 0.2b	1.8 ± 0.1a	27.4 ± 8.8bc
Bermudagrass	66.7 ± 6.7	1.7 ± 0.2a	2.10 ± 0.2a	1.1 ± 0.3ab	64.9 ± 3.2a
Centipedegrass	66.7 ± 24.0	1.9 ± 0.3a	3.43 ± 0.2b	2.1 ± 0.3a	60.5 ± 4.9a
Ryegrass	40.0 ± 0.0	2.2 ± 0.7a	1.96 ± 0.2a	1.0 ± 0.1b	17.2 ± 4.9c
Seashore paspalum	86.7 ± 6.7	2.1 ± 0.2a	3.57 ± 0.2b	1.8 ± 0.1a	49.1 ± 9.1ab
St. Augustinegrass	66.7 ± 6.7	2.2 ± 0.2a	3.18 ± 0.1b	1.4 ± 0.1a	52.2 ± 7.7ab
Zoysiagrass	86.7 ± 6.7	2.0 ± 0.1a	3.04 ± 0.1b	1.8 ± 0.2a	55.3 ± 6.4ab
2006					
Bahiagrass	79.3 ± 7.9	0.028 ± 0.002a	2.62 ± 0.2abc	103.7 ± 11.2abc	48.1 ± 4.6bc
Bermudagrass	66.8 ± 6.7	0.034 ± 0.003a	2.20 ± 0.2c	69.6 ± 8.3c	87.5 ± 4.6a
Centipedegrass	91.5 ± 4.9	0.030 ± 0.002a	2.43 ± 0.2bc	87.6 ± 0.7bc	36.3 ± 4.2c
Seashore paspalum	62.5 ± 10.5	0.031 ± 0.003a	3.35 ± 0.2a	126.2 ± 15.6a	60.8 ± 4.6ab
St. Augustinegrass	87.3 ± 4.3	0.033 ± 0.002a	2.62 ± 0.2abc	92.2 ± 7.9abc	65.0 ± 6.8ab
Zoysiagrass	70.8 ± 7.9	0.030 ± 0.002a	3.13 ± 0.1ab	114.3 ± 8.7ab	80.9 ± 2.8a

[1]Initial grub weight was not significantly different among treatments at α = 0.05 (2005: F = 1.64; df = 6, 69; P = 0.15 and 2006: F = 1.84; df = 5, 143; P = 0.11).

[2]Means within columns with different letters are statistically different at α = 0.05 (2005: F = 8.51; df = 6, 76; P < 0.0001 and 2006: F = 5.04; df = 5, 109; P = 0.0004).

[3]Proportions were calculated by dividing the final weights by initial weights. Means within columns with different letters are statistically different at α = 0.05 (2005: F = 3.89; df = 6, 76; P = 0.002 and 2006: F = 3.95; df = 5, 109; P = 0.0026).

[4]Means within columns with different letters are statistically different at α = 0.05 (2005: F = 6.32; df = 6, 105; P < 0.0001 and 2006: F = 16.52; df = 5, 111; P < 0.0001).

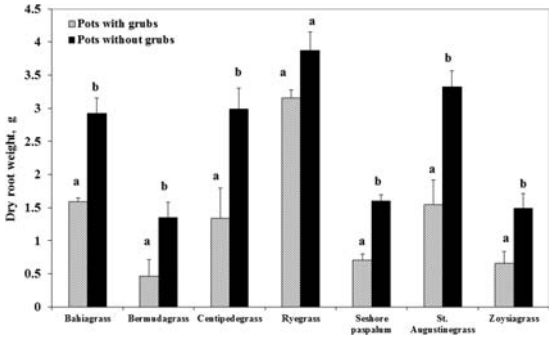

Fig. 1. Reduction of root mass caused by third instar *T. subtropicus* feeding, 2005. Means marked with different letter are different at a = 0.05 ($F = 39.51$; $df = 1, 130$; $P < 0.0001$)

average, clippings collected from the infested pots weighed less than clippings from uninfested control pots, and this difference increased over time. For bahiagrass, bermudagrass, and centipedegrass the main effect of grub presence was significant, whereas the interaction between grub presence and time was not significant. Clipping yield of these grasses changed significantly over time in all pots, but uninfested pots on average yielded more clippings. For zoysiagrass, the main effect of grub presence was not significant although the main effects of time and the interaction of time and grub presence were significant. Thus, decrease in clipping weight over time was more pronounced in the pots with grubs. Only the main effect of time was significant for ryegrass and seashore paspalum, so grass growth changed over time, but variation of clipping yield was not related to grub presence.

Tomarus subtropicus Neonate Survival, Growth, and Development on 6 Warm Season Grasses

On average, 76.3% of grubs survived across treatments and 70.8% of all (94% of survivors) grubs reached the third instar (Table 1). Percentage survival, percent of grubs that reached the third instar, and head capsule width did not differ among the grasses ($F = 1.64$; $df = 5, 23$; $P = 0.20$; $F = 2.17$; $df = 5, 23$; $P = 0.10$; and $F = 1.73$; $df = 5, 109$; $P = 0.13$).

Mean initial first instar weight (0.031 ± 0.01 g) did not correlate with mean grub final weight (2.75 ± 0.86 g) ($r = 0.11$, $P = 0.22$), and was not included in the analysis as a covariate. Final grub weight and proportional weight gain differed among grasses. Similar to the result obtained in the 2005-experiment, grubs feeding on bermudagrass gained less weight (weight gain about 70 times initial weight) when compared to grubs reared on seashore paspalum (weight gain about 126 times initial weight) and zoysiagrass (weight gain about 114 times) (Table 1).

Similar to the 2005-experiment, bermudagrass appeared to be a poorer host for *T. subtropicus* compared to the other warm season grasses. However, *T. subtropicus* larvae are reported to damage bermudagrass (Reinert 1979), so despite the slower grub growth, this grass may still be an acceptable host. Reduced grub growth may be influenced by a smaller root mass in bermudagrass (grubs consumed on average 87.5% of root mass).

TABLE 2. STATISTICS SHOWING EFFECTS OF TIME, GRUB FEEDING, AND THEIR INTERACTION ON CLIPPING YIELD DURING AN 8-WEEK PERIOD IN 2005 AND 2006.

	Grub feeding			Time			Time*Grub		
Grass species	F	*df*	P	F	*df*	P	F	*df*	P
2005									
Bahiagrass	8.50	1, 27	<0.01	2.48	7, 21	0.05	1.05	7, 21	0.43
Bermudagrass	11.22	1, 23	<0.01	44.49	7, 17	<0.01	1.96	7, 17	0.12
Centipedegrass	12.80	1, 23	<0.01	3.20	7, 17	0.02	1.6	7, 17	0.20
Seashore paspalum	3.07	1, 19	0.10	20.12	7, 13	<0.01	2.57	7, 13	0.07
St. Augustinegrass	0.09	1, 27	0.76	2.92	7, 21	0.03	0.67	7, 21	0.74
Zoysiagrass	9.47	1, 23	<0.01	1.99	7, 17	0.11	2.69	7, 17	0.05
2006									
Bahiagrass	1.56	1, 41	0.22	5.43	7, 35	<0.01	1.99	7, 35	0.08
Bermudagrass	8.10	1, 38	<0.01	5.38	7, 32	<0.01	2.47	7, 32	0.04
Centipedegrass	4.66	1, 44	0.04	6.09	7, 38	<0.01	0.36	7, 38	0.92
Seashore paspalum	0.30	1, 39	0.59	6.88	7, 33	<0.01	0.68	7, 33	0.69
St. Augustinegrass	1.49	1, 44	0.01	5.38	7, 32	<0.01	2.47	7, 32	0.04
Zoysiagrass	4.61	1, 46	0.04	9.48	7, 34	<0.01	1.29	7, 34	0.28

Grub movement was also limited, so grubs could not migrate in search of food after the previous source had been exhausted.

Grub feeding caused significant root reduction of all grasses in our study ($F = 70.61$; $df = 6, 287$; $P < 0.0001$) (Fig. 2). The percent of root reduction ranged from 36 to 87% and differed among grasses ($F = 16.52$; $df = 5, 111$; $P < 0.01$) (Table 1). However, the total plant yield was reduced only for bahiagrass and bermudagrass ($F = 22.81$; $df = 11, 287$; $P = 0.001$) (Fig. 3). Root loss does not necessarily reduce aboveground plant growth, and in some cases, minor root damage can lead to increased or compensatory foliage growth (Humphries 1958; Seastedt et al. 1988; Brown & Gange 1990; Bardgett et al. 1999; Blossey & Hunt-Joshi 2003).

Measurements of grass yield over time demonstrated that tested grasses responded differently to *T. subtropicus* herbivory (statistics are summarized in Table 2). Centipede and zoysiagrass tended to yield fewer leaf clippings if infested with grubs, and clipping weights varied over time (the main effects of infestation and time on clipping yield were significant), but effect of grub feeding was not significantly stronger with time (interaction was not significant). The interaction of the 2 factors was significant for bermudagrass and St. Augustinegrass, so grub feeding decreased clipping yield beginning week 5. Grub feeding did not affect clipping yield in pots of bahiagrass and seashore paspalum.

Grub feeding reduced the quality ratings for St. Augustinegrass, bermudagrass, zoysiagrass and centipedegrass, but not for bahiagrass and seashore paspalum (statistics are summarized in Table 3). Differences in grass quality were apparent 4 weeks (St. Augustinegrass, bermudagrass), 6 weeks (zoysiagrass), and 8 weeks (centipedegrass) after grubs were introduced. Grass crowns could be pulled easily from the pots with grubs, but grass remained green in all pots.

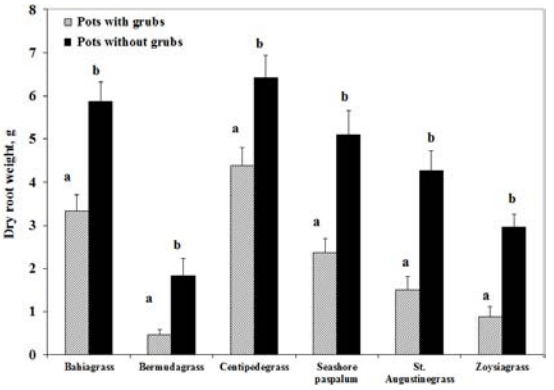

Fig. 2. Reduction of root mass caused by larval *T. subtropicus* feeding, 2006. Means marked with different letters are different at $\alpha = 0.05$ ($F = 70.61$; $df = 1, 287$; $P < 0.0001$)

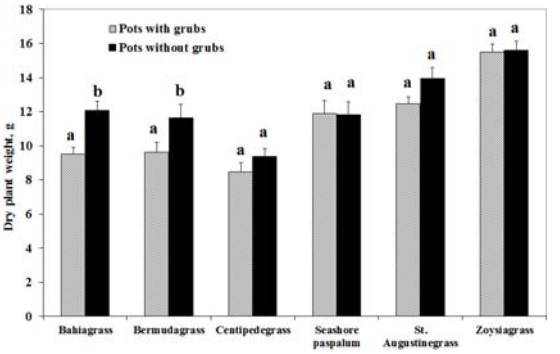

Fig. 3. Effect of grub feeding on total plant yield by warm season grasses, 2006. Means marked with different letter are different at a = 0.05 ($F = 5.51$; $df = 1, 253$; $P = 0.02$)

Our study demonstrated that *T. subtropicus* can successfully survive and develop on bahiagrass, bermudagrass, centipedegrass, zoysiagrass and seashore paspalum, and confirmed that St. Augustinegrass was an adequate host (Reinert 1979), regardless of soil organic content. Quality ratings and clipping yields decreased for most of the turfgrasses after 5 weeks of infestation, but bahiagrass and seashore paspalum were less affected by *T. subtropicus* root feeding, compared to the other grass species. It was previously reported that 3 grubs per 0.1 m^2 could severely damage bermudagrass (Reinert 1979), but the grasses in our study could tolerate approximately 5 and 12 third instar grubs per 0.1 m^2 in 2005 and 2006, respectively, despite >50% root reduction.

Environmental conditions (temperature, photoperiod, herbivore aboveground grazing or mowing, fertilization, and irrigation practices) can significantly affect plant tolerance to root herbivory in addition to plants characteristics and insect density (Ladd & Buriff 1979; Seastedt et al. 1988; Brown & Gange 1990; Potter et al. 1992; Crutchfield et al. 1995; Crutchfield & Potter 1995; Braman & Raymer 2006). For instance, 4 Japanese beetle (*Popillia japonica* Newman) grubs per 15-cm pot (~20 grubs per 0.1 m^2) significantly reduced *Poa pratensis* L. clipping yield in a greenhouse study (Ladd & Buriff 1979), but clipping yield from other field and greenhouse tests was unaffected by 60-90% root reduction from 40-60 grubs per 0.1 m^2 and 24-30 grubs per 0.1 m^2, respectively (Potter et al. 1992; Crutchfield & Potter 1995).

During our experiment, grass was regularly irrigated, and although the roots were dramatically reduced and crowns could be easily removed from infested pots, the foliage remained green. Most third instar-feeding in the field occurs during the

TABLE 3. STATISTICS SHOWING EFFECT OF TIME, GRUB FEEDING, AND THEIR INTERACTION ON TURFGRASS QUALITY IN 2006.

	Grub feeding			Time			Time*Grub		
Grass species	F	*df*	P	F	*df*	P	F	*df*	P
Bahiagrass	0.6	1, 44	0.44	3.90	7, 40	0.15	0.95	7, 40	0.43
Bermudagrass	9.08	1, 44	<0.01	17.07	7, 40	<0.01	5.54	7, 40	<0.01
Centipedegrass	5.71	1, 44	0.02	13.51	7, 40	<0.01	8.66	7, 40	<0.01
Seashore paspalum	0.04	1, 44	0.83	4.61	7, 40	0.01	0.37	7, 40	0.70
St. Augustinegrass	9.08	1, 44	<0.01	17.07	7, 40	<0.01	5.54	7, 40	<0.01
Zoysiagrass	32.23	1, 44	<0.01	14.08	7, 40	<0.01	9.82	7, 40	<0.01

fall (Kostromytska 2007), which coincides with reduced rainfall and slower warm season turfgrass growth. Thus, drought and/or other environmental stresses during this time, in addition to root damage by grubs, may more quickly overwhelm the grass.

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VAGILITY AS A LIABILITY: RISK ASSESSMENT OF THE LEAF-BLOTCHING BUG *EUCEROCORIS SUSPECTUS* (HEMIPTERA: MIRIDAE), A PROSPECTIVE BIOLOGICAL CONTROL AGENT OF THE AUSTRALIAN TREE *MELALEUCA QUINQUENERVIA*

GARY R. BUCKINGHAM[1,*], SUSAN A. WINERITER[1], JASON D. STANLEY[2], PAUL D. PRATT[3] AND TED D. CENTER[3]
[1]USDA-ARS Invasive Plants Research Laboratory, P.O. Box 147100, Gainesville, FL 32614-7100

[2]Division of Plant Industry, Florida Department of Agriculture and Consumer Services, P.O. Box 147100, Gainesville, FL 32614-7100

[3]USDA-ARS Invasive Plant Research Laboratory, 3225 College Ave., Fort Lauderdale, FL 33314

*Retired

ABSTRACT

Melaleuca quinquenervia (Cav.) S.T. Blake (Myrtales: Myrtaceae) forms dense monocultures that displace native vegetation in wetlands of southern Florida, USA. Faunal studies in the tree's native Australian range revealed several prospective biological control agents, including the leaf-blotching bug, *Eucerocoris suspectus* Distant (Hemiptera: Miridae). This herbivore was imported into quarantine to assess risk to Florida native and ornamental species after preliminary Australian studies had indicated that it might be useful. Ornamental *Melaleuca* spp. suffered heavy feeding in no-choice adult feeding trials, with moderate feeding on some native Myrtaceae. Native species sustained light to heavy feeding in multichoice adult feeding trials and in a no-choice nymphal feeding trial. Feeding increased on native species in a large enclosure after *M. quinquenervia* was cut, allowed to dry, and then removed. Nymphs completed development only on *M. quinquenervia* and ornamental bottlebrushes, *Melaleuca* spp. However, inability to fully develop on non-target species is of limited importance as a criterion for release of insects with highly mobile immature stages as compared to less vagile species. Local movement from the host to other plant species could result in unacceptable non-target damage despite seemingly adequate developmental specificity. This insect would clearly harm native and ornamental Myrtaceae and should therefore not be released.

Key Words: Biological control, Hemiptera, *Eucerocoris suspectus*, host range, *Melaleuca quinquenervia*, Miridae, Myrtaceae, risk assessment, weed control

RESUMEN

Melaleuca quinquenervia (Cav.) S.T. Blake (Myrtales: Myrtaceae) forma monoculturas densas que desplazen la vegetación nativa en las tierras humedas en el sur de la Florida, EEUU. Estudios faunísticas realizados en el rango nativo Australiano del árbol revelan varios agentes de control biológico prospectivos, incluyendo un chinche que mancha las hojas, *Eucerocoris suspectus* Distant (Hemiptera: Miridae). Este herbívoro fue importado al laboratorio de cuarentena para evaluar su riesgo hacia las especies nativas de la Florida y ornamentales después de que estudios preliminares en Australia indicaron que esta especie puede ser útil. Especies ornamentales de *Melaleuca* sufrieron niveles fuertes de alimentación en pruebas sin opción de los adultos, con alimentación moderada en plantas nativas de la familia Myrtaceae. Las especies nativas sostuvieron alimentación leve y fuerte en pruebas de opciones multiples de alimentos para los adultos y en pruebas sin opción de alimentos para las ninfas. La alimentación aumentó sobre las especies nativas en un cercado grande después que la *M. quinquenervia* fue cortada, puesta a secar y quitada. Las ninfas completaron su desarrollo solamente sobre *M. quinquenervia* y especies de *Melaleuca* ornamentales. Sin embargo, la incapacidad para desarrollar completamente sobre especies que no son el enfoque es de importancia limitada como un criterio para la liberación de insectos con estadios de immaduros altamente moviles comparado con especies menos moviles. El movimento local de un hospedero a otras especies de plantas puede resultar en daño no aceptable en plantas que son el enfoque a pesar de que la especificidad del desarrollo parece adecuada. Este insecto claramente dañaría las Myrtaceae nativas y ornamentales y por ello no debe ser liberado.

Melaleuca quinquenervia (Cav.) S. T. Blake is a large tree of Australian origin and one of numerous invasive plants threatening the Florida Everglades. This introduced tree forms expansive monocultures and spreads rapidly from prolific seed production. Its presence and rapid spread hinders restoration of many south Florida ecosystems including sawgrass prairies, hardwood hammocks, and even pine uplands (Bodle 1998; Turner et al. 1998). Invaded habitats are transformed into nearly pure stands of *M. quinquenervia* trees, thereby altering the function and structure of these systems.

A biological control program began in 1986 to curtail the *M. quinquenervia* invasion by inhibiting its reproduction. *Eucerocoris suspectus* Distant (Hemiptera: Miridae) seemed an excellent candidate based upon the injury it caused to young shoots (Fig. 1) in Australia (Burrows & Balciunas 1999). It was introduced into quarantine during 1996 to complete host range evaluations by focusing on native and cultivated Myrtaceae. Herein, we report results of host range studies that led us to reject this species.

Burrows & Balciunas (1999) described the biology and life history of *E. suspectus* as follows. Females insert eggs into the young shoots. Nymphal development progresses through 5 instars and requires about 17 d but is influenced by plant quality. A single female produces up to 163 progeny and adults live up to 72 d. Adults and nymphs feed on the sap of young leaves and shoots causing distinctive brown blotches on the foliage. Their dispersive capacity is unknown but both nymphs and adults are very active, making them difficult to contain, and are readily able to disperse onto nearby vegetation.

Materials and Methods

Laboratory Cultures

Dr. Charles Turner and staff of the USDA-ARS Australian Biological Control of Weeds Laboratory collected *E. suspectus* adults near Brisbane, Australia, during Jul and Aug 1996. A shipment arrived in quarantine at Gainesville, Florida on

Fig. 1. A female *Eucerocoris suspectus* and feeding scars on *Melaleuca quinquenervia*.

12 Jul with 17 of 38 adults alive (5 males and 12 females). A second shipment arrived on 31 Aug, which contained 4 of 75 adults alive (not sexed).

Adults were placed on seed-grown *M. quinquenervia* saplings of varying sizes, usually less than 2 m tall. The saplings were sleeved with fine-mesh netting (100 holes/cm^2) and held in air-conditioned quarantine greenhouses. Plants were fertilized with an encapsulated fertilizer, watered regularly, and occasionally sprayed with a soap-vegetable oil mixture to control insect pests. They were not sprayed after the bugs were added. During rearing, adults and/or nymphs, which preferred new growth, were removed and placed on new saplings, depending upon the amount of damage and the amount of remaining leaf material. They were reared continuously from Jul 1996 to Jun 1997, during which time 5 host range studies, designated I-V, were conducted. Each study consisted of 1 or more separate trials designated A-L.

No-choice Adult Feeding and Oviposition Trials (Study I)

Potted test plants (Table 1) 1-2 m tall bearing new growth on the stem tips (hereafter referred to as shoots) were individually caged in quarantine greenhouses in sleeves of nylon netting. Groups of 5 pairs of adults were randomly assigned to the cages. Most plant species were set up within 2 d of the commencement of the study but 3 species were set up 11-13 d later. Each group of plants (n = 3 groups) was assigned a separate control plant of *M. quinquenervia* to comprise trials A, B, and C (Table 1, Test ID). Adults and nymphs were removed at 7-11-d intervals and placed separately on new plants. Each exposed plant was held to assess further nymphal emergence. Adults were transferred to a new plant of the same species as many as 4 times (Table 1). Adult survival was recorded at each plant change. Nymphs were removed, counted, and placed together on a new plant of the same species. The number of shoots bearing damaged leaves and the number of leaves with feeding blotches were recorded for most species. Damage results were categorized by a three-tiered intensity scale: "+" = not damaged; "++" = moderately damaged; and "+++" = heavily damaged. The presence of eggs was noted at the end of the test. Water-filled vials with bouquets of ex-

TABLE 1. RESULTS OF THE NO-CHOICE ADULT FEEDING AND OVIPOSITION TRIALS WITH POTTED MYRTACEAE AND LYTHRACEAE EXPOSED TO *EUCEROCORIS SUSPECTUS* (STUDY I).

Test species[a]	Trial ID[b, c]	No. Plants Tested	Time to 100% Mortality (d)	Feeding Intensity[d]	Nymphs Produced (no.)[e]
Melaleuca citrina (Curtis) Dum. Cours	A	4	27	+++	91
Melaleuca citrina (broad-leaved)	B	4	>42	+++	184
*Melaleuca viminalis (*Sol. ex Gaertn.) Byrnes	A	4	27	+++	181
Melaleuca viminalis 'Little John'	C	4	32	+++	51
Calyptranthes pallens Griseb.*	C	1	9	+	0
*Calyptranthes pallens**	B	1	10	+	0
Calyptranthes zuzygium (L.) Sw.*	C	3	32	++	0
Eugenia axillaris (Sw.) Willd.*	C	1	9	++	0
Eugenia confusa DC.*	B	1	10	+	0
Eugenia foetida Pers.*	C	1	9	++	0
*Eugenia foetida**	B	1	10	++	0
Eugenia uniflora L.	B	2	>10	+++	0
Lagerstroemia indica L. (Lythraceae)	A	1	>25	+	0
Leptospermum scoparium J. R. Forst. & G. Forst.	B	2	20	+	0
Melaleuca quinquenervia (Cav.) Blake	C	4	>42	+++	18[f]
*Melaleuca quinquenervi*a	B	4	>25	+++	401
Melaleuca quinquenervia	A	4	>25	+++	257[g]
Myrcianthes fragrans (Sw.) McVaugh*	B	1	10	++	0
Psidium friedrichsthalianum (O. Berg.) Nied.	C	2	17	+++	0
Psidium cattleianum Sabine	C	3	32	+++	0
Syzygium paniculatum Gaertn.	A	1	10	++	0

[a]Florida natives are indicated with *.

[b]Plants with the same letter had the same *Melaleuca quinquenervia* control plant.

[c]Five pairs of adults on a potted plant covered with mesh sleeve, adults moved weekly to a new plant, old plant was held for nymphal emergence, total plants tested with those adults.

[d]Subjective feeding estimate, compared with feeding on *M. quinquenervia*; at first plant change *M. quinquenervia* reps. had 129-168 leaves with >10 feeding spots.

[e]Total nymphs produced on all plants exposed to that cohort of adults.

[f]Progeny of one female, four of the 5 females were trapped and died in the release vial.

[g]The test ended when all adults were dead on the 3 companion plants. Two females were still alive on *M. quinquenervia*.

cised shoots were tethered to *Melaleuca citrina* (Curtis) Dum.Cours and *Melaleuca viminalis* (Sol. ex Gaertn.) Byrnes at the third and fourth plant changes to provide supplemental food as the original plant material was insufficient following extensive feeding.

No-choice Nymphal Feeding Trials (Study II)

Techniques were similar to those for the previous adult test except that 10 first instars (1.3 to 1.7 mm in length) were placed on the plants instead of adults. Supplemental bouquets of excised shoots in water-filled vials were tethered to test plants that had too few suitable flushing shoots to support the insects after the first week. Four native *Eugenia* spp. were included with *M. quinquenervia* in trial D, and 2 non-target species of *Melaleuca* along with *M. quinquenervia* in trial E (Table 2). Insect survival, the number of adults produced, and the number of leaves attacked were recorded weekly for 28 d. Adults that developed during the test were retained on the plants with the remaining nymphs.

Multi-choice Adult Feeding and Oviposition Trials without *M. quinquenervia* (Study III)

In trial F, 2 bouquets of shoots in water-filled vials of each of 10 test plant species (Table 3) were placed into a glass-topped wooden cage (44.5 x 44.5 × 44.5 cm, l × w × h) in a greenhouse with daily mean temperature 22-24°C (range 19-33°C), and 78-81% RH (range 45-97%). Natural lighting was supplemented with fluorescent lights to maintain a 16L:8D photoperiod. One bouquet of each species was randomized to one of 10 positions in each half of the cage. Ten females and 2 males were released in the cage. Feeding was subjectively estimated on a scale of 0 to 5 from light to heavy after 2 d when the bouquets were replaced and again after 3 d when trial F ended. The plants were examined for eggs at the end of the trial.

In trial G, 1 potted plant of each of 4 species was placed in a cloth screen cage (0.6 × 0.6 × 1.2 m, l × w × h) in a greenhouse. The test species included 3 Myrtaceae, pineapple guava (*Feijoa sellowiana* (O. Berg) O. Berg), bay rum tree (*Pimenta racemosa* (Mill.) J. W. Moore), and java plum (*Syzygium cumini* (L.) Skeels), and 1 Rutaceae, lemon (*Citrus limon* (L.) Burm.f. (pro. Sp.) (*medica* × *aurantifolia*)). Three pairs of adults were released in the cage. Two plants with extensive feeding were removed during the second d and the other 2 plants were left until d 11. The plants were checked for eggs and nymphs when the test ended.

Large Enclosure Multi-choice Adult Feeding Trials with and without *M. quinquenervia* (Study IV)

Potted plants, 1-1.5 m tall, were exposed to adults in a large walk-in screen enclosure (1.8 x 1.5 × 1.8 m, l × w × h) in a greenhouse. Plants were randomized to 1 of 9 positions in 3 rows of 3 each, in trials H and I. A *M. quinquenervia* plant was placed next to the test plant in the center of the enclosure at the start of each trial. The 15 test plant species are listed in Table 4. All were Myrtaceae except *Morella cerifera* (L.) Small (Myricales: Myricaceae), which was considered at risk

TABLE 2. NO-CHOICE NYMPHAL FEEDING AND DEVELOPMENT TRIALS ON NATIVE *EUGENIA* SPP. AND EXOTIC *MELALEUCA* SPP. (STUDY II).

	Survival (%)[a] after:			Leaves attacked (cumulative no.)[c]	
Test Plant	7 d	28 d	No. Adults[b]	7 d	28 d
Trial D					
Eugenia axillaris	0	0	0	20	—
Eugenia confusa	0	0	0	6	—
Eugenia foetida	0	0	0	28	—
Eugenia rhombea (Berg) Krug & Urb.	30	0	1	34	76
Melaleuca quinquenervia	60	30	5	50+	344+
Trial E					
Melaleuca citrina	100	0	7	50	284+
Melaleuca citrina (broad-leaved)	10	0	0	20	20
Melaleuca viminalis	80	40	4	50+	347+
Melaleuca viminalis 'Little John'	100	70	8	50	459
Melaleuca quinquenervia	80	50	6	50+	446+

[a]Ten small nymphs per plant, new adults were left on the plant with the remaining nymphs.
[b]Some adults died before 28 d when trapped in the folds of the mesh sleeve.
[c]Plants with a "+" were difficult to count accurately so the count is a minimum.

TABLE 3. MULTI-CHOICE ADULT FEEDING AND OVIPOSITION TRIAL ON MYRTACEAE WITHOUT *MELALEUCA QUINQUENERVIA* (STUDY III).

	Feeding intensity[a]				
	Day 2[b]		Day 5[c]		
Test species (Trial F)	Bouquet 1	Bouquet 2	Bouquet 1	Bouquet 2	Eggs
Calyptranthes pallens	1	4	4	5	0
Calyptranthes zuzygium	0	0	0	0	0
Eugenia axillaris	2	1	5	4	6
Eugenia confusa	1	1	3	1	0
Eugenia foetida	0	0	1	1	2
Eugenia uniflora	2	3	2	2	0
Leptospermum scoparium	0	0	0	0	0
Myrcianthes fragrans	1	1	3	1	0
Psidium friedrichsthalianum	2	3	3	5	0
Psidium cattleianum	3	1	5	4	1

[a]Ten ♀ ♀ and 2 ♂ ♂ released in the cage. Feeding estimate: 1 = Light, scattered feeding, no large blotches; 2 = Light-medium; 3 = Medium, noticeable feeding, on multiple leaves; 4 = Medium-heavy; 5 = Heavy, some leaves blackened, some abscinded.
[b]Bouquets were randomized in each half of cage and were replaced in d 2.
[c]Test terminated on d 5 when eggs were counted.

because of limited use by other *Melaleuca* herbivores (Wheeler 2005; Pratt et al. 2009). Two specimens of *Eugenia* DC. were placed together at the same position in trial H because they had fewer shoots than the others. Ten pairs of adults were released on the *M. quinquenervia* plant. Damaged leaves were counted daily except for trial H, which was not assessed until the third d. The few damaged leaves on the test plants were removed daily to avoid duplicate counting. Damaged leaves were not removed from *M. quinquenervia* because this would have resulted in total defoliation of the tree. The resultant cumulative counts were therefore underestimates for *M. quinquenervia* inasmuch as most leaves would have been subjected to repeated feeding. The *M. quinquenervia* was cut on the fifth day and the pieces were tied to the trunk to allow the leaves to dry and the bugs to disperse. The dried *M. quinquenervia* was removed on the seventh day and the trial terminated on the tenth day. The plants in trial I were examined for eggs when the test ended.

Melaleuca viminalis and the 2 forms of *M. citrina* were randomized to 9 positions, 3 each, in trial J. Due to a limitation of standard *M. viminalis* plants, 1 *M. viminalis* cultivated variety 'Little John' was also incorporated into the trial. We placed 2 pots together at one position for *M. citrina* and one for *M. viminalis* because they had fewer shoots than the other plants. Eighteen males were released on the ceiling in the center of the cage. Three survivors were removed on the fifth day, but 2 more were found on the seventh day when damaged leaves were counted. Males were used to avoid oviposition in order to preserve the plants for other uses.

No-choice Starvation Trial with Nymphs and Adults (Study V)

Three potted sugarcane plants, *Saccharum officinarum* L. (Cyperales: Poaceae), and 2 potted lemon plants were individually caged in nylon sleeves in the greenhouse (trial K). Three females, 2 males, and 6 medium-sized nymphs were released in each cage where they remained until they died. The number of leaves with feeding spots was counted after all bugs were dead. An additional trial (trial L) was conducted to determine if discoloration observed on the plants in trial K was due to feeding damage. A leaf of a sugarcane plant and a lemon plant was covered with a small net sleeve. One pair of adults and 2 nymphs were released in the small sleeve. Each plant was enclosed in a larger sleeve. All plants in trials K and L were examined for eggs when the test ended.

RESULTS

No-choice Adult Feeding and Oviposition Trials (Study I)

Feeding was moderate to heavy on nearly all plants tested (Table 1). The 2 non-target *Melaleuca* spp., the 2 *Psidium* spp., and *Eugenia uniflora* L. were most heavily attacked. Native Myrtaceae were noticeably damaged but, with the exception of *Calyptranthes zuzygium* (L.) Sw. on which they survived for 32 d, the adult bugs died within 10 d. Nymphs developed and eggs were found only on non-target *Melaleuca* spp. and *M. quinquenervia*. *Lagerstroemia indica* L. suffered only light damage, although some nymphs lived

TABLE 4. WALK-IN ENCLOSURE MULTI-CHOICE ADULT FEEDING TRIALS ON MYRTACEAE WITH AND WITHOUT *MELALEUCA QUINQUENERVIA* (STUDY IV).

Test Species	Leaves with feeding blotches, cumulative tally on day				Eggs
	3	5	7	10	
Trial H. Ten adult pairs, potted plants at 9 positions, *M. quinquenervia* next to central plant					
Calyptranthes pallens	1	1	5	11	
Calyptranthes zuzygium	0	0	0	0	—
Eugenia axillaris	1	3	11	16	—
Eugenia confusa	0	1	14	22	—
Eugenia foetida	8	8	25	36	—
Eugenia rhombea	0	0	4	4	—
Melaleuca quinquenervia	164	293	Drying[a]	Removed[b]	—
Myrcianthes fragrans	2	2	30	36	—
Morella cerifera (L.) Small (Myricaceae)	0	1	1	1	—
Psidium longipes (Berg) McVaugh	0	0	22	22	—
Trial I. Set up same as Trial H but eggs were assessed at the end of evaluation					
Melaleuca citrina					
Melaleuca citrina (broad-leaved)	2	3	13	21	no
Melaleuca viminalis	3	28	104	254	yes
Eucalyptus camaldulensis Dehnh.	0	0	1	1	no
Eucalyptus camaldulensis	4[c]	11[c]	12[c]	23[c]	yes
Leptospermum scoparium	0	0	0	0	no
Melaleuca quinquenervia	258	328	Drying	Removed	—
Psidium friedrichsthalianum	0	0	7	0	no
Psidium cattleianum	0	0	0	0	no
Trial J. Eighteen ♂ ♂, potted plants at 9 positions, 3 of each species					
Melaleuca citrina	—	—	23[d]		—
Melaleuca citrina (broad-leaved)	—	—	38		—
Melaleuca viminalis	—	—	127		—

[a]*Melaleuca quinquenervia* was cut on d 5 and left in cage to dry slowly.
[b]*Melaleuca quinquenervia* was removed from the cage on d 7.
[c]Number of stems fed upon. Feeding was on stems not on leaves.
[d]Total of the 3 positions, 122 leaves attacked on *C. viminalis* were all on 1 plant.

at least 25 d on it. Damage was usually distributed throughout the plant with over 50% of the shoots attacked on 7 of 10 species. In general, survival on most test plants was quite long (Table 1).

No-choice Nymphal Feeding Trials (Study II)

Small nymphs were dead by d 7 on 3 native *Eugenia* spp. in trial D, but 30% survived on *E. rhombea* Ridl. (Table 2). One became an adult, but it and the remaining nymphs were dead by d 28. Only the newest leaves on *E. rhombea* were attacked but they were heavily damaged and abscinded. Similar feeding and damage was observed on the other *Eugenia* spp., with few leaves attacked due to low nymphal survival but heavy damage levels (Table 2). Older leaves were also attacked on *E. axillaris* (Sw.) Willd., but with little damage. On *M. quinquenervia,* 60% of nymphs survived to d 7 and 50% became adults. Two died after being entrapped in the folds of the cloth sleeve, so survivorship would probably have been somewhat greater.

Survival and feeding on non-target *Melaleuca* spp. in trial E were similar to those on *M. quinquenervia* except on the broad-leaved *M. citrina*. All nymphs were alive on d 7 on *M. citrina* and *M. viminalis* "Little John" and 80% were alive on *M. viminalis* and on *M. quinquenervia*. All were dead on *M. citrina* by d 28, but 40% and 70% were alive on the 2 *M. viminalis* plants and 50% on *M. quinquenervia*. Most surviving nymphs developed to adults on both non-target *Melaleuca* spp. and *M. quinquenervia*, and some adults died before 28 d. Feeding on 2 non-target *Melaleuca* spp. was comparable to that experienced by *M. quinquenervia* (Table 2). Feeding on the test plants was usually distributed throughout the canopy, with most foliage damaged at shoot tips.

Multi-choice Adult Feeding and Oviposition Trials without *M. quinquenervia* (Study III)

All species in trial F except *C. zuzygium* and *Leptospermum scoparium* J. R Forst. & G. Forst. sustained damage, but *Eugenia foetida* Pers. was not damaged until after d 2 (Table 3). Most species had light to medium damage by d 2. Four species showed medium to heavy feeding on one bouquet on d 5 (*Calyptranthes pallens* Griseb., *E. axillaris*, *Psidium friedrichsthalianum* (O. Berg.) Nied., and *P. cattleianum* Sabine). Eggs were found on 3 species: the natives *E. axillaris* and *E. confusa* and the cultivated *P. cattleianum.*

Two of the 4 species in trial G were attacked on d 1. Five of 7 leaves on *P. racemosa* and 4 of 5 leaves on *S. cumini* had more than 5 feeding blotches. The trial ended on d 11, with no feeding on lemon but with 14 leaves damaged on *A. sellowiana*, half of which had more than 5 feeding blotches. No live insects were recovered at the end of trial G. Nymphs only emerged from eggs in the stems of *A. sellowiana*. None emerged from stems of *P. racemosa* or *S. cumini*.

Large Enclosure Multi-choice Adult Feeding Trials With and Without *M. quinquenervia* (Study IV)

With *M. quinquenervia* present in trials H and I, there was little feeding on test plants by d 5 except on one of 2 plants of *M. citrina* and 1 plant of *M. viminalis* (Table 4). However, the feeding on both non-target plants was much less than that on *M. quinquenervia*. Feeding increased on most plants after *M. quinquenervia* was cut, but was still minor except on the 2 plants already mentioned. Feeding on *M. viminalis* on d 10 was similar to that on *M. quinquenervia* on d 3. Eggs were found only on *M. citrina*, *M. viminalis* and *M. quinquenervia* in trial I. There was little feeding on the broad-leaved *M. citrina* in this trial, although it was heavily attacked by adults in no-choice trials. Feeding on plants of other non-target species was not particularly damaging, but was noticeable.

Little feeding occurred on either variety of *M. citrina* in trial J. Almost all feeding on *M. viminalis*, 96%, and all on *M. citrina* occurred at one of the 3 positions within the cage. Also, the attacked leaves were on relatively few shoot tips, not widely distributed over the plant (*M. citrina* 3 of 25 tips had feeding, *M. citrina* (broad-leaved), 5 of 10, and *M. viminalis*, 9 of 100).

No-choice Starvation Trial with Nymphs and Adults (Study V)

All insects died on sugarcane plants (n = 3) by the eighth d without feeding in trial K. On lemon plants (n = 2) all insects were dead by the sixth d, but 7 and 8 leaves were damaged on 2 of the plants. This damage was slight, perhaps due to test probing, and all damaged leaves had less than 5 feeding blotches. Brown streaks were observed along the veins on some sugarcane leaves, but when additional insects were confined on new leaves (trial L) to determine if this streaking was symptomatic of feeding damage, none resulted. No eggs were found on any of the test plants.

Discussion

The host range of the *Melaleuca* leaf-blotching bug, *E. suspectus*, may be considered acceptable based on the fact that the insect was able to complete development only on *M. quinquenervia*, other exotic *Melaleuca* spp. and once on *E. rhombea*. However, the vagile nature of this insect would enable it to feed on a wide variety of plants that are not developmental hosts. Feeding damage proved especially troublesome because of the frequency that this insect probed or test fed on non-target plants. Thus, even though *E. suspectus* is stenophagous, it presents a risk to rare endemic plant species such as *E. rhombea* that are sympatric with *M. quinquenervia* in southern Florida. The exact nature of this risk cannot be known without further study but the precautionary principal, which dictates conservative actions, would disqualify release of this insect. We did not test unusually high numbers of insects per cage, for instance, but unacceptably high levels of collateral damage were observed on non-target species in large cage trials. In contrast, and contrary to cage tests, Burrows & Balciunas (1999) observed that adults released on test plants in a shade-house fed and reproduced only on *M. quinquenervia*. Our concerns, however, were confirmed through field observations by personnel at the Brisbane laboratory: damage was observed on mixed Myrtaceae in a garden plot, all stages of *E. suspectus* were found on bottlebrush, *Melaleuca* spp., and damage to guava was severe (Purcell et al. 2000). Bottlebrushes are relatively common ornamentals in Florida and some other states. These were originally placed in the genus *Callistemon* but have since been synonymized with *Melaleuca* (Craven 2006).

These results matched those of Burrows & Balciunas (1999) quite closely in terms of common genera tested in cage tests. They reported noticeable feeding on *Melaleuca* (=*Callistemon*), *Psidium,* and *Syzygium*, as did we. Nymphal survival to adult was 47% on *M. viminalis* in their tests as compared to 50% herein.

Damage from an equal amount of feeding on *Calyptranthes, Eucalyptus, Eugenia*, and *Psidium* was greater than that on *Melaleuca*. The damaged young leaves and young stems dried and abscinded on those genera, as they often did on *Melaleuca*, but there were fewer leaves on non-target plants than on the longer, foliose shoots of

the target weed. Thus, an equal number of attacked leaves among test plants resulted in a disproportionate level of damage on non-target hosts as compared to *Melaleuca*. However, this level of non-target damage was limited to a few test species as many more leaves were usually attacked on *Melaleuca*.

The present data also have relevance to experimental protocols for host range testing. Evaluation of an herbivore's host range is an effort to maximize predictive precision within the bounds of practicality. Multiple replicated experiments provide insight to variation in an herbivore's host preferences, but limited financial resources necessitate abandoning continued testing (replication) at early stages of evaluation for herbivores that demonstrate broad host ranges. Although development of *E. suspectus* appears to be confined to *Melaleuca*, non-host feeding by the highly mobile nymphs and adults is too broad and too damaging for this bug to be used for biological control. Therefore, many tests were terminated with few replicates when it became apparent that this insect was not a suitable candidate for release. Continuation of testing for the sake of additional replicates would have wasted time and resources, so testing was curtailed in favor of more suitable candidates.

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COMPARISON OF SYNTHETIC FOOD-BASED LURES AND LIQUID PROTEIN BAITS FOR CAPTURE OF *ANASTREPHA SUSPENSA* (DIPTERA: TEPHRITIDAE) ADULTS

NANCY D. EPSKY, PAUL E. KENDRA, JORGE PEÑA[1] AND ROBERT R. HEATH
Subtropical Horticultural Research Station, United States Department of Agriculture, Agricultural Research Service, 13601 Old Cutler Road, Miami, FL 33158, U.S.A.

[1]University of Florida, Tropical Research and Education Center, 18905 SW 280th Street, Homestead, FL 33031, U.S.A.

ABSTRACT

Field tests were conducted in south Florida to compare capture of the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in Multilure traps baited with either of the liquid protein baits torula yeast/borax or Nulure/borax, or with food-based synthetic lures including two-component Biolure (ammonium acetate, putrescine) and three-component Biolure (ammonium acetate, putrescine, trimethylamine). The highest relative proportion of females captured was in traps baited with the two-component Biolure (44-61%), intermediate capture was in traps baited with the three-component Biolure (14-24%) or torula yeast/borax (8-25%), and the lowest capture tended to be in traps baited with Nulure/borax (0-19%). Similar results were obtained for capture of males. Tests of the unipak two-component Biolure, which has a reduced ammonium acetate release rate and is a single package with both ammonium acetate and putrescine sections, captured similar numbers of both females and males as Biolure formulated in 2 individual packages. Traps baited with unipak Biolure combined with the addition of a trimethylamine lure captured fewer females than the unipak alone, but this was greater than capture in traps baited with torula yeast/borax. Our studies confirmed that the best lure for *A. suspensa* is ammonium acetate and putrescine. However, *C. capitata*-targeted traps baited with three-component Biolure should be as effective for *A. suspensa* detection and monitoring as traps baited with torula yeast/borax. The unipak two-component Biolure will provide the improved handling that has been requested by users.

Key Words: Caribbean fruit fly, Biolure, ammonium acetate, unipak, torula yeast

RESUMEN

En pruebas de campo realizadas en el sur de Florida, se compararon resultados de captura de la mosca de la fruta del Caribe *Anastrepha suspensa* (Loew), capturadas en trampas Multilure que habian sido cebadas con cebos de proteina liquida de levdura torula/borax o con Nulure/borax, o con atrayentes alimenticios sinteticos los cuales incluian Biolure con dos componentes (acetato de ammonia, putrescina) y Biolure con 3 componentes (acetato de ammonia, putrescina, trimethylamina). El mas alto porcentajge de captura de hembras ocurrio en trampas cebadas con Biolure de dos componentes (44-61%), un nivel de captura intermedio ocurrio en trampas cebadas con Biolure de 3 componentes (14-24%) o con los cebos de levadura torula /borax (8-25%), mientras que la captura mas baja fue en aquellas trampas cebadas con Nulure/borax (0-19%). Se obtuvieron resultados similares en la captura de machos. Pruebas unipak del Biolure de dos componentes, el cual da una tasa de liberacion reducida de acetato de ammonio y el cual es un atrayente individual con secciones de acetato de ammonio y putrescina, no mostro diferencias en captura de machos o hembras entre el Biolure de dos componentes formulado como atrayentes individuales o como unipak. La captura intermedia de las hembras fue obtenida en trampas cebadas con unipak Biolure combinado con un atrayente individual de trimethylamina, pero esta captura fue mayor que la obtenida cuando se usaron trampas cebadas con levadura torula /borax. Nuestros estudios confirmaron que el mejor atrayente para *A suspensa* es el acetato de ammonio y putrescina. Sin embargo, trampas destinadas a atrapar *C. capitata* y cebadas con acetato de amonio, putrescina y trimethylamina deben ser tan efectivas como aquellas trampas cebadas con levadura torula /borax para la deteccion y monitoreo de *A. suspensa*. El unipak con Biolure de dos componentes es igualmente efectivo y dara una mejor facilidad para manipulacion, la cual ha sido pedida por los usuarios.

Translation provided by the authors.

Tephritid fruit flies are among the most important pests of fruits and vegetables in the world, and use of traps and lures are important components of fruit fly pest management programs. Standard trapping protocols have been developed for fruit fly detection and monitoring and, depending on target species, different female-targeted baits may be used (IAEA 2003). Selection of trap and lure depends on the purpose of trapping, availability of materials and cost. Although use of a single trap type that would capture the highest number of females and males of multiple species would have many advantages, species-specific traps that use lures specific to the target fruit fly and environmental conditions are the best systems available currently (Díaz-Fleischer et al. 2009). Among the numerous liquid protein baits, agencies primarily use torula yeast/borax solutions for detecting and monitoring *Anastrepha* spp. and NuLure/borax solutions for the Mediterranean fruit fly, *Ceratits capitata* Weidemann (Epsky et al. 1993; Heath et al. 1993, 1994).

Food-based synthetic attractants have been developed based on volatile chemicals released from liquid protein baits. A two-component Biolure attractant comprised of ammonium acetate and putrescine is used in traps that target *Anastrepha* spp. (Heath et al. 1995; Epsky et al. 1995, Thomas et al. 2001). Addition of trimethylamine is used in traps that target *C. capitata* (Heath et al. 1997), and McPhail-type traps baited with the Biolure three-component attractant are equal to or better than liquid protein-baited traps for capture of *C. capitata* females (Epsky et al. 1999). Capture of *Anastrepha* spp. flies by traps baited with either the two-component or three-component attractant, however, is more variable (IAEA 2007). Initial studies found no difference in capture of the Mexican fruit fly, *Anastrepha ludens* (Loew), in traps baited with Biolure ammonium acetate and putrescine with or without the third Biolure component, trimethylamine (Heath et al. 1997). However, Holler et al. (2006) found that addition of trimethylamine reduced capture of the Caribbean fruit fly, *Anastrepha suspensa* (Loew).

Regulatory agencies deploy McPhail-type traps baited with the three-component Biolure (ammonium acetate, putrescine, and trimethylamine; Suterra LLC, Bend, OR), which is commercially available, primarily to monitor for new infestations of *C. capitata* in areas currently fly-free, and questions remain on the effectiveness of this trapping system for detecting and/or monitoring *A. suspensa*. Due to problems with the deployment of the synthetic attractants as individual lures, several versions of the components combined in a single lure have been developed and tested (Jang et al. 2007; Navarro-Llopis et al. 2008) including two-component and three-component unipak versions of Biolure (Holler et al. 2009). We report results of field tests that were conducted in south Florida to compare capture of *A. suspensa* in traps baited with either of the liquid protein baits or with the Biolure two- and three-component food-based synthetic attractant (both individual lures and unipak lure) to determine relative effectiveness of these standard trapping systems.

Materials and Methods

Traps and Lures

MultiLure traps (Better World Manufacturing Inc., Fresno, CA, USA) were used in all experiments. Liquid protein baits included aqueous solutions of torula yeast/borax (three 5-g pellets, 2.25:2.75 yeast:borax, in 300 mL water) (ERA International, Freeport, NY) and NuLure (Miller Chemical & Fertilizer Co., Hanover, PA) as a 300 mL aqueous solution of 9% NuLure (vol:vol) and 3% borax (wt:vol; sodium tetraborate decahydrate). Synthetic attractants included individual component Biolure formulations of ammonium acetate, putrescine, and trimethylamine, and the unipak two-component Biolure formulation of ammonium acetate and putrescine (Suterra LLC, Bend, OR). The membrane-release area of the ammonium acetate lure in the two-component unipak has been reduced from ~35 mm diam. on individual component lure to ~23 mm diam. on unipak lure. This lowers the release rate of ammonium acetate, which improves capture of the Mexican fruit fly, *Anastrepha ludens* (Loew) but has no effect on capture of *A. suspensa* (Thomas et al. 2008). Traps baited with synthetic lures contained 300 mL 10% polypropylene glycol (vol:vol; LowTox, Prestone, Danbury, CT, USA) aqueous solution to retain captured flies.

Field Tests

Field tests were conducted at the Tropical Research and Education Center (TREC), University of Florida, Homestead, FL. Experiment 1 compared capture of flies in traps baited with (1) NuLure/borax, (2) torula yeast/borax, (3) two-component Biolure (individual ammonium acetate and putrescine lures), and (4) three-component Biolure (individual ammonium acetate, putrescine, and trimethylamine lures). Experiment 2 compared capture of flies in traps baited with (1) torula yeast/borax, (2) two-component Biolure, (3) unipak two-component Biolure, and (4) unipak two-component Biolure plus trimethylamine individual Biolure. Tests were conducted in two hosts, Surinam cherry, *Eugenia uniflora* L., and guava, *Psidium guajava* L., for experiment 1; and in one host, guava, for experiment 2. For the tests in Surinam cherry, all 4 treatments were placed around the periphery of a large tree in fruit. There were 3 blocks (replicates) of traps, with 2 m

between traps within a block and 10 m between blocks. For the tests in guava, there was 1 trap per tree with the traps placed in 3 rows (blocks/replicates) of trees. There were at least 10 m between rows and 10 m between traps within a row. For both experiments, traps were sampled every 7 d, and numbers of male and female flies were recorded. Traps were sampled for 4 wk per sampling period, for a total of 4 sampling periods in experiment 1 (sample periods 1-2 in Surinam cherry, sample periods 3-4 in guava), and 1 sampling period in experiment 2. The protein bait solutions were replaced every 7 d, but the synthetic lures were not replaced during a sampling period. A complete randomized design was used for trap placement at the start of each sampling period. Traps were rotated sequentially to the next position within a block at time of sampling, so that all treatments were in all positions within each sampling period. Trees were in fruit throughout both experiments, but the tests in Surinam cherry were conducted toward the end of the fruiting season (18 Jun to 13 Aug, 2008) and the experiments in guava were conducted in the beginning of the fruiting season (experiment 1 - Jun 25 to Aug 20, 2008; experiment 2 - Aug 27 to Sep 17, 2009). Fruiting in guava trees began earlier in 2008 than in 2009 because the trees were trimmed in spring 2009.

Statistical Analysis

Effect of treatment and either sample period (experiment 1) or sample week (experiment 2) were analyzed by two-way ANOVA in a factorial model with interaction (Proc GLM, SAS Institute 2000) followed by LSD mean separation ($P = 0.05$) for significant factors. When necessary, data were transformed prior to analysis to satisfy conditions of equal variance (Box et al. 1978). Numbers of total flies per trap per day and percentage females for flies captured in traps baited with the Biolure two-component attractant were analyzed as assessments of population level. Summary statistics are presented as average ± standard deviation.

Results

Experiment 1

Number of total *A. suspensa* captured per trap per day in traps baited with the two-component Biolure was affected by sampling period ($P = 9.08$, $df = 3, 44$; $P < 0.0001$; log $(x + 1)$ transformed data). Numbers of *A. suspensa* in traps in Surinam cherry decreased from 4.0 ± 4.4 flies per trap per day in sampling period 1 to 2.0 ± 2.3 flies per trap per day in sampling period 2. Numbers in traps in guava increased from 0.4 ± 0.2 in sampling period 3 to 5.8 ± 5.0 in sample period 4. Percentage of females captured in these traps was also affected by sampling period ($P = 3.27$, $df = 3, 48$; $P = 0.0321$; square-root $(x + 0.5)$ transformed data). All captures were female biased, and percentage of females captured during sampling periods 1, 2, 3 and 4 were 86.6 ± 10.6, 80.8 ± 14.5, 60.8 ±32.0, and 76.1 ± 16.7, respectively.

Numbers of female and male flies per trap per block were converted to relative trapping efficiency to facilitate comparisons among the range of population levels tested during the different sampling periods (Epsky et al. 1999). There was a significant interaction between sampling period and treatment for female ($F = 3.17$; $df = 9, 32$; $P = 0.0075$) but not for male ($F = 0.97$; $df = 9, 32$; $P = 0.4825$) relative trapping efficiencies. Therefore, one-way analyses were used to test effect of treatment within each sampling period for females and over all sampling periods for males. Traps baited with the two-component Biolure had the highest relative trapping efficiency for females for all sampling periods (Table 1). The next highest relative trapping efficiencies were for traps baited with the three-component Biolure and with torula yeast/borax solution. Relative trapping efficiency in traps baited with NuLure/borax was significantly less than capture in traps with the three-component attractant for the sampling periods in which the population was the lowest (sample periods 2 and 3), Treatment also had an effect on capture of males ($F = 24.24$; $df = 3, 44$; $P < 0.0001$). The highest relative trapping efficiency of males was 64.5 ± 20.2% in traps baited with the two-component Biolure, and this was higher than traps baited with the three-component Biolure, NuLure/borax or torula yeast/borax (12.4 ± 10.9, 12.2 ± 12.7, and 10.9 ± 10.5, respectively).

Experiment 2

Number of total *A. suspensa* captured per trap per day in traps baited with the two-component Biolure was affected by sample week ($P = 23.83$, $df = 3, 8$; $P = 0.0003$; log $(x + 1)$ transformed data). Numbers increased from 6.5 ± 0.6 in week 1 to 29.0 ± 6.2 in week 4. Percentage of females captured in these traps was not affected by sampling period ($P = 1.62$, $df = 3, 8$; $P = 0.2591$; square-root $(x + 0.5)$ transformed data). Overall, captures were female biased, but the percentage of females captured decreased slightly from 74.7 ± 8.9 in week 1 to 65.9 ± 5.1 in week 4.

As in experiment 1, numbers of female and male flies per trap per block were converted to relative trapping efficiency for subsequent analysis. There was no interaction between treatment and sample week, so data from all sample weeks were pooled and effect of treatment was analyzed with one-way ANOVA. Both individual and unipak two-component Biolure formulations captured more females than traps baited with torula yeast/

TABLE 1. RELATIVE TRAPPING EFFICIENCY (%) FOR CAPTURE OF FEMALE *ANASTREPHA SUSPENSA* IN FIELD TESTS CONDUCTED IN HOMESTEAD, FL. MULTILURE TRAPS WERE USED FOR ALL BAITS AND EACH SAMPLE PERIOD WAS 4 WEEKS. SURINAM CHERRY WAS AT THE END OF THE FRUITING SEASON IN SAMPLE PERIOD 2 AND GUAVA WAS AT THE BEGINNING OF THE FRUITING SEASON IN SAMPLE PERIOD 3.

	Surinam cherry**		Guava	
Bait*	Sample period 1	Sample period 2	Sample period 3	Sample period 4
Two-component BioLure	44.1 ± 4.5 a	60.2 ± 10.2 a	60.9 ± 12.8 a	60.9 ± 20.5 a
Three-component BioLure	19.4 ± 8.5 b	24.2 ± 13.9 b	14.3 ± 9.4 b	23.7 ± 15.8 b
Torula yeast/borax	17.3 ± 7.2 b	10.1 ± 4.3 bc	24.8 ± 12.6 b	7.5 ± 2.2 b
NuLure/borax	19.2 ± 12.8 b	5.5 ± 2.9 c	0 c	8.0 ± 4.4 b
F	4.22	21.56	27.17	11.06
df	3,8	3, 8	3,8	3,8
P	0.0459	0.0003	0.0002	0.0032

*Ammonium acetate and putrescine alone (two-component Biolure) or with trimethylamine (three-component Biolure) in traps with 300 mL 10% propylene glycol solution, 3 torula yeast/borax pellets in 300 mL water, 9% NuLure and 3% borax in 300 mL water.

**Means within a column followed by the same letter are not significantly different (LSD mean separation test on square root (x + 0.5)-transformed data, non-transformed mean ± standard deviation presented).

borax (Fig. 1 solid bars; F = 7.73; df = 3, 44; P = 0.0003), with intermediate capture with unipak two-component Biolure plus trimethylamine. There was no difference in number of males captured in traps baited with two-component Biolure or unipak two-component Biolure (Fig. 1 shaded bars; F = 3.37; df = 3, 44; P = 0.0268), and both treatments captured more males than traps baited with either with unipak Biolure plus trimethylamine or torula yeast/borax.

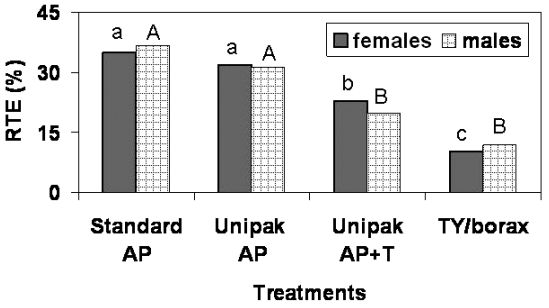

Fig. 1. Relative trapping efficiency (%) for capture of female (solid bars) and male (shaded bars) *Anastrepha suspensa* in field tests conducted in Homestead, FL. Multilure traps were used for all baits and traps were sampled for 4 weeks. Treatments included two-component Biolure as individual ammonium acetate and putrescine lures (Standard AP), unipak two-component Biolure (Unipak AP), unipak two-component Biolure plus trimethylamine individual Biolure (Unipak AP + T) in traps with 300 mL 10% propylene glycol solution, and 3 torula yeast/borax pellets (TY/borax) in 300 mL water. Bars headed by the same lowercase (females) or uppercase (males) letter are not significantly different (LSD mean separation test on square root (x + 0.5)-transformed data, non-transformed means presented).

DISCUSSION

Glass McPhail traps baited with torula yeast/borax solution have been the standard trapping system for *Anastrepha* spp. since early studies found that torula yeast performed better than a number of other yeast formulations (Lopez et al. 1971), and most studies have evaluated liquid protein baits in these traps. Torula yeast/borax captured more *A. suspensa* than NuLure/borax in tests with glass McPhail traps (Epsky et al. 1993). Plastic McPhail traps baited with two-component Biolure captured more flies than glass McPhail traps baited with either torula yeast/borax or two-component Biolure in tests in Florida in Surinam cherry, loquat (*Eriobotrya japonica* [Thunb.] Lindl.), and guava (Thomas et al. 2001; Hall et al. 2005). Tests in grapefruit, *Citrus paradisi* Macfady, in Florida found that captures in Multilure traps baited with either two-component Biolure or torula yeast/borax were higher than in traps baited with NuLure/borax (Thomas et al. 2008). Tests in sapodilla, *Manilkara zapota* Van Royen, and mamey sapote, *Pouteria sapota* (Jacq.), in Puerto Rico, however, found more *A. suspensa* were captured in Multilure traps baited with torula yeast/borax than with two-component Biolure (Pingel et al. 2006). In the only previous test of three-component Biolure, Holler et al. (2006) found that the highest capture was in plastic McPhail traps baited with two-component Biolure, and that there was no difference between capture in plastic McPhail traps baited with three-component Biolure or glass McPhail traps baited with torula yeast/borax. Results from that study, in which traps were placed in scattered wild guava trees, were the same as the results from our study.

Unipak versions of two-component and three-component Biolure have been shown to be equal

to the same components formulated in single lure formulations for capture of sterile *C. capitata* released in Florida and for capture of *A. suspensa* in traps placed in backyard plantings of host fruit trees in Sarasota/Bradenton and in Ft. Pierce (Holler et al. 2009). We recorded similar results for *A. suspensa* in our tests in south Florida. Additionally, we found that the unipak two-component in combination with trimethylamine may be better than torula yeast/borax for *A. suspensa* monitoring. This may be due to the lower release rate of ammonia from the unipak two-component formulation. The greater amount of ammonia from the individual lure formulation used in experiment 1, when combined with the amines from the trimethylamine lure, may have been repellant to *A. suspensa*.

Our studies confirmed that the best lure for *A suspensa* is two-component Biolure ammonium acetate and putrescine, however, *C. capitata*-targeted traps baited with three-component Biolure ammonium acetate, putrescine and trimethylamine should be as effective as traps baited with torula yeast/borax for *A. suspensa* detection and monitoring. The unipak two-component Biolure is equally effective and will provide the improved handling that has been requested by users.

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FOOD-BASED LURE PERFORMANCE IN THREE LOCATIONS IN PUERTO RICO: ATTRACTIVENESS TO *ANASTREPHA SUSPENSA* AND *A. OBLIQUA* (DIPTERA: TEPHRITIDAE)

DAVID A. JENKINS[1], NANCY D. EPSKY[2], PAUL E. KENDRA[2], ROBERT R. HEATH[2] AND RICARDO GOENAGA[1]
[1]USDA-ARS, Tropical Agriculture Research Station, 2200 Ave. P.A. Campos, Mayaguez, PR 00680-5470

[2]USDA-ARS-Subtropical Horticulture Research Station, 13601 Old Cutler Road, Miami, FL 33158

ABSTRACT

Lures based on odors released by hydrolyzed protein were assessed for their attractiveness to *Anastrepha obliqua* and *A. suspensa* at 3 locations in Puerto Rico in Aug through Oct 2009. Lures compared included ammonium acetate combined with putrescine, hydrolyzed corn protein (Nulure) with borax, freeze-dried Nulure, freeze-dried Nulure in combination with ammonium acetate, freeze-dried Nulure in combination with ammonium acetate and putrescine, and the Unipak lure, a single lure containing ammonium acetate and putrescine. Where the distribution of trapped flies departed significantly from what would be expected given an equal attraction of the baits, Nulure and freeze-dried Nulure always attracted fewer flies than the other baits tested, regardless of species, sex, or location. Although all of the baits or bait combinations containing ammonium acetate attracted more flies than the Nulure or freeze-dried Nulure baits, there was a distinct trend of ammonium acetate and putrescine and the Unipak lures to attract more flies after the 4th week of the study and for the freeze-dried Nulure with ammonium acetate or in combination with ammonium acetate and putrescine to attract more flies in the 1st 4 weeks of the study. This trial is unique in that it was conducted in orchards of carambola, *Averrrhoa carambola* (Oxalidaceae), a poor host for both fly species. Our results are compared with other studies on lures of *A. obliqua* and *A. suspensa* and the implications for monitoring/detecting pest Tephritidae are discussed.

Key Words: ammonium acetate, putrescine, Nulure, McPhail trap

RESUMEN

Trampas que trabajan a base de olores liberados por proteína hidrolizada se evaluaron como atrayentes de las moscas *Anastrepha obliqua* y *A. suspensa* en tres localidades en Puerto Rico durante agosto a octubre de 2009. Las trampas utilizadas en el estudio incluyeron acetato de amonio en combinación con putrescina, proteína hidrolizada de maíz (NuLure) con bórax, NuLure liofilizado en combinación con acetato de amonio y putrescina, y la trampa Unipak la cual contiene acetato de amonio y putrescina en una sola mezcla. Las trampas NuLure y NuLure liofilizada atrajeron menos moscas que el resto de las trampas irrespectivamente de la especie, sexo, o localidad. Aunque todas las trampas o combinaciones de estas con acetato de amonio atrajeron más moscas que las trampas NuLure o NuLure liofilizada, hubo una clara tendencia de las trampa de acetato de amonio y putrescina y la trampa Unipak a atraer más moscas después de la cuarta semana a partir de comenzado el estudio y de las trampas NuLure liofilizadas con acetato de amonio o en combinación con acetato de amonio y putrescina a atraer más moscas en las primeras cuatro semanas del estudio. Este estudio es único en que se llevó a cabo en huertos de carambola *Averrrhoa carambola* (Oxalidácea), un cultivo que es un pobre hospedero de ambas especies de moscas. Nuestros resultados se comparan con otros estudios con trampas de *A. obliqua* y *A. suspensa* y las implicaciones para el monitoreo y detección de plagas Tephritidae son discutidos.

Translation provided by the authors.

Although less than 10% of the 199 described species of *Anastrepha* are considered economically important (White & Elson-Harris 1992; Aluja 1994; Norrbom 2004), the occurrence of any of these economically important species in a region has a negative impact on growers. Growers may be restricted from exporting their produce to certain markets, or may have to subject their fruit to expensive post-harvest sterilization measures (Simpson 1993).

The island of Puerto Rico contains populations of 2 economically important species; the Caribbean fruit fly, *A. suspensa* (Loew) and the West Indian fruit fly, *A. obliqua* (Macquart) (Jenkins & Goenaga 2008). Although there are populations of *A. suspensa* in Florida, there are no populations of *A. obliqua* there, making it risky to transport some Puerto Rican produce to Florida. Establishment of *A. obliqua* in Florida could jeopardize mango and other subtropical fruit crops.

Regulatory agencies spend considerable effort and expense monitoring large areas for these and other potentially invasive *Anastrepha* spp. (Anonymous 2010). The need for effective monitoring/detection devices has resulted in a long history of studies on attractants for *Anastrepha* spp. (Heath et al. 1993). Females of all frugivorous species of Tephritidae that have been studied, including *Anastrepha* spp., are anautogenous, i.e., they need to consume protein as adults for ovary development (Drew & Yuval 2000). Exploiting this need for protein, a variety of potential lures based on odors released from hydrolyzed proteins have shown some degree of attractiveness, including ammonia (released from ammonium acetate, ammonium bicarbonate and urine) (Bateman & Morton 1981; Burditt et al. 1983), and hydrolyzed torula yeast (Lopez et al. 1971; Burditt 1982). For many years hydrolyzed torula yeast in a liquid suspension, along with borax to reduce cadaver decay, was used in 1 piece glass McPhail traps to monitor and detect populations of *Anastrepha* spp. (Anonymous 1989; Heath et al. 1993). A series of modifications to the trap and the lures have improved the utility and effectiveness of the trap (Epsky et al. 1993; Heath et al. 1995; Thomas et al. 2001). Heath et al. (1995) identified some common volatiles from baits and decomposing fruit that were attractive to *A. suspensa*. These included ammonia, acetic acid (both released from ammonium acetate) and putrescine. Although not attractive when deployed alone (Heath et al. 2004), putrescine has been shown to be a potent synergist to ammonium acetate for capture of both *A. ludens* and *A. supensa* (Kendra et al. 2008). Thomas et al. (2001) pointed out that the design of the 1-piece glass McPhail trap was difficult to service, especially with the new lures, and prone to damage. A 2- piece plastic version of the McPhail trap has since been widely adopted by regulatory agencies. However, despite many studies, no single bait has been identified to satisfy the needs of regulatory agencies. Ideally, a bait would be easy to apply, long-lasting, attractive to target species (often multiple target species; regulatory agencies in Florida are currently monitoring for *A. obliqua* and the Mediterranean fruit fly, *Ceratitis capitata* Wied., among many others) combined with low non-target attractiveness.

APHIS-PPQ in Puerto Rico currently deploys a battery of traps and lures to detect and monitor pest Tephritidae; trimethylamine and ammonium acetate plus putrescine are used in Multilure traps (2-piece plastic McPhail traps) to detect *C. capitata*; methyl eugenol is used in Jackson traps (tent-shaped sticky cards) to detect Oriental fruit flies, *Bactrocera dorsalis* (Hendel) and carambola fruit flies, *B. carambolae* Drew & Hancock, and Cuelure is used in Jackson traps to detect melon fruit fly, *B. cucurbitae* (Coquillett), and Queensland fruit flies, *B. tryoni* (Froggatt) (Anonymous 2010). In addition, torula yeast is still used at some trap sites (Saez, personal communication).

Current lures for detecting/monitoring pest *Anastrepha* spp. include Nulure (Miller Chemical & Fertilizer, Hanover, PA.), a hydrolyzed corn protein lure (Gilbert et al. 1984), a freeze-dried preparation of Nulure (Heath et al. unpublished), ammonium acetate combined with putrescine (Biolure, Suterra LLC, Bend, Oregon), and the Unipak (Suterra LLC), a single bait dispenser containing ammonium acetate and putrescine (Holler et al. 2009). Our objective in this study was to compare these lures, as well as certain combinations (freeze-dried Nulure combined with ammonium acetate, or combined with both ammonium acetate and putrescine) for relative attractiveness to populations of *A. suspensa* and *A. obliqua* in Puerto Rico. We chose to conduct these trials in carambola, *Averrhoa carambola* (Oxalidaceae), because orchards of this fruit were available to the researchers in 3 different regions of Puerto Rico. Additionally, this is a poor host of both *A. obliqua* and *A. suspensa*; collections of thousands of carambola fruit yielded no pupae of *A. supensa* and relatively few pupae of *A. obliqua*, principally when preferred hosts, such as mango, were not available (Jenkins & Goenaga 2008). Most lure trials are conducted in orchards of preferred hosts; by conducting these trials in a poor host environment we evaluated efficacy of lures for detection of pest *Anastrepha* at low population levels.

Materials and Methods

Study Sites

Field trials were conducted in Sep and Oct of 2009, a time we had determined to be peak season for both fly species (Jenkins, unpublished). All trap blocks were set in experimental orchards of carambola located at the USDA-ARS Tropical Agriculture Research Experimental Station in Isabela, PR, and at the University of Puerto Rico Agricultural Experiment Stations in Corozal and Juana Diaz, PR. All orchards were planted in 1999 and were composed of 10 rows, each row containing 22 trees. Trees were planted in a quincunx system 3.7 m apart with 5.5 m between rows. All of the 6 internal rows had 9 varieties of tree planted randomly throughout the row. The varieties, grafted onto Goldenstar rootstock, were Arkin, B-10, B-16, B-17, Kajang, Kari, Lara, Sri-Kembangan, and Thai Knight. The 2 rows of trees on either side of these 6 internal rows were composed entirely of Arkin grafted onto Goldenstar rootstock. The first 2 trees and the last 2 trees of each row were also Arkin grafted onto Goldenstar rootstock. We have never recovered *A. suspensa* from thousands of carambola fruit and relatively low numbers of *A. obliqua* have been recovered

from carambola fruit (Jenkins & Goenaga 2008); nonetheless past experience has demonstrated that both species can be trapped in relative abundance from orchards of this fruit with no demonstrable effects of fruit variety on trap catch (Jenkins, unpublished).

Traps and Lures

All baits were tested using plastic 2-piece Multilure™ traps (Better World Manufacturing, Inc., Fresno, CA). Commercial lures (Suterra, LLC, Bend, OR) consisted of ammonium acetate and putrescine (Biolure MFF), and the newly formulated Unipak.

A total of 6 lures or lure combinations were tested in each block as follows:

1. Ammonium acetate + putrescine (=AAPt)
2. Nulure with borax
3. Freeze-dried Nulure 7 (=FDN7)
4. Freeze-dried Nulure 7 + ammonium acetate (=FDN7 + AA)
5. Freeze-dried Nulure 7 + ammonium acetate + putrescine (=FDN7 + AAPt)
6. Unipak

For all treatments except the 9% Nulure with borax, the trap fluid consisted of 200 mL of a 10% solution of propylene glycol (Qualichem Technologies, GA) and water. The trap fluid for the 9% Nulure lure (18 mL) consisted of 3% Borax (6 g) mixed with 182 mL of water. The Nulure and freeze-dried Nulure (9 g) were dissolved in the respective trap fluid. Nulure and freeze-dried Nulure baits were changed every 2 weeks (3 times during the study). The ammonium acetate and putrescine lures were changed every 4 weeks (once during the study).

Trap Block Design

Traps were deployed in 3 rows of each orchard. Rows with traps were at least 2 rows from the orchard border and separated by at least 1 trap-less row from another row with traps. All 6 treatments were represented in each of the 3 rows. Trees with traps were separated from other trees with traps by at least 1 tree. Traps were rotated to subsequent positions within rows each time they were checked. Traps at all sites were checked for fruit flies on Monday and Friday of each week between 24 Aug 2009 and 16 Oct 2009. All fruit flies were returned to the laboratory for identification and stored in 95% EtOH.

Statistical Analyses

The large number of independent variables we were comparing combined with the low number of replicates we were forced to use made the use of an ANOVA unsuitable for our purposes. Chi-square analyses were used to compare the observed number of flies of each species and sex trapped in each treatment to the expected number of flies under the assumption that flies would be equally distributed among the treatments if there was no difference in attraction. These analyses were conducted for each sex of each species for each week of the study (a total of 8 weeks) and for the total number of flies trapped throughout the study for each site. Chi-square probabilities exceeding 0.05 were labeled as insignificant. When the total number of flies trapped during a given week or at a given site was less than 30, i.e., an expected distribution among the treatments would be less than 5 (30 flies divided by 6 treatments = 5), the result was regarded as too weak to make inferences, even if the analyses indicated significant departure from the null hypothesis (= there was no difference in the distribution of flies among the treatments).

Results

Too few *A. suspensa* were caught in traps at the Isabela site (9 females and 1 male, total) to merit analysis. A total of 294 *A. obliqua* flies were caught in Isabela, 167 of which were females (58.0%) and 127 of which were males (42.0%). Throughout the 8 weeks of the trial, the percentage of females trapped averaged 57.9% ± 4.7 (SEM). Of the 294 *A. obliqua* trapped at the Isabela site during the experiment, 23% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 19% were in traps baited with freeze-dried Nulure and ammonium acetate, 19% in traps baited with UniPak lures, 17% were in traps baited with ammonium acetate plus putrescine, 14% were in traps baited with freeze-dried Nulure, and 7% in traps baited with Nulure. Chi-square analyses indicated that the distribution of *A. obliqua* (combined sexes) among the treatments departed significantly from the null hypothesis, although this was not true for every week of the study (Table 1).

A total of 231 *Anastrepha* spp. individuals were trapped at the Corozal site. One hundred and fifty nine (69%) of these were identified as *A. obliqua*, of which 109 (69%) were females and 50 (31%) were males. Of the 72 *A. suspensa* identified from traps at Corozal, 54 (75%) were female and 18 (25%) were male. Throughout the 8 weeks of the trial, the percentage of female *A. obliqua* trapped averaged 70.2% ± 3.0 (SEM) and the percentage of female *A. suspensa* trapped averaged 75.3% ± 3.8 (SEM).

Of the 159 *A. obliqua* trapped at the Corozal site during the experiment, 30% were in traps baited with ammonium acetate and putrescine, 28% were in traps baited with freeze-dried Nu-

TABLE 1. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE ISABELA SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

		Number of flies															
		AAPt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UniPak		Total flies			
Week	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	χ^2 value	χ^2 prob
1	*A. obliqua*	0	1	3	5	1	3	7	3	2	2	0	2	13	16	12.6	0.0280
	A. suspensa	0	0	0	1	0	0	0	0	0	0	0	0	0	1	NA	NA
2	*A. obliqua*	3	3	3	1	3	8	4	14	11	16	4	3	28	45	31.8	<0.0001
	A. suspensa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
3	*A. obliqua*	4	1	2	0	4	1	3	3	9	8	6	3	28	16	18.7	0.0020
	A. suspensa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
4	*A. obliqua*	2	5	2	2	4	3	6	1	2	0	3	4	19	15	4.1	0.5320
	A. suspensa	0	0	0	0	0	0	0	1	0	0	0	0	0	1	NA	NA
5	*A. obliqua*	0	1	0	0	0	1	1	3	2	1	0	0	3	6	9.0	0.1090
	A. suspensa	0	1	0	0	0	0	0	1	0	2	0	0	0	4	NA	NA
6	*A. obliqua*	3	1	1	0	2	3	3	1	2	6	5	10	16	21	19.3	0.0020
	A. suspensa	0	0	0	0	0	0	1	1	0	0	0	0	1	1	NA	NA
7	*A. obliqua*	1	4	2	0	0	1	2	4	1	3	0	9	6	21	9.2	0.1000
	A. suspensa	0	0	0	0	0	0	0	1	0	1	0	0	0	2	NA	NA
8	*A. obliqua*	9	13	0	2	2	4	0	1	2	2	1	5	14	27	28.3	<0.0001
	A. suspensa	0	0	0	0	0	0	0	0	0	0	0	0	0	0	NA	NA
Total	*A. obliqua*	22	29	13	10	16	24	26	30	31	38	19	36	127	167	25.4	<0.0001
	A. suspensa	0	1	0	1	0	0	1	4	0	3	0	0	1	9	NA	NA

lure and ammonium acetate and putrescine, 13% were in traps baited with UniPak lures, 12% were in traps baited with freeze-dried Nulure and ammonium acetate, 11% were in traps baited with freeze-dried Nulure, and 6% were in traps baited with Nulure.

Of the 72 *A. suspensa* trapped at the Corozal site during the experiment, 33% were in traps baited with ammonium acetate and putrescine, 26% were in traps baited with UniPak lures, 25% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 8% were in traps baited with freeze-dried Nulure and ammonium acetate, 6% were in traps baited with freeze-dried Nulure, and 1% were in traps baited with Nulure. As at the Isabela site, ammonium acetate and putrescine, freeze-dried Nulure combined with ammonium acetate or combined with ammonium acetate and putrescine, and the Unipak trapped more flies than the Nulure or the freeze-dried Nulure (Table 2). This was true for both *A. obliqua* and *A. suspensa*.

A total of 157 *Anastrepha* spp. individuals were trapped at the Juana Diaz site. Ninety four (59.9%) of these were identified as *A. suspensa*, of which 78 (83.0%) were female and 16 (17%) were male. A total of 63 (40.1%) *A. obliqua* were trapped at the Juana Diaz site, of which 49 (77.8%) were female and 14 (22.2%) were male. Throughout the 8 weeks of the trial, the percentage of female *A. obliqua* trapped averaged 80.0% ± 4.2 (SEM) and the percentage of female *A. suspensa* trapped averaged 75.3% ± 7.3 (SEM).

Of the 63 *A. obliqua* trapped at the Juana Diaz site during the experiment, 24% were in traps baited with freeze-dried Nulure combined with ammonium acetate and putrescine, 21% were in traps baited with freeze-dried Nulure, 17% were in traps baited with UniPak lures, 16% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 13% were in traps baited with ammonium acetate and putrescine, and 10% were in traps baited with Nulure.

Of the 94 *A. suspensa* trapped at the Juana Diaz site during the experiment, 27% were in traps baited with freeze-dried Nulure and ammonium acetate, 26% were in traps baited with ammonium acetate and putrescine, 21% were in traps baited with freeze-dried Nulure and ammonium acetate and putrescine, 16% were in traps baited with UniPak lures, 6% were in traps baited with Nulure, and 4% were in traps baited with freeze-dried Nulure. Generally, too few flies were captured of either species to make a confident analysis except when captures were summed for the duration of the experiment (Table 3). No significant departures from the null hypothesis were detected in the distribution of *A. obliqua* flies among the different baits.

At all 3 sites there was a consistent temporal pattern in capture; freeze-dried Nulure combined with ammonium acetate or combined with ammonium acetate and putrescine caught more flies in the first 4 weeks, whereas ammonium acetate plus putrescine or the Unipak lures caught more flies after the fourth week (Tables 1-3). The only exception occurred at the Juana Diaz site when the ammonium acetate and putrescine combination caught more *A. suspensa* than expected in the first week (Table 3).

Discussion

For all locations, species and sexes, where a Chi-square analysis detected significant departure from equal distribution among the different baits (and at least 30 flies were captured) Nulure or freeze-dried Nulure consistently attracted the fewest flies. This would suggest that the higher attractiveness of the Unipak, ammonium acetate and putrescine lures and the freeze-dried Nulure in combination with either ammonium acetate or ammonium acetate and putrescine is attributable to the common factor of these lures, namely, the presence of ammonium acetate in all of these lures. However, bait attractiveness was not constant over time, with a general trend of freeze-dried Nulure in combination with ammonium acetate or in combination with ammonium acetate and putrescine attracting more flies in the first 4 weeks of the study and ammonium acetate and putrescine or Unipak lures attracting more flies in the fourth week and later. This appears to be consistent with the anecdotal reporting that freshly opened ammonium acetate lures are less attractive than those that have been out a week or more (Thomas et al. 2008). This is potentially due to the dosage of ammonia released; Thomas et al. (2008) demonstrated that higher doses of the ammonia significantly reduced capture of *A. suspensa* and *A. ludens* compared to lower doses. Also, Kendra et al. (2005) demonstrated that increased doses of ammonia decreased the capture of female *A. suspensa* with undeveloped ovaries. However, fresh ammonium acetate and putrescine lures were placed in the field on the 5th week of this study, approximately when they began to catch more flies. Also, the freeze-dried Nulure combined with ammonium acetate or ammonium acetate and putrescine caught more flies early in the study, suggesting that the freshly opened ammonium acetate packages are not too strong, or that combined with the freeze-dried Nulure, the ammonium acetate packages are attractive at higher doses.

Many studies have been conducted on the attractiveness of certain baits, but comparing these studies in a meaningful manner is difficult and subject to speculation. This is because these studies are often conducted in different regions, trap different species of flies, different strains of flies (wild versus lab-reared), test different combina-

TABLE 2. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE COROZAL SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

		Number of flies															
		AAPt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UniPak		Total flies			
Week	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	χ^2 value	χ^2 prob
1	*A. obliqua*	0	1	0	1	2	0	1	6	0	6	0	0	3	14	15.1	0.0100
	A. suspensa	0	0	0	0	0	0	0	0	1	1	0	2	1	3	8.0	0.1560
2	*A. obliqua*	0	1	2	0	0	0	0	3	0	4	1	0	3	8	5.9	0.3150
	A. suspensa	0	0	0	0	0	0	1	2	0	0	0	0	1	2	15.0	0.0100
3	*A. obliqua*	3	4	2	1	0	6	3	5	7	16	0	0	15	32	51.5	<0.0001
	A. suspensa	0	1	0	0	1	0	0	1	0	5	0	1	1	8	10.3	0.0660
4	*A. obliqua*	6	9	0	2	0	0	0	0	3	3	0	3	9	17	37.2	<.0001
	A. suspensa	1	4	0	0	0	1	0	0	2	2	1	2	4	9	27.3	<0.0001
5	*A. obliqua*	0	3	0	0	1	2	0	1	3	1	0	2	4	9	5.0	0.4160
	A. suspensa	0	4	0	0	0	1	0	1	1	1	0	3	1	10	5.9	0.3150
6	*A. obliqua*	2	1	0	0	0	2	0	0	0	0	0	4	2	7	17.0	0.0050
	A. suspensa	2	2	0	0	0	1	0	0	0	2	0	0	2	5	11.0	0.0510
7	*A. obliqua*	3	3	0	1	0	0	0	0	0	0	1	5	4	9	42.7	<.0001
	A. suspensa	3	3	1	0	0	0	0	0	0	2	2	5	6	10	17.8	0.0030
8	*A. obliqua*	4	8	0	0	2	3	0	0	1	0	3	2	10	13	27.9	<.0001
	A. suspensa	1	3	0	0	0	0	0	1	0	1	1	2	2	7	9.0	0.1090
Total	*A. obliqua*	18	30	4	5	5	13	4	15	14	30	5	16	50	109	48.3	<0.0001
	A. suspensa	7	17	1	0	1	3	1	5	4	14	4	15	18	54	37.5	<0.0001

TABLE 3. NUMBER OF FLIES CAPTURED BY WEEK AND BY BAIT AT THE JUANA DIAZ SITE. CHI-SQUARE ANALYSES WERE PERFORMED ON COMBINED SEXES WITHIN A SPECIES.

		Number of flies															
		AAPt		Nulure		FDN7		FDN7 + AA		FDN7 + AAt		UniPak		Total flies			
Week	Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	χ^2 value	χ^2 prob
1	*A. obliqua*	0	3	0	1	0	3	1	2	1	3	0	0	2	12	4.9	0.4332
	A. suspensa	1	9	2	3	0	0	0	4	1	6	1	1	5	23	13.6	0.0190
2	*A. obliqua*	0	0	0	0	1	0	0	0	0	2	0	0	1	2	7.0	0.2206
	A. suspensa	1	3	0	0	0	0	1	7	0	7	0	2	2	19	17.0	0.0050
3	*A. obliqua*	0	1	0	1	2	2	1	3	1	2	1	2	5	11	3.5	0.6234
	A. suspensa	0	4	0	1	0	2	0	8	0	2	2	1	2	18	9.4	0.0940
4	*A. obliqua*	0	1	1	0	0	0	1	1	0	5	1	0	3	7	9.2	0.1013
	A. suspensa	0	0	0	0	1	1	1	3	0	1	0	3	2	8	8.0	0.1560
5	*A. obliqua*	0	0	0	0	0	1	1	0	0	0	0	5	1	6	16.4	0.0057
	A. suspensa	1	1	0	0	0	0	0	0	0	0	1	2	2	3	10.6	0.0600
6	*A. obliqua*	0	1	0	0	0	0	0	0	0	1	0	0	0	2	4.0	0.5494
	A. suspensa	0	1	0	0	0	0	0	0	0	2	0	1	0	4	5.0	0.4160
7	*A. obliqua*	0	0	1	2	0	0	0	0	0	0	0	2	1	4	10.6	0.0599
	A. suspensa	0	1	0	0	0	0	0	0	0	0	1	0	1	1	4.0	0.5490
8	*A. obliqua*	0	2	0	0	1	3	0	0	0	0	0	0	1	5	14.0	0.0156
	A. suspensa	1	1	0	0	0	0	1	0	0	1	0	0	2	2	5.0	0.4160
Total	*A. obliqua*	0	8	2	4	4	9	4	6	2	13	2	9	14	49	5.1	0.4038
	A. suspensa	4	20	2	4	1	3	3	22	1	19	5	10	16	78	25.9	<0.0001

tions of lures, are conducted in orchards of different crop species, and at different times of the year, all of which can impact the outcome. Nonetheless, it is useful to summarize these studies because baits will be used in a variety of conditions/locations/seasons to monitor/detect a variety of pest Tephritidae.

Regionally, the experiments most similar to the present study are those of Pingel et al. (2006) comparing the attractiveness of ammonium acetate plus putrescine with torula yeast plus borax in commercial orchards of 3 crop species in southern Puerto Rico, coinciding climactically and geographically with our Juana Diaz site. Conducted in Apr to May of 2002, their study found that the torula yeast outperformed the ammonium acetate and putrescine combination in orchards of mamey sapote and sapodilla, but the ammonium acetate and putrescine lure attracted more flies than the torula yeast in carambola orchards. The difference between the effectiveness of the 2 lures in the different orchards is striking and they point out the preponderance of *A. obliqua* in the carambola orchard (94% trapped flies in the carambola orchard were *A. obliqua*) whereas the other orchards had higher relative populations of *A. suspensa*. *Anastrepha suspensa* was more abundant in the carambola orchard at the Juana Diaz site during our study.

In a Colombian mango orchard *A. obliqua* was caught in traps baited with Nulure and borax more frequently than in traps baited with ammonium acetate with putrescine, torula yeast, or ammonium bicarbonate with putrescine (Epsky et al. 2003). However, in another study in a Mexican *Pouteria sapota* (Sapotaceae) orchard, traps baited with ammonium acetate with putrescine caught more *A. obliqua* than traps with the other baits, and in a Mexican mango orchard Nulure and ammonium acetate with putrescine both caught more *A. obliqua* than traps baited with other lures (Epsky et al. 2003). Furthermore, traps baited with torula yeast in Costa Rica and Honduras caught more *A. obliqua* than traps baited with the other lures. *Anastrepha suspensa* does not occur in any of the locations of the cited trial and so no comparison can be made with the *A. suspensa* results of our study. A similar study indicated that ammonium acetate was the best lure for detection of *A. suspensa* in Florida but that Nulure or torula yeast were the best lures for *A. obliqua* in the Dominican Republic, based on traps in mango orchards (Thomas et al. 2008).

In a study conducted in cages in Mexico, Díaz-Fleischer et al. (2009) found ammonium acetate and putrescine were more attractive to *A. obliqua* than Nulure, but the ammonium acetate and putrescine was not as attractive to *A. ludens*. *Anastrepha ludens* is an economic pest which regulatory agencies in the United States would like to be able to detect. They also found that attractiveness varied according to whether the flies tested were wild or reared in the laboratory for several generations.

There is strong evidence that the attractiveness of a particular lure to a given fly is based on that fly's physiological state, usually the stage of ovary development and a possible explanation for our results may be that populations varied physiologically over the duration of the experiment. Electroantennagram studies on *A. suspensa* indicated that immature females (females with little ovary development) were more responsive to ammonia and to ammonium bicarbonate lures, while females with mature ovaries were more responsive to putrescine and to carbon dioxide (Kendra et al. 2005; Kendra et al. 2009). Diaz-Fleischer et al. (2009) found that diet of the target fly did influence subsequent capture in traps, with more protein-starved individuals being captured by protein baits.

Nulure, followed by freeze-dried Nulure, consistently attracted the fewest flies in our study, regardless of species, sex, or location. This contrasts with the results obtained by Thomas et al. (2008) in mango orchards in Dominican Republic, where *A. obliqua* was most attracted to Nulure and torula yeast baits. It is conceivable that Nulure would attract more flies in different seasons. Liquid lures, including Nulure and torula yeast, have been shown to be more attractive in the dry season than in the wet season (Heath et al. 1997) and the Thomas et al. study was conducted at the beginning of the wet season.

Diaz-Fleischer et al. (2009) recently concluded that "there is no magic fruit fly trap," based on the complex interactions of fly species, physiological state and bait "preference," aggravated by low trap efficiency. One particular short-coming was the number of flies that entered a trap and successfully escaped from it. It is certainly true that these interactions are complex and that no single bait or trap will suffice for all target species in all regions. It has long been known that different tephritid species respond differently to hydrolyzed protein from different sources; *A. ludens* was more attracted to hydrolyzed cottonseed oil than to hydrolyzed corn protein (Lopez and Becerril 1967). *Anastrepha striata, A. serpentina, A. obliqua* and *A. balloui* preferred baits of hydrolyzed soy protein to torula yeast hydrolysate (Jiron and Soto-Manitiu 1989). Despite all of the improvements to the traps themselves and the lures, estimates of percent capture have not changed over more than 20 years; Calkins et al. (1984) and Diaz-Fleischer et al. (2009) came up with estimates of about 10% of the available population. Kendra et al. (2010) were able to recapture up to 35% of released *A. suspensa*, though. The results of this study confirm what has been suspected; that trap baits will have to be tailored based on regional and seasonal use.

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HOST PREFERENCE BY *DIACHASMIMORPHA LONGICAUDATA* (HYMNEOPTERA: BRACONIDAE) REARED ON LARVAE OF *ANASTREPHA FRATERCULUS* AND *CERATITIS CAPITATA* (DIPTERA: TEPHRITIDAE)

Sergio M. Ovruski, Laura P. Bezdjian, Guido A. Van Nieuwenhove, Patricia Albornoz-Medina and Pablo Schliserman

Laboratorio de Investigaciones Ecoetológicas de Moscas de la Fruta y sus Enemigos Naturales (LIEMEN). Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI) - CCT Tucumán - CONICET. Avda. Belgrano y Pje. Caseros, (T4001MVB) San Miguel de Tucumán, Tucumán, Argentina

Abstract

The preferences of *Diachasmimorpha longicaudata* (Ashmead) for larvae of *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) were evaluated under laboratory conditions in no-choice and dual-choice tests, based on percent parasitism, proportion of emerged parasitoids, proportion of female offspring, and number of parasitoid female visits to and ovipositor probes on the artificial oviposition device as different measures of host preference. In no-choice tests *D. longicaudata* females did not demonstrate a significant preference between *C. capitata* and *A. fraterculus* larvae. Nevertheless, *D. longicaudata* females showed a strong preference for *A. fraterculus* larvae in dual-choice test. Although female biased parasitoid progeny resulted in all assays, significantly more *D. longicaudata* female offspring emerged from *A. fraterculus* pupae than from *C. capitata* pupae. Thus, this study confirmed that both *C. capitata* and *A. fraterculus* are appropriate host for rearing *D. longicaudata*, but also provided evidence that female parasitoid progeny yield can be substantially improved by using *A. fraterculus* larvae as the host instead of *C. capitata* larvae.

Key Words: fruit flies, parasitoids, host preference, biological control, Argentina

Resumen

Se evaluó la preferencia de *Diachasmimorpha longicaudata* (Ashmead) por larvas de *Anastrepha fraterculus* (Wiedemann) y *Ceratitis capitata* (Wiedemann) bajo condiciones de laboratorio en situaciones de elección y no-elección. Las variables consideradas para el análisis fueron el porcentaje de parasitismo, la proporción de parasitoides emergidos, la proporción de descendientes hembras, el número de hembras que visitaron la unidad artificial de oviposición y el número de hembras que realizaron pruebas con el ovipositor en la unidad. Los resultados de los ensayos de no-elección mostraron que las hembras de *D. longicaudata* no tienen una significativa preferencia por las larvas de una u otra especie de tefrítido. No obstante, en el ensayo de elección, las hembras del parasitoide manifestaron una significativa preferencia por las larvas de *A. fraterculus*. En todos los ensayos realizados, la proporción de descendientes hembras de *D. longicaudata* obtenida fue superior a la de los machos, aunque significativamente más hembras del parasitoide se obtuvieron de puparios de *A. fraterculus*. El presente estudio confirma que tanto las larvas de *C. capitata* como las de *A. fraterculus* son adecuadas para criar *D. longicaudata* en laboratorio, aunque también señala que el empleo de larvas de *A. fraterculus* mejoran sustancialmente la producción de descendientes hembras del parasitoide.

Translation provided by the authors.

The South American fruit fly, *Anastrepha fraterculus* (Wiedemann), and the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) are 2 of the major pests currently affecting fruit crops in Argentina (Guillén & Sánchez 2007). Early biological control attempts to suppress both tephritid pest species resulted in the use of exotic parasitoids (Ovruski et al. 2000). *Diachasmimorpha longicaudata* (Asmead) is 1 of 5 exotic parasitoids introduced into Argentina from Costa Rica and México (Ovruski et al. 2003). It was originally collected in the Malaysia-Philippine region and is a solitary, koinobiont, larval-prepupal endoparasitoid of several tephritid species (Montoya et al. 2000). At present, *D. longicaudata* is considered 1 of the most significant biological control agents for augmentative releases against economically important fruit fly species in several Latin American countries (Montoya et al. 2007; Paranhos et al. 2008; López et al. 2009).

Although small scale releases of *D. longicaudata* were made in the Citrus-growing areas of northern Argentina during the 1960s (Ovruski et al. 2000), the permanent establishment of this

opiine parasitoid on *A. fraterculus* has been verified as a direct result of early classical biological control programs (Oroño & Ovruski 2007).

Currently, the suitability for successfully rearing *D. longicaudata* on larvae of either *C. capitata* or *A. fraterculus* is being studied in the PROIMI insectary in San Miguel de Tucumán—Argentina, as part of an augmentative release program against both tephritid fruit fly species. Therefore, the study here presented was conducted to evaluate the effects of both *C. capitata* and *A. fraterculus* on parasitism, parasitoid emergence, and sexual ratio of offspring in *D. longicaudata* under laboratory conditions. Furthermore, both the number of visiting and oviposition events was documented to assess the parasitoid female preference for 1 or the other host tephritid species.

Materials and Methods

The study was performed at the Biological Control Division of Planta Piloto de Procesos Industriales Microbiológicos y Biotecnología (PROIMI) located in San Miguel de Tucumán, Argentina. The colony of *D. longicaudata* was originally established in 1999 with individuals imported from México (Ovruski et al. 2003), where this colony had been reared in the laboratory on *Anastrepha ludens* (Loew) larvae (Montoya et al. 2000). First, *D. longicaudata* was successfully reared at the PROIMI laboratory on late-third instars of *C. capitata*. Then, in 2005 a second colony of *D. longicaudata* was established on late-third instars of *A. fraterculus*. Parasitoid colonies were held in cubical Plexiglas cages (30 cm) covered by organdy screen on both lateral sides, at a capacity of 300 pairs per cage at 25 ± 1°C; 75 ± 5% RH, and 12:12 (L:D) h photoperiod. The parasitoid rearing cage was provided with water and honey every other day. The general *C. capitata* and *A. fraterculus* rearing procedures were carried out as described by Ovruski et al. (2003) and by Vera et al. (2007), respectively. Both *A. fraterculus* and *C. capitata* puparia were selected from different samples and weighed for host quality evaluation.

Each species of fruit fly was exposed to 10 mated *D. longicaudata* females in cubical Plexiglas cages (30 cm) under both dual-choice and no-choice assays. In the choice assay, an oviposition unit (an organdy screen-covered petri dish, 8 cm diameter, 0.8 cm deep) containing 300 laboratory-reared third-instars of *A. fraterculus* (11 d old) was placed on the floor of the test cage along with another oviposition unit containing 300 laboratory-reared third-instars of *C. capitata* (6 d old). Larvae of both fruit fly species were placed in the units with artificial diet (brewer yeast + wheat germ + sugar + water). Oviposition units were positioned in the central part of the test cage; each unit was placed 1 cm from the side wall and separated by 10 cm from the other unit. In the no-choice assays, an identical oviposition unit containing 300 third-instars of *A. fraterculus* (or 300 third-instars of *C. capitata*) was placed on the floor of the central part of the test cage away from the walls. All female parasitoids used in experiments were 7-8 d old and deprived of any host larvae before testing. The females used in no-choice tests came either from parasitized puparia of *A. fraterculus* or from parasitized puparia of *C. capitata*. In the choice assay, 5 females stemming from parasitized puparia of *A. fraterculus* and 5 females stemming from parasitized puparia of *C. capitata* were used jointly. This combination of parasitoids from different origins was used so as to ameliorate a possible conditioned response by the previous experience with the host on which it was reared (Godfray 1994). Two control tests (no parasitoids) were made to determine both natural *A. fraterculus* and *C. capitata* mortality and emergence rates. Each test, including control treatments, was replicated 22 times. Each replicate lasted 24 h. All assays were conducted in the laboratory under the environmental conditions described previously.

Behavioral observations can be used to provide evidence of host preference for solitary parasitoids (Mansfield & Mills 2004). For this reason, upon release of parasitoids into each test cage, the number of female visits to and ovipositor probes in the oviposition units was recorded. Odor concentrations of host fruit (Messing & Jang 1992) or oviposition-deterring pheromone of tephritid fly (Prokopy & Webster 1978) were not considered in the assays because oviposition units with artificial diet were used. The female parasitoids were observed once every 15 min during the first 3 h and each observation lasted 30 s (Duan & Messing 2000a). A visit was recorded each time a female arrived on the oviposition unit after release. An ovipositor probe was confirmed each time a female parasitoid inserted its ovipositor through the top organdy screen of the oviposition dish. After the 3-h observations, all oviposition units remained exposed to female parasitoids for 21 h to finish a 24-h period (Duan & Messing 2000a). Then, all oviposition dishes were removed from the cages, and fly larvae were directly transferred into plastic cups (7 cm diameter, 6.7 cm deep) containing a 2 cm-vermiculite layer on the bottom as pupation medium. Later, each cup was tightly covered with a piece of organdy cloth on the top. Thus, fly pupae were held within plastic cups with moist, sterilized vermiculite until eclosion. After that, the number and sex of the emerged parasitoids, the number of emerged flies, and the number of uneclosed puparia were checked. Uneclosed puparia were dissected 2 weeks after emergence of the last adult parasitoid in each cup to check for the presence or absence of recognizable immature parasitoid stages (larvae, prepupae, or pupae) and/or fully developed pherate-adult parasitoids.

Both the parasitism percentage and the number and sex ratio of emerged parasitoid progeny were used as 3 suitable variables to measure host preference, in addition to the behavioral observations (Mansfield & Mills 2004). Parasitism percentage was calculated by dividing the total number of emerged and unemerged parasitoids into the total number of larvae exposed in the oviposition unit. The proportion of emerged parasitoids was calculated as the total number of emerged offspring divided by the total number of recovered pupae. The proportion of emerged flies was computed as the total number of retrieved adult flies divided by the total number of recovered pupae. The proportion of dead pupae was determined as the total number of pupae that did not yield flies or parasitoids divided into the sum of eclosed and uneclosed puparia.

Data on parasitism, parasitoid and fly emergences, sexual ratio of parasitoid offspring (as proportion of females), pupal mortality, and the number of female visits to and ovipositor probes on the artificial oviposition device were analyzed by a 2-sample unpaired *t*-test ($P = 0.05$) in no-choice assays, and by a paired *t*-test ($P = 0.05$) in the choice assay. Moreover, the numbers of emerged adults and dead pupae recorded from each fruit fly species per assay were statistically compared with control treatments by means of one-way analyses of variance ($P < 0.05$). Means were separated with a Tukey honest significant difference test (HSD) ($P = 0.05$). The proportion data were transformed to arcsine square root before analysis. All untransformed means (± SEM) were presented in the text. Pupal weight difference between *A. fraterculus* and *C. capitata* was analyzed by a Mann-Whitney Rank Sum test ($P = 0.05$).

Results

From the dual-choice test, significantly higher parasitism and emerged adult parasitoid percentages were recorded from *A. fraterculus* than from *C. capitata* (Table 1). When these 2 fruit fly species were analyzed in the no-choice tests, there was no significant difference for either of these 2 measures of host preference (Table 1). Sex ratios were female biased when *D. longicaudata* was reared from either host fruit fly species. However, the proportion of female offspring was always significantly higher when the parasitoid was reared on *A. fraterculus* than on *C. capitata* (Table 1).

The proportion of emerged *A. fraterculus* and *C. capitata* adults was significantly different between dual-choice, no-choice, and no-exposure control tests ($F_{(2, 63)} = 260.0, P < 0.0001$ for *A. fraterculus*; $F_{(2, 63)} = 311.8, P < 0.0001$ for *C. capitata*, Table 2). A significantly higher proportion of *A. fraterculus* adults were recovered from the no-choice test than from dual-choice test (Table 2). In contrast, significantly

Table 1. Mean (±SEM) percentage parasitism by *Diachasmimorpha longicaudata*, and proportion of adult parasitoids and female progeny emerged from *Ceratitis capitata* and *Anastrepha fraterculus* for both dual-choice and no-choice tests.

	Dual-choice test			No-choice test		
Fly species	% Parasitism	% emerged adult parasitoids	% parasitoid female progeny	% Parasitism	% emerged adult parasitoids	% parasitoid female progeny
C. capitata	17.4 ± 1.5 a	13.3 ± 1.1 a	50.7 ± 2.8 a	37.6 ± 2.0 a	32.1 ± 1.5 a	55.0 ± 1.1 a
A. fraterculus	35.5 ± 2.1 b	25.3 ± 2.3 b	82.4 ± 1.5 b	43.2 ± 2.2 a	36.3 ± 1.8 a	79.5 ± 1.6 b
	paired-t = 7.60	paired-t = 5.86	paired-t = 5.86	unpaired-t = 1.90	unpaired-t = 1.95	unpaired-t = 12.5
	df = 21.0	df = 21.0	df = 21.0	df = 42.0	df = 42.0	df = 42.0
	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$	$P = 0.0643$	$P = 0.0585$	$P < 0.0001$

Values in the same column with the same latter are not significantly different (paired and unpaired *t*-test, $P = 0.05$).

TABLE 2. MEAN (±SEM) PROPORTION OF EMERGED ADULTS AND DEAD PUPAE FROM *CERATITIS CAPITATA* AND *ANASTREPHA FRATERCULUS* RECORDED IN CHOICE, NO-CHOICE, AND CONTROL TESTS.

Tests	% emerged *A. fraterculus* adults	% emerged *C. capitata* adults	% dead *A. fraterculus* pupae	% dead *C. capitata* pupae
Dual-choice	17.3 ± 1.8 a	63.2 ± 1.7 a	34.2 ± 1.4 a	25.5 ± 1.1 a
No-choice	24.6 ± 2.6 b	25.1 ± 1.9 b	32.3 ± 2.0 a	37.4 ± 2.5 b
Control	82.4 ± 1.3 c	85.3 ± 1.6 c	17.6 ± 1.3 b	14.7 ± 1.6 c

Values in the same column with the same latter are not significantly different (Tukey`s test, $P < 0.05$).

2.5-times greater proportion of *C. capitata* adults emerged from the dual-choice test than from no-choice test (Table 2). The significantly lowest proportion of dead fly pupae was recorded from no-exposure control tests ($F_{(2, 63)} = 28.6$, $P < 0.0001$ for *A. fraterculus*; $F_{(2, 63)} = 38.9$, $P < 0.0001$ for *C. capitata*, Table 2). Significantly greater proportion of dead *C. capitata* pupae was recorded from no-choice tests than from dual-choice tests (Table 2).

Under both dual- and no-choice conditions, the mean numbers of *D. longicaudata* female visits to the oviposition units containing *A. fraterculus* larvae were significantly similar to those of parasitoid visits to the oviposition units containing *C. capitata* larvae (paired-t = 1.89, df = 21.0, P = 0.0732 for dual-choice test; unpaired-t = 0.47, df = 42.0, P = 0.6435 for no-choice test; Fig. 1 A). Similarly, in the no-choice assays, there were no significant differences in the mean numbers of parasitoid females probing the oviposition artificial devices (unpaired-t = 0.58, df = 42.0, P = 0.5631; Fig. 1 B). In contrast, in the dual-choice test, a significantly greater number of *D. longicaudata* females were observed probing the oviposition unit containing *A. fraterculus* larvae that the device containing *C. capitata* larvae (paired-t = 5.54, df = 21.0, $P < 0.0001$; Fig. 1 B).

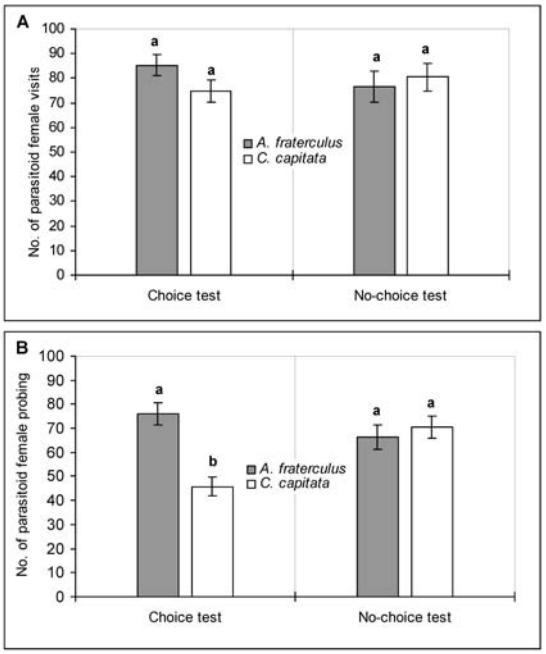

Fig. 1 (A and B). Mean (± SEM) (A) number of *D. longicaudata* female visits to, (B) and ovipositor probes on the oviposition units containing artificial diet plus third-instars of *A. fraterculus* or *C. capitata* recorded in no-choice and dual-choice tests. Bars in each graph followed by the same letter indicate no significant differences [unpaired t-test (P = 0.05) in the no-choice tests, and paired t-test (P = 0.05) in the dual-choice test]

DISCUSSION

While *D. longicaudata* attacked both *C. capitata* and *A. fraterculus* larvae at similar rates when only 1 of the species was present, they preferred *A. fraterculus* when provided a choice. This divergence may be suggestive of the relative host size differences. For example, *A. fraterculus* larvae used as host in this study were twice as large as *C. capitata* larvae (T = 60100.0, $P < 0.0001$, n = 200). Previous studies conducted by Messing et al. (1993), Cancino et al. (2002) and López et al. (2009), found that *D. longicaudata* females prefer large hosts. Eben et al. (2000) also pointed to the progeny sex ratio as a measure of host larva preference in *D. longicaudata*. These authors found that *D. longicaudata* reared from a larger species, *A. ludens* (Loew), in mango (*Mangifera indica* L.) had a much higher proportion of female progeny than those parasitoids that had developed in a smaller species, *A. obliqua* (Macquart), infesting the same fruit.

Behavioral observations provided further evidence for a preference for *A. fraterculus* over *C. capitata* larvae. In dual-choice tests *D. longicaudata* females are more likely to exhibit oviposition behaviors on devices containing *A. fraterculus.* However, Silva et al. (2007) found that *D. longicaudata* females did not discriminate between the volatiles produced by *C. capitata* or *A. fraterculus* larvae. In contrast to the present study, the larvae exposed by Silva et al. (2007) were feeding inside infested guava fruits (*Psidium guajava* L.). It has been repeatedly demonstrated that *D. longicaudata* females respond to fruit volatiles, especially from rotting fruits (Greany et al. 1977; Leyva et

al. 1991; Messing & Jang 1992; Purcell et al. 1994; Eben et al. 2000; Carrasco et al. 2005). Chemical cues derived from fermentation of the artificial rearing medium can be exploited for host searching by *D. longicaudata* (Duan & Messing 2000b). However, it is possible that differences in host larval substrates might have influenced *D. longicaudata*'s host detection ability.

Duan & Messing (2000b) found that *C. capitata* larvae outside of the substrate on which they fed generated vibration and chemical cues that stimulated oviposition in *Diachasmimorpha tryoni* Cameron, another generalist opiine fruit fly larval parasitoid (Wharton 1989). In the case of *D. longicaudata,* chemical cues produced by *C. capitata* larvae had little influence on probing behavior (Duan & Messing 2000b). However, it is possible that *D. longicaudata* females may respond more positively to chemical cues of *A. fraterculus* larvae than to those from *C. capitata* larvae. In addition to larval frass, other parts of the host larva such as hemolymph, alimentary canal, fat bodies, labial glands, and mandibular glands may be the source of 1 or more kairomones that stimulate oviposition movements in larval parasitoid species (Arthur 1981). Therefore, additional research should be performed to further define specificity of *D. longicaudata* female responses to chemical cues from both *A. fraterculus* and *C. capitata* larvae. Based on this requirement, we plan to conduct a second series of future experiments with *D. longicaudata* and 2 neotropical opiine fruit fly larval parasitoids.

Although dual-choice test results obtained in the present study provide reliable information on host rank order preferences for *D. longicaudata*, the ecological considerations on preference cannot be conjectured from this data. Therefore, we are currently verifying the host preference by *D. longicaudata* in field-cage tests using different host fruit species which are commonly infested by *C. capitata* and/or *A. fraterculus* larvae in the field.

Finally, this study confirmed previous data indicating that both *C. capitata* (Ovruski et al. 2003; Viscarret et al. 2006) and *A. fraterculus* (Ovruski et al. 2007) are suitable hosts for laboratory rearing of *D. longicaudata* in Argentina. It also provided evidence that female parasitoid progeny yield can be highly improved by using *A. fraterculus* larvae as host instead of *C. capitata* larvae.

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A NEW METHOD FOR QUANTIFYING COLOR OF INSECTS

MATTHEW S. LEHNERT[1,2], MURAT O. BALABAN[3] AND THOMAS C. EMMEL[2]
[1]Department of Entomology and Nematology, University of Florida, Bldg. 970 Natural Area Drive, P.O. Box 110620, Gainesville, FL 32611-0620

[2]McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, S.W. 34th Street and Hull Road, P.O. Box 112710, Gainesville, FL 32611-2710

[3]Fishery Industrial Technology Center, University of Alaska Fairbanks, 118 Trident Way, Kodiak, AK 99615

ABSTRACT

We describe a method to quantify color in complex patterns on insects, using a combination of standardized illumination and image analysis techniques. Two color comparisons were investigated: (1) the percentage of blue in the submarginal band of the hindwing in yellow and dark morph females of *Papilio glaucus* L., and (2) the percentage of orange hues in the wings of 2 putative subspecies of Eastern Tiger Swallowtail, *P. g. glaucus* L. and *P. g. maynardi* Gauthier. Live specimens were photographed in a light-box with standardized lighting and a color standard. Digital images were processed in LensEye® software to determine the percentage of selected colors. No significant differences were found in the percentage of blue between yellow and dark morph females, but the percentage of orange hues between *P. g. glaucus* and *P. g. maynardi* differed significantly. Color quantification can be a useful tool in studies that require color analysis.

Key Words: color analysis, color quantification, butterfly comparison, digital image, *Papilio glaucus*

RESUMEN

Se describe un método para cuantificar el color en los patrones complejos de los insectos, utilizando una combinación de iluminación estandarizada y de la técnica de análisis de imagen. Se investigaron dos comparaciones de color: (1) el porcentaje de azul en la banda submarginal de las alas posteriores en las hembras de forma amarilla y de forma oscura de *Papilio glaucus* L. y (2) el porcentaje de tonos de color anaranjado en las alas de dos subespecies putativos de *Papilio glaucus, P. g. glaucus* L. y *P. g. maynardi* Gauthier. Se tomaron fotos de especimenes vivos en una caja de luz con iluminación estandarizada y un estándar de color. Las imágenes digitales fueron procesadas usando el programa LensEye ® para determinar el porcentaje de los colores seleccionados. No se encontraron diferencias significativas en el porcentaje de color azul en las hembras de forma amarilla y de forma oscura, pero el porcentaje de tonos anaranjados entre *P. g. glaucus* y *P. g. maynardi* diferían significativamente. Cuantificación del color puede ser una herramienta útil en los estudios que requieren de un análisis de color.

Color and color patterns have been used to study a wide range of ecological and evolutionary topics, including sexual selection (Punzalan et al. 2008), aposematism (Brower 1958), industrial melanism (Kettlewell 1961), and mimicry (Jiggins et al. 2001; Saito 2002). Color is used in the classification of organisms to verify species and population properties, and subspecies (Brower 1959). The color of butterfly life stages and wings is used to understand evolutionary-developmental patterns and phenotypic plasticity (Starnecker & Hazel 1999; Nice & Fordyce 2006; Otaki 2008). However, most of these studies are hindered in their ability to quantify color.

When reporting quantified colors, RGB (red, green, blue) and L*, a*, and b* values (L* = lightness, scale: 0-100; a* = green to red, scale: -120-120; and b* values = blue to yellow, scale: -120-120) are typically used. RGB are digitally represented by 256 values each, meaning a total of more than 16 million possible color combinations (Balaban 2008), but the colors produced by these values are typically non-uniform and do not correlate well to human vision (Pedreschi et al. 2006). However, L*, a*, and b* values are combined together to represent a color that can be used in a comparative context to other similar colors (Pedreschi et al. 2006), and do account for the way humans perceive color.

Existing methods for quantifying color include simple visual estimates, with or without the use of a book of color standards for reference such as Munsell's (1976), spectrophotometry (Stevens et al. 2007), color software with RGB applications

(Villafuerte & Negro 1998), and colorimetery (Yagiz et al. 2009). Human vision is color biased (Wyszecki & Stiles 1982); factors such as lighting condition, illumination, and color are context-dependent (Endler 1990; Zuk & Decruyenaere 1994), and make color difficult to quantify. Specimens need to be nearly homogenous in color and have an almost flat surface to be accurately represented with colorimetry (Balaban 2008; Yagiz et al. 2009), and common image software such as Adobe Photoshop® has limitations when standardizing or calibrating a digital image and when quantifying the color patterns of complex images with large color variation.

Our objective was to introduce the use of image analysis with the LensEye® software as a tool to quantify the color of insects. LensEye® software was developed specifically for color quantification purposes, which makes it more user-friendly than other general color analysis programs such as Adobe Photoshop®. LensEye® has been used in food and agricultural sciences (Balaban 2008; Yagiz et al. 2009), but its application to entomological studies is novel. To illustrate this process, the wing colors of male and female Eastern Tiger Swallowtail butterflies, *Papilio glaucus* L., were analyzed in 2 comparisons: (1) the percentage of blue on the hindwing between yellow and dark morph females of *P. glaucus*, and (2) the percentage of orange hues between males of the 2 subspecies *P. g. glaucus* L. and *P. g. maynardi* Gauthier. In the first comparison, we predicted that the percentage of blue on the hindwing would be similar in yellow and dark morph females, because to our knowledge no previous reports have suggested a larger amount of blue in either morph. In the second comparison, we expected that males of *P. g. maynardi* would have a significantly larger percentage of the wings represented by high a* and b* values when compared with *P. g. glaucus*, as a combination of these values (reds and yellows) likely produces the orange hues that are diagnostic for this subspecies. To our knowledge, this is the first report of color quantification of tiger swallowtail butterflies.

Materials and Methods

Study Species and Specimen Preparation

The Eastern Tiger Swallowtail, *Papilio glaucus* L. (Lepidoptera: Papilionidae), is a large multi-colored butterfly found throughout the eastern half of the USA (Scriber 1996). Females are polymorphic and are either yellow with black stripes or melanic (Clarke & Sheppard 1962; Scriber 1996; Scriber et al. 1996); both forms have blue scales along the submarginal region of the dorsal side of the hindwings. Currently, 2 putative subspecies are recognized, *P. g. glaucus* and *P. g. maynardi*; the latter has a unique orange background color rather than the yellow found on the *glaucus* subspecies (Maynard 1891; Scriber 1986). *Papilio g. maynardi* is primarily found in Florida, but occasionally is found in other southeastern states (Maynard 1891; Brower 1959; Scriber 1986; Lindroth 1991). Ten yellow females and 10 dark morph females of *P. glaucus* were captured from Cedar Key and Lake Placid, Florida to compare the percentage of blue in the hindwings between these morphs. To compare the percentage of orange hues between the 2 subspecies, 10 males were collected from La Fayette, Georgia, and 10 males from Lake Placid, Florida, to represent the *P. g. glaucus* and *P. g. maynardi* subspecies, respectively. All specimens were captured during Apr-Jun, 2008, representing what is likely the spring brood of *P. glaucus* in these regions.

All butterflies were captured with a butterfly net and placed into glassine envelopes for transport. The live adults of *P. glaucus* were cooled in a walk-in refrigerator at 4°C, removed from the glassine envelopes, and their wings spread at 4°C on white Styrofoam® to expose the dorsal side of the wings, positioned as if prepared for a professional insect collection. Spreading was facilitated with insect pins placed near the costal and A1 veins of the forewing and the anal vein and distal portion of M3 vein of the hindwing proximal to the tail. No pins were inserted into the body. Once a butterfly was spread, it was removed from the walk-in refrigerator and walked to the equipment for color analysis.

Protocol for Color Analysis

Each butterfly was placed individually in a light-box with D65 standardized lighting (Luzuriaga et al. 1997), and a Labsphere® (North Sutton, NH) yellow color standard was placed next to the butterfly. Inside the light-box, a Nikon D200 digital camera was fastened to a stand approximately 0.3 m tall so that the camera faced down, and was fixed at a specific height and connected to a computer by a USB cable (camera specifications listed in Table 1). The light-box door was closed and a photograph was taken of the butterfly. Once in the light-box, it took less than 30 sec to process an individual butterfly. The computer used Camera Control-Pro® software (Nikon, Tokyo, Japan) to control the act of taking a photograph with the camera; therefore, a photograph could be taken from the computer while the camera was enclosed within the light-box, and the picture would upload onto the computer. Two types of software were used for color analysis: Adobe Photoshop 6.0® (Adobe Systems Inc, San Jose, California) used for image adjustments, modifications, and edits, and LensEye® (Engineering and CyberSolutions, Gainesville, Florida), used for color quantification and analysis).

TABLE 1. CAMERA SPECIFICATION USED FOR COLOR ANALYSIS OF LEPIDOPTERAN WINGS.

Image Quality	Compressed RAW (12-bit)
Image Size	Large (3872 × 2592)
Lens	VR 18-200 mm F/3.5-5.6 G
Focal Length	35 mm
Sensitivity	ISO 100
Optimize Image	Custom
High ISO NR	Off
Exposure Mode	Manual
Metering Mode	Multi-Pattern
Shutter Speed	1/3 sec - F/11
Exposure Comp.: (in Camera)	0 EV
Focus Mode	AF-S
Exposure Comp.: (by Capture NX)	0 EV
Sharpening	Auto
Tone Comp.	Auto
Color Mode	Model
Saturation	Normal
Hue Adjustment	0
White Balance	Direct Sunlight

The digital photographs (JPEG) (Fig. 1a) were cleaned in Adobe Photoshop 6.0® to isolate the images necessary for color analysis. The "eraser" tool was used to remove insect pins, feces, and additional artifacts created during photographing. The image of the Labsphere® color standard was cleaned by selecting the "elliptical marquee" tool that was used to highlight a yellow circular area within the color standard, which was moved with the "move" tool to the left of the butterfly, and the remainder of the color standard was erased. This process created 2 final images: a butterfly and a yellow circle. The image resolution was adjusted to 700 pixels wide by selecting "Image" in the main toolbar, then "resize" and "image size", and saved as a 24 bit BMP image (Fig. 1b). Females of *P. glaucus* were cleaned with the use of the "eraser" tool until only one hindwing remained. Males of *P. g. glaucus* and *P. g. maynardi* were cleaned so the entire butterfly (minus antennae) remained.

Cleaned images were opened and analyzed in LensEye® software. In Lenseye®, the objects of interest were separated from the background by designating the background color to consist of any pixel with RGB colors between 220 and 255, and the "16 colors per axis (4096 color blocks)" option was selected. This color information was displayed as the "% of total object area." Objects smaller than a user-selected threshold of 100 pixels were ignored, ensuring only the butterfly and color standard would be analyzed. In the color calibration option, the L*, a*, and b* values of the color standard were entered (L*, a*, and b* value of 90.17, -3.27, and 74.30, respectively), and the image was calibrated by selecting the "Process Image" tab. The software then calculated the average L*, a*, and b* values of the color standard from the uncalibrated image, and adjusted the color of each pixel in the image so that the average color of the standard in the image would equal that of the given reference values; this process calibrated all objects in the image (Fig. 1c). A spreadsheet was produced listing the percentage of each color (color ID#) and the average and standard deviation of the L*, a*, and b* values based on each pixel in the object. Each color ID # has a unique L*, a*, and b* value (Table 2), and the in-

TABLE 2. SELECTED COLOR ANALYSIS RESULTS FROM LENSEYE® SOFTWARE OF LEPIDOPTERAN WINGS.

Color ID#[1]	Color Standard	Butterfly[2]
3472	0	1.534
3488	0	2.076
3744	0	8.74
3745	0	1.805
3762	0	0.271
Lab L*	90.17	71.61
StdDev L*	0.37	2.84
Lab a*	-3.27	14.18
StdDev a*	0.71	1.72
Lab b*	74.3	78.42
StdDev b*	3.12	6.93
NBS name	brilliant yellow	strong orange yellow

[1]Each Color ID# represents a specific color (available in the software) with a unique L*, a*, and b* value.

[2]The numbers represent the percentage of each color (Color ID#) in the image. Percentages do not equal 100, because this is only a selected portion of the entire spreadsheet from the analysis. The numbers that correspond to the Lab L*, Lab a*, and Lab b* represent the average L*, a*, and b* value of the image. The NBS name represents the name of the color using the average L* a* and b* values.

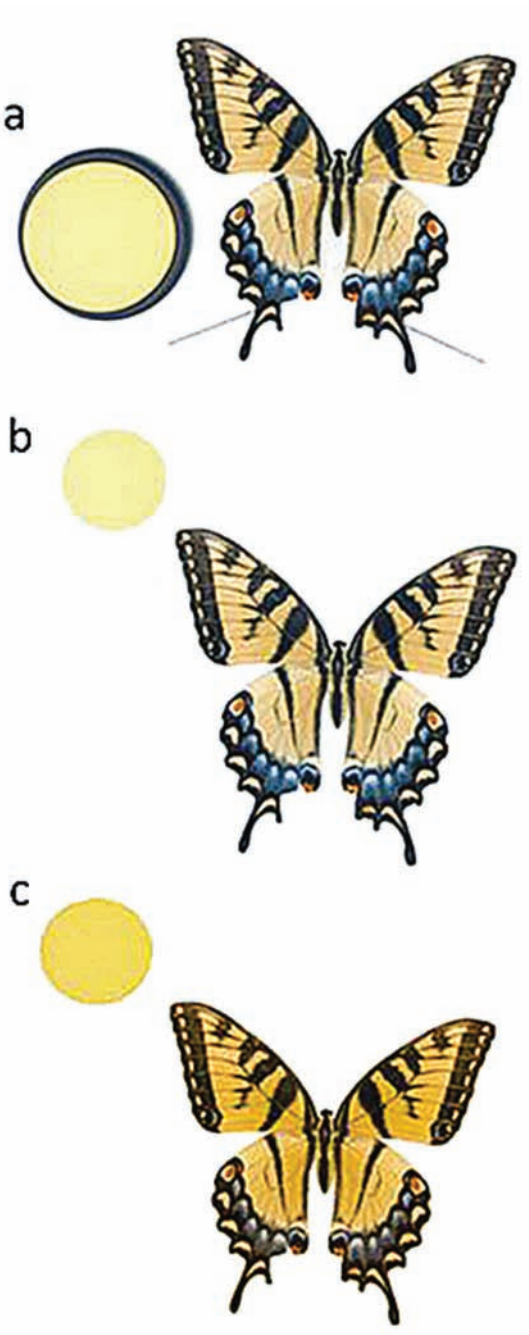

Fig. 1. Example of sequential images produced during color analysis. The raw image of the butterfly and color standard (a) is saved as a JPEG and opened in Adobe Photoshop® where it is cleaned and saved as a bitmap image 700 pixels wide (b). The cleaned image is opened in Lenseye® and calibrated and the colors quantified (c).

formation for each color was provided in the "color block information" in the software.

Both comparisons required the use of the "color contours" option in LensEye® software. For the first comparison, the most abundant colors of blue (color ID #) were selected from the spreadsheet and the L*, a*, and b* values of these colors were searched for in the "color block information" option. To analyze the calibrated image, it had to be reopened and reprocessed in LensEye®. The "show contours" option was selected revealing a table with options for selecting thresholds, where the blue L*, a*, and b* values were entered. On the image, the L*, a*, and b* contour settings were manipulated by interactively adjusting them and evaluating the quantity of blue pixels that were highlighted in the image to find the range of blue color values that encompassed the entire blue area on the butterfly. After 2 images of both yellow and dark morph females were manipulated, the following settings were deemed best suited for the task: L* contour greater than 20, a* contour less than 19, b* contour less than 25. These threshold values were entered for each of the 20 images and the software selected all the pixels that met the above criteria (all blue areas were highlighted in red, Fig. 2A). The percentages of blue colors of the total wing area were recorded for each image by selecting the "report contour" option.

For the second comparison, we used a male of *P. g. maynardi* from Lake Placid, Florida, to determine color composition to represent the *maynardi* subspecies. The image of this male was calibrated to receive the spreadsheet with the color ID # information, and the color moderate-orange-yellow (L*, a*, and b* values equal to 70, 9, and 60, respectively) was chosen to represent the threshold to distinguish *P. g. maynardi* from *P. g. glaucus*. This color was chosen because it was the lightest orange hue represented by the specimen in the image, and we also wanted to include darker hues of orange in our analysis, as these colors also may be present on the wings of *P. g. maynardi*. Calibrated images of the males were reopened in LensEye® and reprocessed. The "show contours" option was selected and the L*, a*, and b* contour values were entered into the threshold space. All values greater than the chosen threshold values were highlighted, because these values (higher a* and b* values) would represent darker orange colors in the butterfly wings than the moderate-orange-yellow color (Fig. 2B). The "report contour" option was chosen to record the percentage of wing area highlighted.

Statistical Analysis

We used a Welch's t test (two-tailed; $P = 0.05$) to evaluate differences in the percentage of blue between yellow and dark morph females, and the percentage of orange on the wings of males of the 2 subspecies.

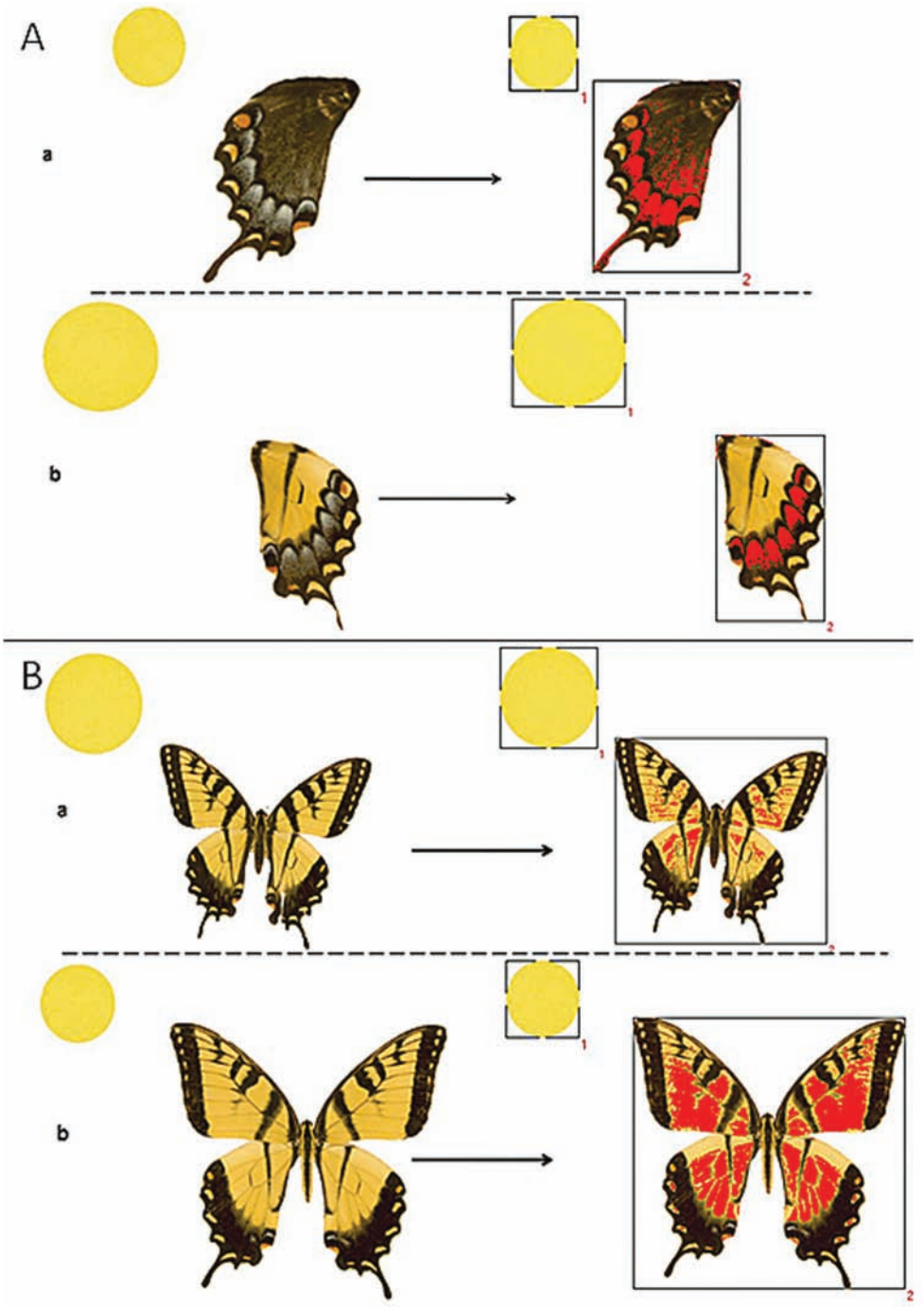

Fig. 2. Example of images used to determine the percentage of blue on hindwings of females of *P. glaucus* (A), and to study color differences between subspecies of *P. glaucus* (B), with designated L*, a*, and b* color values. In image A, the dark morph female (a) has more blue extending proximally from the submarginal band compared with the yellow morph female (b). The regions of blue interpreted by Lenseye® using specified values are highlighted in red by the software. The percentage of blue in image (a) and (b) is 21.4% and 12.8%, respectively. In image B, the same threshold for colors with a higher L*, a*, and b* value than moderate-orange-yellow were used for all males of *P. glaucus*. *Papilio g. glaucus* (a) has less orange than *P. g. maynardi* (b), as indicated by the red. Image (a) and (b) have 5.0% and 20.6% of the wings at or above the designated threshold. Both sets of images (A and B) display the calibrated image on the left and the analyzed image on the right.

Results

The dark morph and yellow females did not differ significantly in the percentage of blue on the hindwing (mean ± SE) (16.98 percent ± 3.10 and 14.2 percent ± 1.4, respectively) ($t = 1.5858$; $df = 18$; $P = 0.1411$). However, the pattern of blue differed between the morphs (Fig. 2A). All yellow females had blue scales restricted to the submarginal area of the hindwing, resulting in less than 20% of blue color on the hindwing, which was similar to some dark females, but other dark females had blue that continued proximally and became more random and scattered, resulting in a larger variation of blue color in these morphs. Four of the dark morph females had over 20% of blue scales on the hindwing, synonymous with the scattered blue scale phenotype, but the large variation in this morph led to an average quantity of blue not significantly different from that of the yellow morph.

Males of *Papilio glaucus maynardi* from Lake Placid, Florida, had significantly more orange than the butterflies from La Fayette, Georgia (9.97 percent ± 2.18 and 0.52 percent ± 0.90, respectively) ($t = 4.007$; $df = 18$; $P = 0.0021$), 80% of the analyzed *P. glaucus* from La Fayette had 0% of the wings at or above the designated L*, a*, and b* threshold used to represent moderate-orange-yellow. Although *P. g. maynardi* from Lake Placid, Florida, was visually distinct from the northern subspecies, the range of orange hues on the wings would have been difficult to quantify without a computer vision system and image analysis software. Lenseye® highlighted only the areas of the wings we were interested in analyzing. Even small patches of blue in the hindwing were highlighted, verifying the software's sensitivity to interpreting specified colors in an intricate color pattern.

Discussion

The application of image analysis software and our methods open a new avenue for quantifying color that could influence understanding of color components in ecological and evolutionary systems. For instance, color associated with the effects of temperature or host plant (phenotypic plasticity) (Price 2006), range distributions of hybrid zones (Blum 2002; Gay et al. 2008), floral color changes in response to insect pollination (Paige & Whitham 1985), and seasonal polyphenisms (Hazel 2002) can be quantified. This study also provides a means to analyze color of live specimens, which could have important implications to studies of endangered species. In this study, the butterflies seemed unaffected by the method, and were capable of flight, copulation, and oviposition after the study, verified by additional studies (M.S.L., unpublished data). Our methods also provide a protocol to quantify museum specimens, for instance, in studying how color dynamics of populations have shifted over time.

Our method allows the use of thresholds to study colors of interest and to determine their percentage compared with the rest of the image. For example, the blue scales scattered over the hindwing of a dark morph female were quantified, even though these small blue spots were on a black background. Additionally, similar, but different, colors (yellow-orange) were quantified to distinguish 2 entities. *Papilio glaucus maynardi* is relatively unstudied, and there are conflicting reports concerning its distribution (Forbes 1960; Harris 1972; Howe 1975; Mather & Mather 1985; Scriber 1986; Lindroth et al. 1988). Our method could provide a means to determine its distribution. Other aspects of its evolutionary history could be addressed, such as determining if the subspecies represent a color cline or a rapid shift in color, suggesting similar dynamics of a narrow hybrid zone where one phenotype rapidly shifts to the other.

The primary limitation of our method, and other color quantification methods, is that standardized lighting is necessary; therefore, these methods would not be reliable in all situations, such as comparing the color of butterfly wings from photographs taken outdoors under different lighting conditions. We addressed this issue by using a light-box with standardized lighting. Other source and processing errors may have occurred, such as instrumental inaccuracies of the light-box, camera, and software; however, to minimize these errors we used the same camera and light specifications for each individual. In addition, there may be a source error in that populations of *P. glaucus* may experience a seasonal polyphenism, which could alter our interpretations of the data sets. We addressed this issue by collecting the individuals from the various locations during a similar time period.

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THE LARGE DECAPITATING FLY *PSEUDACTEON LITORALIS* (DIPTERA: PHORIDAE): SUCCESSFULLY ESTABLISHED ON FIRE ANT POPULATIONS IN ALABAMA

SANFORD D. PORTER[1], L. C. "FUDD" GRAHAM[2], SETH J. JOHNSON[3], LARRY G. THEAD[4] AND JUAN A. BRIANO[5]
[1]Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608

[2]Department of Entomology and Plant Pathology, Auburn University, 301 Funchess Hall, Auburn, AL 36849-5413

[3]Department of Entomology, 400 Life Sciences Building, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

[4]Biological Control of Pests Research Unit, USDA-ARS, P.O. Box 67, Stoneville, MS 38776
Current Address: 10303 Wildcat Road, Collinsville, MS 39325

[5]South American Biological Control Laboratory, USDA-ARS, Bolivar 1559 (1686) Hurlingham, Buenos Aires, Argentina

ABSTRACT

The large fire ant decapitating fly, *Pseudacteon litoralis* Borgmeier, from northeastern Argentina was successfully released as a self-sustaining biocontrol agent of imported fire ants in south central Alabama in 2005. Five years later, this fly is firmly established at the original release site and has expanded outward at least 18 km. Nevertheless, populations remain very low considering *P. litoralis* is one of the most abundant fire ant decapitating flies in large areas of its range in South America. The reasons for low densities and why we were only able to establish this fly at 1 of 9 release sites in 4 states (2003-2006) are unknown, but problems with host-matching, release procedures, weather conditions, and competition with previously released decapitating flies are discussed as possible factors.

Key Words: *Solenopsis invicta*, biological control, low population density

RESUMEN

La mosca grande del norte y centro-este de Argentina, *Pseudacteon litoralis* Borgmeier, decapitadora de la hormiga de fuego (hormiga brava), fue liberada exitosamente como agente de control biológico de la hormiga de fuego importada en el sur-centro de Alabama en 2005. Cinco años después, esta mosca se encuentra firmemente establecida en ese sitio y se ha expandido al menos 18 km; sin embargo, las poblaciones permanecen muy bajas considerando que *P. litoralis* es una de las moscas decapitadoras de hormiga de fuego más abundante en su área de América del Sur. Se desconocen las razones de las bajas densidades y el por qué del establecimiento de esta mosca en sólo uno de los nueve sitios de liberación en cuatro estados (2003-2006), pero se discuten como posibles factores los problemas de correspondencia de hospederos, procedimientos de liberación, condiciones climáticas y competencia con moscas decapitadoras liberadas previamente.

Translation provided by the authors.

The decapitating fly *Pseudacteon litoralis* Borgmeier (Fig. 1) is a parasitoid of the red imported fire ant, *Solenopsis invicta* Buren, the black imported fire ant, *Solenopsis richteri* Forel, and 3 other species of *saevissima* complex fire ants in southern Brazil, Paraguay, and northern Argentina (Patrock et al. 2009). *Pseudacteon litoralis* is the largest of the common *Pseudacteon* species that attack fire ants and specializes in parasitizing the largest sizes of fire ant workers (Morrison et al. 1997). It is active throughout the daylight hours, but prefers dawn and especially dusk (Pesquero et al. 1996). As with several other *Pseudacteon* phorids (e.g., *P. tricuspis* and *P. nocens*), sex is probably determined environmentally, primarily by the size of the host, rather than genetically like most other insects (Morrison et al. 1999). Males of *P. litoralis* are not attracted to fire ant mounds like *P. tricuspis* and *P. obtusus* (Porter & Pesquero 2001; Calcaterra et al. 2005). In the lab, mating appeared to occur on and around black objects in the top of the large attack boxes (SDP, unpubl. obs.). This fly is one of the most abundant fire ant decapitating flies throughout much of its range in South America both numerically and spatially (Calcaterra et al. 2005; Patrock et al. 2009, personal observations, SDP). Like other species in the genus, *P. litoralis* is

Fig. 1. Female *Pseudacteon litoralis* fly preparing to oviposit in the thorax of a fire ant worker.

highly host-specific (Porter & Gilbert 2004; Weissflog et al. 2008) probably because these flies use fire ant alarm pheromones to find their hosts (Vander Meer & Porter 2002) and also because of their highly specialized life history of decapitating fire ant workers and then pupating inside their empty head capsules (Porter et al. 1995).

The characteristics discussed above made *P. litoralis* an attractive target for release as a self-sustaining or classical fire ant biological control agent. The objectives of this paper are to document the release and establishment of *P. litoralis* in south central Alabama and to describe the fate of 8 additional field releases conducted in Florida, Mississippi, and Louisiana from the spring of 2003 to the summer of 2006.

Materials and Methods

The original source population for the *P. litoralis* flies discussed in this paper was from several sites just off Route 11 about 6 kilometers south of San Justo, Santa Fe, Argentina (30.550°S, 60.607°W). About 1,800 fire ant workers parasitized with *P. litoralis* were brought back to Gainesville, FL in Apr 2001. The fire ants at the collection sites were *S. invicta*, although probably not the same biotype as that found in the United States (Ross & Trager 1991; Caldera et al. 2008). By the summer of 2001 the newly established *P. litoralis* laboratory colony had dropped to about 1000 individuals (about 20-30 pupae per day, assuming a 40-d life cycle) and remained at this level through the end of 2001, after which numbers began to gradually increase. In the winter of 2002, 100 or so males were added to the San Justo colony from a collection site on the Paraguay River near Herradura, Formosa, Argentina (26.514°S, 58.284°W). The *S. invicta* ants at this site were probably more similar to the U.S. biotype, but still not quite the same. By the time releases had begun in the spring of 2003 the colony was producing about 500 pupae per day. Maximum production was about 1,000 pupae per day in Jan 2006.

Releases were conducted at sites where fire ants were abundant (Table 1). We selected sites with a large percentage of monogyne colonies because monogyne or single-queen fire ant colonies have a higher percentage of the larger workers preferred by *P. litoralis* females (Morrison et al. 1997). Most sites were near water sources and had patches of tall grass or shrubbery that was assumed to help protect fly pupae from being killed in the sun. All of the sites were pastures except the Florida Ironwood Golf Course (Table 1) which was a mixture of fairways, lake edges, and service roads along drainage canals. The Alabama release site (Table 1) was drenched by Hurricane Dennis just before the final groups of parasitized ants were released in Jul 2005.

Table 1. Field release data for the fire ant decapitating fly *Pseudacteon litoralis*.

Site	County, State	Start Date	Duration (days)	Number Released	Fate
Mickle Farm	Alachua, FL	May 2003	~3	~150[a]	Failed
Morrill Farm	Alachua, FL	15 May 2003	12	2,400[a]	Failed
Whitehurst Farm- A[f]	Marion, FL	15 Sep 2003	21	4,500[b]	Failed[c]
Knox Site[d,f]	Clay, MS	4 Aug 2004	20	6,400[b]	Failed
Whitehurst Farm- B[f]	Levy, FL	25 Apr 2005	27	5,200[b]	Failed
Ironwood Golf Course[f]	Alachua, FL	10 May 2005	32	4,800[b]	Failed[c]
Biddle Farm	Wilcox, AL	21 Jun 2005	18	4,600[b]	Established
Idelwilde Res. Station	E. Feliciana, LA	15 May 2006	20	5,200[b]	Failed
Morrill Farm[f]	Alachua, FL	18 May 2006	44	17,200[e]	Failed

[a]Adult flies released over disturbed mounds.
[b]Estimated parasitized fire ant workers.
[c]First-generation adult flies recovered at release site.
[d]Fire ants at this site were primarily hybrids (black × red); ants at all the other sites were the red imported fire ant, *S. invicta*.
[e]Adult flies emerged from pupae in shaded emergence box in field.
[f]Sites where *P. curvatus* flies were established prior to the release of *P. litoralis*, *P. tricuspis* was previously established at all sites except the Mississippi site.

Competing *P. tricuspis* flies were present at all of the *P. litoralis* release sites except the Mississippi site where *P. tricuspis* had been unable to establish on the hybrid fire ants (Table 1). At the time *P. litoralis* was released, *P. curvatus* flies were not present at the Mickle and Morrill release sites in Florida, the Louisiana site, or the Alabama site (until 2007).

The *P. litoralis* flies were released at the first 2 sites (Table 1) as adult flies over disturbed fire ant mounds as was the procedure for *P. tricuspis* (Porter et al. 2004). However, only a few of the females were observed to hover over and attempt to oviposit in the disturbed workers. The next 6 releases (Table 1) were conducted by releasing workers parasitized in the laboratory back into their mother colonies as described for *P. curvatus* (Vazquez et al. 2006). The hope was that emerging females would naturally mate with nearby males and then be attracted to attack fire ant workers. At the final site (Table 1), pupae on moist plaster trays were placed inside a large emergence box (61 by 41 by 51 cm; height, width, depth) in the field. This was done several days before the pupae were due to emerge. The box was shaded to prevent overheating and placed on a stand coated with Fluon to limit access for ants and other arthropods. Upon emergence, the flies flew to the light and exited through window screen that protected the pupae from access of larger organisms. Average emergence rates of adult flies from pupae in this box was 84%, a value comparable to that achieved with good rearing procedures in the laboratory.

Initial surveys to determine whether the flies had established were usually conducted in the late afternoon or early evening by disturbing several mounds at or near the release site and aspirating all flies that were attracted to the mounds (Porter et al. 2004; Vazquez et al. 2006). Beginning in 2006, most surveying in Florida was accomplished with sticky traps (baited with live ants) supplemented by aspiration (Puckett et al. 2007; Porter 2010). Sticky traps baited with either live ants or freeze killed ants were also tried in Alabama in 2008. We did not conduct prerelease surveys to detect the presence of *P. litoralis* at our release sites because none of the 20 or so South American *Pseudacteon* species that attack red imported fire ants have ever been found in North America (unless they were intentionally released) despite extensive collections and observations over many years (Porter et al. 2004; Patrock et al. 2009; Porter 2010; Plowes et al. 2011).

Results

The decapitating fly *P. litoralis* only became established at the release site in Alabama (Table 1). This site was a series of small weedy pastures encircled by trees and shrubbery (~7 ha). Releases were conducted in overgrown areas near the tree lines of the pastures. The first *P. litoralis* fly was recovered at this site on 20 Jun 2006. This collection occurred a year after the release even though sampling had been conducted several times previously in both 2005 and 2006. The next flies were detected a year later on 23 Jul (2 flies) and 31 Jul 2007 (7 flies). In 2008 (Jun and Jul) 3 years after the release, *P. litoralis* flies were collected with aspirators at 5 sites: the release site (1 fly), 6 km south (1), 11 km south (2), 6 km west (1), and 18 km west (1). In the summer 2008 (Jun and Jul), sticky traps were placed every half mile along road right-of-ways for 10 miles in each of the 4 cardinal directions (80 total traps) for the sole purpose of monitoring *P. litoralis* expansion. This was repeated 3 times. Many *P. curvatus* and *P. tricuspis* flies were found on the traps, but no *P. litoralis* flies. In Jun 2009, single flies were collected 2, 6, and 14 km north of the release site. In Jul and Aug 2010, a total of 7 flies were collected on 3 different occasions at the release site. Throughout this period, abundance of *P. litoralis* was always low; *P. litoralis* was not collected at most of the sites surveyed, and they were generally found in only a small fraction of disturbed mounds inspected. However, 113 flies were aspirated at the release site in the early morning on 16 Sep 2010, an abundance that is equivalent to high densities of this species in South America. To date, all *P. litoralis* in Alabama have been collected with aspirators.

First generation, field-reared *P. litoralis* females were found about 6 weeks after 2 of the 6 Florida releases (Table 1). Unfortunately, repeated monitoring (2003-2010) failed to detect any additional flies, including in the fall of 2010 when 4 sites near each of the 3 major release areas were checked twice for *P. litoralis* flies (Sep and Oct, 74 total mounds). The Louisiana site was first sampled 4 months after the release (Sep 2006). This release site was rechecked twice in 2009 (Apr and Sep) and twice in 2010 (Apr and Sep) without finding *P. litoralis*. Five other sites were sampled near the release site (1.6-5.2 km away) in 2009 (Apr and Sep) and again in 2010 (Apr and Sep). Ten mounds were inspected at each of the Louisiana sample sites, but no *P. litoralis* flies were collected even though both *P. curvatus* and *P. tricuspis* flies were collected. Flies also were not detected at the Mississippi site which was checked 11 times after the release (Sept-Nov, 2004) and once in Jul 2005, almost a year after the release. Three locations near the Mississippi site were checked in Sep 2010, but only a few dozen *P. curvatus* flies were found.

Discussion

The large decapitating fly, *P. litoralis*, is firmly established on red imported fire ants in south

central Alabama. Populations of this species are generally low, but they have survived through 5 winters and they have expanded at least 18 km from the release site. This makes *P. litoralis* the third decapitating fly species released and successfully established on imported fire ant populations in the United States. The first 2 *Pseudacteon* species, *P. tricuspis*, and *P. curvatus* were released at numerous sites across the Southeast and currently cover about 65% and 90% of the imported fire ant range in the United States, respectively, (Callcott et al. 2011). A fourth *Pseudacteon* species, *P. obtusus,* has been established in Texas and Florida (Gilbert et al. 2008; SDP) and a fifth very small species, *P. cultellatus,* is currently being released in Florida (SDP). In addition to the flies mentioned above, several other parasitic arthropods (Williams et al. 2003), 2 species of mermithid nematodes (Poinar et al. 2007), 2 species of microsporidian pathogens, and at least 3 kinds of viruses, are being investigated as potential fire ant biocontrol agents (Oi & Valles 2009).

The expansion rate of *P. litoralis* from the release site in Alabama has proven difficult to monitor because low densities make this fly difficult to detect at sample sites. Despite low densities, the rate of expansion for *P. litoralis* in Alabama is similar to expansion rates reported for *P. tricuspis* in Texas and Louisiana, but probably less than the very abundant *P. curvatus* in Florida and Mississippi (Henne et al. 2007; Porter 2010). The low densities of *P. litoralis* at sites in Alabama is curious because *P. litoralis* is consistently one of the most abundant decapitating flies across most of its range in South America both numerically and spatially (Calcaterra et al. 2005; Patrock et al. 2009). The large number of flies recently collected (Sep 2010) from the release site is encouraging, but it is unknown whether this represents a new trend or is just a temporal quirk.

The apparent failure to establish *P. litoralis* at the other 8 sites was disappointing. We made releases at sites with a variety of habitats and climates in hopes that variety would increase the probability of success. The Mississippi site was chosen in hopes that the flies might do better on the *S. invicta* x *S. richteri* hybrid fire ants found at that site.

It is possible that populations have been established at some sites listed in Table 1, but densities are still too low to be easily detected, as has occurred on several occasions with *P. curvatus* (Graham et al. 2003; Vazquez et al. 2006). Nevertheless, this possibility seems unlikely at the Florida, Louisiana, and probably Mississippi sites considering the frequency and duration of the sampling efforts in those areas.

Repeated failures to establish *P. litoralis* in the field is reminiscent of failures to establish *P. curvatus* collected from black fire ants in South America on red fire ants in the United States (Graham et al. 2003; Callcott et al. 2011). Perhaps a biotype of *P. litoralis* better adapted to the biotype of red imported fire ants found in the United States would have been more successful. However, we tried twice to establish additional laboratory colonies of *P. litoralis* from flies collected along the Parana River near Herradura, Formosa, Argentina (Apr 2003, 314 flies; Dec 2005, 1400 flies). Unfortunately, both attempts failed as did other attempts to culture *P. litoralis* flies collected in Saõ Paulo State, Brazil (1997) and the Corrientes area of Argentina (2004-2006). Exactly why we were able to culture the flies collected from San Justo, but not the *P. litoralis* flies collected elsewhere is unknown, although it may be related to problems with mating since the adult females seemed to be attracted normally to the fire ant workers we provided to them in the laboratory attack boxes.

While poor host matching may have been a problem, other factors may also have been important in the failure of *P. litoralis* to establish at some of release sites, especially since they did establish in Alabama and thus should have been able to be established elsewhere on *S. invicta* fire ants. Competition with previously released species is one likely explanation. Our colleagues in Texas provide strong evidence that the presence of *P. curvatus* at their release sites greatly diminished the success rate of establishing *P. obtusus* (Plowes et al. 2011). Similarly in Florida, competition between *P. curvatus, P. tricuspis,* and the recently released *P. obtusus* appears to be greatly reducing *P. tricuspis* populations (SDP and Lu, unpublished). However, competition with *P. curvatus* was not a problem with the first 2 releases in Florida or with the releases in Alabama and Louisiana because *P. litoralis* was released at these sites before *P. curvatus* was present.

Poor weather conditions may have been another factor at some of the failed sites. Examination of release records for *P. tricuspis* (Callcott et al. 2011) indicates that summer releases were about half as successful as releases in the spring or fall. Five of the 9 *P. litoralis* releases, including the successful one in Alabama (Table 1), were at least partly carried out during hot summer months (although rain and clouds from Hurricane Dennis likely reduced negative impacts of summer heat for the Alabama release). Another possible problem is that U.S. fire ant populations may not have enough major workers to sustain large numbers of *P. litoralis*, but intercontinental comparisons of worker polymorphism have not been done to see if this is a real concern. Certainly, U.S. fire ant colonies do have many workers in the size range which *P. litoralis* prefers to parasitize (Porter & Tschinkel 1985; Morrison et al. 1997; Morrison et al. 1999). Poor release technique is another explanation. This would certainly seem to be true for the first 2 releases, because the adult

flies did not show much interest in the disturbed fire ant mounds and very few flies were used at the first site. The large release box used in the last release was an effort to try something different than what had previously been done. The lack of any first-generation field-reared flies at this release site was disappointing considering the number of flies released and the extended period of the release.

In the fall of 2006, we made the decision to focus on other biocontrol agents with higher probabilities of success. Nevertheless, *P. litoralis* is firmly established in Alabama and will presumably expand into other states. While *P. litoralis* was locally abundant on one occasion in 2010, it failed at most of the release sites and remained rare in Alabama over most of the last 5 years, a curious situation considering *P. litoralis* is one of the most abundant species of fire ant decapitating flies throughout most of its range in South America (Calcaterra et al. 2005; Patrock et al. 2009).

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HOST SPECIFICITY OF *ANTHONOMUS TENEBROSUS* (COLEOPTERA: CURCULIONIDAE), A POTENTIAL BIOLOGICAL CONTROL AGENT OF TROPICAL SODA APPLE (SOLANACEAE) IN FLORIDA

J. MEDAL[1], N. BUSTAMANTE[1], E. BREDOW[2], H. PEDROSA[2], W. OVERHOLT[1], R. DÍAZ[1] AND J. CUDA[1]

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

[2]Universidade Federal do Paraná, Curitiba, Brazil

ABSTRACT

Multiple-choice and no-choice tests were conducted at the Florida Department of Agriculture quarantine facility to determine the host specificity of the South American flower bud weevil, *Anthonomus tenebrosus* Boheman, intended for biological control of the exotic weed tropical soda apple (TSA), *Solanum viarum* Dunal in Florida, USA. Ninety-one plant species in 21 families were included in multiple-choice feeding and oviposition experiments, including the target weed and the 6 major cultivated Solanaceae: bell pepper (*Capsicum annuum* L.), chili pepper (*C. frutescens* L.), tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.). Plant bouquets with flower-buds of 8 to 10 randomly selected plant species, always including TSA (*S. viarum*) were exposed to 10-20 *A. tenebrosus* adults for 1 to 2 weeks. Oviposition and feeding were observed twice a week. No-choice host-specificity tests were also conducted with *A. tenebrosus* adults using potted flowering plants. Ten adults were exposed to 29 plant species individually tested for 1 to 2 weeks. Plant species in each test were replicated 3 or 4 times. All tests showed that *A. tenebrosus* fed and laid eggs only on the target weed. No eggs were deposited on any of the other of the 91 plant species tested. Host-specificity tests indicated that a host range expansion of *A. tenebrosus* to include any of the crops, and native Solanaceae, and non-solanaceous plants tested is highly unlikely. A petition for field release in the USA was submitted to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) in Oct 2007.

Key Words: host-specificity tests, weed biological control, *Solanum viarum*, Solanaceae

RESUMEN

Pruebas de ovoposición y alimentación (con y sin elección), se realizaron para evaluar la especificidad del picudo del botón floral, de origen suramericano, *Anthonomus tenebrosus* Boheman, como agente potencial para control biológico de bola de gato, *Solanum viarum* Dunal en los Estados Unidos. Las pruebas se efectuaron en la cuarentena del Departamento de Agricultura de la Florida. Noventa y una especies de plantas, en 21 familias, fueron incluidas en las pruebas de especificidad de múltiples elección, incluyendo la maleza objetivo y las seis plantas cultivadas pertenecientes a la familia Solanaceae más importantes: chile dulce (*Capsicum annuum* L.), chile (*Capsicum frutescens* L.), tomate (*Lycopersicon esculentum* Mill.), tabaco (*Nicotiana tabacum* L.), berenjena (*Solanum melongena* L.), y papa (*Solanum tuberosum* L.). En cada prueba se utilizaron racimos florales de ocho a diez plantas escogidas al azar incluyendo siempre la planta objetivo las cuales fueron expuestas a 10-20 adultos de *A. tenebrosus* por una a dos semanas. Registros de alimentación y ovoposición fueron realizados dos veces por semana. Pruebas de alimentación/ovoposición sin elección fueron también realizadas usando plantas en floración. Diez adultos fueron expuestos a 29 especies de plantas en forma individual por una a dos semanas. Cada prueba tuvo tres o cuatro repeticiones. Las pruebas mostraron que *A. tenebrosus* se alimentó y colocó posturas solo en bola de gato. Ninguna postura fué depositada en las otras 90 especies de plantas evaluadas. Las pruebas indicaron que la posibilidad de *A. tenebrosus* de llegar a ser una plaga de las Solanaceae cultivadas es muy remota. La solicitud al comité TAG para liberar el picudo en los Estados Unidos fue presentada en octubre 2007.

Tropical soda apple (TSA), *Solanum viarum* Dunal (Solanaceae), is an invasive weed native to southeastern Brazil, northeastern Argentina, Paraguay, and Uruguay that has invaded Florida grasslands and natural ecosystems. In 1988, TSA was first reported in the USA in Glades County, Florida (Coile 1993; Mullahey & Colvin 1993); the introduction pathway is unknown. In 1993, a survey of beef cattle operations in south Florida estimated 157,145 ha of infested pasture land, twice the infestation present in 1992 (Mullahey et al. 1994). The infested area increased to more than

303,000 ha in 1995-96 (Mullahey et al. 1998). Currently, more than 404,000 ha are believed to be infested in Florida (Medal et al. 2010b). Due, at least in part, to favorable environmental conditions, the lack of natural enemies (herbivores and pathogens), and seed dispersal by wildlife and cattle feeding on the fruits. TSA has been spreading rapidly and has been observed in the majority of the counties in Florida and also in Alabama, Georgia, Louisiana, Mississippi, North Carolina, Pennsylvania, South Carolina, Tennessee, Texas, and Puerto Rico (Bryson & Byrd Jr. 1996; Dowler 1996; Mullahey et al. 1993, 1998; Medal et al. 2003, 2010a). Although TSA has been reported in Pennsylvania and Tennessee, it is highly probable that does not overwinter in these states. Patterson (1996) studied the effects of temperatures and photoperiods on TSA in controlled environmental chambers and speculated that the range of TSA could expand northward into the midwestern US. *S. viarum* was placed on the Florida and Federal Noxious Weed Lists in 1995.

TSA typically invades improved pastures, where it reduces livestock carrying capacity. Foliage and stems are unpalatable to cattle; dense stands of the prickly shrub prevent access of cattle to shaded areas, which results in summer heat stress (Mullahey et al. 1998). TSA control costs for Florida ranchers were estimated at $6.5 to 16 million annually (Thomas 2007), and economic losses from cattle heat stress alone were estimated at $2 million (Mullahey et al. 1998). TSA is a reservoir for at least 6 crop viruses (potato leafroll virus, potato virus Y, tomato mosaic virus, tomato mottle virus, tobacco etch virus, and cucumber mosaic virus) and the early blight of potato and tomato fungus, *Alternaria solani* Sorauer (McGovern et al. 1994a, 1994b; McGovern et al. 1996). In addition, major insect pests utilize TSA as an alternate host; including Colorado potato beetle, *Leptinotarsa decemlineata* (*Say);* tomato hornworm *Manduca quinquemaculata* (Haworth); tobacco hornworm, *M. sexta* (L.); tobacco budworm, *Helicoverpa virescens* (Fabricius); tomato pinworm, *Keiferia lycopersicella* (Walsingham); green peach aphid, *Myzuz persicae* (Sulzer); silverleaf whitefly biotype B of *Bemisia tabaci* (Gennadius); soybean looper, *Pseudoplusia includens* (Walker); and the southern green stink bug, *Nezara viridula* (L.) (Habeck et al. 1996; Medal et al. 1999; Sudbrink et al. 2000). TSA also reduces biodiversity in natural areas, ditch banks, and roadsides by displacing native vegetation (Langeland & Burks 1998). TSA interferes with restoration efforts in Florida by invading areas that are reclaimed following phosphate mining operations (Albin 1994).

TSA Management practices in Florida pastures primarily involve herbicide applications and mowing (Sturgis & Colvin 1996; Mislevy et al. 1996, 1997; Akanda et al. 1997). Herbicides or mowing provide temporary weed suppression at an estimated cost of $61 and $47 per ha, respectively (Thomas 2007). However, application of these control methods is not always feasible in rough terrain or inaccessible areas.

In June 1994, the first exploration for TSA natural enemies in South America was conducted by University of Florida and Brazilian researchers (Medal et al. 1996). Sixteen species of insects were found attacking the weed during this 2-week survey. Host specificity tests were initiated in 1997 by J. Medal (University of Florida) in collaboration with the Universidade Estadual Paulista, Jaboticabal campus, Brazil, and the USDA Biological Control Laboratory in Hurlingham, Buenos Aires province, Argentina, and in Stoneville, MS. The South American leaf-feeder *Gratiana boliviana* (Chrysomelidae) was approved for field release in Florida in summer 2003. In total, at least 230,000 beetles have been released in 39 Florida counties since the summer 2003. The beetles established at almost all the release sites in central/south Florida and they are having extensive defoliations and reducing the weed fruit production on TSA plants (Medal & Cuda 2010; Medal et al. 2010a; Overholt et al. 2009, 2010).

A second potential TSA biocontrol agent is the flower-bud weevil *Anthonomus tenebrosus* Boheman (Coleoptera: Curculionidae). This insect was collected on TSA in Rio Grande do Sul, Brazil (29.66465°S, 50.80171°W) by the late Daniel Gandolfo and Julio Medal in April 2000. The identity of *A. tenebrosus* was confirmed by Drs. Wayne Clark (Auburn University, AL) and Germano Rosado Neto (Universidade Federal do Paraná in Curitiba, Brazil). Voucher specimens of *A. tenebrosus* are deposited at Auburn University, Alabama, at the Universidade Federal do Paraná - Curitiba campus, Brazil, and at the Florida State Collection of Arthropods, Division of Plant Industry in Gainesville, Florida. This species does not have a common name in South America. The only known *A. tenebrosus* host plants in South America are *S. viarum* and *S. acculeatisimum*.

The biology of *A. tenebrosus* was studied by Davis (2007) at the quarantine facility in Gainesville, Florida. Eggs are inserted individually into TSA flower-buds, and hatch in 3-5 days. Larvae are cream-colored with a yellowish brown head capsule. They feed on the contents of the flower-bud, and this feeding prevents the flower-bud from opening. There is typically 1 larva, but occasionally 2 larvae in a single flower-bud. As larval feeding progresses, the flower-bud senesces and drop from the plant. Three larval stadia are completed in 7-13 days. The pupal stage is completed in 3-7 days inside the fallen flower bud. Pupae resemble the adult in form; they are cream-colored but darken shortly before eclosion. Emerging adults chew their way out of the flower-bud. De-

velopment from egg to adult stage lasts 11-69 days. Longer developmental times are apparently not associated with seasonal differences as they occurred throughout the year. Adults can live up to 210 days under laboratory conditions. Adult size appears to be related to food abundance during development rather than beetle sex. Copulation has been observed a few hours after adult emergence and throughout the oviposition period. At least 7-8 generations per year can occur under laboratory conditions (temperature 24° ± 3C, relative humidity 50-70%) conditions.

Materials and Methods

Host Specificity Tests

Laboratory host specificity tests with *A. tenebrosus* adults were conducted from May 2000 to January 2003 at the Florida Department of Agriculture and Consumer Services-Division of Plant Industry quarantine facility in Gainesville, Florida. Open field host-specificity tests were conducted at the Universidade Federal do Paraná Agricultural Experiment Station in Paraná state, Brazil from Oct 2005 to Mar 2007. For Florida tests, *A. tenebrosus* adults were collected from TSA plants in Rio Grande do Sul, Brazil and introduced onto caged plants of TSA plants growing in 1-gallon pots to establish a laboratory colony in quarantine.

In this article we report the results of various host-specificity tests with the flower-bud weevil *A. tenebrosus,* to assess its possible use as biological control agent of the non-native weed tropical soda apple.

Multiple-Choice Feeding and Oviposition Tests

Ninety-one plant species in 21 families were included in the feeding oviposition preference tests at the Gainesville quarantine (Table 1). Tested plants included 53 species in the family of the target weed (Solanaceae), 26 of which were from the genus *Solanum* and 27 from 14 other genera that include plants of agricultural or ecological importance. Ten species represented 5 families (Boraginaceae, Convolvulaceae, Ehretiaceae, Nolanaceae, Polemoniaceae) very close related to Solanaceae within the order Polemoniales (Heywood 1993) were also included. Twenty-eight plant species representing 15 families, most of them with economic and/or environment value in North America, were also tested. The target weed (*S. viarum*), and other 9 plant species in Solanaceae were tested at least 3 times (Table 1). These included the natives *Solanum donianum* Walpers, listed as a threatened plant in Florida (Coile 1998), and *S. americanum* Mill, 2 non native-weeds (*S. tampicense* Dunal, *S. torvum* Sw.), and the 5 major cultivated *Solanaceae* (bell pepper, *Capsicum annuum* L., tomato, *Lycopersicon esculentum* Mill., tobacco, *Nicotiana tabacum* L., eggplant, *Solanum melongena* L., and potato, *Solanum tuberosum* L.). Bouquets of leaves and flower-buds of 8 to 10 plant species, always including TSA were simultaneously exposed to 10-20 *A. tenebrosus* adults (approximately 50% males and 50% females) in clear plastic round containers (26 cm diameter by 9 cm height, with four 4-7 cm diameter vents drilled along the sides of the container to allow for air circulation). At the beginning of each test, the insects were placed at the bottom center of each container to allow them to choose any tested plants. Plant species in each test were replicated 3-4 times (1 replication of tested plants in each separate container). Bouquets were exposed to *A. tenebrosus* adults for 1 to 2 weeks. Observations of oviposition and feeding were made twice a week, and consumed bouquets were replaced as needed. Flower-buds were checked for oviposition and eggs were counted weekly. On the last day of each experiment, flower-buds were scored for feeding damage, and eggs laid on them were counted. Leaf and flower bud area consumed was visually estimated using a scale from 0 to 5 (0 = no feeding, 1 = probing or <5% of area consumed, 2= light feeding or 5-20% of the area, 3 = moderate feeding or 21-40%, 4 = heavy feeding or 41-60%, and 5 = intense feeding or >60% of the area consumed).

No-Choice Adult Feeding and Oviposition Tests

No-choice host specificity tests were also conducted with *A. tenebrosus* adults at the Gainesville-quarantine facility using potted plants (20-60 cm height) in cages. Cages were made of clear-plastic cylinders (15 cm diam, 50-60 cm height), with a mesh screen at the top and covering 6 circular holes (6 cm diam) located in pairs at the bottom, middle, and upper part of the cylinder to allow for air circulation. *A. tenebrosus* adults were exposed to 29 plant species in 3 families, including the native *S. donianum*, and all major cultivated Solanaceae (Table 2). Five to 7 plant species with flower-buds were individually tested each time due to limited cage numbers. Plants were exposed to 10 *A. tenebrosus* adults (5 males, 5 females) for 1 to 2 weeks; each test plant was replicated 3 or 4 times. Adults were F_2 or F_3 progeny from adults originally collected in southern Brazil and reared in quarantine on TSA. Adults had either recently eclosed from pupae or were still young less than 1 week old. Plants were replaced as needed. At the end of the testing periods, feeding and oviposition were recorded.

First Field Experiment in Brazil

A multiple-choice, open field experiment was conducted at the Universidade Federal do Paraná, Agriculture Experimental Farm 'Canguiri'. *A.*

TABLE 1. *ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Category 1. Genetic types of the target weed species found in North America					
SOLANACEAE					
Tribe Solaneae					
Genus *Solanum*					
Subgenus *Leptostemonum*					
Section Acantophora					
Solanum viarum Dunal	Tropical soda apple	40	430	3-5	5-9
Category 2. Species in the same genus as the target weed, divided by subgenera (if applicable).					
Tribe Solaneae					
Genus *Solanum*					
Subgenus *Leptostemonum*					
Section Acantophora					
Solanum capsicoides All.	Red soda apple	12	160	0	0
Section Lasiocarpum					
Solanum quitoense Lam.	Naranjilla	4	40	0	0
Solanum pseudolulo Heise	Falso lulo	4	40	0	0
Solanum sessiliflorum Dunal	Cocona nightshade	4	40	0	0
Section Micracantha					
Solanum jamaicense Mill.	Jamaican nightshade	3	30	0	0
Solanum tampicense Dunal	Wetland nightshade	10	110	1	0
Section Melongena					
Subsection Lathyrocarpum					
Solanum carolinense L.	Horse nettle*	4	40	0	0
Solanum dimidiatum Raf.	Western horsenettle*	3	30	0	0
Solanum elaeagnifolium Cav.	Silverleaf nightshade*	4	40	0	0
Subsection Melongena					
Solanum melongena L.	Eggplant				
Cv. 'Black Beauty'		7	70	0	0
Cv. 'Classic'		4	40	0	0
Cv. 'Market'		7	70	0	0
Cv. 'Asian Long Purple'		3	35	0-1	0
Section Persicariae					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. *(CONTINUED) ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Subgenus Leptostemonum					
Solanum bahamense	Bahama nightshade	7	75	0	0
Section Torva					
Solanum torvum Sw.	Turkey berry	12	140	0	0
Solanum verbascifolium L.	Mullein nightshade*	3	30	0	0
Subgenus *Solanum*					
Solanum americanum Mill.	American nightshade*	10	100	0	0
Solanum diphyllum L.	2-leaf nightshade*	3	30	0	0
Solanum erianthum Don.	Potato tree*	3	30	0	0
Solanum jasminoides Paxt.	White potato vine	7	75	1	0
Solanum mauritianum Scop.	Earleaf nightshade	4	40	0	0
Solanum nigrescesns Mart. & Gal	Divine nightshade*	3	30	0	0
Solanum nigrum L.	Black nightshade*	4	50	0	0
Solanum pumillum Dunal	Rock outcrop Solanum*	3	30	0	0
Solanum seaforthianum Scop.	Brazilian nightshade	3	30	0	0
Solanum tuberosum	L. Potato	8	95	0	0
Category 3. Species in other genera in the same family as the target weed, divided by subfamily (if applicable).					
Genus *Acnistus*					
Acnistus australe (Griseb.) Griseb.	Acnistus	3	30	0	0
Genus *Iochroma*					
Iochroma sp.	Iochroma	3	30	0	0
Genus *Physalis*					
Physalis angulata L.	Cutleaf groundcherry	3	30	0	0
Physalis arenicola Kearney	Cypresshead*	3	30	0	0
Physalis crassifolia Benth	Yellow groundcherry*	3	30	0	0
Physalis gigantea L.	Strawberry groundcherry	3	30	0	0
Physalis ixocarpa Brot.	Tomatillo	3	30	0	0
Physalis pubescens L	Husk tomato*	3	30	0	0
Physalis walteri Nutt.	Walter's groundcherry*	3	30	0	0
Tribe Daturae					
Genus *Brugmansia*					
Brugmansia sanguinea (Ruiz & Pav.) Don	Angel's trumpet	3	30	0	0
Genus *Datura*					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. *(CONTINUED) ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Datura discolor Bernh	Desert thorn-apple*	3	30	0	0
Datura metel L.	Devil's trumpet	3	30	0	0
Datura meteloides D.	Devil's weed*	3	30	0	0
Datura stramonium L.	Jimson weed*	3	30	0	0
Tribe Lycieae					
Genus *Lycium*					
Lycium carolinianum Walter	Carolina desert-thorn*	3	30	0	0
Lycium fremontii Gray.	Fremont desert thorn*	3	30	0	0
Genus *Lycopersicon*					
Lycopersicon esculentum Mill.	Tomato	12	130	0	0
Tribe: Nicandreae					
Genus: *Nicandra*					
Nicandra physaloides (L.) Gaertn.	Apple of Peru	3	30	0	0
Tribe Nicotianae					
Genus *Nicotiana*					
Nicotiana tabacum L.	Tobacco	8	100	0	0
Nicotiana rustica L.	Aztec tobacco	7	75	0	0
Nicotiana sylvestris Speg. & Comes	Woodland tobacco	3	30	0	0
Genus *Nierembergia*					
Nierembergia scoparia Sendtri	Broom cupflower	3	30	0	0
Tribe Salpiglossidae					
Genus *Salpiglossis*					
Salpiglossis sinuata Ruiz & Pav	Painted tongue	3	30	0	0
Genus *Schizanthus*					
Schizanthus spp.	Butterfly flower	3	30	0	0
Tribe Solandeae					
Genus *Solandra*					
Solandra glandiflora Swartz	Showy chalicevine	3	30	0	0
Category 4. Threatened and endangered species in the same family as the target weed divided by subgenus, genus, and subfamily.					
Section Torva					
Solanum donianum Walpers	Mullein nightshade*	9	90	0	0
Category 5. Species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to the target weed.					
BORAGINACEAE					

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. *(CONTINUED) ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Heliotrope sp.	Heliotrope	3	30	0	0
Myosotis alpestris Schmidt	Forget-Me-Not*	3	30	0	0
Convolvulus purpurea L.	Morning-glory*	3	30	0	0
Ipomoea batata (L.) Lam.	Sweet-potato	3	30	0	0
Evolvulus muttallianus	Shaggy dwarf morning-glory*	3	30	0	0
EHRETIACEAE					
Cordia sebestena L.	Largeleaf geigertree*	3	30	0	0
NOLANACEAE					
Nolana paradoxa Lindl.	Chilean bellflower	3	30	0	0
POLEMONIACEAE					
Cobaea scandens Cav.	Catedral bells	3	30	0	0
Gilia tricolor Benth	Bird's-eye gilia*	3	30	0	0
Phlox panuculata L.	Fall phlox*	3	30	0	0
Category 6. Species in other orders that have some morphological or biochemical similarities to the target weed or that share the same habitat.					
ANACARDIACEAE					
Mangifera indica L.	Mango	3	30	0	0
Pistacia vera L.	Cultivated pistachio	3	30	0	0
APIACEAE					
Daucus carota L.	Carrot	3	30	0	0
ASTERACEAE					
Helianthus annuus L.	Common sunflower*	3	30	0	0
Lactuca sativa L.	Lettuce	3	30	0	0
CAMPANULACEAE					
Campanula persicifolia L	Peachleaf bellflower*	3	30	0	0
CRUCIFERAE					
Brassica oleracea L. var. botrytis	Broccoli	3	30	0	0
CUCURBITACEAE					
Citrullus lanatus (Thumb)	Watermelon	3	30	0	0
Cucurbita sativus L.	Cucumber*	3	30	0	0
ERICACEAE					
Vaccinium ashei Rende	Rabbiteye blueberry*	3	30	0	0
FABACEAE					
Glycine max (L.) Merrill	Soybean	3	30	0	0

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 1. *(CONTINUED) ANTHONOMUS TENEBROSUS* ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE MULTIPLE-CHOICE TESTS.

Plant Family Species	Common Names *indicates native species	No. of Plants	No. of Insects	Feeding Score[1]	Eggs Laid per Female
Phaseolus vulgaris L.	Kidney bean	3	30	0	0
LOBELIACEAE					
Lobelia cardinalis L.	Cardinalflower*	3	30	0	0
LOGANIACEAE					
Buddleia davidii Franch	Butterfly bush	3	30	0	0
POACEAE					
Oryza sativa L.	Rice	3	30	0	0
Saccharum officinarum L.	Sugarcane	3	30	0	0
Zea mays L.	Corn*	3	30	0	0
ROSACEAE					
Fragariax ananassa Duchesne	Garden strawberry	3	30	0	0
Malus pumilla Mill.	Paradise apple*	3	30	0	0
Rosa sp.	Miniature rose	3	30	0	0
Rubus betulifolius Small	Blackberry*	3	30	0	0
RUTACEAE					
Citrus sinensis (L.) Osbeck	Sweet orange	3	30	0	0
Citrus limon (L.) Burm.	Lemon	3	30	0	0
Citrus paradise Mcfady	Grapefruit	3	30	0	0
Murraya paniculata (L.) Jacq.	Orange Jasmine	6	60	0	0
SCROPHULARIACEAE					
Antirrhinum majus L.	Garden snapdragon	3	30	0	0
Nemensia strumosa Benth	Capejewels	3	30	0	0
Category 7. Any plant on which close relatives of the biological control agent (within the same genus) have been found or recorded to feed/ or reproduce.					
MALVACEAE					
Gossypium hirsutum L.	Cotton	10	100	0	0
SOLANACEAE					
Genus *Capsicum*					
Capsicum annuum L.	Bell pepper	8	80	0	0
Capsicum frutescens L.	Chili pepper	4	40	0	0
Genus Solanum					
Solanum sisymbriifolium Lam.	Sticky nightshade	3	30	1	0

*0 = No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Solanaceous taxonomic categories were taken from the Radboud University of Nijmegen, Netherland website (www.bagard.sci.kun.nl). Most of the plant common names are from: http:/www.plants.usda.gov.

TABLE 2. ANTHONOMUS TENEBROSUS ADULT FEEDING AND OVIPOSITION ON SELECTED PLANTS IN QUARANTINE NO-CHOICE TESTS.

Plant family Species	Common names (*indicates native Species)	No. of Plants	No. of Insects	Feeding Score*	Eggs/female
SOLANACEAE					
Capsicum annuum	Bell pepper	9	90	0	0
Capsicum frutescens	Chili pepper	7	70	0	0
Lycopersicon esculentum	Tomato	9	90	0	0
Nicotiana tabacum	Tobacco	7	70	0	0
Nierembergia scoparia	Broom cupflower	3	30	0	0
Physalis crassifolia	Yellow groundcherry*	3	30	0	0
Solanum americanum	American nightshade*	3	30	0	0
Solanum capsicoides	Red soda apple	3	30	0	0
Solanum carolinense	Horse nettle*	3	30	0	0
Solanum citrullifolium	Watermelon nightshade*	3	30	0	0
Solanum dimidiatum	Western horsenettle*	3	30	0	0
Solanum diphillum	2-leaf nightshade	3	30	0	0
Solanum donianum	Mullein nightshade	7	70	0	0
Solanum elaeagnifolium	Silverleaf nightshade*	3	30	1	0
Solanum heterodoxum	Melonleaf nightshade*	3	30	0	0
Solanum jamaicense	Jamaican nightshade	3	30	0	0
Solanum jasminoides	White potato vine*	3	30	0	0
Solanum melongena	Eggplant				
cv. Black Beauty		3	30	0	0
cv. Classic		3	30	0	0
cv. Market		3	30	0	0
cv. Asian Long Purple		9	90	0	0
Solanum nigrescens	Divine nightshade*	3	30	0	0
Solanum pumilum	Rock-outcrop Solanum*	3	30	0	0
Solanum ptycanthum	Wonder berry*	3	30	0	0
Solanum retroflexum	Sunberry*	3	30	0	0
Solanum scabrum	Garden huckleberry*	3	30	0	0
Solanum tampicense	Wetland nightshade	7	70	1	0
Solanum torvum	Turkeyberry	7	70	1	0
Solanum tuberosum	Potato	9	90	0	0
Solanum viarum	Tropical soda apple	9	90	3-5	4-11
MALVACEAE					
Gossypium hirsutum L.	Cotton	9	90	0	0
CONVOLVULACEAE					
Ipomoea batata (L.) Lam.	Sweet-potato	9	90	0	0

*0=No feeding, 1 = Probing (<5% of flower bud/leaf area), 2 = Light (5-20%), 3 = Moderate (21-40%), 4 = Heavy (41-60%), 5 = Intense (>60% area). Most of the plant common names are from: http:/www.plants.usda.gov.

tenebrosus adults were collected in Rio Grande do Sul state in Dec 2005. These insects were reared in screened cages (0.6 × 0.6 × 0.9 m) at the Neotropical Biological Control Laboratory in Curitiba on TSA plants growing in 1-2 gallon pots to provide progeny weevils for the experiment. One hundred *A. tenebrosus* adults recently emerged from pupae were released in the field (30 × 20m^2) with 5 plant species (TSA, eggplant cv. 'Black Beauty', bell-pepper, potato, and tomato). Seven plants of each species tested (35 plants/plot, 1m between plants, 35m^2/plot, 4 plots, 10m between plots) were randomly assigned in each of the experimental plots following a Complete Block Randomized Experimental Design with 4 replications. Test plants (n = 140) were transplanted in Oct 2005, and insects were released when plants were flowering during the last week of Dec 2005 on the ground approximately 1m from any plant. All plants were thoroughly examined weekly from 22 Dec 2005 to 31 Mar 2006, and number of adults, feeding, and number of egg on the plants were recorded.

Second Field Experiment in Brazil

Another multiple-choice, open field experiment exposing *A. tenebrosus* adults to flowering eggplant cv.' Black Beauty', tomato, potato, and

bell pepper, but not TSA, was conducted at the Universidade Federal do Paraná, Agriculture Experimental Farm 'Canguiri', Brazil from Dec 2006 to Mar 2007. Control plots with flowering TSA plants alone, were also established at the Neotropical Biological Control Laboratory in Curitiba located approximately 45 km from the cultivated crop plots to prevent plant species interference. Distance between plants was similar to the first experiment. Field collected *A. tenebrosus* weevils were released into crop and TSA plots (80 and 76 adults, respectively). Beetles were randomly released in groups (6-10) on the ground but not on any test plant. Evaluations (visual estimation of number of insects and feeding) were made weekly checking thoroughly each of the plants tested.

Results and Discussion

Multiple-Choice Feeding-Oviposition Tests

In the quarantine multiple-choice tests, *A. tenebrosus* adults fed moderately to intensively (>20% of the area offered) on *S. viarum,* the target weed (Table 1). Weevils did some probing or exploratory feeding (<5% of the area offered) on *S. tampicense* Dunal (an exotic weed of Mexico-Central America-Caribbean origin and established and expanding in central-south Florida), on *S. sisymbriifolium* Lam., and *S. jasminoides* Paxt. (weeds of South-American origin also present in Florida), and on eggplant cv. 'Asian Long Purple' (crop of economic importance). No feeding was observed on any of the other 86 plant species in 21 families that were tested. *A. tenebrosus* adults lay from 5 to 9 eggs inside TSA flower-buds during the 1-2 week period of the test (Table 1). No eggs were deposited on any of the other 90 plant species tested, including the threatened *S. donianum*. Although minor *A. tenebrosus* feeding occurred on eggplant in quarantine, this insect has never been recorded attacking eggplant in South America. Expanded host ranges of weed-biocontrol insects under confined laboratory conditions have been reported by South African researchers (Neser et al. 1988; Hill & Hulley 1995; Olckers et al. 1995; Hill & Hulley 1996; Olckers 1996). They indicated that almost all agents tested for biocontrol of *Solanum* weeds have fed on closely related plant species that are never attacked under natural conditions. For example, *Gratiana spadicea* (Klug) (Coleoptera: Chrysomelidae) screened as a potential biocontrol agent of *S. sisymbrifolium* in South-Africa (Hill & Hulley 1995), fed and completed development on eggplant in the laboratory. In 1994, this insect was released in South-Africa based mainly on the lack of records as an eggplant pest in South America. *Gratiana spadicea* is established on *S. sisymbriifolium* with no reports of attacks in South African eggplant fields. Multiple choice tests conducted at the USDA-South American Biological Control Laboratory in Hurlingham, Argentina with *Anthonomus sisymbrii* Hustache by late Daniel Gandolfo, showed this weevil fed and lay eggs on eggplant and potato, although the number of eggs lay on these crops was significantly lower than on TSA. He also reported that 75% of the eggs on potato and 25% on eggplant were abnormally oviposited outside the flower-buds. Gandolfo indicated that *A. sisymbrii* could use eggplant, and possibly other *Solanum*, at least for feeding purposes that may result in an economic impact (Gandolfo et al. 2004). The only known natural hosts of *A. sisymbrii* are *S. sisymbrifolium*, *S. viarum*, and *S. aculeatissimum* (Medal unpublished data). To corroborate the specificity and safety of *A. tenebrosus*, a weevil related to *A. sisymbrii,* 2 open field experiments (discussed later) were conducted in Brazil, which indicated that *A. tenebrosus* did not represent a threat to eggplant and other economic crops tested under natural conditions and it is safe as a biological control agent of TSA.

No-Choice Adult Feeding Tests

Starvation (no-choice) tests with *A. tenebrosus* adults exposed to individual potted plants (29 species in 3 families) in quarantine cages indicated that this insect fed and laid eggs (range: 4-11; average, 8 eggs per female) only on TSA (Table 2). Feeding on TSA was moderate to intense (>21% of the area offered) compared to a probing or exploratory feeding (<5%) observed on *S. elaeagnifolium*, *S. tampicense*, and on *S. torvum*. No eggs were laid on any of the 28 non-target plant species tested including 4 eggplant cultivars ('Black Beauty', 'Classic', 'Market', 'Asian Long Purple').

First Field Experiments in Brazil

In the open-field planted with TSA, bell-pepper, tomato, potato, and eggplant, *A. tenebrosus* adults (100) fed and laid eggs, and larvae developed only on TSA. A total of 83 eggs, 21 larvae, and 51 adults of *A. tenebrosus* were recorded on TSA plants. Feeding on TSA flower-buds was moderate to heavy (21 to 50% of the area). No feeding was observed on any of the Solanaceous crops tested. This field test confirms that *A. tenebrosus* feeds and develops only on TSA and does not represent a threat to eggplant, tomato, potato, or bell-pepper.

Second Field Experiment in Brazil

The field test exposing A. *tenebrosus* adults to eggplant, tomato, potato, and bell-pepper, showed that no adults or immature stages were found on these crops tested when TSA plants were not

present. In a separate plot, *A. tenebrosus* feeding on TSA flower buds was moderate (21-30%), contrary to no-feeding on the crops tested. A total of 124 eggs, 72 larvae, and 45 adults of *A. tenebrosus* were recorded on TSA plants. This test showed that *A. tenebrosus* adults fed and laid eggs, and larvae developed only on TSA, with no utilization of eggplant, potato, tomato, and bell pepper when TSA is not present.

The laboratory and open-field experiments indicated that no *A. tenebrosus* feeding damage and reproduction on the native solanaceous plants and crops tested are likely to occur. It is expected that this weevil will complement the TSA damage by *G. boliviana* in south and central Florida.

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EFFECT OF ORGANIC MULCHES ON SOIL SURFACE INSECTS AND OTHER ARTHROPODS

HARSIMRAN K. GILL[1], ROBERT MCSORLEY[1] AND MARC BRANHAM[1]
[1]Entomology and Nematology Department, University of Florida, Gainesville, Florida 32611-0620, USA

ABSTRACT

Four different types of organic mulches were evaluated for their effects on soil surface insects and related arthropods. Field experiments were conducted in fall 2007 and 2008 near Citra, Florida. In both the years, five treatments were compared: cowpea (*Vigna unguiculata* (L.) Walp.) mulch, sunn hemp (*Crotalaria juncea* L.) mulch, sorghum-sudangrass (*Sorghum bicolor* Moench × *S. sudanense* ((Piper)] Stapf) mulch, pine bark nuggets, and unmulched control. Data were collected on insects and other arthropods using pitfall traps. Results indicate that organic mulches can affect a wide range of different insects. Diptera, dominated by *Asyndetus* spp. (Dolichopodidae), were most dense in pine bark plots in both years. Populations of small plant-feeding insects such as Aphididae, Thripidae, and Aleyrodidae were most dense in cowpea and unmulched control plots in one season. It is possible that these insects were affected by weed growth in cowpea and control plots. Ants, which tend or feed on small plant feeders, were fairly abundant in these plots as well, as were predatory beetles. Some groups, such as Collembola (mainly Isotomidae), spiders, and Orthoptera (Acrididae and Gryllidae) were unaffected by mulches.

Key Words: cover crop residue, organic mulch, insect community, pine bark

RESUMEN

Se evaluaron cuatro diferentes tipos de coberturas orgánicas por sus efectos sobre los insectos y artrópodos relacionados de la superficie del suelo. Se realizaron los experimentos de campo en el otoño del 2007 y 2008 cerca de Citra, Florida. En ambos años, se compararon cinco tratamientos: el mantillo de caupí (*Vigna unguiculata* (L.) Walp.), el mantillo de cáñamo sunn (*Crotalaria juncea* L.), el mantillo de sorgo-pasto de Sudán (*Sorghum bicolor* Moench * *S. sudanense* (Piper) Stapf), pedazos de la corteza de pino, y sin cobertura (control). Se utilizaron trampas de caída para obtener los datos de los insectos y de los otros artrópodos. Los resultados indican que el mantillo orgánico puede afectar a una amplia gama de diferentes insectos. Los Diptera, dominado por las especies de *Asyndetus* (Dolichopodidae), fueron más densas en parcelas de corteza de pino en ambos años. Las poblaciones de insectos que se alimentan de plantas pequeñas, tales como Aphididae, Thripidae y Aleyrodidae eran más densas en caupí y parcelas sin cobertura (control) en una temporada. Es posible que estos insectos fueron afectados por el crecimiento de malezas en las parcelas de caupí y del control. Las hormigas, que atienden o se alimentan de insectos que se alimentan de plantas pequeñas, fueron bastante abundantes en estas parcelas, al igual que los escarabajos depredadores. Algunos grupos, como los colémbolos (principalmente Isotomidae), arañas, y ortópteros (Acrididae y Gryllidae) no fueron afectados por las coberturas.

Use of cover crop residues as organic mulches has a number of advantages to farming systems such as reducing soil erosion, conserving soil moisture, moderating soil temperature, improving infiltration of water, and providing a slow-release source of nutrients (Gruda 2008; Hatwig & Ammon 2002; Hatwig & Hoffman 1975; Powers & McSorley 2000; Snapp et al. 2005; Westerman & Bicudo 2005). Plant mulches can be an effective way to provide shelter for predatory insects (Johnson et al. 2004) and to control weeds (Reeleder et al. 2004; Teasdale et al. 2004). Mulches can help to maintain soil moisture required for plant vigor and to promote plant tolerance to the attack of insect pests (Johnson et al. 2004).

Cover crops and intercrops have been used as living mulches for managing some insect pests. Alfalfa (*Medicago sativa* L.) and kura clover (*Trifolium ambiguum* M. Bieb.) mulches increased predator populations to manage European corn borer (*Ostrinia nubilalis* Hübner) (Prasifka et al. 2006). Eggs and larval densities of pest caterpillars were higher in broccoli (*Brassica oleracea* L. var. *botrytis*) monoculture when compared to broccoli with undersown mulches like strawberry clover (*Tribolium fragiferum* L.), white clover (*Tribolium repens* L.), and yellow sweet clover (*Melilotus officinalis* L.) (Hooks & Johnson 2004). Alfalfa living mulch increased predators to manage outbreaks of the invasive soybean aphid, *Aphis glycines* Matsumura (Schmidt et al. 2007).

While these examples suggest that living mulches may offer resources to support preda-

tors, non-living mulches derived from killed cover crops, hay from cover crops, or composted waste products may offer benefits as well. In sweetpotato (*Ipomoea batatas* (L.) Lam.), higher numbers of fire ants, rove beetles, and carabid beetles were captured using pitfall traps in plots covered with killed-cover crop (Jackson & Harrison 2008). Also, the injury level from soil insect pests to roots of sweetpotato was lower in killed-cover crop plots than in conventional plots. In an apple (*Malus domestica* Borkh.) orchard, the dominance of several carabid species depended on different factors including sampling dates and different types of ground cover including plastic mulch and straw mulch (Miñarro & Dapena 2003). Predation of beet armyworm, *Spodoptera exigua* (Hübner), pupae was 33% greater in cover crop mulch as compared with conventional production plots (Pullaro et al. 2006). Mulch from sunn hemp (*Crotalaria juncea* L.) hay was effective in reducing incidence of lesser cornstalk borer, *Elasmopalpus lignosellus* (Zeller) on bean (*Phaseolus vulgaris* L.) (Gill et al. 2010).

Changes in cropping systems affect insect pests and their natural enemies (Hummel et al. 2002). Organic mulches might provide hiding places to harbor populations of natural enemies. Different types of cover crops harbor distinctive complexes of beneficial insects, pest arthropods, and their diverse trophic relationships (Bugg & Waddington 1994). Many previous studies that used mulches for the management of insect pests focused especially on flying insects moving into mulched areas (Brown & Tworkoski 2004; Gill et al. 2010; Hooks & Johnson 2004; Prasifka et al. 2006; Pullaro et al. 2006; Reeleder et al. 2004; Schmidt et al. 2007; Tremelling et al. 2002). The effects of mulches on insects and other soil arthropods living on the soil surface is a relatively less explored area.

More information is needed on arthropods that are active on the soil surface where the mulches occur, and how different materials on the soil surface affect these arthropods. To answer these questions, the present study was designed with main objective to determine the impact of mulches on the community of arthropods that live and move on the soil surface. The purpose was to obtain an overview of various arthropod groups that were active on the soil surface, rather than focusing on selected key species.

Materials and Methods

Field experiments were conducted in fall 2007 and 2008 at the University of Florida Plant Science Research and Education Unit (29°24'N, 82°9'W), Citra, Florida. The soil at the experimental site was Arredondo sand (95% sand, 2% silt, 3% clay) with 1.5% organic matter (Thomas et al. 1979).

Fall 2007

The experimental field was sprayed with glyphosate (Roundup®, Monsanto, St. Louis, Missouri) to kill weeds on Sep 26 followed by rototilling on Oct 3. Average soil moisture measured gravimetrically before planting was 6.1%. Five treatments compared were: cowpea (*Vigna unguiculata* (L.) Walp.) mulch; sunn hemp mulch; sorghum-sudangrass (*Sorghum bicolor* Moench × *S. sudanense* (Piper) Stapf) mulch; pine bark nuggets as mulch (HTC Hood Timber Co., Adel, GA); and unmulched control. Treatments were arranged in a randomized complete block design with five replications (total of 25 plots). Individual plots for each treatment were 3.0 m long and 2.4 m wide and the distance between plots was 3.0 m. All plots were planted with 'Roma II' bush beans (*Phaseolus vulgaris* L.) on Oct 4. Seeds were spaced 10 cm apart at a rate of 30 seeds per row, in two rows per plot.

The mulches used were readily available or easily supplied by cover crop residues. Cover crop mulches were obtained from crops of 'Iron and Clay' cowpea, 'Tropic Sun' sunn hemp, and 'Growers Choice' sorghum-sudangrass planted in early Jul. Mulches were obtained from these cover crops (prior to flowering) planted near the experimental site. To obtain mulches, these cover crops were harvested on Oct 11 by clipping plants at the base, removing above-ground biomass, and applying it to the plots The resulting mulches (3-5 cm deep) were a composite of leaves and stems and were applied by hand over the entire plot, next to the rows of bean plants. Therefore, except for the plant rows, the entire plot was covered with mulch. Mulches were applied only once at the start of experiment on Oct 11, using the following amounts of material: cowpea (18.1 kg fresh wt/ plot), sunn hemp (15.9 kg fresh wt/ plot), and sorghum-sudangrass (17.7 kg fresh wt/plot). The pine bark nuggets (29.8 kg fresh wt/plot) were not obtained from cover crops, but were purchased locally. Plots were irrigated as needed using drip irrigation, and no insecticides were applied during the course of the experiment.

Fall 2008

The experiment was repeated at the same site in the fall 2008, with the same treatments. Experimental procedures remained the same with a few minor changes. The experimental field was sprayed with glyphosate to kill weeds in the first week of Sep followed by rototilling on Sep 16. Average soil moisture measured gravimetrically at planting was 6.9%. Beans were planted on Oct 7. Cowpea (12.7 kg fresh wt/plot), sunn hemp (15.9 kg fresh wt/plot), sorghum-sudangrass (13.6 kg fresh wt/plot), and pine bark nuggets (29.8 kg fresh wt/plot) were applied on Oct 9. Early frost in

each season caused severe damage to the bean plants, so that crop harvests were not possible.

Data collection

Insects were collected on several sampling dates in both seasons (Oct 24, Nov 6, Nov 20, Dec 3, and Dec 17 in 2007; Oct 13, Oct 28, Nov 9, and Nov 24 in 2008). Pitfall traps were used for capturing insects that run or move on the soil surface (Borror et al. 1989). A plastic sandwich container (14 cm × 14 cm × 4 cm) was used as a pitfall trap. One pitfall trap was placed in the middle of each plot, and buried so that the upper edge was flush with the soil surface. The traps were filled three quarters with water, along with 3 to 4 drops of dish detergent (Ultra Joy®, Procter and Gamble, Cincinnati, Ohio) to break surface tension, ensuring that the insects would remain in the trap. Pitfall traps were set out in the morning (9:00 am) and collected at approximately the same time (9:00 am) the next day (which was recorded as the sampling date). The traps were brought to the laboratory, kept in a cold room at 10°C, and contents transferred and stored in 70% ethanol in vials. Insects and related arthropods were identified to order and family levels using a dissecting microscope.

Data analysis

All statistical analyses were performed using the Statistical Analysis System (SAS) package (version 9.1; SAS Institute, Cary, North Carolina). Data for each dependent variable (insect groups) were analyzed across all sampling dates in each year using repeated measures (PROC MIXED procedure of SAS) to examine the effects of treatment, sampling date, and interactions between treatments and sampling dates. Since no interactions were found, data were pooled across sampling dates for calculations of means and standard errors of the means. When treatment effects were significant ($P \leq 0.05$), least square means (LS) values were computed to compare means of mulch treatments.

Results

Fall 2007

Diptera were affected ($P \leq 0.05$) by mulches, and were more common in pine bark mulch than in sunn hemp and sorghum-sudangrass (Table 1). Diptera consisted mainly of Dolichopodidae (43.9%, *Asyndetus* spp.) followed by Mycetophilidae (fungus gnats) and other micro-dipterans (37.1%) and other Diptera (19.0%). Cicadellidae and small plant-feeding insects were not significantly ($P \leq 0.05$) affected by treatment, but Cicadellidae showed some an interesting trend ($P \leq$

TABLE 1. EFFECT OF MULCH TREATMENTS ON ARTHROPOD TAXA (NUMBERS/PITFALL TRAP) THROUGHOUT THE SEASON IN CITRA, FLORIDA, 2007.

Treat[1]	Hymenoptera[2]	Collembola[2]	Homoptera[2]	Diptera[2]	Orthoptera[2]	Araneae	Coleoptera[2]	Others[2]
CP	6.48 ± 1.51 a	58.52 ± 8.76 a	1.48 ± 0.29 a	5.56 ± 0.71 ab	1.28 ± 0.37 a	0.52 ± 0.13 a	2.16 ± 0.50 a	1.64 ± 0.34 a
SH	5.08 ± 1.42 a	138.20 ± 42.39 a	1.04 ± 0.30 a	4.12 ± 0.67 b	0.60 ± 0.16 a	0.76 ± 0.18 a	1.48 ± 0.32 a	0.72 ± 0.20 a
SO	6.96 ± 3.22 a	70.00 ± 11.53 a	1.32 ± 0.30 a	4.32 ± 0.68 b	0.84 ± 0.21 a	0.68 ± 0.15 a	1.80 ± 0.33 a	0.92 ± 0.36 a
PB	3.00 ± 0.64 a	84.36 ± 30.78 a	0.88 ± 0.22 a	7.32 ± 0.99 a	1.24 ± 0.25 a	1.08 ± 0.57 a	0.96 ± 0.20 a	1.04 ± 0.26 a
C	2.52 ± 0.51 a	86.80 ± 27.82 a	2.80 ± 1.03 a	5.28 ± 0.86 ab	1.00 ±0.45 a	0.48 ± 0.13 a	1.52 ± 0.37 a	1.44 ± 0.35 a
P > F	0.2638	0.4128	0.0899	0.0111	0.4928	0.5136	0.1918	0.2272
F value	1.42	1.04	2.34	4.32	0.88	0.84	1.69	1.55

[1]Treatments CP = cowpea, SH = sunn hemp, SO = sorghum-sudangrass, PB = pine bark, C = unmulched control

[2]Hymenoptera = Formicidae; Collembola = Isotomidae and Sminthuridae; Homoptera = Cicadellidae; Diptera = Dolichopodidae, Mycetophilidae and micro-dipterans; Orthoptera = Acrididae and Gryllidae; Coleoptera = Staphylinidae, Carabidae, Elateridae, and Chrysomelidae; others = Aphididae, Aleyrodidae, and Thripidae

Data are means ± standard error of 25 replications (data pooled across 5 sampling dates). Means in columns for each sampling date followed by the same letters do not differ significantly based on least square means ($P \leq 0.05$).

0.10) toward greater abundance in unmulched control plots. Small plant-feeding insects consisted of aphids (72.7%, Aphididae), whiteflies (24.3%, Aleyrodidae), and thrips (3.0%, Thripidae). The numbers of Formicidae (mixture of *Pheidole* spp., and *Dorymyrmex* spp.), Collembola (Isotomidae with a few Sminthuridae), Orthoptera (mixture of *Melanoplus* spp., *Dichromorpha* spp., and *Gryllus* spp.), Araneae, and Coleoptera (Staphylinidae, Carabidae, Elateridae, and Chrysomelidae) did not differ among treatments (Table 1). In addition, the few micro-Hymenoptera (mainly small parasitoid wasps) collected were also not affected by treatments (data not shown). Beetles collected were from the families Staphylinidae (23.4%), Carabidae (12.2%, *Anisodactylus* spp.), Elateridae (14.2%, *Conoderus* spp.), and Chrysomelidae (48.4%, *Altica* spp.), but none of these individual families were significantly ($P \leq 0.05$) affected by treatments. A few specimens of other plant-feeding insects were occasionally recovered at low levels in pitfall traps, including cutworms (Noctuidae), plant hoppers (Fulgoridae), spittlebugs (Cercopidae), and stink bugs (Pentatomidae), but none were affected by treatments ($P \geq 0.10$).

Fall 2008

In this season, Diptera were more common in pink bark mulch than in sunn hemp and unmulched control plots (Table 2). Diptera consisted mainly of Dolichopodidae (81.1%) followed by fungus gnats (Mycetophilidae) and other micro-dipterans (3.7%) and other Diptera (15.2%). Formicidae were affected by mulches, and were greatest in cowpea plots. Total numbers of beetles were greater in unmulched control and cowpea than in sorghum-sudangrass. Beetles collected were from the families Staphylinidae (71.7%), Carabidae (21.6%), Elateridae (3.2%), and Chrysomelidae (3.4%), but none of these individual families were significantly ($P \leq 0.05$) affected by treatments. Small plant-feeding insects were most abundant in cowpea and unmulched control plots. Small plant-feeding insects consisted of aphids (73.0%, Aphididae), whiteflies (26.0%, Aleyrodidae), and thrips (0.9%, Thripidae). The numbers of Cicadellidae, Araneae, Collembola (mostly Isotomidae), and Orthoptera (Acrididae and Gryllidae) collected did not differ among treatments (Table 2).

TABLE 2. EFFECT OF MULCH TREATMENTS ON ARTHROPOD TAXA (NUMBERS/PITFALL TRAP) THROUGHOUT THE SEASON IN CITRA, FLORIDA, 2008.

Treat[1]	Hymenoptera[2]	Collembola[2]	Homoptera[2]	Diptera[2]	Orthoptera[2]	Araneae	Coleoptera[2]	Others[2]
CP	12.00 ± 1.80 a	22.20 ± 2.87 a	3.90 ± 0.99 a	9.85 ± 1.94 ab	2.95 ± 0.85 a	0.45 ± 0.15 a	2.25 ± 0.70 ab	6.00 ± 1.27 a
SH	4.49 ± 0.96 b	27.00 ± 6.29 a	2.05 ± 0.52 a	6.60 ± 1.23 b	1.70 ± 0.41 a	1.00 ± 0.74 a	1.20 ± 0.28 bc	1.70 ± 0.52 bc
SO	3.75 ± 0.95 b	28.00 ± 4.66 a	3.10 ± 0.63 a	10.10 ± 1.92 ab	1.65 ± 0.47 a	1.15 ± 0.46 a	1.15 ± 0.29 c	3.00 ± 0.62 bc
PB	3.80 ± 0.72 b	28.60 ± 3.68 a	1.75 ± 0.34 a	14.30 ± 2.08 a	1.50 ± 0.48 a	0.40 ± 0.15 a	1.30 ± 0.42 bc	1.10 ± 0.23 c
C	5.10 ± 0.84 b	38.70 ± 8.59 a	3.90 ± 1.00 a	7.65 ± 1.35 b	2.10 ± 0.56 a	0.25 ± 0.10 a	2.50 ± 0.55 a	4.15 ± 0.88 ab
$P > F$	<0.0001	0.4777	0.2364	0.0050	0.1500	0.5105	0.0439	<0.0001
F value	14.76	0.91	1.51	5.17	1.90	0.85	2.99	12.88

[1]Treatments CP = cowpea, SH = sunn hemp, SO = sorghum-sudangrass, PB = pine bark, C = unmulched control

[2]Hymenoptera = Formicidae; Collembola = Isotomidae; Homoptera = Cicadellidae; Diptera = Dolichopodidae, Mycetophilidae and micro-dipterans; Orthoptera = Acrididae and Gryllidae; Coleoptera = Staphylinidae, Carabidae, Elateridae, and Chrysomelidae; others = Aphididae, Aleyrodidae, and Thripidae.

Data are means ± standard error of 20 replications (data pooled across 4 sampling dates). Means in columns for each sampling date followed by the same letters do not differ significantly based on least square means ($P \leq 0.05$).

DISCUSSION

The arthropods recovered during this study encompassed a variety of trophic groups and feeding habits (Table 3). Effects of treatments on different insect groups varied, but some interesting patterns were evident. Several insect groups, including ants, beetles, and small plant feeding in-

TABLE 3. ARTHROPODS AND THEIR FEEDING HABITS IN THE NATURAL ENVIRONMENT.

Arthropods	Feeding habits	References
Hymenoptera (Formicidae: *Pheidole, Dorymyrmex*)	Mainly predators of small invertebrates Some ants feed on plant sap, nectar, honeydew or fungi	Wilson 2005 Triplehorn & Johnson 2005
Collembola (Isotomidae and Sminthuridae)	Usually fungi associated with decaying vegetation	Coleman et al. 2004
Homoptera (Cicadellidae)	Mainly herbivores feeds on plant sap	Redak et al. 2003
Diptera (Dolichopodidae: *Asyndetus*, and Mycetophilidae)	Dolichopodidae mainly predators of small invertebrates Mycetophilidae feed on fungus	Ulrich & Schmelz 2001 Triplehorn & Johnson 2005
Orthoptera (Gryllidae: *Gryllus*, and Acrididae: *Melanoplus*, *Dichromorpha*)	Generalist herbivores, feed on most kinds of vegetation including weeds	Capinera 1993
Araneae	Generalist predators of small-sized arthropods	Riechert & Lockley 1984
Coleoptera: (Staphylinidae),	Most Staphylinidae are facultative predators	Frank & Thomas 2010
Carabidae: (*Anisodactylus* spp.),	*Anisodactylus* spp. are typically predators but granivory has been recently reported	Sasakawa 2009
Elateridae: (*Conoderus* spp.),	*Conoderus* spp. eat seeds, and feed on stem and roots of seedlings and lead to weak plant stand	Mossler 1993,
and Chrysomelidae: (*Altica* spp.)	*Altica* spp. generally feed on different kinds of plants	Jenkins et al. 2009
Others (Aphididae, Aleyrodidae, and Thripidae)	Plant feeders	Triplehorn & Johnson 2005

sects (aphid, whiteflies, and thrips), were highest in unmulched control or cowpea plots in one season. It is possible that weeds (including nutsedges, grasses, and broadleaf) in unmulched control and cowpea plots may have led to the higher numbers of small plant-feeding insects in these plots. Cowpea mulch degraded quickly and allowed the emergence of weeds after 3-4 weeks. At this time, broadleaf weeds covered about 10% of the surface area in unmulched control and cowpea plots, but <5% in other plots. Broadleaf weeds consisted of Florida pusley (*Richardia scabra* L.), eveningprimrose (*Oenothera laciniata* Hill.), and cudweed (*Gnaphalium* spp.).

Beetles are the largest and most diverse group of insects, and varied in their response to treatment over the two seasons, reaching highest numbers in cowpea plots in 2008. The majority of these were Staphylinidae and Carabidae, which are predators, and the increased abundance of potential prey insects (Aphididae, Cicadellidae etc.) in unmulched control plots may have stimulated these predatory beetles as well (Table 3). Ants have been observed to feed on or tend sucking insects such as aphids and whiteflies (Borror et al. 1989), so their increased numbers may be related to the other insects in unmulched control and cowpea plots. This effect was observed by Pullaro et al. (2006) who recorded a greater number of fire ants in plots with killed-cover crop mulch compared with conventional plots. Flies were most common in pine bark plots in both years, possibly because pine bark was the only mulch that did not degrade as fast as others (C:N ratio = 208:1), and may have served as cover for these insects and their larvae. This mulch may have provided favorable habitat for long-legged flies (Dolichopodidae) that typically inhabit organic debris and feed on small invertebrates on the soil surface (Borror et al. 1989; Triplehorn & Johnson 2005; Ulrich & Schmelz 2001). Collembola were unaffected by treatments, with similar levels in mulched and unmulched plots. This was unexpected since the degradation of mulch could provide a continuous supply of organic matter. Generally, Collembola are cryptozoic and feed on fungi associated decaying organic matter (Coleman et al. 1996; Powers & McSorley 2000).

We were surprised to find a number of aphids, whiteflies, and thrips in pitfall traps. The pitfall trap is the one of the most commonly used methods to sample insects and other arthropods on the soil surface (Southwood & Henderson 2000). On the other hand, small plant feeders such as aphids, whiteflies, and thrips are typically sampled by other methods such as sticky cards rather than pitfall traps (Southwood & Henderson 2000). However, small numbers of them will fall from vegetation into pitfall traps as well (Tremelling et al. 2002). Future studies should anticipate presence of some small plant feeders in pitfall traps, which could probably be better explained by concurrent sampling of above-ground vegetation by other methods.

Conclusions

The present study suggests that insects varied in their responses to different mulches. During both years, flies (mainly Dolichopodidae) were found in highest numbers in pine bark plots throughout the season. Several other groups were affected indirectly due to the effects of mulches on weed growth. Weed growth in unmulched control and cowpea plots may have led to increased populations of small plant feeders such as aphids, thrips, and whiteflies. Ants that tend or feed on small plant feeders were more abundant in these plots as well, as were predatory beetles in 2008. Some groups, such as Collembola, spiders, and parasitoid wasps, were unaffected by mulches, while others such as leafhoppers showed only minimal trends.

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TWO NEW SPECIES OF DRYINIDAE (HYMENOPTERA: CHRYSIDOIDEA FROM NANLING NATIONAL NATURE RESERVE, CHINA

ZAIFU XU[1], MASSIMO OLMI[2] AND JUNHUA HE[3]
[1]College of Natural Resources and Environment, South China Agricultural University Guangzhou, Guangdong 510642, P. R. China

[2]Corresponding author. Department of Plant Protection, University of Tuscia, Via San Camillo de Lellis, I-01100 Viterbo, Italy

[3]Institute of Insect Sciences, Zhejiang University, Hangzhou 310029, P. R. China

ABSTRACT

Anteon nanlingense **sp. nov**. and *Anteon longum* **sp. nov.** are described from Nanling National Nature Reserve (Guangdong, P.R. China). A check-list of Dryinidae from Nanling National Nature Reserve is presented.

Key Words: Dryinidae, *Anteon nanlingense*, *Anteon longum*, new species, Nanling Nature Reserve, China

RESUMEN

Se describen por primera vez a *Anteon nanlingense* sp. nov. y *Anteon longum* sp. nov. ambos colectados en la Reserva Natural Nanling (Guangdong, P.R. China); asimismo, se realiza un listado de los Dryinidae presentes en dicha reserva.

Translation provided by the authors.

Dryinidae (Hymenoptera: Chrysidoidea) are parasitoids of Hemiptera: Auchenorrhyncha (Guglielmino & Olmi 1997, 2006, 2007). The species of Dryinidae inhabiting China have been studied in the last 10 years mainly by He & Xu (2002), Xu, He & Olmi (2001) and Xu, Olmi & He (2006a, 2006b, 2006c, 2007, 2008, 2009a, 2009b, 2009c, 2010, 2011). With approximately 126 described species, *Anteon* Jurine, 1807, is 1 of the largest genera of the Oriental region. Two additional new species of *Anteon* are described herein. They were collected in 1 of the most interesting protected areas of P.R. China, i.e., Nanling National Nature Reserve. This large park includes the highest mountain of Guangdong Province, Mt. Shikengkong (1902 m). This paper presents a revised check-list of Dryinidae inhabiting Nanling National Nature Reserve.

MATERIALS AND METHODS

The descriptions follow the terminology used by He & Xu (2002) and Olmi (1984, 1994, 1999). The measurements reported are relative, except for the total length (head to abdominal tip, without the antennae), which is expressed in mm. In the descriptions, POL is the distance between the inner edges of the lateral ocelli; OL is the distance between the inner edge of a lateral ocellus and the median ocellus; OOL is the distance from the outer edge of a lateral ocellus to the compound eye; OPL is the distance from the posterior edge of a lateral ocellus to the occipital carina; TL is the distance from the posterior edge of an eye to the occipital carina.

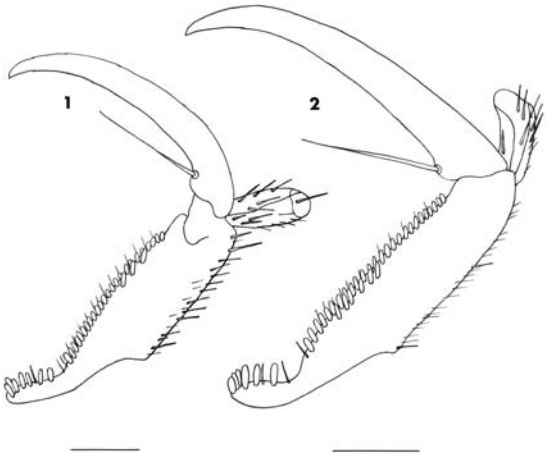
The material studied in this paper is deposited in the Hymenoptera Collection of South China Agricultural University, Department of Entomology, Guangzhou, Guangdong, P. R. China (SCAU).

SYSTEMATIC ACCOUNTS

Anteon nanlingense **sp. nov.** (Fig. 1)

Material examined: Holotype: Female, P.R. CHINA, Guangdong Prov., Nanling National Nature Reserve, 4-5.X.2004, Zaifu Xu (SCAU).

Description. Holotype female; Macropterous; length 2.4 mm; head black, except mandibles, clypeus and anterior half of face are testaceous; ventral side of head black, except a median testaceous stripe; antenna testaceous; prothorax testaceous; rest of mesosoma black; petiole black; gaster testaceous, except some brown areas on dorsal side; legs testaceous-whitish. Antenna clavate; antennal segments in following proportions: 10:4:10:7:6.5:7:7:6:6:8.5. Head shiny, smooth, punctate, without sculpture among punc-



Figs. 1 and 2. Chelae of holotypes of *Anteon nanlingense* sp. nov. (1) and *Anteon longum* sp. nov. (2). Scale bars 0.10 mm for 1 and 0.11 mm for 2.

tures; anterior half of face rugose; frontal line complete; face with 2 lateral longitudinal keels around orbits and directed towards antennal toruli; POL = 5; OL = 4; OOL = 5; OPL = 3.5; TL = 3; greatest breadth of posterior ocellus much shorter than OPL (2:3.5); occipital carina complete. Pronotum shiny, smooth, with anterior surface weakly rugose; posterior surface smooth, finely punctate, without sculpture among punctures; posterior surface shorter than scutum (8:12.5), more than twice as broad as long (18:8); pronotal tubercles reaching tegulae. Scutum shiny, finely punctate, without sculpture among punctures. Notauli incomplete, reaching approximately 0.9 length of scutum. Scutellum and metanotum shiny, without sculpture. Propodeum reticulate rugose, with a strong transverse keel between dorsal and posterior surface; posterior surface with 2 longitudinal keels and median area shiny, as rugose as lateral areas, with some smooth areas. Forewing hyaline, without dark transverse bands; distal part of stigmal vein much shorter than proximal part (5:9). Fore tarsal segments in following proportions: 8:2.5:2.5:4:16; fore tarsal segment 2 curved into a hook. Segment 5 of fore tarsus (Fig. 1) with basal part slightly longer than distal part (10:7). Enlarged claw (Fig. 1) with a proximal prominence bearing a long bristle. Segment 5 of fore tarsus (Fig. 1) with 2 rows of 3 + 24 lamellae; distal apex with a group of 8 lamellae.

Male. Unknown.

Hosts. Unknown.

Etymology. This species is named after its occurrence in Nanling National Nature Reserve, China.

Remarks. *Anteon nanlingense* resembles *A. xuexini* Xu, He & Olmi, 2001, from P.R. China, Zhejiang Prov. However, in *A. nanlingense* the prothorax is testaceous, the notauli reach approximately 0.9 length of scutum and the anterior half of the face is dull and rugose, whereas in *A. xuexini* the prothorax is black, the notauli reach 0.6-0.7 length of scutum and the anterior half of the face is smooth and punctate. Following the above description of *A. nanlingense*, the key to the females of Oriental *Anteon* presented by Xu, He & Olmi (2001) can be modified by replacing couplet 11 as follows:

11 Scutellum testaceous-reddish . . . . . *A. subdignum* Olmi

— Scutellum black . . . . . 11'

11' Anterior half of face dull, rugose, posterior half punctate, without sculpture among punctures; prothorax testaceous; notauli reaching approximately 0.9 length of scutum . . . . . *A. nanlingense* **sp. nov.**

— Face completely finely punctate, smooth; prothorax black; notauli reaching approximately 0.6- 0.7 length of scutum . . . . . *A. xuexini* Xu, He & Olmi

Anteon longum sp. nov. (Fig. 2)

Material examined: Holotype: female, P.R. CHINA, Guangdong Prov., Nanling National Nature Reserve, 4-5.X.2004, Zaifu Xu (SCAU).

Description. Holotype female, Macropterous, length 3.1 mm; head black, except mandibles testaceous; antenna testaceous; mesosoma black; gaster brown; legs testaceous. Antenna clavate; antennal segments in following proportions: 12:6:10:8:7:8:7:7:7:10. Head shiny; face rugose, mainly on lateral regions, with a large area in front of anterior ocellus smooth, punctate and without sculpture among punctures; vertex weakly rugose behind posterior ocelli and on temples; frontal line complete; face with 2 lateral keels near orbits directed towards antennal toruli; anterior third of face and clypeus densely hairy; rest of head almost hairless; POL = 5; OL = 4; OOL = 4; OPL = 5; TL = 5; greatest breadth of posterior ocellus shorter than OPL (3:5); occipital carina complete. Pronotum shiny, with anterior surface rugose; posterior surface shiny, punctate, without sculpture among punctures, shorter than scutum (9:16), more than twice as broad as long (22:9); pronotal tubercles reaching tegulae. Scutum, scutellum and metanotum shiny, smooth, finely punc-

tate, without sculpture among punctures. Notauli incomplete, reaching approximately 0.7 length of scutum. Propodeum with a strong transverse keel between dorsal and posterior surface; dorsal surface reticulate rugose; posterior surface with 2 complete longitudinal keels and median area as rugose as lateral areas. Forewing hyaline, without dark transverse bands; distal part of stigmal vein much shorter than proximal part (5:11). Fore tarsal segments in following proportions: 8:2.5:2.5:6:19. Enlarged claw (Fig. 2) with a proximal prominence bearing a long bristle. Segment 5 of fore tarsus (Fig. 2) with basal part slightly longer than distal part (11:8), with 2 rows of approximately 6 + 27 lamellae; distal apex with a group of about 7 lamellae.

Male. Unknown.

Hosts. Unknown.

Etymology. This species is named after the conspicuous length of the holotype.

Remarks. *Anteon longum* resembles *A. acre* Olmi, 1991, from Vietnam and Taiwan. However, in *A. longum* the posterior surface of pronotum is longer than half of scutum and OPL is longer than OOL, whereas in *A. acre* the posterior surface of pronotum is shorter than half of scutum and OPL is much shorter than OOL. Following the above description of *Anteon longum*, the key to the females of Oriental *Anteon* presented by Xu, He & Olmi (2001) can be modified by replacing couplet 56 as follows:

56 Segment 4 of fore tarsus as long as segment 1. . . . . *A. insertum* Olmi

— Segment 4 of fore tarsus shorter than segment 1 . . . . . 56'

56' Posterior surface of pronotum shorter than half of scutum; head with OPL much shorter than OOL . . . . . *A. acre* Olmi

— Posterior surface of pronotum longer than half of scutum; head with OPL longer than OOL . . . . . *A. longum* **sp. nov.**

Check-list of Dryinidae of Nanling National Nature Reserve

This check-list is the result of many years of research by 1 of the authors (Prof. Zaifu Xu) in Nanling National Nature Reserve. The following 28 species were found:

Aphelopinae
Aphelopus maculiceps Bergman, 1957
Aphelopus nepalensis Olmi, 1984
Aphelopus taiwanensis Olmi, 1991
Aphelopus zhaoi Xu, He & Olmi, 1998

Conganteoninae
Fiorianteon rugosum Olmi, 1991

Anteoninae
Anteon bauense Olmi, 1984
Anteon borneanum Olmi, 1984
Anteon chaoi Xu & He, 1997
Anteon fidum Olmi, 1991
Anteon hirashimai Olmi, 1993
Anteon insertum Olmi, 1991
Anteon lankanum Olmi, 1984
Anteon lini Olmi, 1996
Anteon longum, new species
Anteon nanlingense, new species
Anteon songyangense Xu, He & Olmi, 1998
Anteon thai Olmi, 1984
Anteon wengae Xu, Olmi & He, 2006b
Anteon yasumatsui Olmi, 1984

Dryininae
Dryinus adgressor Xu, Olmi & He, 2006c
Dryinus chenae Xu, Olmi & He, 2007
Dryinus indianus (Olmi, 1984)
Dryinus irregularis Olmi, 1984
Dryinus punctulatus Xu, Olmi & He, 2008
Dryinus pyrillivorus Olmi, 1986
Dryinus sinicus Olmi, 1987
Dryinus stantoni Ashmead, 1904

Gonatopodinae
Neodryinus grandis Xu, Olmi & He, 2011

Conclusions

Nanling National Nature Reserve is a large mainly mountainous area covered with dense forests. This range, separating Guangdong and Hunan Provinces, hosts populations of temperate and tropical species. This environment explains why the above check-list is composed mainly of 3 genera of Dryinidae: *Aphelopus* Dalman, 1823, *Anteon* Jurine, 1807, and *Dryinus* Latreille, 1804. Notably These genera include species with macropterous females that parasitize mainly forest leafhoppers and planthoppers. Cicadellidae: Typhlocybinae are parasitized by *Aphelopus*; Cicadellidae: Deltocephalinae, Eurymelinae, Iassinae, Idiocerinae, Ledrinae, Macropsinae and Tartessinae are parasitized by *Anteon*; many families of Fulgoromorpha (Acanaloniidae, Cixiidae, Flatidae, Fulgoridae, Issidae, Lophopidae, Ricaniidae and Tropiduchidae) are parasitized by *Dryinus* (Guglielmino & Olmi 1997, 2006, 2007). The subfamily Gonatopodinae, characterized mainly

by apterous females, parasitizes Hemiptera feeding on herbaceous plants, so that the species usually do not live in forests and prefer grasslands. Among the few genera of Gonatopodinae with macropterous females, *Neodryinus* Perkins, is better adapted to live in forests, because the species parasitize Flatidae, Nogodinidae and Ricaniidae, which feed both on herbaceous plants and on shrubs and trees. Currently Nanling National Nature Reserve is known to host 28 of the 193 dryinid species listed in China by He & Xu (2002).

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DELIVERY SYSTEM USING SODIUM ALGINATE VIRUS LOADED PELLETS TO RED IMPORTED FIRE ANTS (*SOLENOPSIS INVICTA*, HYMENOPTERA: FORMICIDAE)

DANIELLE M. TUFTS[1,3], KYLE SPENCER[1], WAYNE B. HUNTER[2] AND BLAKE BEXTINE[1]
[1]University of Texas—Tyler, Biology Department, 3900 University Blvd., Tyler, TX 75799

[2]ARS, USDA, U.S. Horticultural Research Laboratory, 2001 South Rock Road, Fort Pierce, FL 34945

[3]Current Affiliation: School of Biological Sciences, University of Nebraska-Lincoln, 323 Manter Hall, Lincoln, NE 68588

ABSTRACT

Microencapsulation as a delivery mechanism of SINV-1 and other molecules such as dsRNA, offers an approach to *Solenopsis invicta* Buren management that is target specific and fits current approaches to baiting ants with toxins and/or RNA-interference. The delivery method presented here targets ground dwelling, foraging ants with an ant-infecting virus which is specific to the genus, *Solenopsis*. Endemic ant-infecting viruses, like *S. invicta* viruses (SINV-1, SINV-2, and SINV-3) are being evaluated for efficacy in *S. invicta* population suppression. In this study, SINV-1 (TX5 strain) was extracted from *S. invicta* colonies and microencapsulated in sodium alginate pellets. Pellets containing extracted whole virions were offered to confirmed non-infected *S. invicta* colonies. Colonies were sampled every 5 d and tested by reverse transcription polymerase chain reaction (RT-PCR) for presence of viral RNA. The longevity of control and viral pellets were also evaluated. Within 30 d, post-feeding of virus, 35% of *S. invicta* colonies acquired SINV-1 infection ($P = 0.03$). Thus, microencapsulation as a delivery mechanism was successful to deliver SINV-1 to *S. invicta* colonies. Future incorporation of this economically affordable method can be implemented to deliver biological agents for specific ant species and to augment current approaches that bait ants. While a virus was used to demonstrate delivery, an adequate and affordable virus production system still needs to be developed before a viral strategy can be adopted as a tool for biological control of fire ants.

Key Words: ant management, anti-infecting virus, *Solenopsis invicta* virus (SINV), microencapsulation, mortality, biological control

RESUMEN

Microencapsulación como un mecanismo para entregar el virus SINV-1 y otras moléculas como dsARN, ofrece una aproximación al manejo de *Solenopsis invicta* Buren que es un objetivo específico y se ajusta a los enfoques actuales de cebos para hormigas tóxicos y / o interferencia de ARN. El método de entrega presentado aqui se enfoca sobre hormigas que hábitan el suelo o que forrajean y utiliza un virus que infecta específicamente a hormigas del género *Solenopsis*. Los virus endémicos que infectan las hormigas, como los virus de *S. invicta* (SINV-1, SINV-2 y SINV-3) estan siendo evaluados para su eficacia en suprimir poblaciones de *S. invicta*. En este estudio, el virus SINV-1 (cepa TX5) fue extraido de colonias de *S. invicta* y microencapsulado en granulos de alginato de sodio. Se ofrecieron granulos con los virus encapsulados a las colonias de *S. invicta* que fueron confirmadas de no estan infectadas. Las colonias fueron examinadas cada cinco dias y probadas usando la reacción reversa de la transcripción de la cadena polimerasa (RT-RCP) para la presencia de ARN viral. La longevidad de los granulos virales y su control fueron evaluados. En un período 30 días, posterior a la ingestión de la virus, el 35% de las colonias de *S. invicta* adquireron una infección de SINV (P = 0,03). Por lo tanto, la microencapsulación como un mecanismo para entregar el SINV-1 a las colonias de *S. invicta* fue exitosa. La incorporación de este método economicamente asequible puede ser implimentado en el futuro para entregar agentes biológicos para especies específicas de hormigas y para aumentar los métodos actuales que usan cebo para controlar las hormigas. Aunque el virus fue usado para demonstrar la entrega, todavia se necesita desarollar un sistema de producción adecuada y asequible antes que una estrategia viral pueda ser adoptada como una herramienta de control biológico para las hormigas de fuego.

The red imported fire ant (*Solenopsis invicta* Buren) invaded North America in the 1930s and since then has become a serious threat to humans and devastated many endemic wildlife species

(Taber 2000). *Solenopsis invicta* colonies were quickly established in the USA because of their aggressive behavior, large colony size, and lack of natural predators (Taber 2000). Therefore, a safe and species specific method for reducing ant colony size and population density of *S. invicta* is desperately needed.

A positive, single-stranded RNA virus in the Family *Dicistroviridae* was reported to only infect ants in the S*olenopsis* genus (Valles et al. 2007). The *S. invicta* viruses (SINV-1, SINV-2, and SINV-3) and genotypes (SINV-1A and SINV-1 TX5) infect all stages of development and caste members (Valles et al. 2004; Valles & Strong 2005; Valles et al. 2007; Valles & Hashimoto 2009; Tufts et al. 2010). Rapid replication of SINV-1 (TX5) can quickly produce an infected fire ant colony. Although acute mortality observed within field populations of ants under natural viral infection has not been significant, when the entire colony becomes infected under controlled conditions the colony dies (Valles et al. 2004). Other examples of insects which became infected with single stranded RNA (ssRNA) viruses related to SINV-1 also showed increased mortality and colony collapse, e.g., leafhoppers (Hunter et al. 2006; Hunnicutt et al. 2006) and honey bees (*Apis mellifera* L.) (Cox-Foster et al. 2007). These studies suggest that the effect of high virus titers or multiple virus infections may be required to cause colony collapse. A virus closely related to SINV-1, the Israeli acute paralysis virus (IAPV), infects honey bees in approximately 90% of apiaries (Johnson 2010). However, only in conjunction with other pathogens (i.e. other viruses or parasites) did it produce colony collapse (Cox-Foster et al. 2007; VanEngelsdorp et al. 2009).

Fire ants, which are also hymenopterans, are under increased stress from the implementation of several biological control agents, e.g., phorid flies (*Pseudacteon* spp.; Graham et al. 2003), fungi (*Beauveria bassiana*; Baird et al. 2007), and microsporidian protozoan (*Thelohania solenopsae*; Oi & Williams 2002; Oi & Valles 2008). These natural enemies continue to be investigated with limited success with respect to their effective use in reducing fire ant populations; therefore, the addition of multiple viral pathogens may increase the efficacy of these currently used approaches. Costly, chemical control programs have been successful, but often displace fire ant colonies to surrounding regions, with re-colonization occurring as the chemicals break down and fire ant populations increase.

The entomopathogenic fungus, *B. bassiana*, was microencapsulated in sodium alginate pellets and tested as a potential biological control method with success (Bextine & Thorvilson 2002). *Beauveria bassiana* was delivered to *S. invicta* colonies by workers that had fed on pellets. The objective of this study was to develop an effective delivery method that would transfer viral infection to *S. invicta* colonies. Thus, SINV-1 (TX5) was encapsulated in pellets and the delivery method assessed. While not tested directly, the potential of this strategy is to provide the means to infect the majority of ants with a high titer of virus, consequently inducing colony collapse.

Materials and Methods

Colony Collection and Viral Detection

Solenopsis invicta colonies were collected during May and Aug 2008 from Smith, Cherokee, Hunt, and Gregg Counties, Texas. A total of 58 colonies were collected from Smith County, 15 colonies from Cherokee County, 9 colonies from Hunt County, and 7 colonies from Gregg County; all colonies were of the polygyne phenotype. To rigorously evaluate variability of acceptance of these pellets by fire ants, ant colonies were collected from widely disparate locations to increase the probability of including behavioral and genetic variations in the ant colonies tested. All colonies collected were tested for the presence of the *Solenopsis invicta* virus - 1 (SINV-1) by reverse transcription polymerase chain reaction (RT-PCR) and gel electrophoresis. RNA was extracted from entire *S. invicta* colonies (workers, queens, and brood) using TRIzol® reagent (Invitrogen, Carlsbad, CA) following manufacturer's protocol. Samples were then tested for virus using a SuperScript One-Step Reverse Transcriptase PCR (RT-PCR) kit (Invitrogen, Carlsbad, CA). RT-PCR was completed using a specific primer set (p62 and p63; Valles & Strong 2005) for a short segment (326 bp) of SINV-1 and performed in duplicate (Tufts et al. 2010). Purified, active virus was extracted from colonies which tested positive for SINV-1 using a modified protocol by Hunter, USDA, ARS (Tufts et al. 2010).

Development of Sodium Alginate Pellets

The virus extract was microencapsulated using a 1% sodium alginate suspension (Bextine & Thorvilson 2002). The solution was prepared by dissolving 2.5 g sodium alginate (Spectrum, Gardena, CA) and 2 g corn meal (Quaker Oats Co., Chicago, IL) in a solution of 10 mL 95% ethanol and 8.5 ml purified virus extract, the mix was then brought to a final volume of 100 mL using autoclaved, nano-pure filtered water. The gelatinous solution was mixed vigorously for 10 min and then slowly dripped into an aqueous solution of 0.25 M calcium gluconate with a sterile 10 mL disposable pipette. After 5 min, the pellets were strained out of the gluconate solution by a sieve and allowed to dry at 22°C on 2 sheets of wax paper for 24 h. Control pellets were produced in the same manner, substituting autoclaved, nano-pure

filtered water for viral extract. Pellets were observed to shrink substantially from their original size over the following 24 h and were stored in airtight plastic vials at 22°C, in the dark. Dried pellets had an average weight of 5.7 mg and an average diameter of 2 mm (n = 20).

Introduction of Pellets to Laboratory Colonies

All colonies were tested by RT-PCR for the presence or absence of SINV, protocols as stated above. A sample of 20 worker ants from each colony was tested for SINV presence, for a total of 1780 ants. Ten of the established laboratory colonies which were negative for SINV infection were randomly chosen; 5 with brood present and 5 colonies without brood. All 10 treated colonies were offered 5 virus pellets. An additional 5 colonies, which also tested SINV negative, were chosen and offered 5 control pellets. All pellets (control and treated) were coated with 200 µL of Vienna sausage liquid (Libby's, Chicago, IL) and placed approximately 16 cm away from the brood box (14 cm × 10 cm × 4 cm). A circle drawn around the pellets on the underside of the ant's observation tray (57.5 cm × 41 cm × 14.5 cm) delineated pellet movement. Throughout the experimental trial period (30 d) 2 samples of 10 ants each (a combination of foragers and workers) were collected every 5 d from each colony (control and treated), resulting in a total of 120 ants tested per colony. RNA was extracted and analyzed immediately for the presence or absence of virus using specific primer sets (p62 and p63; Valles & Strong 2005). Chi-square analysis of SINV positive ants between those with or without brood was performed with pooled data from the experimental groups.

The experiment was replicated with pellets (control and treated) that were stored in dark, air tight containers at room temperature (22°C) for 12 mo. Fifteen new colonies were collected, screened for SINV presence, and established as before. The experimental period for the aged pellets was 35 d. At the conclusion of the second replicated experiment, queens, current brood, and males from 2 infected colonies were sacrificed and tested for the presence of virus.

Results

Of the 89 colonies collected only 26 were found to be positive for SINV, 13 from Smith County, 9 from Cherokee County, 2 from Hunt County, and 2 from Gregg County. Overall ant colonies within 2 counties in Texas (Smith and Cherokee, Co) showed a higher incidence of SINV-1 in Cherokee County with 9 of 15 colonies testing positive for SINV (60%), while Smith County had 13 of 58 colonies test positive (22.4%). Sample sizes for Hunt and Gregg, Co were not large enough to make statistical inferences.

Ants quickly accepted the pellets and within 24 h of pellet introduction, some colonies had moved most of the pellets into the brood box. By d 15 all colonies including control colonies (100%) had moved all 5 pellets into their brood boxes. By d 5, post-feeding one set of forager/worker ants (20 individuals) tested positive for SINV. By d 10, post-feeding 2 additional colonies (40 individuals) tested positive, and by d 15 post-feeding, forager/worker groups in 4 of the 10 colonies (80 individuals) had tested positive for SINV (Fig. 1). Additionally, none of the control colonies tested positive for SINV infection, during or after the experiment. After 30 d the experiment was terminated, no additional colonies tested positive for SINV after d 15, resulting in a 40% infection rate of foragers/workers tested for the 10 experimental colonies. The presence or absence of

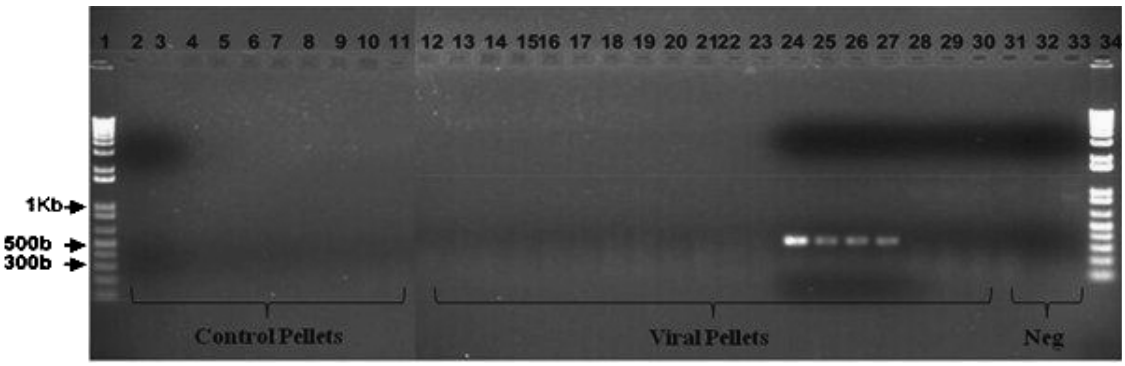

Fig. 1. Gel electrophoresis of the RT-PCR from d 15 post-feeding. This gel illustrates 2 samples as duplicates in neighboring lanes, thus displaying 2 of the 4 colonies that were SINV-1 positive over the duration of the testing period (30 d). Each sample depicts 10 forager/worker ants that were offered 2 d old pellets. A 1Kb ladder (TrackIt™ 1Kb Plus DNA Ladder, Invitrogen, Cat. no. 10488-085) (lanes 1 and 34), ants given control pellets for 5 colonies (lanes 2-11), ants given viral pellets for 10 colonies (lanes 12-31), lanes 24-27 illustrate positive detection of virus for two colonies (~300bp), and negative controls for the RT-PCR (lanes 32-33). Multiple gels are shown.

brood in a colony did not appear to have an effect on colony infection or detection of SINV.

For the experiment with aged pellets, it was found that 30% of tested foragers/workers from the colonies tested positive for SINV 5 d post-feeding. No additional colonies tested positive for SINV over the duration of the trial (Fig. 2) and no control colonies were found to be SINV positive. Upon termination of the second experiment, queens, remaining brood, and males did not test positive for the presence of virus. Based on Chi-square analysis, a significant difference was observed between the virus exposed and unexposed colonies ($\chi^2 = 4.565$, $df = 1$, $P = 0.03$).

Discussion

We have successfully demonstrated an inexpensive method to produce pellets containing ant-infecting virus for delivery to fire ants. Pellets with attractive flavor and virus were produced by a simple method which provides potential application for increasing the efficacy of currently used biological control agents of *S. invicta*. The virus containing pellets provide substantial evidence that this method may have potential, after further refinement, as an effective tool to introduce SINV and other viruses to fire ants in the field. The longevity of these viral pellets was shown to be at least 1 year when storing pellets in dark, airtight plastic containers, at room temp (22°C). Delivery of virus to multiple colonies for both the freshly prepared and extendedly stored pellets (2 d and 12 mo, respectively) was successful. A crude virus preparation was used to produce these pellets, therefore a precise virus titer was not obtained, calculations based on nanodrop readings showed a 139.5 ng of protein/µL of inoculum was used. Even though a total of 600 ants were sampled in virus exposed colonies, detection of SINV-1 was sporadic when sampling workers that may or may not have yet been exposed to the virus through food sharing, i.e., trophallaxis. A plausible explanation for this anomaly is that SINV-1 was only being ingested and not transferred at 100% success from queen to offspring or worker to worker. However, trials were terminated after 30 d and infections may have persisted. Long term trials are needed but were beyond the current scope of this trial. External contamination is not a likely candidate for explaining infection because colonies that tested positive for SINV-1 remained positive throughout the experimental period. If external contamination were contributing to the detection of infection we would expect more colonies to have tested positive randomly or only during a single sampling time. Queens, males, and brood tested negative for the presence of virus; increasing the likelihood that SINV was not being transferred by contact between individuals but by ingestion alone. An individual may have become infected with the virus and died, but due to rapid mortality under laboratory conditions the virus may not have spread to nest mates. Conversely, the experiment clearly demonstrated that delivery of virus to foragers in a colony using this pellet formulation method was possible.

Future studies will evaluate combinations of microencapsulated virus with the fungus, *Beauveria bassiana*, previously shown to also reduce *S. invicta* colonies (Bextine & Thorvilson 2002) and various chemical control agents (e.g. Amdro (Tetrahydro-5,5-dimethyl-2(1H)-pyrimidinone [3-[4(trifluoromethyl)phenyl]-1-[2-[4-(trifluoromethyl)phenyl]ethyenyl]-2-propenylidene]hydrazone), and Over-and-Out (5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1,R,S)-(trifluromethyl) sulfinyl)-1-H-pyrazole-3-carbonitrile)). Given that SINV-1 is taxonomically related to IAPV, adding additional immune stressors to *S. invicta* colonies may be able to induce a 'colony collapse' effect, thus effectively decreasing populations of *S. invicta*.

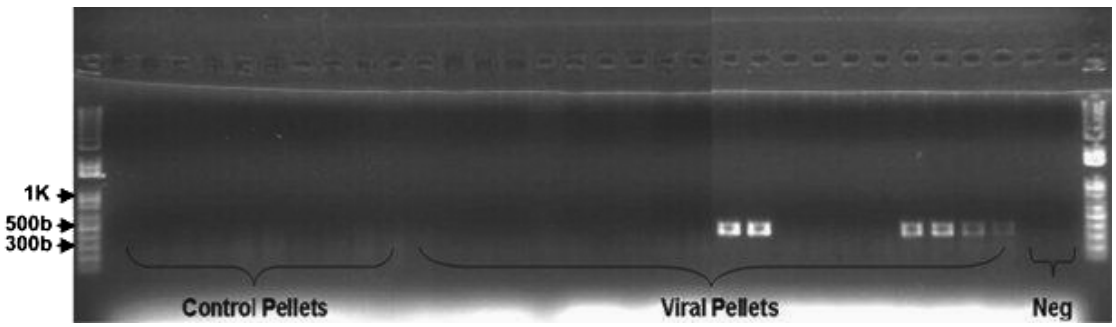

Fig. 2. Gel electrophoresis of the RT-PCR from d 5 presented in duplicate, illustrating 3 different colonies with positive bands for viral infection. Each sample depicts 10 forager/worker ants that were offered 12-month-old pellets. A 1Kb ladder (TrackIt™ 1Kb Plus DNA Ladder, Invitrogen, Cat. no. 10488-085) (lanes 1 and 34), ants given control pellets for 5 colonies (lanes 2-11), ants given viral pellets for 10 colonies (lanes 12-31), lanes 22-23 and 28-31 confirm positive detection of virus for 3 colonies (~300bp), and negative controls for the RT-PCR (lanes 32-33). Multiple gels are shown.

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SUGARCANE PLANTING DATE IMPACT ON FALL AND SPRING SUGARCANE BORER (LEPIDOPTERA: CRAMBIDAE) INFESTATIONS

J. M. BEUZELIN[1], A. MÉSZÁROS[1], W. AKBAR[1,2] AND T. E. REAGAN[1]
[1]Department of Entomology, Louisiana Agricultural Experiment Station,
Louisiana State University Agricultural Center, Baton Rouge, LA 70803, USA

[2]Current address: Monsanto Company, 700 Chesterfield Pkwy West GG3E, Chesterfield, MO 63017, USA

ABSTRACT

In a two-year field study, sugarcane was planted on 4 dates ranging from the first week of Aug to the third week of Nov, reproducing sugarcane phenologies associated with planting and harvesting operations in Louisiana. Sugarcane planted in early Aug offered an extended period of plant availability for sugarcane borer, *Diatraea saccharalis* (F.), infestations during the fall. Periodic sampling throughout the fall showed that early Aug plantings had higher ($P < 0.05$) *D. saccharalis*-caused deadheart densities than later planted sugarcane. Destructive sampling conducted in early Oct showed that Aug plantings harbored greater deadheart densities ($P < 0.05$ in fall 2007) and *D. saccharalis* infestations ($P < 0.05$ in fall 2006 and 2007) than Sep plantings. Data from this study suggest a potential for increased *D. saccharalis* overwintering populations in early plantings associated with greater infestations during the fall. However, differences in deadhearts and *D. saccharalis* infestations in deadhearts were not detected ($P > 0.05$) during the spring. Three commercial sugarcane cultivars ('L 99-226', 'L 97-128', 'HoCP 95-988') were studied. Differences in *D. saccharalis* injury or infestations as affected by cultivar were detected ($P < 0.05$) only in early Oct 2007 when 'HoCP 95-988' harbored 2.3-fold greater infestations than 'L 99-226'.

Key Words: *Diatraea saccharalis* (F.), cultural practices, sugarcane IPM

RESUMEN

En un estudio de campo de dos años, se sembro caña de azúcar en cuatro fechas desde la primera semana de agosto hasta la tercera semana de noviembre, que reproduce la fenología de la caña de azúcar asociada con las operaciones de siembra y cosecha en Louisiana. La caña de azúcar sembrada en el principio de agosto ofreció un período extenso de disponibilidad de la planta para infestaciones por el barrenador de la caña, *Diatraea saccharalis* (F.), durante el otoño. Muestras periódicas tomadas durante el otoño mostró que las siembras del principio de agosto tuvieron una mayor densidad ($P < 0.05$) de cañas con corazones muertos causados por *D. saccharalis* que la caña de azúcar sembrada mas tarde. El muestreo destructivo realizado en el principio de octubre mostró que las siembras de agosto albergaba mayores densidades de corazones muertos ($P < 0.05$ en el otoño de 2007) e infestaciones de *D. saccharalis* ($P < 0.05$ en el otoño de 2006 y 2007) que las siembras de septiembre. Los datos de este estudio sugieren un posible aumento de poblaciones invernantes de *D. saccharalis* en siembras tempranas asociadas a una mayor infestación durante el otoño. Sin embargo, no se detectaron ($P > 0.05$) diferencias en los corazones muertos y las infestaciones de *D. saccharalis* en los corazones muertos durante la primavera. Se estudiaron tres variedades comerciales de caña de azúcar ('L 99-226', 'L 97-128', 'HoCP 95-988'). Se detectaron ($P < 0.05$) diferencias en el daño causado por *D. saccharalis* o de infestaciones afectadas por el tipo de variedad solamente al principio de octubre del 2007, cuando 'HoCP 95-988' albergaba las infestaciones de 2.3 veces mayor que la 'L 99-226'.

The sugarcane borer, *Diatraea saccharalis* (F.), historically has been the most damaging arthropod in Louisiana sugarcane (hybrids of *Saccharum* L. spp.) (Hensley 1971; Reagan 2001). With the widespread use of susceptible high-yielding sugarcane cultivars, current *D. saccharalis* management is achieved by judiciously timed chemical control of economically damaging infestations, conservation of natural enemies, and cultural practices (Posey et al. 2006; Beuzelin et al. 2009, 2010).

In Louisiana, sugarcane is grown in a 4- to 6-year rotation cycle, i.e., 3 to 5 crops are harvested from a single planting and are followed by a fallow period (Salassi & Breaux 2002). Sugarcane vegetative seed pieces are planted from Aug to Oct, with the traditional peak in Sep. However, as farms grow larger and more diversified, planting operations have become less flexible due to simultaneous harvesting and planting activities (Garrison et al. 2000). In addition, late season production of sugarcane seed pieces has become more challenging due to early lodging of recently developed cultivars. Therefore, producers currently plant both earlier and later in the growing season

(Garrison et al. 2000; Viator et al. 2005b). Planting borer-free sugarcane seed pieces is a recommended *D. saccharalis* management tactic to reduce overwintering populations (LSU AgCenter 2010). Because of the onset of low temperatures beginning about mid-Nov, the growing and milling seasons are approximately 9 months and 3 to 4 months, respectively. Thus, harvest in Louisiana begins in Sep and is completed by early Jan. Sugarcane stalks are harvested close to the soil surface, and growers may leave post-harvest crop residue in the field. *Diatraea saccharalis* larvae infesting crop residues at that time are exposed to cold temperatures and natural enemies, which increase overwintering mortality (Kirst & Hensley 1974, Bessin & Reagan 1993). Sugarcane stubble in fallow fields should be plowed out as quickly as possible to reduce the number of overwintering larvae (LSU AgCenter 2010). For non-fallow fields, burning of crop residue occurs mostly in the early spring.

With standard sugarcane management practices, early planting typically provides a better root establishment and higher yields (Viator et al. 2005a). Viator et al. (2005b) conducted a study to determine how Aug, Sep, and Oct planting dates impacted the yield of 5 sugarcane cultivars in Louisiana. Plant cane sugar yields for cultivar 'LCP 85-384' did not differ with planting dates, whereas for 'HoCP 85-845' and 'CP 70-321' sugar yields were higher for the Aug planting date. Charpentier & Mathes (1969) reported that fields planted in Aug show increased *D. saccharalis* infestations because they are highly suitable for moth oviposition. Fall sugarcane shoots (plant cane crop) and fall stubble (ratoon cane crop) are not considered to be *D. saccharalis* overwintering habitats but can serve as means of entry for larvae into seed pieces and stubble portions underground where overwintering occurs (Kirst 1973). The earlier sugarcane is planted or harvested, the greater the period of time during the late summer and fall that shoots are available for *D. saccharalis* oviposition and larval establishment. Early planted and early harvested fields may, therefore, represent a substantial refuge for overwintering *D. saccharalis*, and serve as a source of borers in the spring. Two field experiments were conducted between 2006 and 2008 to determine the effect of sugarcane field phenology associated with planting and harvesting dates on *D. saccharalis* infestations from the fall to the spring.

Materials and Methods

Planting Date Experiment 2006-2007

A field experiment was conducted from 2006 to 2007 near Patoutville (N 29.872°, W 91.744°) in Iberia Parish, LA. A randomized split-plot complete block design with 10 blocks (1 replication per block) was used. Each block was 36.9 m long and 11.0 m wide (6 rows) with 4 main plots, each containing 2 subplots. The range of phenological conditions occurring throughout the Louisiana sugarcane industry was mimicked by assigning early Aug, early Sep, early Oct, and late Nov planting dates to main plots. Each main plot was 6.4 m long and 11.0 m wide (6 rows), separated by a 1.2-m gap. Subplots were planted either with cultivar 'L 97-128' (*D. saccharalis* susceptible, White et al. 2008) or 'L 99-226' (*D. saccharalis* moderately resistant, White et al. 2008). Each subplot was 6.4 m long and 3 rows wide. Sugarcane was planted as whole stalks on Aug 4, Sep 2, Oct 5, and Nov 22 at a density of 6 stalks per 6.4-m row. For each subplot, sugarcane density (shoot counts) and growth (height) were recorded from the center row during subsequent planting dates. On the third planting date (Oct), the number of *D. saccharalis*-caused deadhearts was recorded from the center row of each subplot for the first and second planting dates. Deadhearts are shoots with dead whorl leaves caused by herbivores damaging the apical meristem before above ground internodes are formed (Bessin & Reagan 1993). Insects such as the lesser cornstalk borer (*Elasmopalpus lignosellus* (Zeller) Lepidoptera: Pyralidae) and wireworms (Coleoptera: Elateridae) also cause deadhearts in sugarcane. Therefore, only deadhearts exhibiting entrance holes and frass characteristic of *D. saccharalis*, but no silken tubes (characteristic of *E. lignosellus*), were recorded. Additionally, a 2.1-m long section of row was randomly selected from 1 outer row of each subplot, and plants from this section were destructively sampled for *D. saccharalis*. The number of injured shoots, injured shoots turned into deadhearts, as well as the abundance and size of *D. saccharalis* immatures found within the injured shoots were recorded. The size of *D. saccharalis* larvae was visually determined, with small, intermediate, and large larvae corresponding approximately to first-second, third, and fourth-fifth instars, respectively. On the fourth planting date (Nov), the number of *D. saccharalis*-caused deadhearts was recorded from the center row of each subplot from the first, second, and third planting dates. The following spring (May 18 and Jun 7), numbers of shoots and deadhearts found in the center row were recorded. Deadhearts were collected and dissected for *D. saccharalis* immatures, whose number and size were recorded.

Planting Date Experiment 2007-2008

A second field experiment was conducted from 2007 to 2008 near Bunkie (N 30.950°, W 92.163°) in Avoyelles Parish, LA. A randomized split-plot complete block design with 4 blocks (1 replication per block) was used. Each block was 53.6 m long

and 14.6 m wide (8 rows), and contained 4 main plots, 1 for each planting date. Main plots were 12.5 m long and 14.6 m wide (8 rows), separated by a 1.2-m gap. Subplots were planted with cultivar 'HoCP 95-988' (*D. saccharalis* susceptible, White et al. 2008) or 'L 99-226'. Each subplot was 12.5 m long and 7.3 m wide (four rows). Sugarcane was planted as whole stalks, at a density of 14 to 20 stalks per 12.5-m row, on Aug 6, Sep 5, Oct 10, and Nov 21. Sugarcane emergence and growth data collection was conducted on the 2 center rows of each subplot in the same manner as that of the 2006-2007 experiment. On the third planting date, the number of *D. saccharalis*-caused deadhearts was recorded from the 2 center rows of each subplot from the first and the second planting dates. Additionally, sugarcane shoots for each subplot were examined from one randomly selected outer row. The number of injured shoots, injured shoots turned into deadhearts, and the abundance and size of *D. saccharalis* immatures found within the injured shoots were recorded. On the fourth planting date, the number of *D. saccharalis*-caused deadhearts was recorded from the 2 center rows of each subplot from the first, second, and third planting dates. The following spring (May 12 and 28), numbers of shoots and deadhearts found in the 2 center rows were recorded. Deadhearts were collected and dissected for *D. saccharalis* immatures, with immature number and larval size recorded.

Data Analyses

Data from experiments initiated in 2006 and 2007 were analyzed separately. Analyses of variance (ANOVAs) were conducted with Proc GLIMMIX (SAS Institute 2008), and linear regressions were conducted by Proc REG (SAS Institute 2008). Data collected in early Oct from destructive sampling (*D. saccharalis*-caused deadheart, *D. saccharalis*-injured shoot, and *D. saccharalis* immature counts), and data collected during the spring (shoot, *D. saccharalis*-caused deadheart, and *D. saccharalis* immature counts) were compared in two-way ANOVAs with planting date and cultivar as factors. Shoot count, plant size, and deadheart count data collected from periodic sampling of subplot center rows during the fall were compared by three-way repeated measures ANOVAs with planting date, cultivar, and observation date as factors. A variance component covariance structure was used to model the effects of repeated measures. In the experiment initiated in 2007, each of the 2 subplot center rows was considered a sampling unit. The Kenward-Roger adjustment for denominator degrees of freedom was used in all the ANOVA models to correct for inexact F distributions (Proc GLIMMIX, SAS Institute 2008). When ANOVA effects were detected ($P < 0.05$), least square means were separated by the least significant difference (LSD, $\alpha = 0.05$). Least square means ± standard errors on a per hectare basis are reported.

Linear regressions were conducted to determine whether a relationship between *D. saccharalis* and deadheart counts (recorded from destructive sampling in early Oct) was detected. In addition, linear regressions between fall (late Nov) and spring deadheart counts (recorded from subplot center rows) were conducted to investigate the relationship between end and beginning of the year *D. saccharalis* infestations in newly planted sugarcane.

Results

Sugarcane Availability

Planting date, observation date, and planting date by observation date interaction effects were detected ($P < 0.05$) for plant availability estimates (shoot density and plant height) from periodic sampling during the fall of 2006 and 2007 (Table 1). In 2006, differences in shoot densities between cultivars 'L 99-226' and 'L 97-128' were not detected ($F = 0.00$; $df = 1,54$; $P = 0.984$). August plantings had 33,178 ± 1,764 shoots/ha (LS mean ± SE) by early Sep. In early Oct, Sep plantings had emerged with 47% lower shoot densities (Fig. 1) than the Aug plantings. In late Nov, the Oct plantings had the lowest shoot densities, 5.1-fold and 2.9-fold less than Aug and Sep plantings, respectively. Plant height followed a pattern similar to that observed for shoot density (Fig. 1). In early Sep, Aug plantings measured 47.0 ± 1.3 cm (LS mean ± SE). By late Nov, the Oct plantings had the smallest plants, 3.7-fold and 2.3-fold smaller than Aug and Sep plantings, respectively. In addition to a numerical trend ($F = 3.19$; $df = 1,27$; $P = 0.085$) for 'L 99-226' plants being taller than 'L 97-128' plants, a significant cultivar by planting date two-way interaction was detected ($F = 7.87$; $df = 2,27$; $P = 0.002$). 'L 99-226' plants from Aug plantings were 9% taller than 'L 97-128' plants whereas cultivar differences were not detected in other plantings. Whereas shoots growing from the first 3 plantings were available during the fall, shoots from the Nov plantings did not emerge until the following year (Fig. 1).

Shoot density and plant height during the fall of 2007 showed patterns comparable to those observed in 2006, with early plantings having increased availability and the last planting not emerging until the following year (Fig. 1). In early Sep, the Aug plantings had 53,808 ± 2,538 shoots/ha that measured 50.7 ± 1.9 cm. In late Nov, Aug plantings shoot density was 1.4-fold and 10.9-fold greater than that of Sep and Oct plantings, respectively. August plantings were 1.9-fold and 5.9-fold taller than those from Sep and Oct plantings, respectively. Shoot density and plant height

TABLE 1. SELECTED STATISTICAL COMPARISONS FOR SHOOT DENSITIES, PLANT HEIGHT, AND DEADHEART DENSITIES FROM SUGARCANE PLANTED ON 4 DATES RANGING FROM EARLY AUG TO LATE NOV, 2006 AND 2007.

	Fall 2006			Fall 2007		
Comparison	F	*df*	P > F	F	*df*	P > F
Shoot density						
Planting date	746.46	2,54	<0.001	504.34	2,18	<0.001
Observation date	993.33	2,108	<0.001	541.07	2,84	<0.001
Planting date × Observation date	105.03	4,108	<0.001	115.35	4,84	<0.001
Plant height						
Planting date	1047.71	2,18	<0.001	853.93	2,6	<0.001
Observation date	1141.93	2,108	<0.001	890.50	2,108	<0.001
Planting date × Observation date	74.33	4,108	<0.001	113.46	4,108	<0.001
Deadheart density						
Planting date	54.23	2,54	<0.001	11.67	2,9	0.003
Observation date	20.81	1,54	<0.001	13.13	1,42	<0.001
Planting date × Observation date	4.20	2,54	0.020	8.49	2,42	<0.001

were also affected by cultivar ($F = 5.41$; $df = 1,18$; $P = 0.032$ and $F = 49.99$; $df = 1,9$; $P < 0.001$, respectively), with 'L 99-226' showing greater density (13%) and height (23%) than 'HoCP 95-988'. However, two-way and three-way interactions involving cultivar effects also were detected ($P < 0.05$). Although 'L 99-226' generally had higher shoot densities than 'HoCP 95-988' (Fig. 1), the cultivar by collection date interaction ($F = 3.38$; $df = 2,84$; $P = 0.039$) and the planting date by collection date by cultivar ($F = 12.34$; $df = 4,84$; $P < 0.001$) interaction showed that differences in shoot density between 'L 99-226' and 'HoCP 95-988' at each collection date changed to varying extents for each planting date (Fig. 1). For Aug plantings, 'L 99-226' had 50% higher shoot densities than 'HoCP 95-988' in early Sep; however, differences were not detected (LSD $P > 0.05$) during later sampling. For Sep plantings, 'L 99-226' had 39 and 31% higher shoot densities than 'HoCP 95-988' in early Oct and late Nov, respectively. For Oct plantings, differences in shoot densities between 'L 99-226' and 'HoCP 95-988' in late Nov were not detected (LSD $P > 0.05$). The cultivar by collection date ($F = 4.66$; $df = 2,108$; $P = 0.011$), cultivar by planting date ($F = 9.45$; $df = 2,9$; $P = 0.006$), and the three-way ($F = 2.95$; $df = 4,108$; $P = 0.023$) interactions showed that differences in plant height between 'L 99-226' and 'HoCP 95-988' at each collection date changed to varying extents for each planting date (Fig. 1). For Aug plantings, 'L 99-226' was 35, 22, and 13% taller than 'HoCP 95-988' in early Sep, early Oct, and late Nov, respectively. For Sep plantings, 'L 99-226' was 24 and 26% taller than 'HoCP 95-988' in mid-Oct and late Nov, respectively. For Oct plantings, 'L 99-226' was 51% taller than 'HoCP 95-988' in late Nov.

Diatraea saccharalis Fall Infestations

Planting date, collection date, as well as planting date by observation date two-way interaction effects were detected ($P < 0.05$) for *D. saccharalis*-caused deadheart densities from periodic sampling during the fall of 2006 and 2007 (Table 1). Differences in deadheart densities as affected by sugarcane cultivar were not detected ($F = 0.26$; $df = 1,54$; $P = 0.614$ in 2006 and $F = 0.51$; $df = 1,9$; $P = 0.492$ in 2007). In early Sep, deadhearts in Aug plantings were not observed in 2006 and 2007 (Fig. 2). In early Oct, Aug plantings had higher deadheart densities than Sep plantings (4,313 vs. 43 and 1,093 vs. 0 deadhearts/ha in 2006 and 2007, respectively). In late Nov 2006, Oct plantings had the lowest deadheart densities, 37.8-fold and 9.8-fold less than Aug and Sep plantings, respectively. September plantings had intermediate deadheart densities, 3.9-fold less than Aug plantings (Fig. 2). *Diatraea saccharalis* adult emergence holes, indicating life cycle completion, were observed in deadhearts from sugarcane planted in Aug (641 ± 1,069 exit holes/ha [mean ± SD]). In late Nov 2007, deadhearts were not observed in Oct plantings whereas early Sep plantings had 13.0-fold less deadhearts than Aug plantings (Fig. 2).

In early Oct 2006, after shoot examination and destructive sampling from border rows of Aug and Sep plantings, differences in deadheart densities were not detected (Table 2). Even in the absence of deadheart symptoms, some sugarcane shoots were injured with *D. saccharalis* feeding signs in leaf sheaths and boring into the stem. The density of these non-deadheart injured sugarcane shoots was greater (2.3-fold) in Aug vs. Sep plantings (Ta-

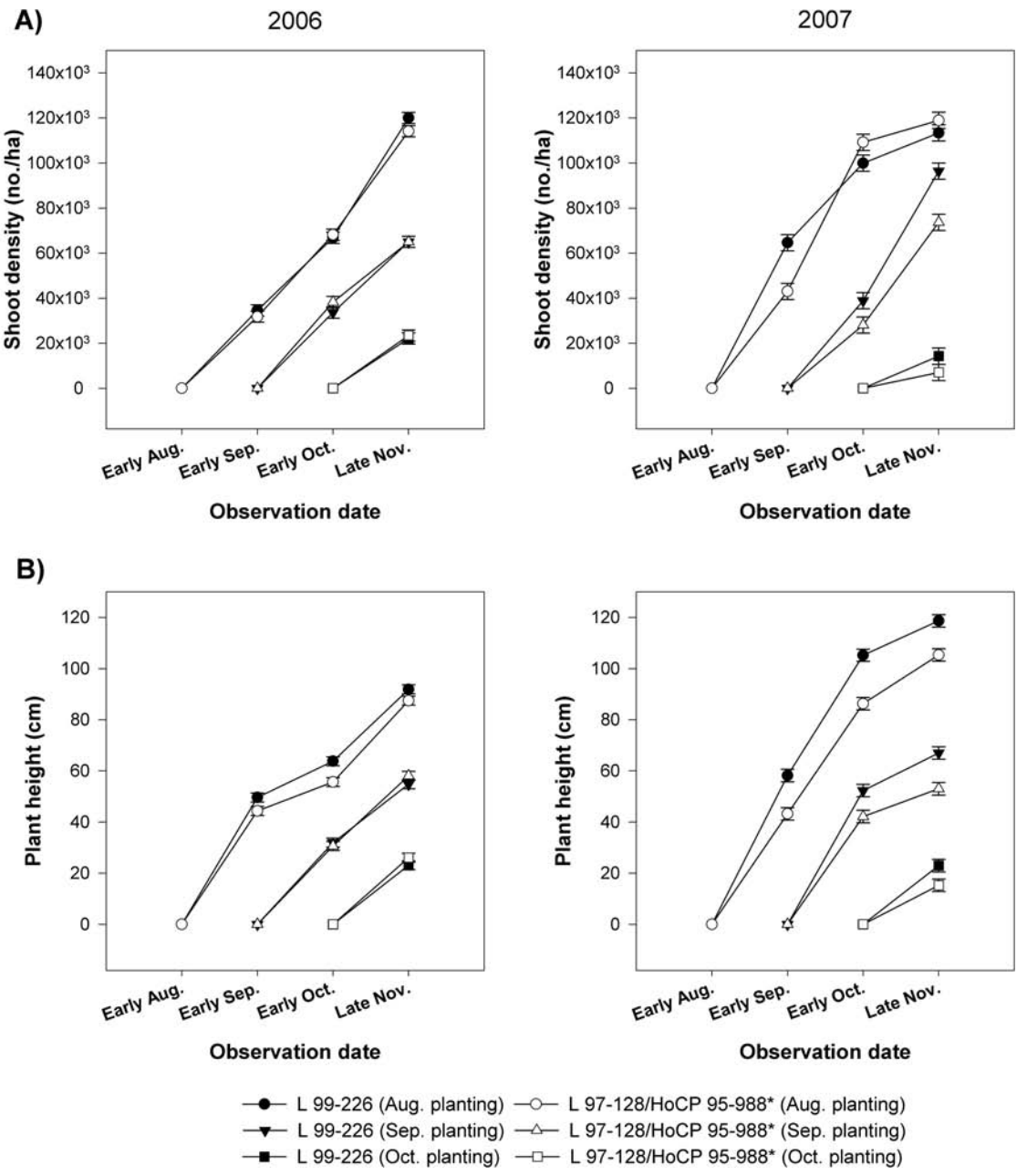

Fig. 1. A) Sugarcane shoot density (LS means ± SE) and B) plant height (LS means ± SE) during the fall from planting date field experiments in Patoutville, LA (2006) and Bunkie, LA (2007). *Cultivar 'L 97-128' for 2006 plantings and 'HoCP 95-988' for 2007 plantings.

ble 2). In addition, there were differences in *D. saccharalis* infestations (Table 2), with Aug plantings harboring 4.7-fold more borers than Sep plantings. Differences between cultivars 'L 99-226' and 'L 97-128' for deadheart densities, non-deadheart injured shoot densities, and *D. saccharalis* infestations were not detected ($P >$ 0.05, Table 2). Among the *D. saccharalis* larvae that were collected in Aug and Sep plantings, 25 and 27% were small, 40 and 18% were intermediate, 35 and 55% were large, respectively. A linear regression ($F = 9.09$; $df = 1,38$; $P = 0.005$; $R^2 = 0.193$) showed that *D. saccharalis* infestations in early Oct (dependent variable) were

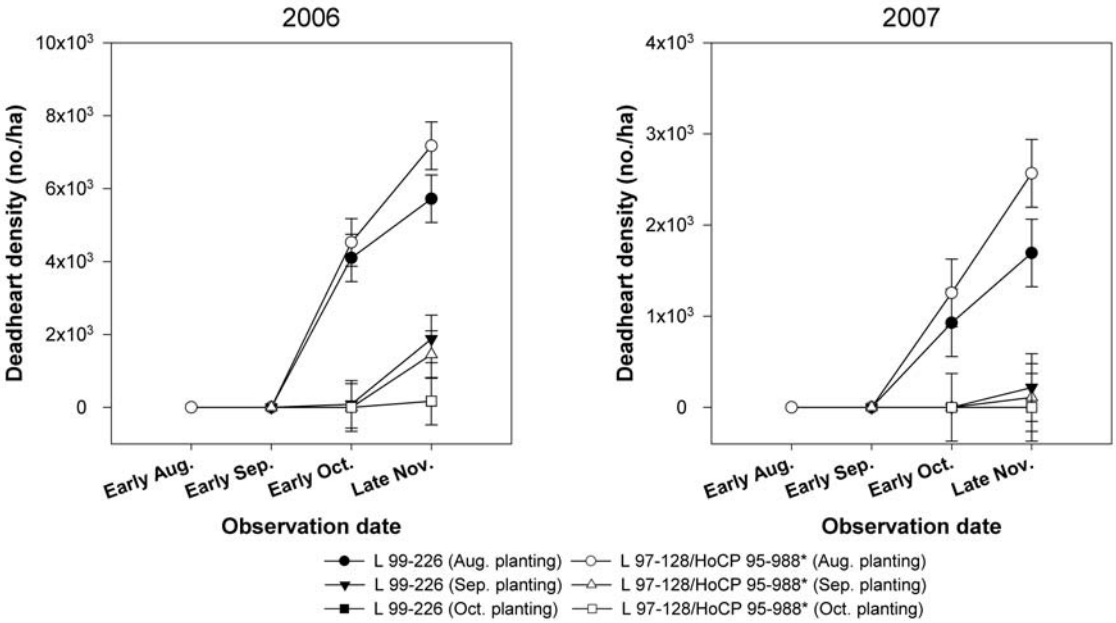

Fig. 2. *Diatraea saccharalis*-caused deadheart densities (LS means ± SE) during the fall in sugarcane from planting date field experiments in Patoutville, LA (2006) and Bunkie, LA (2007). *Cultivar 'L 97-128' for 2006 plantings and 'HoCP 95-988' for 2007 plantings.

positively correlated with deadheart densities (slope: 0.694, 95% C.I. = 0.228, 1.161; intercept: 0.655, 95% C.I. = -0.331, 1.642).

In early Oct 2007, shoot examination and destructive sampling from border rows showed that more *D. saccharalis*-caused deadhearts (24.0-fold) occurred in Aug than in Sep plantings (Table 2). There was a numerical trend for greater deadheart differences between Aug and Sep plantings in cultivar 'HoCP 95-988' ($P < 0.10$ for the planting date by cultivar two-way interaction, Table 2) than in 'L 99-226'. More *D. saccharalis* larvae were collected in Aug than in Sep plantings (19.0-fold), and in 'HoCP 95-988' than in 'L 99-226' (2.3-fold). The significant ($P < 0.05$) planting date by cultivar interaction showed that differences in *D. saccharalis* infestations between Aug and Sep plantings occurred to a greater extent in cultivar 'HoCP 95-988' than in 'L 99-226' (Table 2). Among the *D. saccharalis* larvae that were collected from Aug plantings, 3, 11, and 86% were small, intermediate, and large, respectively. All larvae recovered from Sep plantings were large. A linear regression ($F = 241.60$; $df = 1,14$; $P < 0.001$; $R^2 = 0.945$) showed that *D. saccharalis* infestations in early Oct (dependent variable) were positively correlated with deadheart densities (slope: 0.500, 95% C.I. = 0.431, 0.569; intercept: 0.158, 95% C.I. = -0.396, 0.712). Destructive sampling data collected in Oct 2006 did not differentiate *D. saccharalis* in deadhearts from *D. saccharalis* in non-deadheart injured shoots. However, data from 2007 showed that 68% of recovered borers were infesting deadhearts from the Aug planting date. Despite the presence of deadhearts, all *D. saccharalis* larvae collected from the Sep planting date were feeding in non-deadheart injured shoots.

Diatraea saccharalis Spring Infestations

Differences in sugarcane shoot densities during the spring changed with planting dates (Table 3, Fig. 3). During the spring of 2007 and 2008, sugarcane planted in Aug (2006 and 2007, respectively) had higher shoot densities than that planted in Sep (14 and 25%, respectively), Oct (51 and 76%, respectively), and Nov (87 and 97%, respectively). Sugarcane planted in Sep (2006 and 2007) had higher shoot densities than that planted in Oct (33 and 41%, respectively) and Nov (65 and 58%, respectively). However, the effect of planting dates during the spring of 2007 occurred to a different extent in 'L 99-226' vs. 'L 97-128' (Fig. 3), as shown by the significant two-way planting date by cultivar interaction (Table 3). In addition, shoot densities in 'L 99-226' plots were 30% higher than those in 'HoCP 95-988' plots during the spring of 2008 (Fig. 3).

Differences in deadheart densities and *D. saccharalis* infestations from deadhearts during the spring were not detected among planting dates (Table 3). Among *D. saccharalis* immatures infesting deadhearts during the spring of 2007, 25% were intermediate, 71% were large, and 4% were

TABLE 2. DEADHEART DENSITIES, NON-DEADHEART INJURED SHOOT DENSITIES, AND *D. SACCHARALIS* INFESTATIONS (LS MEAN/HA ± SE) OBSERVED IN EARLY OCT FROM SUGARCANE PLANTED IN EARLY AUG AND EARLY SEP 2006 AND 2007.

	Fall 2006			Fall 2007		
Sugarcane	Deadheart density	Non-deadheart injured shoot density	*D. saccharalis* density	Deadheart density	Non-deadheart injured shoot density	*D. saccharalis* density
Planting date						
Early Aug	1,196 ± 384	2,306 ± 422 a	2,220 ± 541 a	3,933 ± 990 a	819 ± 326	2,076 ± 432 a
Early Sep	1,068 ± 384	982 ± 422 b	470 ± 541 b	164 ± 990 b	55 ± 326	109 ± 432 b
F[1]	0.06	4.92	5.24	7.25	3.59	12.46
P > F	0.817	0.033	0.034	0.036	0.155	0.039
Cultivar						
'L 99-226'	1,110 ± 331	1,708 ± 422	1,324 ± 481	1,475 ± 786	492 ± 274	656 ± 362 b
'L 97-128'/'HoCP 95-988'[2]	1,153 ± 331	1,580 ± 422	1,366 ± 481	2,622 ± 786	382 ± 274	1,530 ± 362 a
F[3]	0.01	0.05	0.01	2.57	0.32	8.73
P > F	0.911	0.831	0.943	0.160	0.595	0.026
Planting date × Cultivar						
Early Aug						
'L 99-226'	1,110 ± 468	2,477 ± 597	2,050 ± 680	2,622 ± 1,112	874 ± 354	1,093 ± 480 b
'L 97-128'/'HoCP 95-988'[2]	1,281 ± 468	2,135 ± 597	2,391 ± 680	5,244 ± 1,112	765 ± 354	3,059 ± 480 a
Early Sep						
'L 99-226'	1,110 ± 468	939 ± 597	598 ± 680	328 ± 1,112	109 ± 354	219 ± 480 b
'L 97-128'/'HoCP 95-988'[2]	1,025 ± 468	1,025 ± 597	342 ± 680	0 ± 1,112	0 ± 354	0 ± 480 b
F[3]	0.11	0.13	0.26	4.25	0.00	13.64
P > F	0.739	0.723	0.615	0.085	1.000	0.010

[1]*df* = 1,18; 1,36; 1,18; 1,6; 1,3; and 1,3, respectively,
[2]Cultivar 'L 97-128' for fall 2006 and 'HoCP 95-988' for fall 2007,
[3]*df* = 1,18; 1,36; 1,18; 1,6; 1,6; and 1,6, respectively.
LS means in columns followed by the same letter are not different (LSD, $\alpha = 0.05$).

TABLE 3. STATISTICAL COMPARISONS FOR SHOOT DENSITIES, DEADHEART DENSITIES, AND *D. SACCHARALIS* INFESTATIONS IN DEADHEARTS FROM SUGARCANE PLANTED ON 4 DATES RANGING FROM EARLY AUG TO LATE NOV.

	Spring 2007			Spring 2008		
Comparison	F	*df*	P > F	F	*df*	P > F
Shoot density						
Planting date	38.43	3,27	<0.001	19.26	3,24	<0.001
Cultivar	5.50	1,36	0.025	13.58	1,24	0.001
Planting date × Cultivar	15.62	3,36	<0.001	0.52	3,24	0.675
Deadheart density						
Planting date	0.80	3,72	0.497	1.51	3,9	0.277
Cultivar	1.08	1,72	0.303	0.49	1,44	0.486
Planting date × Cultivar	0.55	3,72	0.647	2.07	3,44	0.118
D. saccharalis density						
Planting date	1.16	3,36	0.337	0.97	3,9	0.448
Cultivar	0.28	1,36	0.601	0.00	1,44	1.000
Planting date × Cultivar	1.54	3,36	0.221	1.75	3,44	0.170

pupae. Pupae were recovered from deadhearts collected from Sep and Nov plantings. Among *D. saccharalis* larvae infesting deadhearts during the spring of 2008, 26% were intermediate and 74% were large. No pupae were recovered. Linear regressions conducted on data from experiments initiated in 2006 and 2007 did not detect a correlation ($F = 0.30$; $df = 1,78$; $P = 0.583$; $R^2 = 0.004$ and $F = 3.74$; $df = 1,62$; $P = 0.058$; $R^2 = 0.057$, respectively) between deadheart densities observed during the fall (late Nov) and the subsequent spring (May-June).

DISCUSSION

In this two-year study, sugarcane was planted on 4 dates from the first week of Aug to the third week of Nov to reproduce sugarcane phenologies associated with planting and harvesting operations in Louisiana. Because several crops are harvested from a single planting, 25-30% of the Louisiana sugarcane production area is replanted each year with vegetative seed pieces produced from the harvest of 6.5% of the acreage (Legendre & Gravois 2001, 2006, 2010). This study showed that sugarcane fields planted (or harvested) in early Aug offer an extended period of plant availability for *D. saccharalis* infestations, with higher shoot densities and taller plants (increased biomass) than fields planted (or harvested) later in the summer or fall. Late Nov plantings did not produce vegetation until the following spring, suggesting that sugarcane fields planted (or harvested) after late Nov preclude the growth of a

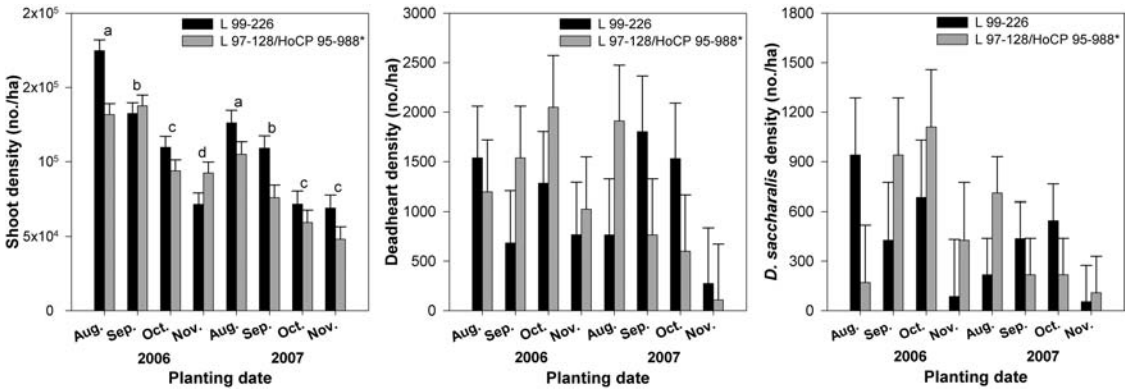

Fig. 3. Shoot densities, deadheart densities, and *D. saccharalis* infestations in deadhearts (LS means ± SE) during the spring from sugarcane planted on 4 dates ranging from early Aug to late Nov, 2006 and 2007. Planting dates within a year followed by the same letter are not different (LSD, $\alpha = 0.05$). *Cultivar 'L 97-128' for 2006 plantings and 'HoCP 95-988' for 2007 plantings.

suitable host substrate for *D. saccharalis* oviposition.

Sampling throughout the fall showed that early Aug plantings had higher *D. saccharalis* deadheart densities than later planted sugarcane. This suggests that sugarcane earlier availability and greater biomass associated with early plantings increased *D. saccharalis* infestations. Destructive sampling conducted in early Oct confirmed that greater deadheart densities were associated with higher *D. saccharalis* infestations. Although Charpentier & Mathes (1969) commented that Aug planting dates were associated with increases in *D. saccharalis* infestations in Louisiana, our study is the first to quantify and compare fall infestations in newly planted sugarcane under current Louisiana production practices. Data from this study suggested a potential for increased *D. saccharalis* overwintering populations in early plantings associated with greater infestations during the fall. However, differences in deadhearts and *D. saccharalis* infestations in deadhearts were not detected during the spring. Four to 5 overlapping *D. saccharalis* generations occur annually in Louisiana (Hensley 1971). After being induced within the first 2 larval stadia (Roe et al. 1984), *D. saccharalis* enters a form of diapause as a large larva, with a peak incidence (63 to 71% of field populations) between Oct and Dec under Louisiana conditions (Katiyar & Long 1961). Although crop residues that are left in the field after harvest may initially be infested with larvae, they decay rapidly and do not serve as habitat for overwintering *D. saccharalis* populations (Kirst & Hensley 1974). The main overwintering habitats are underground portions of vegetative seed pieces and stubble. Because *D. saccharalis* larvae can use fall shoots to gain access to their underground overwintering habitat (Kirst & Hensley 1974) and greater fall infestations were found in early plantings, differences in deadhearts and *D. saccharalis* infestations were expected during the spring.

Deadheart incidence estimates the level of *D. saccharalis* infestations that occur during the spring in sugarcane (Bessin & Reagan 1993). *Diatraea saccharalis* larvae found in spring deadhearts from our study were a combination of intermediate and large larvae, indicating that both overwintering and first generation borers were infesting the deadhearts. Although deadhearts provide appropriate estimates for *D. saccharalis* spring infestations, they were not adequate for determining infestations that had successfully overwintered in newly planted sugarcane. In addition, the small size of our experimental plots likely increased the redistribution rate of adults among plots in the late fall and spring, thus mitigating potential differences in overwintering larval infestations. Red imported fire ants (*Solenopsis invicta* Buren), the primary *D. saccharalis* natural enemies in Louisiana sugarcane (Bessin & Reagan 1993; Beuzelin et al. 2009), were not artificially suppressed and may also have increased variability in spring *D. saccharalis* infestations. Some overwintering mortality factors (i.e., temperature, flooding) likely impacted overwintering populations to the same extent regardless of *D. saccharalis* densities. However, density dependent mortality factors (i.e., predation, parasitism) may have decreased infestations to a greater extent in more heavily infested sugarcane. Because of methodological weaknesses and potential interactions among overwintering mortality factors, a better assessment of overwintering populations should have been conducted during the winter and spring. During the experiment initiated in 2006, destructive sampling of underground seed pieces was conducted in Jan from 2.1-m long sections of border row for each subplot. Only one overwintering *D. saccharalis* larva was recovered and sampling was extremely labor intensive. The use of field cages collecting moths emerging from overwintering larvae may assist in better determining the role of sugarcane phenology during the fall on *D. saccharalis* overwintering populations (e.g., Kfir et al. 1989).

Although a practice of some insect pest management programs (Pedigo 2002), the manipulation of planting dates is more often associated with the agronomic management of crops. Because sugarcane stalks are the shortest in Aug, greater areas have to be harvested for seed piece production to achieve optimal planting rates. However, seed pieces are easier to harvest and plant in Aug before sugarcane stalks bend due to lodging (Viator et al. 2005a, 2005b). In addition, early planted sugarcane tends to produce higher yields (i.e., cane tonnage, sucrose concentration, sugar yield) associated with better root establishment (Viator et al. 2005a, 2005b; Hoy et al. 2006). Nevertheless, the effect of planting dates on yields is dependent on cultivar, with cultivar-specific optimal planting dates. Different cultivars may also show varying degrees of yield response to planting dates. In addition, planting date effects on yields vary with planting methods (Viator et al. 2005a; Hoy et al. 2006). In our study, sugarcane was planted as whole stalks. Louisiana growers also plant sugarcane as billets (stalk sections of 50-60 cm, Viator et al. 2005a). The yield response to planting dates of billet- vs. whole stalk-planted sugarcane seems less consistent (Viator et al. 2005a; Hoy et al. 2006). Whereas early planted sugarcane may increase regional *D. saccharalis* populations during the spring, better root establishment and greater biomass may help compensate for borer injury during the spring, which might help protect yields. Early planting dates have also been reported to reduce losses associated with root injury from wireworms (Charpentier & Mathes 1969).

'L 99-226', 'L 97-128', and 'HoCP 95-988' are 3 commercial sugarcane cultivars, respectively, grown over 11, 17, and 5% of the Louisiana sugarcane production area (Legendre & Gravois 2010). These cultivars have shown varying levels of resistance to *D. saccharalis* (White et al. 2008) and differences in shoot population and growth during the fall and spring were observed in our study. However, differences in *D. saccharalis* injury or infestations as affected by cultivar were only detected in early Oct 2007 when 'HoCP 95-988' harbored greater (2.3-fold) infestations than 'L 99-226'. In a previous study, Bessin & Reagan (1993) observed greater deadheart densities in 'CP 61-37' (*D. saccharalis* susceptible) than in 'CP 70-330' (resistant) during the spring. Cultivar resistance to *D. saccharalis* has traditionally been determined based on measures of mature stalk injury (% bored internodes), adult production (number of moth exit holes in stalks), and tolerance to injury (% yield loss relative to % bored internodes) (Bessin et al. 1990; White et al. 2008). When comparing 10 sugarcane cultivars with varying levels of resistance, White & Dunckelman (1989) found limited differences in *D. saccharalis* deadheart injury. However, the percentages of deadhearts were typically consistent with resistance rankings based on independent assessment of stalk injury levels (% bored internodes). Although differences in *D. saccharalis* resistance levels may not be observed when deadhearts occur (i.e., early in sugarcane phenology before the formation of elongated internodes), the potential of cultivars with increased resistance to minimize fall and spring borer infestations deserves further research.

Diatraea saccharalis infestations in newly planted sugarcane and stubble growth during the fall do not contribute directly to economic damage and have not been considered in management (Hensley 1971). *Diatraea saccharalis* late summer and fall populations are the source for overwintering borers, which will emerge in the spring the following year and cause economic damage. Our study showed that early planting and harvesting enhance late summer and fall *D. saccharalis* populations, thus having the potential for enhancing overwintering populations and subsequent economic damage. In areas where *D. saccharalis* is a severe problem, when susceptible cultivars are planted, or when insecticides cannot be applied, optimization of planting dates (e.g., Sep) may help minimize *D. saccharalis* population build-up.

Acknowledgments

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THE DIFFERENTIAL GRASSHOPPER (ORTHOPTERA: ACRIDIDAE)—ITS IMPACT ON TURFGRASS AND LANDSCAPE PLANTS IN URBAN ENVIRONS

JAMES A. REINERT[1], WAYNE MACKAY[2], M. C. ENGELKE[1] AND STEVE W. GEORGE[1]
[1]Texas A&M AgriLIFE Research & Extension Urban Solutions Center, 17360 Coit Road, Dallas, TX, 75252-6599, U.S.A.

E-mail: j-reinert@tamu.edu

[2]Mid-Florida Research & Education Center, 2725 S. Binion Road, Apopka, FL, 32703-8504 U.S.A.

ABSTRACT

The differential grasshopper, *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae), frequently migrates from highway rights-of-way, pastures, and harvested fields to feed in urban/suburban landscapes and retail/wholesale nurseries across the southern and southwestern U.S.A., as these areas dry down during hot dry summers. Nine selected turfgrasses and 15 species of landscape plants were evaluated for their susceptibility or resistance to this grasshopper. Grasshoppers were collected from stands of Johnsongrass, *Sorghum halepense*, which was used as a standard host for comparison in both experiments. Based on feeding damage, number of grasshopper fecal pellets produced, and their dry weight, *Zoysia matrella* cv. 'Cavalier' was the least preferred grass followed by *Buchloe dactyloides* cv. 'Prairie' and *Z. japonica* cv. 'Meyer'. *Festuca arundinacea* was significantly the most preferred host and sustained the most feeding damage, followed by *Poa pratensis* × *P. arachnifera* cv. 'Reveille' and 2 *Cynodon* spp. cultivars, 'Tifway' and 'Common'. Among the landscape plants, *Hibiscus moscheutos* cv. 'Flare', *Petunia violacea* cv. 'VIP', *Phlox paniculata* cv. 'John Fanick', *Tecoma stans* cv. 'Gold Star', and *Campsis grandiflora* were the least damaged or most resistant. *Plumbago auriculata* cv. 'Hullabaloo', *Glandularia hybrid* cv. 'Blue Princess', *Canna* × *generalis,* Johnsongrass, and *Cortaderia selloana* cv. 'Pumila' sustained the most damage. Based on the number of fecal pellets produced and their weights, *Canna* × *generalis* and *Glandularia hybrid* cv. 'Blue Princess' were the most preferred landscape plants tested.

Key Words: turfgrass, lawns, landscape plants, nursery plants, host plant resistance, *Melanoplus differentialis*

RESUMEN

El chapulín diferencial, *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae), frecuentemente emigra desde los derechos de vía, pasturas y terrenos cosechados hacia jardines urbanos y viveros comerciales en busca de alimento, principalmente donde las áreas comienzan a secarse en el verano del sur y sureste de Estados Unidos La susceptibilidad o resistencia a la alimentación de chapulines fue evaluada en nueve pastos para césped y otras quince plantas ornamentales. Los chapulines se colectaron en Johnsongrass, *Sorghum halepense*, el cual se uso como un hospedero estándar en ambos experimentos. Con base a los datos del daño al alimentarse, numero y peso de las heces fecales producidas, *Zoysia matrella* cv. 'Cavalier' es el menos preferido, seguido de *Buchloe dactyloides* cv. 'Prairie' y *Z. japonica* cv. 'Meyer'. El mas preferido significativamente, con el mayor daño al alimentarse fue *Festuca arundinacea* seguido de *Poa pratensis* × *P. arachnifera* cv. 'Reveille' y dos pastos de *Cynodon* spp. cv. 'Tifway' y 'Common'. En el grupo de plantas ornamentales, *Hibiscus moscheutos* cv. 'Flare', *Petunia violacea* cv. 'VIP', *Phlox paniculata* cv. 'John Fanick', *Tecoma stans* cv. 'Gold Star', y *Campsis grandiflora* presentaron la mayor resistencia. *Plumbago auriculata* cv. 'Hullabaloo', *Glandularia hybrid* cv. 'Blue Princess', *Canna* × *generalis*, Johnsongrass, y *Cortaderia selloana* cv. 'Pumila' presentaron el mayor daño significativamente. Con los parámetros de numero y peso de heces fecales, *Canna* × *generalis* y *Glandularia hybrid* cv. 'Blue Princess' fueron las plantas mas preferidas.

Translation provided by Carlos Campos, Texas A&M AgriLIFE Res. & Ext. Center, Dallas, TX

The differential grasshopper, *Melanoplus differentialis* (Thomas) (Orthoptera: Acrididae), does not fly long distances like the migratory grasshopper, *Melanoplus sanguinipes* (Fabricius) (Shotwell 1930). However, as highway rights-of-way, pastures, and harvested fields dry down during hot dry summers, *M. differentialis* adults fly from them to nearby urban/suburban landscapes and retail/wholesale nurseries to consume the foliage of turfgrasses and many landscape plants

across the Southern U.S.A. Based on limited surveys during summers and autumns since 1998, we have recorded the differential grasshopper as the most frequently encountered species occurring in urban areas of Dallas, Texas. *M. differentialis* is also 1 of the most important grasshopper species causing economic injury to corn, wheat, alfalfa, and several other field crops (Anonymous 1994; Isely 1944; Harvey & Thompson 1993). A single adult of this species feeding on a small potted or landscape plant can defoliate it practically overnight, and the invasion of many adults can devastate an entire landscape after just a few days and nights of feeding. Such sudden damage to nursery production can render the planting stock unsellable for the remainder of the season. The extremely hot and dry summers in the Southern and Southwestern U.S.A. create ideal conditions for extensive outbreaks across many states. Dense migrating populations do not occur every year, but when conditions are right, large and quite devastating populations do occur across the region. As pastures and field crops are either harvested or desiccated from drought in late summer and early autumn, *M. differentialis* readily disperse into plant nurseries and the urban landscapes in search of food (Reinert et al. 2001). As a result, extensive damage is common on many landscape plant species, and effective grasshopper control strategies for the urban landscape, and especially plant nurseries, are often required to protect valuable plants that contribute significantly to high property values (Merchant & Cooper 2010; Reinert et al. 2001; Royer & Edelson 2004).

Several studies have been conducted to determine the feeding preferences of selected species of grasshoppers on various grasses and herbaceous plants; however, most of them have dealt with range or pasture grasses, weeds, and cultivated field crops. Isely (1938) determined that the short-horned grasshoppers (Acrididae), including *M. differentialis*, have mandible patterns possessing both graminivorous and forbivorous characteristics, which allows them to readily feed on both grasses and forbs.

Specific host feeding studies have also been conducted with *M. differentialis*. Isely (1944) fed nymphs of *M. differentialis* on 2 native grasses (*Andropogon saccharoides* Swartz and *Sprorobolus heterolepis* A. Gray) and on Johnosongrass, *Sorgham halepense* (L) Pers; bermudagrass, *Cynodon dactylon* L. Pers; and corn, *Zea mays* L. He also fed them on 5 weeds: *Helianthus annuus* (L.) (Asteraceae); common sunflower, *Ambrosia aptera* (DC) (Asteraceae); giant ragweed, *Lactuca virose* (L.) (Asteraceae); wild lettuce, *Gaillardia pulchella* (Four.) (Asteraceae); and *Parthenium hysterophorus* (L.) (Asteracaeae) that were commonly present in stands of Johnsongrass. Isely (1944) did not report on the preference of 1 grass or herb over another, but only that *M. differentialis* matured an average of 12 d faster in cages with forbs than in cages with only grasses. In another set of studies with 12 species of plants in Maryland, *M. differentialis* showed a strong preference for common dandelion, *Taraxacum officinale* F. H. Wigg. (Asteraceae). *Plantago rugellii* Dcne. (Plantaginaceae); *Dactylis glomerata* L.; and *Cyperus strigosus* L. (Cyperaceae) also served as good hosts (Kaufmann 1968). Goldenrod, *Solidago altissima* L. (Asteraceae), was only nibbled by the grasshoppers (Kaufmann 1968). Kaufmann also showed that this grasshopper could develop and reproduce by feeding only on species of Poaceae; but development was slower and adults were smaller than when they fed on both grasses and forbs.

M. differentialis also showed a preference for some corn hybrids over others in choice field experiments (Brunson & Painter 1938; Harvey & Thompson 1993). Even though under field conditions *M. differentialis* feeds heavily on alfalfa, *Medicago sativa* L. (Fabaceae), it was found to be an inadequate host for complete development (Barnes 1963). *M. differentialis* showed strongest preference for the common sunflower, *Helianthus annuus* L. (Asteraceae) compared to the following offered food plants: fava bean, *Faba vulgaris* Moench. (Fabaceae); kale, *Brassica oleracea* L. (Brassicaceae); and tomato, *Solanum Iycopersicum* L. (Solanaceae) (Howard 1995). However, in another test *M. differentialis* preferred giant ragweed, *Ambrosia trifida* L. (Asteraceae), over sunflower (Lewis 1984). Host preference has also been shown with other *Melanoplus* species (Bailey & Mukerji 1976; Fielding & Brusven 1992; Hinks et al. 1990; Hinks & Olfert 1993; Johnson & Mündel 1987; Porter & Redak 1997). Damage to seedlings in a pine nursery was reported by Feaver (1985), but no other literature on the preferences of *M. differentialis* for either turfgrasses or landscape plants has been found.

Mulkern (1967) reviewed the literature on preference for food plants by grasshoppers and concluded that they are selective feeders with definite preferences, especially in choice experiments when they are confined on 2 or more species of plants. Only limited published documentation exists on grasshopper damage to urban landscapes and gardens. Lists of the preferred plants based upon landscape observations when *M. differentialis* nymphs and adults were feeding, and control strategies have been developed by Cooperative Extension Specialists in Texas (Merchant & Cooper 2010), Oklahoma (Royer & Edelson 2004), and Kansas (Bauernfeind 2005).

Knowing the host feeding preferences for this frequent pest in urban landscapes can help the nurseryman and landscape manager determine which plants will serve as good indicators as they develop monitoring strategies for their pest man-

agement program. Additionally, this information can serve as a guide for plant selection for landscape plantings in areas with a higher potential for *M. differentialis* invasion and outbreaks.

This study was initiated to test our hypothesis that some turfgrasses and landscape plants are more preferred than others by *M. differentialis*, and secondly to determine if any of the commonly planted turfgrasses or landscape plants exhibit resistance to this pest. A diverse selection of landscape plants from 13 plant families and 9 turfgrasses was chosen to help identify preferences among the plant groups used in the urban landscape.

Materials and Methods

A representative collection of 9 of the most commonly used cultivars and species of turfgrasses (family Poaceae) in the arid Southwestern U.S.A. and 15 species of landscape plants (in 13 families) found either growing in the landscape or in container nurseries at the Texas AgriLIFE Research & Extension Center, Dallas, Texas was selected for this study. Two no-choice feeding experiments were conducted, the first compared 9 selected turfgrasses and a second study compared 15 species of landscape plants (Table 1). Johnsongrass, *S. halepense*, was included in both experiments as a standard host plant, since the grasshoppers used in these experiments were collected from this host. Johnsongrass is a common food source for *M. differentialis* (Isely 1944), and because it is fairly drought resistant, this grasshopper species tends to aggregate on it as the other plant materials begin to desiccate during the summer heat and drought stress period.

For each test plant in each replicate, leaves or terminal shoots were clipped from the grasses or landscape plant and transported to the laboratory in a cooled ice chest. Adequate plant material

Table 1. Turfgrasses and landscape plants evaluated in feeding study for host preference/resistance to the differential grasshopper.

Plants Family	Plant/Cultivar	Genus and Species
	Turfgrasses (Experiment 1)	
Poaceae	'Common' Bermudagrass	*Cynodon dactylon* (L.) Pers.
Poaceae	'Tifway' Bermudagrass	*Cynodon dactylon* × *C. transvaalensis* Burtt-Davy
Poaceae	'Prairie' Buffalograss	*Buchloe dactyloids* (Nutt.) Engelm
Poaceae	'Raleigh' St. Augustinegrass	*Stenotaphrum secundatum* (Walt.) Kuntze
Poaceae	'Meyer' Zoysiagrass	*Zoysia japonica* Steud
Poaceae	'Cavalier' Zoysiagrass	*Zoysia matrella* (L.) Merr.
Poaceae	'Tejas' Texas Bluegrass	*Poa arachnifera* Torr.
Poaceae	'Reveille' TX x KY Bluegrass	*Poa pratensis* L. × *P. arachnifera* Torr.
Poaceae	Tall Fescue	*Festuca arundinacea* Schreb.
Poaceae	Johnsongrass	*Sorghum halepense* (L.) Pers.
	Landscape Plants (Experiment 2)	
Apocynaceae	'Hardy Red' Oleander	*Nerium oleander* L.
Bignoniaceae	Chinese Trumpet Vine	*Campsis grandiflora* K. Schum
Bignoniaceae	'Gold Star' Esperanza	*Tecoma stans* (L.) Juss. ex Kunth
Cannaceae	Red Canna	*Canna * generalis* L. H. Bailey
Convolvulaceae	'Marguerite' Ornamental Sweet Potato	*Ipomoea batatas* (L.) Lam.
Lythraceae,	Crape Myrtle	*Lagerstroemia fauriei* Koehne
Malvaceae	'Flare' Perennial Hibiscus	*Hibiscus moscheutos* L.
Nyctaginaceae	Bougainvillea	*Bougainvillea spp.* Comm. ex Juss.
Poaceae	'Pumila' Dwarf Pampas Grass	*Cortaderia selloana* (Schult. & Schult. f.) Asch. & Graebn.
Poaceae	Johnsongrass	*Sorghum halepense* (L.) Pers
Polemoniaceae	'John Fanick' Perennial Phlox	*Phlox paniculata* L.
Plumbaginaceae	'Hullabaloo' Blue Plumbago	*Plumbago auriculata* Lam.
Rosaceae	'Climbing Pinkie' Rose	*Rosa* sp.
Solanaceae	'VIP' Petunia	*Petunia violacea* Lindl.
Verbenaceae	'Blue Princess' Perennial Verbena	*Glandularia hybrida* (Groenland & Rümpler) G. L.Nesom & Pruski (formerly *Verbena hybrida*)
Verbenaceae	Lantana	*Lantana horrida* Kunth (Synonym of *Petunia integrifolia* (Hook.) Schinz & Thell.)

(leaves or shoots) to support 1 adult grasshopper for at least 2 d of feeding on the turfgrasses and 3 d on the landscape plants was initially caged with each adult *M. differentialis* in a 9 cm diam × 20 mm deep plastic feeding chamber (Petri dish). Each feeding chamber was provided with 2 water-saturated, 7 cm diam filter paper discs to maintain plant turgidity. Both feeding studies consisted of 1 grasshopper per feeding chamber, with 3 chambers per experimental unit and 8 replications for a total of 24 grasshoppers per test plant. These chambers were observed daily for feeding activity and the weight and production of fecal pellets was recorded.

After 2 d exposure to the test turfgrasses, each grasshopper was moved to a new feeding chamber and several parameters were assayed to determine feeding activity: a) the amount of feeding was rated on a scale of 1-5, 1 = little or no feeding, and 5 = near complete consumption of the plant material; b) fecal pellets were counted; and c) fecal pellets were oven dried (72 h at 70°C) and weighed. The grasshoppers tested on the turfgrasses were again placed on fresh samples of the respective grasses for an additional 6 d (8 d total) of feeding. Grasshoppers were held initially for 3 d on the test landscape plants before these parameters were assayed. Grasshoppers held on the landscape plants were reestablished in the test chambers for an additional 11 d (14 d total) of feeding on each plant species. For both experiments, cages were opened every 2-3 d, so that fecal pellets and decaying plant material could be removed and fresh plant material added to insure that the grasshoppers always had adequate fresh plant material on which to feed. After feeding for 8 and 14 d on turfgrasses and landscape plants, respectively, all remaining fecal pellets were counted, oven dried, and weighed.

Adult differential grasshoppers for these studies were individually collected with a sweep net from large stands of Johnsongrass growing wild in highway and railroad rights-of-ways in Denton, County, Texas, U.S.A. and stored in cooled ice chests for transport to the laboratory. Grasshoppers were held with no food and only water for 72 h to allow them to eliminate any waste from plants on which they had been feeding. Grasshoppers that appeared healthy were then used to establish the tests. Female grasshoppers were randomly chosen for all 8 replicates with the turfgrasses. For the landscape plant experiment, females were used for the first 7 replicates; but since there were not enough females to complete replicate 8, only males were used for this last replicate.

Statistical Analysis

Data for the following parameters were recorded: feeding damage, number, and weight of fecal pellets after 2 d of feeding on each of the turfgrasses; the same 3 parameters after 3 d of feeding on each of the landscape plants; the number and weight of fecal pellets produced after 8 d of feeding on each of the turfgrasses; and the same 2 parameters after 14 d of feeding on each of the landscape plants and they were analyzed by Analysis of Variance (ANOVA) (PROC GLM) for a randomized complete block design to test the differences between test plants. Means were compared at the 5% level of significance using Waller-Duncan k-ratio (k = 100) t test (SAS Institute 2009).

Results and Discussion

Turfgrasses

The feeding response by the differential grasshopper on 9 turfgrasses is presented in Figs. 1 and 2. *Zoysia matrella* cv. 'Cavalier' was the least preferred cultivar of turfgrass with a mean damage rating of 0.79 on the scale of 1 to 5, with 1 = little or no damage, and 5 = near complete consumption of the plant material (Fig. 1). *Buchloe dactyloides* cv. 'Prairie' and *Z. japonica* cv. 'Meyer' were the next 2 least damaged grasses with damage ratings of 1.17 and 2.13, respectively. *Festuca arundinacea* (tall fescue) sustained the most feeding damage and was the most preferred grass (rating of 4.62), followed with significantly less feeding damage by *Poa pratensis* × *P. arachnifera* cv. 'Reveille', *Cynodon dactylon* × *C. transvaalensis* cv. 'Tifway', *C. dactylon* cv. 'Common' (each with damage ratings ≥3.50) (Fig. 1). Feeding on Johnsongrass was also high with a damage rating of 3.24.

When the number of fecal pellets and their weight were compared for each grass after 2 d of feeding, the response among the various grasses was very similar to the results for actual feeding damage ratings (Fig. 2). Grasshoppers feeding on *Zoysia* cv. 'Cavalier' only produced an average of 5.33 fecal pellets (Fig. 2A) during the first 2 d at a weight of 15.57 mg (Fig. 2B). The weight of fecal pellets (24.79 mg) produced on *Buchloe* cv. 'Prairie' was not significantly different from that produced on *Zoysia* cv. 'Cavalier'. In contrast, grasshoppers feeding on *F. arundinacea* produced an average of 29.62 fecal pellets at a mean weight of 80.32 mg. Significantly fewer fecal pellets (21.91, 22.85, 20.15, and 17.78) were produced by grasshoppers feeding on *P. pratensis, P. arachnifera* cv. 'Reveille', *Cynodon* cv. 'Tifway', *Cynodon* cv. 'Common', and *P. pratensis* cv. 'Tejas1', respectively than on *F. arundinacea* (Fig. 2A) and the 2-d fecal pellet weight produced on each of these grasses exceeded 54 mg (Fig. 2B). The number of fecal pellets produced during the first 2 d on *Zoysia* cv. 'Meyer' was not much greater than produced on *Buchloe* cv. 'Prairie';

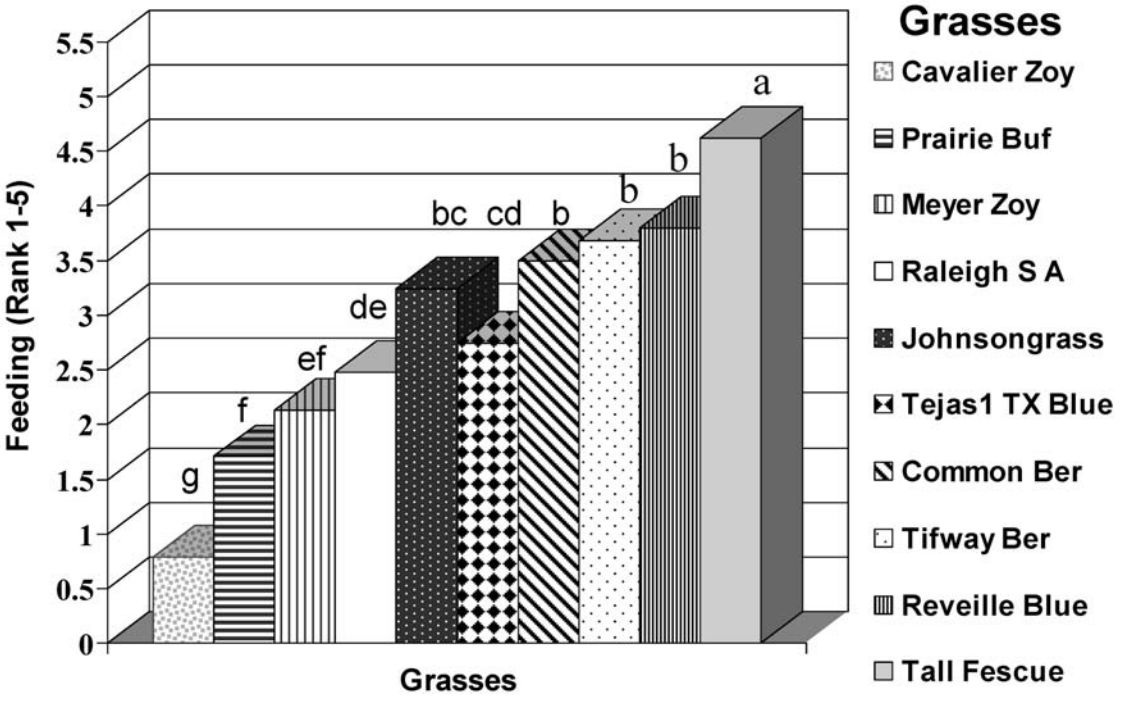

Fig. 1. Feeding damage by differential grasshoppers on 9 turfgrass cultivars and Johnsongrass during the first 2-d feeding period. Damage was rated on a scale of 1-5; where 1 = little or no feeding, and 5 = near complete consumption of the available plant material. The order of cultivars listed at the right side of the graph corresponds to the bars from left to right. Bars for each plant with the same letters above them are not significantly different by Waller-Duncan k-ratio (k = 100) t test (P = 0.05).

however, their weight was more than doubled at 50.76 mg. Even though the damage rating was relatively high on Johnsongrass, the number of fecal pellets (11.96) and their weight (29.94 mg) was unexpectedly low compared to the number and weight of fecal pellets produced on the less damaged *Zoysia* cv. 'Meyer' and *Stenotaphrum secundatum* cv. 'Raleigh'. Production on Johnsongrass was considerably lower than the number and weight of pellets produced on 'Reveille' hybrid bluegrass or on *Cynodon* cvs. 'Tifway' or 'Common', which had similar damage ratings.

After 8 d of feeding, *Zoysia* cv. 'Cavalier' and *Buchloe* cv. 'Prairie' were still significantly the most resistant with the lowest mean number of fecal pellets produced per day (2.05 and 2.78, respectively) (Fig. 2C) and mean weights of 5.78 and 6.77 mg, respectively (Fig. 2D). The number of pellets and their weight were ca. one-half that of the next 2 grasses, *Zoysia* cv. 'Meyer' and *Stenotaphrum* cv. 'Raleigh' with numbers of pellets >5 and weights >12 mg. Johnsongrass continued to be in the midrange of damage with a mean of 5.57 fecal pellets weighing 13.72 mg per day. Isely (1944) also reported Johnsongrass as a good host, especially when it was growing in mixed stands with common sunflower, giant ragweed, and wild lettuce. Tall fescue continued to be significantly the most preferred host with the highest average number of fecal pellets (12.24) and highest weight (31.39 mg) per day over the 8-d feeding period. Regardless of the grass, *M. differentialis* produced more fecal pellets weighing more per day during the first 2 d of feeding than they did daily during the remaining feeding period. This higher level of feeding is probably due to the fact that we starved the grasshoppers for a 72-h period before the initial 2-d feeding period. No literature was found that characterized the preferential feeding behavior of *M. differentialis* for one turfgrass in preference to another.

Landscape Plants

Feeding responses of *M. differentialis* on the 15 landscape plants compared with Johnsongrass are presented in Figs. 3 and 4. The ratings of feeding damage during the first 3 d the grasshoppers were confined on the plant material show that the least visual feeding damage occurred on *Hibiscus*

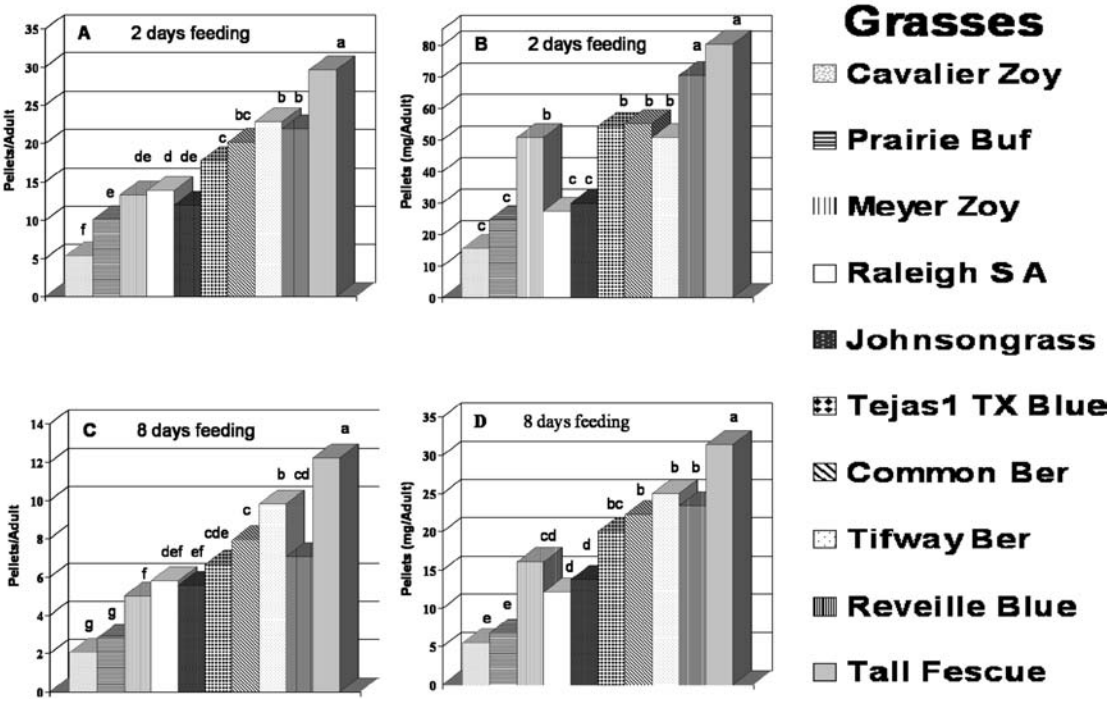

Fig. 2. Number of fecal pellets (A) and their dry weight (B) produced by differential grasshoppers feeding on 9 turfgrass cultivars and Johnsongrass during the first 2-d; Number of fecal pellets (C) and their dry weight (D) after the 8-d feeding period. The order of grass cultivars listed at the right side of the graph corresponds to the bars from left to right. Bars for each plant with the same letters above them are not significantly different by Waller-Duncan k-ratio (k = 100) t test (P = 0.05).

moscheutos cv. 'Flare' (1.96), *Petunia violacea* cv. 'VIP' (2.02), *Phlox paniculata* cv. 'John Fanick' (2.00), *Tecoma stans* cv. 'Gold Star' (2.46), and *Campsis grandiflora* (2.48) (Fig. 3). Conversely, the highest amount of feeding per adult *M. differentialis* occurred on *Plumbago auriculata* cv. 'Hullabaloo' (3.83), *Glandularia hybrida* cv. 'Blue Princess' (3.77), red *Canna* × *generalis* (3.67), Johnsongrass (3.45), and *Cortaderia selloana* cv. 'Pumila' (3.43), with these 5 plants grouped in the top statistical separation. Since *M. differentialis* normally feeds on both grasses and herbs, it was no surprise that Johnsongrass and pampas grass along with several of the landscape plants were among the test plants showing the most feeding damage. When the number and dry weight of fecal pellets per grasshopper for the first 3-d feeding period were examined, *Ipomoea batatas* cv. 'Marguerite', *Bougainvillea* sp., and *Lantana horrida* were also grouped in the same statistical separation of least fed upon plants (nonpreferred) (Fig. 4). Based on these 2 parameters, red *Canna* and *Glandularia* cv. 'Blue Princess' were the most preferred hosts with the highest number of fecal pellets (*Glandularia* = 25.5; *Canna* = 21.75) (Fig. 4A) and the highest fecal pellet weights (*Canna* = 62.0 mg; *Glandularia* = 41.56 mg) (Fig. 4B).

After 14 d of continual feeding, the same 5 cultivars continued to exhibit the least feeding (resistant) based on the number and dry weight of fecal pellets per grasshopper per day of feeding (Fig. 4C and 4D). Red *Canna*, *Glandularia* cv. 'Blue Princess', *Plumbago* cv. 'Hullabaloo', and *Cortaderia* cv. 'Pumila' continued to be among the preferred hosts. The number and weight of fecal pellets produced on red *Canna* during the last 11 d of the trial were significantly reduced compared to the feeding exhibited during the first 3 d. The lower number on red *Canna* can partially be explained by the large amount of fluids present in the *Canna* leaves which caused the grasshoppers to produce very watery fecal pellets that were difficult to distinguish and did not hold together.

Nerium oleander cv. 'Hardy Red' emerged as the most preferred host with nearly 8 fecal pellets (weighing 15 mg) produced per grasshopper per day. Conversely, *M. differentialis* feeding on the 5 aforementioned resistant plants produced fewer than 4 fecal pellets and less than 6 mg of dry pellet weight per day of feeding. The strong feeding

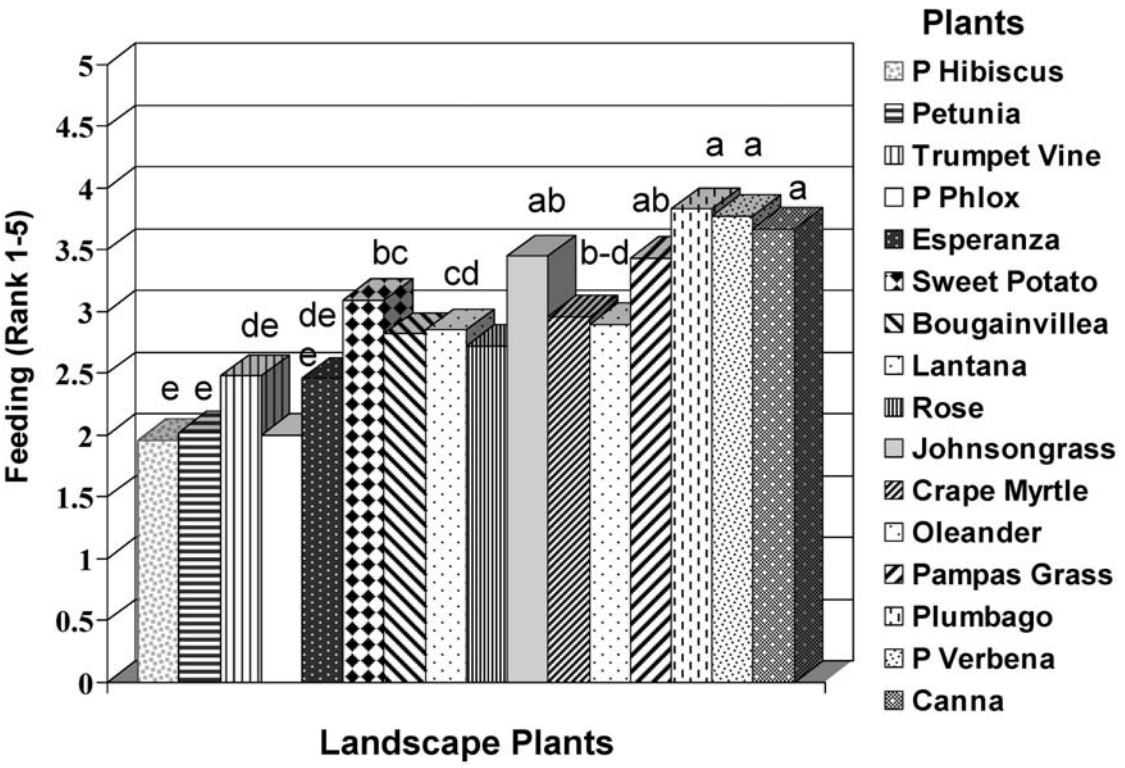

Fig. 3. Feeding damage by differential grasshoppers on 14 landscape cultivars and Johnsongrass during the first 3-d feeding period. Damage was rated on a scale of 1-5; where 1 = little or no feeding, and 5 = near complete consumption of the available plant material. The order of landscape cultivars listed at the right side of the graph corresponds to the bars from left to right. Bars for each plant with the same letters above them are not significantly different by Waller-Duncan k-ratio (k = 100) t test (P = 0.05).

preference by the grasshoppers for *Nerium* was unexpected, because the presence of glucosides in both fresh and dry foliage of *Nerium* makes it extremely toxic to man and animals (Muenscher 1948). This was the first report of the ability to tolerate glucosides expressed by an Orthopteran. The ability of *M. differentialis* to detoxify plant secondary metabolites was first reported by Snyder et al. (1998). He showed that *M. differentialis* can tailor its detoxification enzymes (a variety of microsomal cytochrome P450s and several cytosolic detoxification enzymes) to the profile of secondary metabolites in its diet. All previous work with Orthoptera had dealt with detoxification of synthetic pesticides. This phenomenon had been well documented for several species of Lepidoptera (Berenbaum 1991).

Most previous research showed that several plants in the Asteraceae were good hosts for *M. differentialis* (Howard 1995; Isely 1944; Kaufmann 1968; Lewis 1984). However, these previous works provide little insight as to which other families of herbaceous plants that was tested in this experiment (Table 1) would serve as hosts for this grasshopper. This experiment shows that *M. differentialis* will feed on a wide range of herbaceous plants from a diverse group of plant families.

The differential grasshopper is a significant pest of several field crops but it also causes significant economic damage in urban/suburban landscapes and in plant nurseries. This paper characterizes the level of damage for a select group of turfgrasses and landscape plants commonly used in Southern landscapes. Knowing which plants are most susceptible to damage should be useful information for home owners and managers of parks and other public and private grounds to aid them in choosing plants that are less likely to be damaged. This knowledge can be especially important to nursery plant growers and for wholesale and retail nurseries to more closely monitor certain plant species that are more subject to damage, or to simply avoid handling these species; especially during *M. differentialis* outbreak years. This type of information is necessary for the development of comprehensive IPM programs for urban landscapes and plant nurseries.

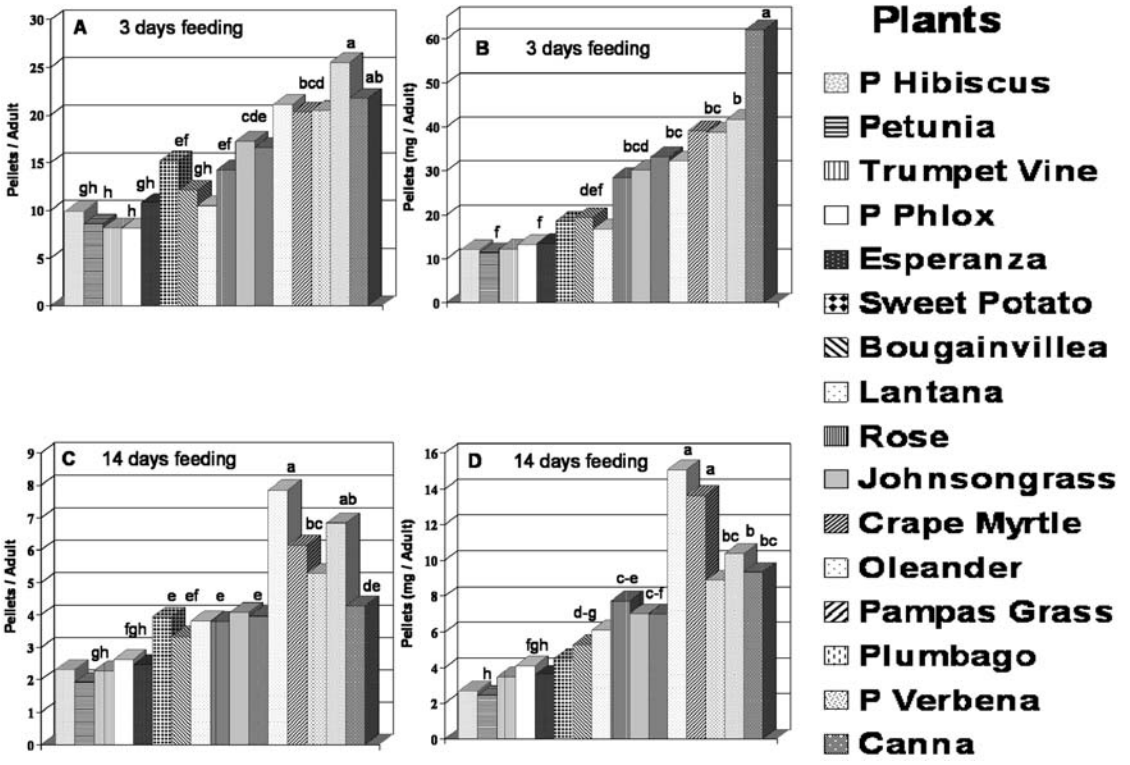

Fig. 4: Number of fecal pellets (A) and their dry weight (B) produced by differential grasshoppers feeding on 14 landscape cultivars and Johnsongrass during the first 3-d; Number of fecal pellets (C) and their dry weight (D) after the 14-d feeding period. The order of landscape cultivars listed at the right side of the graph corresponds to the bars from left to right. Bars for each plant with the same letters above them are not significantly different by Waller-Duncan k-ratio (k = 100) t test (P = 0.05).

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EFFICACY OF SINGLE AND DUAL GENE COTTON *GOSSYPIUM HIRSUTUM* EVENTS ON NEONATE AND THIRD INSTAR FALL ARMYWORM, *SPODOPTERA FRUGIPERDA* DEVELOPMENT BASED ON TISSUE AND MERIDIC DIET ASSAYS[1]

J. Scott Armstrong, John J. Adamczyk, Jr. and Shoil M. Greenberg
USDA–ARS, Beneficial Insect Research Unit, Kika de la Garza Subtropical Agricultural Research Center, Weslaco, TX 78596

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Abstract

We evaluated mortality and developmental parameters of fall armyworms, *Spodoptera frugiperda* (J. E. Smith), to the single *Bacillus thuringiensis (Bt) cotton trait,* Bollgard® and dual Bt cotton traits (Bollgard II® and WideStrike™) by using a cotton leaf-tissue assay and by incorporating lyophilized cotton tissue into a meridic diet. Bioassays were conducted for both neonate and 3rd instars. Leaf tissue bioassays indicated that Bollgard II® and WideStrike™ are highly effective against fall armyworm neonates by causing mortality and by retarding development parameters such as larval weight, pupal duration, and time to adulthood. Bollgard® was not significantly different from non-transgenic cotton in terms of mortality or feeding, with the exception of the non–Bt (PhytoGen 425RF), which had an inherent form of resistance that is not associated with a transgenic event. Third instars evaluated with lyophilized diet bioassays were not as affected by the Bt traits to the same degree as neonates; however, larval weights were lower, and developmental parameters such as time to pupation and time to adulthood were longer. The duration of pupal development was significantly longer for 3rd instars that survived the highest dose of 5,000 µg of WideStrike™ cotton tissue. Sublethal doses for Bollgard II® and WideStrike™ were generally observed at 500 to 5,000 µg of lyophilized cotton tissue per mg of meridic diet, depending upon the variable (time to pupation, pupal duration, time to adult emergence) measured.

Key Words: *Bacillus thuringiensis,* Cry1Ac, Cry2Ab, Cry1F + Cry1Ac, GMO, PIP, transgenic

Resumen

Evaluamos los parámetros de mortalidad y desarrollo del gusano cogollero, *Spodoptera frugiperda* (J. E. Smith), hacía una sola cepa algodonera de *Bacillus thuringiensis (Bt),* Bollgard® y una cepa algodoner dobre de Bt (Bollgard II® y WideStrike™) usando tejido de las hojas de algodón e incorporando tejido de algodón liofilizado en una dieta méridica. Se realizaron bioensayos en larvas recién nacidas y del tercer instar. Los bioensayos con tejidos de hojas indican que Bollgard II® y WideStrike™ son muy efectivos contra las larvas recién nacidas de cogollero al causar mortalidad y por demorar los parámetros de desarrollo como el peso de las larvas, la duración de la etapa de la pupa y el tiempo de llegar a la etapa del adulto. Bollgard® no fue significativamente diferente que el algodón no transgénico en terminos de la mortalidad y alimentación, con la excepción de (PhytoGen 425RF) sin Bt, que tenia una forma natural de resistencia que no fue asociada con un evento transgénico. Los instares de tercer estadio que fueron evaluados con bioensayos de dietas liofilizados no fueron afectadas por las características de Bt al mismo grado que las larvas recién nacidas; sin embargo, el peso de las larvas fue menor, y los parámetros de desarrollo como el tiempo de la pupación y el tiempo para llegar al estado del adulto fueron mas largos. La duracióon del desarrollo de las pupas fue significativamente mas larga para los instares de tercer estadio que sobrevivieron la dosis mas alta de 5,000 µG de WideStrike™ del tejido de algodón. Las dosis subletales para Bollgard II® y WideStrike™ fueron observadas generalmente a los 500 to 5,000 µg de tejido de algodon liofilizad por mg de dieta méridica, dependiendo sobre las variables medidas (tiempo de pupación, duración del estadio pupal, tiempo del emergencia del adulto).

Translation provided by the authors.

The fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith), has increased in importance as a pest of cotton, *Gossypium hirsutum* (L.), and other crops in the southern cotton belt over the last 5 years (Leonard et al. 2006). However, the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), remain important heliothine pests that cause the

most damage and yield loss throughout the cotton belt (Williams 2009). Fall armyworms do not diapause or overwinter in temperate climates (Luginbill 1928; Pair et al. 1986; Raulston et al. 1986; Pair et al. 1991), but populations survive the winter in northern Mexico and southern Texas, Florida where they build on corn (*Zea mays* L.). Subsequent generations migrate north as the season progresses (Pair et al. 1991). Because of the transient nature and unpredictable infestations of FAW in cotton, it has been difficult to develop management strategies and tools for control. Insecticides have been used almost exclusively for FAW control in cotton with marginal, or below acceptable results. Both insecticide resistance (Yu et al. 2003) and the inherent disruption of beneficial insects can occur depending upon the insecticide mode of action. Foliar insecticides applied to young larval infestations are much more efficacious as opposed to later instars (Mink & Luttrell 1989). Larger, mature larvae are harder to control with insecticides because of their inherent behavior of being lower in the cotton canopy and because they burrow into cotton fruit (Ali et al. 1990), reducing exposure to insecticides.

More recent technology for control of lepidopterous pests has been the development of transgenic cotton varieties with *Bacillus thuringiensis* Berliner subsp. kurstaki (Bt), Bollgard®, which codes for δ-endotoxin Cry1Ac endotoxin protein; Bollgard II® which encodes δ-endotoxin Cry1Ac + Cry2Ab proteins; and WideStrike™ which encodes δ-endotoxin Cry1F + Cry1Ac endotoxins. Bollgard® cotton does not provide adequate mortality to FAW but does inhibit feeding and larval development (Adamczyk et al. 1998; Stewart et al. 2001). Improvements in mortality and subsequent control were made with the release of Bollgard II® in commercial cotton varieties (Stewart et al. 2001; Chitkowski et al. 2003). However, the newer transgenic cotton lines with WideStrike™ technology are currently being evaluated for FAW control in cotton. Even though the Rio Grande Valley of Texas has been documented as the corridor for FAW migration to the central U.S. (Pair et al. 1991), transgenic cotton varieties with Bt technology have just recently been adopted by producers from the southernmost growing regions in Texas. A survey by Cattaneo (2006) reports that of the 633,792 ha of cotton planted in the Rio Grande Valley in 2006, 2.6% had Bollgard® technology, while 2.4% had Bollgard II® technology. Our goal in this research was to evaluate the efficacy and sublethal effects of available Bt technologies on FAW, including the more recently released WideStrike™. The dual-trait technologies may provide a more ecologically sound option for control of FAW, especially during active boll weevil eradication where natural enemies may be negatively impacted by the repeated applications of ultra low volume malathion.

Materials and Methods

Cotton Leaf Tissue Bioassays

Cotton varieties, FiberMax 800 BGRR (Bollgard®), FiberMax 800 BGIIRR (Bollgard II®), PhytoGen 425RF (non-BT), and PhytoGen 485 WRF (WideStrike™) were planted in 8.5-L pots with Sunshine® potting mix in the greenhouse on 29 Sep 2006. When the plants were 80 d of age, fully expanded cotton leaves from nodes 6-8 from were removed and placed in 15-cm diameter Petri dishes lined with Whatman® (Buffalo, NY) filter paper. A few drops of water were added to each dish to maintain the cotton leaves and humidity.

Fall armyworms used in the assay originated from larvae collected from sudan-sorghum (*Sorghum* spp. hybrid) on the USDA-ARS research farm near Delta Lake, Hidalgo, Co., TX, in Jun 2006 and were determined to be the "corn" host strain by R. Nagoshi, USDA-CMAVE, Gainesville, FL. The larvae were reared to adults in an environmental growth chamber maintained at 28.5°C, 65% RH, and a 14:10 h (L:D) photophase on artificial diet (King & Hartley 1985) and were the F6 generation at the time of these assays.

For each cotton variety, there were 10 replicates of a cotton leaf in a Petri-dish, with 5 neonates placed on each leaf. Survivorship/mortality was determined 4, 7 and 10 d after infestation. On the 10th d of the trial, surviving larvae were weighed. The same procedures were used evaluating 3rd instars, with the exception that only 1 larva was placed in each Petri dish to prevent cannibalism, and 3 replicates of 10 larvae were used for each trait. Cotton leaf tissue was collected again from nodes 6-8 to replace the originals after 4 d because >50% of the leaf tissue was consumed in the control.

Percentage mortality for neonates was analyzed with the GLIMMIX procedure of Mixed model analysis; degrees of freedom were calculated by the Satterthwaite method (SAS, 2003, version 9.2, SAS Institute Inc., Cary, NC), and means were separated with LS MEANS ($\alpha = 0.05$) option. Percentage mortality for 3rd instars fed was analyzed by the PROC FREQ procedure (SAS Institute), with differences in percentage mortality determined by the Chi-square test because of the wide range (0% to 100%) of mortality from the bioassays. The relative frequency of Log10 3rd instar weights were plotted for comparisons.

Meridic Diet Bioassays

Leaf tissue from the same cotton varieties used for the cotton leaf tissue bioassays described above were incorporated into a meridic diet. A minimum of 50 g of whole cotton leaf tissue from node 6-8 of the plant were collected on 29 Oct 2006, placed in paper bags, and stored at -80°C.

The tissue was then lyophilized (Freezemobile, The Virtis Company Inc. Gardiner, NY) and ground through a 20-mesh screen in a Thomas-Wiley Mill (Thomas Scientific, Swedesboro, NJ) on 6 Dec 2006. On 20 Mar 2007, 3 L of a soy-flour and wheat-germ meridic diet (King & Hartley 1985) were prepared at the USDA-ARS insectary, Weslaco, TX and mixed with lyophilized cotton tissue from each variety of non-Bt cotton (FiberMax 800RR and PhytoGen 425RF) and Bt cotton (FiberMax 800BG (Bollgard®), FiberMax 800BGII® (Bollgard II®), PhytoGen 485WRF (WideStrike™) at dosages of 5, 50, 500, and 5000 µg of tissue for each mg of diet. The powdered lyophilized tissue from the cotton varieties was mixed into warm diet with a high speed blender. Diet cups (15 mL, Anderson Tool and Die, Linden, NJ) were filled with 10 mL of warm diet and allowed to cool before placing a single neonate in each of 30 cups for each of the 4 dosages.

Two separate bioassays were conducted for neonates and 3rd instars placed in the diet cups with paper lids. A single neonate was placed in a single diet cup containing 0, 5, 50, 500, and 5000 µg of tissue for 30 replicates of each treatment. Mortality was evaluated at 4, 7, 10, and 14 d from the time larvae were placed on the diet. The larvae were then observed every 24 h for pupation. Pupae were weighed and placed in individual diet cups and the day of adult emergence was recorded. The same procedures were followed for 3rd instars with the exception that larvae were weighed 10 d from the time of being placed on diet. Mortality data were analyzed by the PROC FREQ procedure (SAS 2003) and compared with the Chi-square Likelihood ratio test ($\alpha = 0.05$). Larval weights, time to pupation, and time to adult emergence in days were transformed to the log Poisson distribution because of skewness. Following transformation, the data were analyzed by GLIMMIX mixed model analysis with residuals estimated by the PL method for fitting the data to the linear model. Cotton trait and dose of lyophilized tissue means were estimated with the LS means statement and adjusted and separated by Tukey's ($\alpha = 0.05$) test for determining significance. Significant interactions for cotton trait and dosage of lyophilized tissue were further examined with the SLICE option of the LS MEANS statement in which cotton trait by dosage sliced by cotton trait and the cotton trait by dosage sliced by dosage were examined.

Results

Cotton Leaf Tissue Bioassays

Larval mortality of FAW neonates evaluated on cotton leaf tissue was significantly different at 4 ($F = 24.2$; $df = 4, 45$; $P < 0.001$); 7 ($F = 25.5$; $df = 4, 45$; $P < 0.001$) and 10 d ($F = 28.6$; $df = 4, 45$; $P < 0.001$) (Fig. 1). Bollgard II® and WideStrike® caused near 80% mortality at 4 d and increased to >95% mortality by 10 d. The Bollgard® trait was less effective in killing neonates, and was not significantly different from both non-Bt cottons at 4, 7, and 10 d.

Third instars were highly susceptible to WideStrike® where 100% mortality occurred by 7 and 10 d (Fig. 2). Bollgard II® was responsible for 35% mortality at 7 d and 50% mortality by 10 d, and this mortality was significantly higher than 10 d mortality for larvae reared on non-Bt cotton (FM 800RR).

The relative frequency (log10) of 3rd instars weighed at 10 d showed a distinct distribution of weights for the survivors (Fig. 3). The weights of larvae reared on Bollgard II® were significantly lower and skewed to the left (Fig. 3). The heavier weights of those reared on Bollgard® were more centrally distributed, and non-Bt weights were skewed to the right, averaging 160 mg more than the Bollgard II®, and 120 mg more than the Bollgard® trait respectively. This is an indication that leaf consumption and feeding on Bollgard II® may occur, but the toxins reduce growth and development.

Meridic Diet Bioassays

Mortality for neonate FAW placed on meridic diet was significantly higher for WideStrike™ with mean mortality of 25% for the 500 µg of tissue (Likelihood ratio Chi-square = 39.73; $df = 4$; $P < 0.0001$) and 47% for the 5000 µg of tissue incorporated into diet (Likelihood ratio Chi-square = 145.53.73; $df = 4$; $P < 0.0001$). Mortality for all other entries, including Bollgard II®, was less than 11%. Larval weights for FAW neonates measured after 14 d of feeding were significantly affected by cotton trait ($F = 25.89$; $df = 4, 285$; $P < 0.0001$), dosage of lyophilized tissue ($F = 10.56$; $df = 3, 285$; $P < 0.0001$) and the interaction of cotton trait by dosage ($F = 8.42$; $df = 12, 285$; $P < 0.0001$) for surviving larvae (Fig. 4). At the lower dosages of 5 and 50 µg, feeding on the non-Bt PHY 425RF suppressed weights of larvae resulting in no significant differences from the Bollgard II® or WideStrike™ traits. At 500 and 5000 µg of incorporated diet, larvae feeding on Bollgard II and WideStrike were significantly smaller ($F = 18.32$; $df = 2, 285$; $P < 0.0001$) than those feeding on non-Bt (PhytoGen 425RF). The Bollgard® trait did not significantly reduce larval weights for those at 5 to 500 µg; however at 5000 µg, weights were significantly lower ($F = 12.32$; $df = 2, 285$; $P < 0.0001$) than the non-Bt cottons, but significantly higher ($F = 9.32$; $df = 2, 285$; $P < 0.001$) than the weights for the Bollgard II® or WideStrike™ traits. The non-BT PhytoGen 425RF appears to have some inherent form

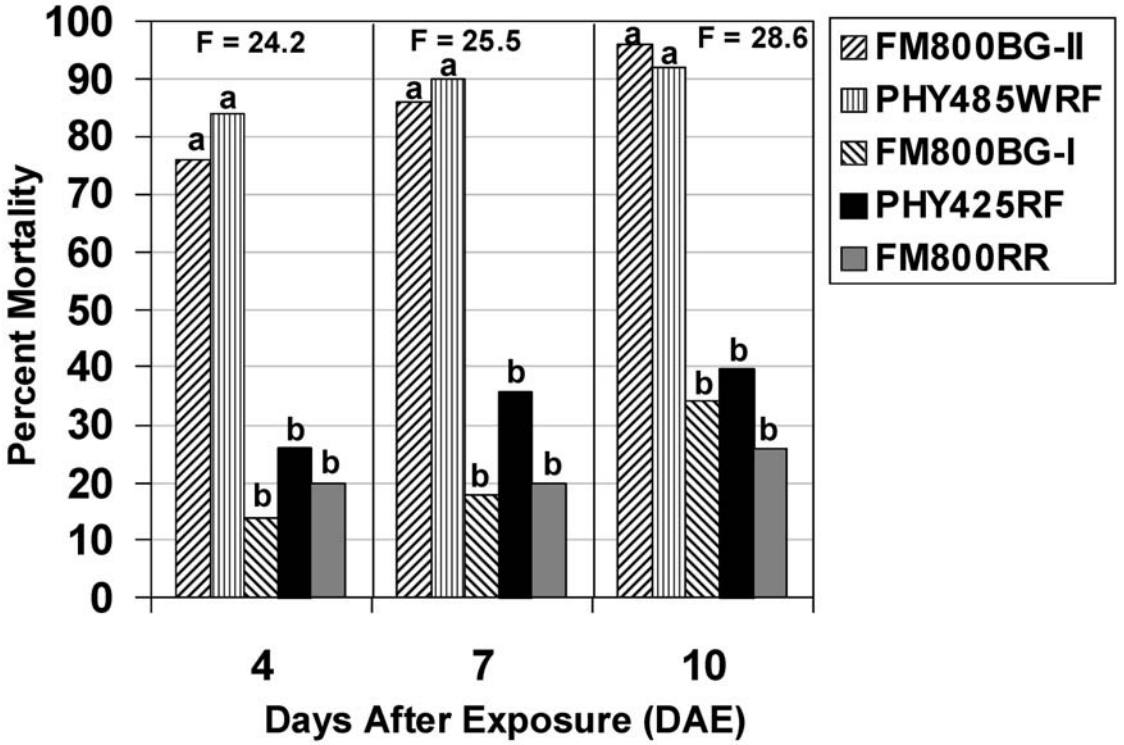

Fig. 1. Larval mortality of fall armyworms placed in Petri dishes as neonates and evaluated for mortality after feeding on Bollgard® (FM 800BGRR), Bollgard II® (FM 800BGII®), WideStrike™ (PHY 485WRF), non-Bt PHY 425RF and non-Bt FM 800RR cotton leaves removed from nodes 6-8.

of resistance that has not been identified, but the detrimental effects of the endotoxins of Bollgard II® and WideStrike™ surpass whatever the effects of the unknown resistance are at the 500 and 5000 µg dosages. The test effects for cotton trait by dosage, sliced by dosage, were significant for 5 µg (F = 3.20; df = 4, 285; P = 0.0137), 50 µg (F = 8.52; df = 4, 285; $P < 0.0001$), 500 µg (F = 12.05; df = 4, 285; $P < 0.0001$) and 5,000 µg (F = 25.78; df = 4, 285; $P < 0.0001$) as were the slice effects of cotton trait by dose, sliced by trait for Bollgard® (F = 3.05; df = 3, 285; P = 0.0288); Bollgard II®, (F = 21.62; df = 3, 285; $P < 0.0001$); PHY 425RF, (F = 3.71; df = 3, 285; P = 0.0121); and WideStrike™ (F = 21.62; df = 3, 285; $P <$ 0.0001), but what we considered one of the non-Bt controls (FM 800RR) in this study was not significant (F = 0.45; df = 3, 285; P = 0.726) when cotton trait by dose was sliced by cotton trait, indicating that dosage of lyophilized tissue containing not Bt trait had no affect on larval growth and development based on larval weights.

There was a significant response in pupal duration for the neonates based on trait (F = 9.34; df = 4, 285; $P < 0.0001$), dosage of trait (F = 2.82; df = 3, 285; P = 0.0394) and for the cotton by dosage interaction (F = 2.88; df = 12, 285; P = 0.0009). The trend in significance was that with increasing dosage of trait, especially the Bollgard II® and WideStrike™. Mean development time for pupae increased from 5 to 8 d when compared to the non Bt FM 800RR and PhytoGen 425RF, respectively, (Fig. 5). The test effects for cotton variety by dose, sliced by dose for pupal duration were not significant for any of the cotton varieties at 5 µg, but all other doses (50 µg, F = 4.56, df = 4, 285, P = 0.0106; 500 µg, F = 4.56; df = 4, 285; P = 0.0014; and 5000 µg, F = 8.94; df = 4, 285; $P <$ 0.0001) extended time to pupation (Fig. 5).

The time in days for neonates to develop to adulthood followed a similar pattern as time in pupation (Fig. 6); however, only the main effect of cotton variety (F = 2.57; df = 4, 130; P = 0.0410) was statistically significant, whereas dosage (df = 3, 130; F = 0.44; P = 0.7271) and cotton by dosage (F = 0.91; df = 12, 130; P = 0.5364) were not. The main effects for the 10-d weights of 3rd instars reared on meridic diet were significant for trait (F = 5.30; df = 4, 162; P = 0.0005); dosage of tissue incorporated into diet (F = 4.89; df = 3,162; P = 0.0028) and the

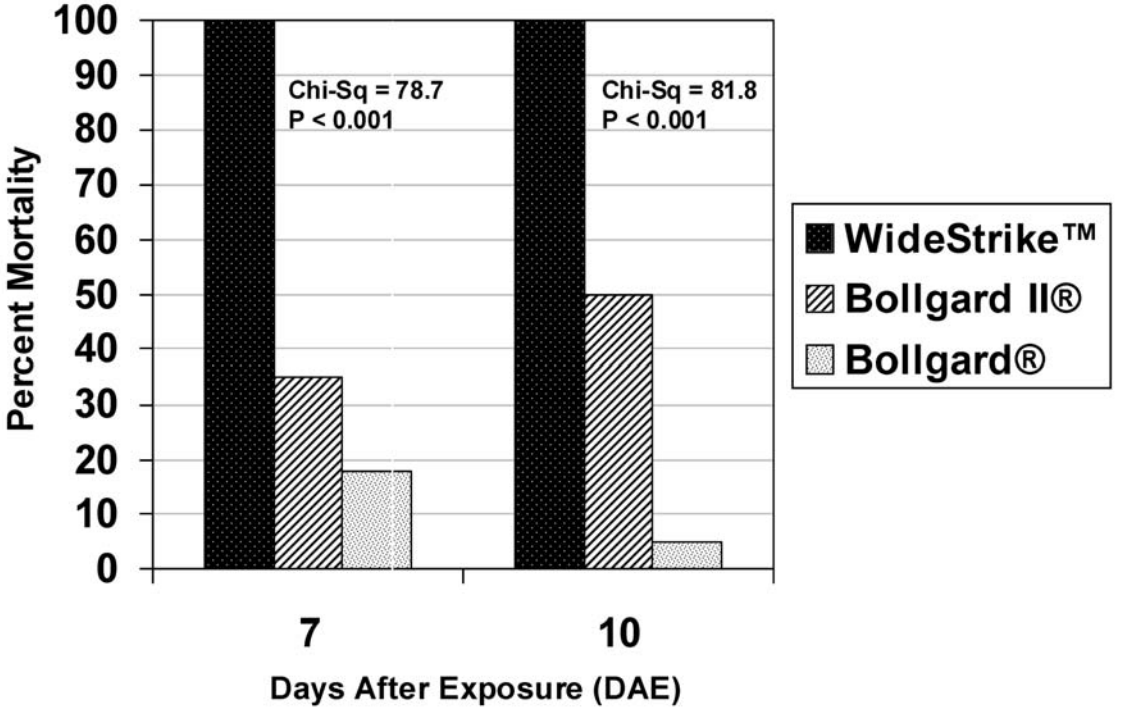

Fig. 2. Larval mortality of 3rd instar fall armyworms at 7 and 10 d after feeding on Bollgard II® (FM 800BGRR), Bollgard II® (FM 800BGII®), and WideStrike™ (PhytoGen 485WRF) cotton leaves removed from nodes 6-8. The non-Bt variety FM 800RR is not shown, and mortality was ≤5%.

cotton trait by dose (F = 2.82; df = 12,162; P = 0.0015) interaction (Fig. 7). Interestingly, the slice effects of cotton by dose, sliced by dose, were only significant for 5000 µg (F = 10.01; df = 4, 162; P < 0.0001), but the cotton trait by dose, sliced by dose was significant for Bollgard II (FM 800BG®II) (F = 4.36; df = 3, 162; P = 0.0055) and WideStrike (PHY 485WRF®) (F = 8.16; df = 3, 162; P < 0.0001). Third instars developed well on the non-Bt (PHY 425RF) incorporated diet based on larval weights, which were significantly higher than all other varieties at the dosages of 500 and 5000 µg, especially when compared to the neonates (Fig. 4). The undetermined form of inherent resistance exhibited in PHY 425RF for neonates does not appear to affect the older larvae.

There were so few 3rd instars that succumbed in the meridic diet study that no data analyses could be conducted. There was no mortality across all treatments at d 4, a total of 4 dead (1.9%) on d 7, and 16 dead (7.6%) after 10 d. The models for the main effects of cotton trait, dosage, and their interactions were not significant for time to pupation, pupal weights, and time to adult emergence for 3rd instars reared on the meridic diet containing lyophilized cotton tissue (Fig. 8). The main effects of cotton trait was significant (F = 3.21; df = 4; 102; P = 0.0159) for the number of days required for pupal development. Fall armyworm reared on the WideStrike™ took on average 5 d longer across all doses when compared to all other cotton types. However the dose or the trait by dose interaction was not significant.

Discussion

Fall armyworm infestations are sporadic, unpredictable, and hard to control in cotton, even with improvements and changes in the expression *Bacillus thuringiensis* endotoxins. It is apparent that one of the more significant improvements in controlling FAW comes from the development of the combination Cry1Ab and Cry1F expression in cotton varieties. In these assays, WideStrike™ provided the most significant effects on FAW mortality and development in terms of larval weights, development times, pupal weights, and successful development to adulthood.

Although we did not quantify the Cry proteins used in the leaf tissue and meridic diet assays,

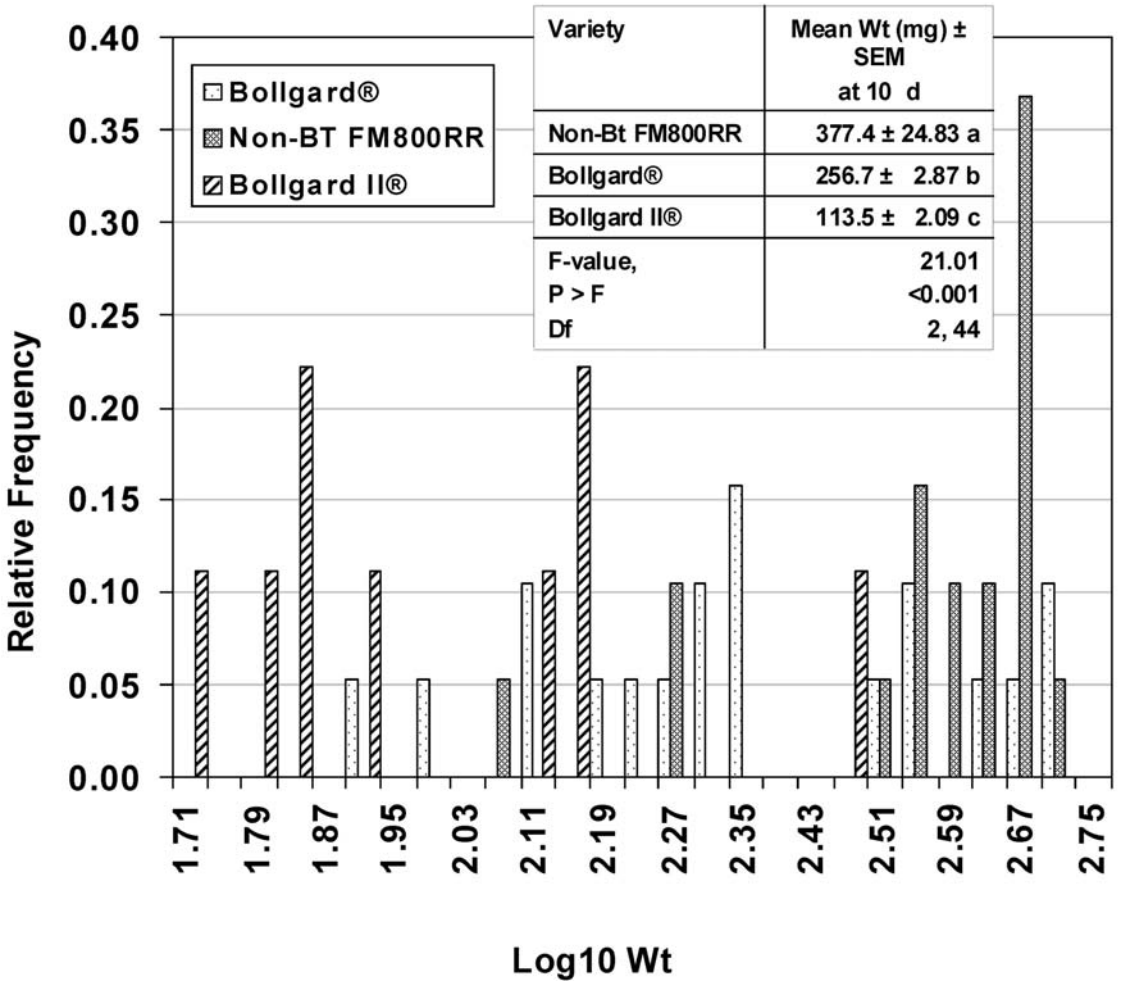

Fig. 3. Relative frequencies and mean live-weights of 3rd instar fall armyworm survivors after 10 d of feeding on non-Bt FM 800RR, Bollgard® (FM 800BG), and Bollgard II® (FM 800BG II) cotton leaves. There were no survivors in the WideStrike™ (PHY 485WRF).

the results give us some summary observations that are of interest when comparing fresh tissue assays with lyophilized tissue incorporated into meridic diet. Fresh cotton tissue from the middle nodes containing tissue from the Bollgard® II and Widestrike® traits can provide close to or above 80% mortality to neonates following 4 d of exposure to leaf-tissue, and near 100% mortality at 7 and 10 d days after exposure. However, when 3rd instars were used in the assays, the WideStrike™ Cry1F combined with the Cry2Ab provides a lethal combination from the fresh leaf tissue, and significantly separates itself from all other technology by providing 100% mortality 7 and 10 d after exposure. Other studies specific to the single and dual traits for Bt for FAW control have reported similar results with middle to upper leaf tissue (Siebert et al. 2008; Adamczyk et al. 2008); however, it has been more recently established that Cry1F is expressed in higher amounts (>85 ppm) collected from the 6th week of flowering from mature leaf tissue collected from the 8th node (Siebert et al. 2009). In addition, the levels of Cry1F were generally higher in all parts of the cotton plant with the exception of flowers. The fact that Cry1F expression increases in older leaf tissue could be advantageous from the standpoint that egg masses are oviposited on the leaves and neonates feed on these leaves before moving to the fruiting structures (Ali et al. 1990).

By comparison, the meridic diet assays as a general rule approached a lethal status for neonates between 500 to 5000 µg of leaf tissue in µg/mL of diet for Bollgard II® and Widestrike in

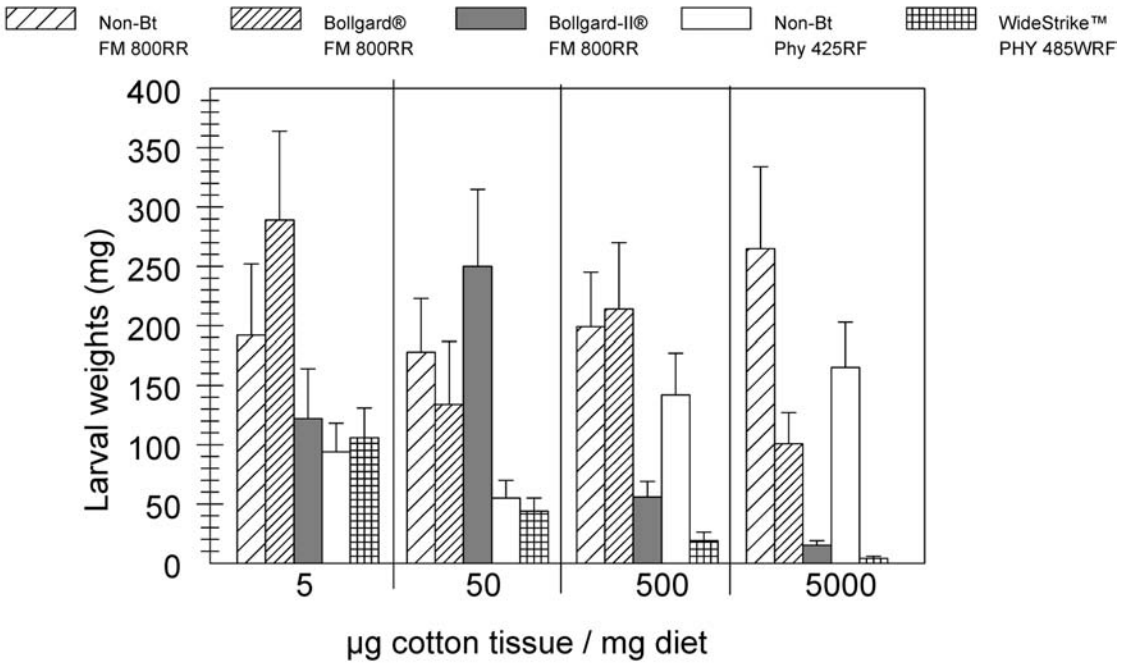

Fig. 4. Larval weights of fall armyworms after being reared from neonates on meridic diet incorporated with lyophilized cotton tissue for 14 d.

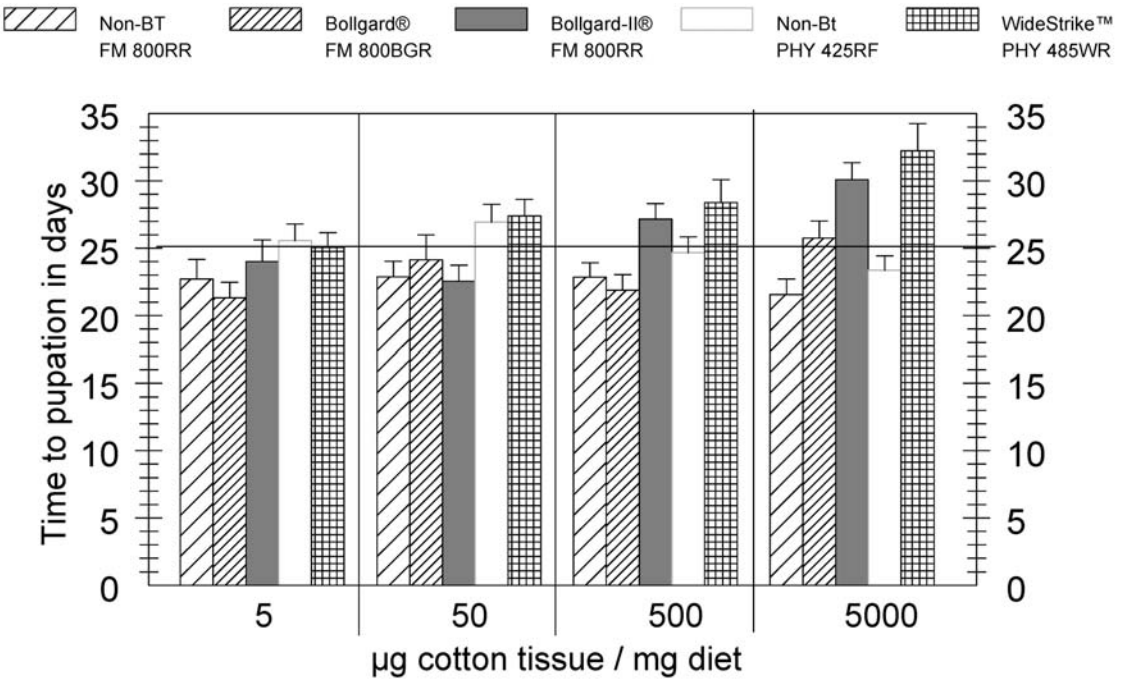

Fig. 5. Time in days to pupation for fall armyworm larvae reared from neonates on lyophilized cotton tissue incorporated into meridic diet for non-Bt FM 800RR, Bollgard® (FM 800BGRR), Bollgard-II®FM 800BGII, non-Bt PHY 425RF, and WideStrike™ (PHY 485WRF). The horizontal line across all doses and varieties represents the overall mean in pupal duration (d) for the assay.

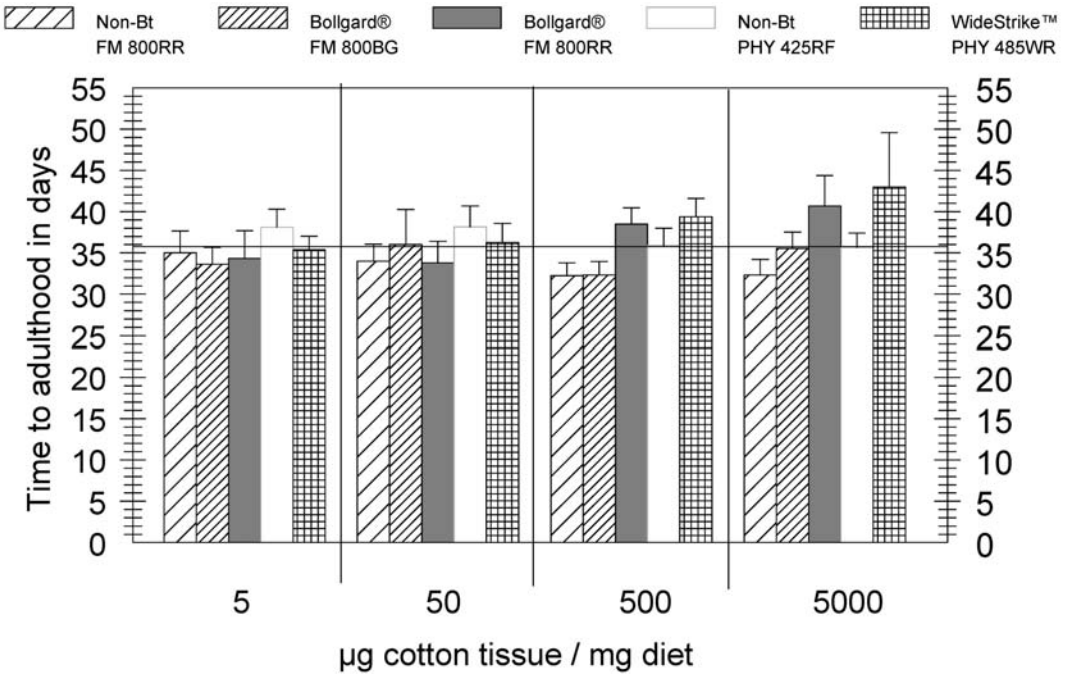

Fig. 6. Time in days to adulthood for fall armyworm larvae that were reared from neonates on lyophilized cotton tissue incorporated into meridic diet for non-Bt FM 800RR, Bollgard® (FM 800 BGRR), Bollgard-II® FM 800BGII, non-Bt PhytoGen 425RF, and WideStrike™ (PHY 485WRF).

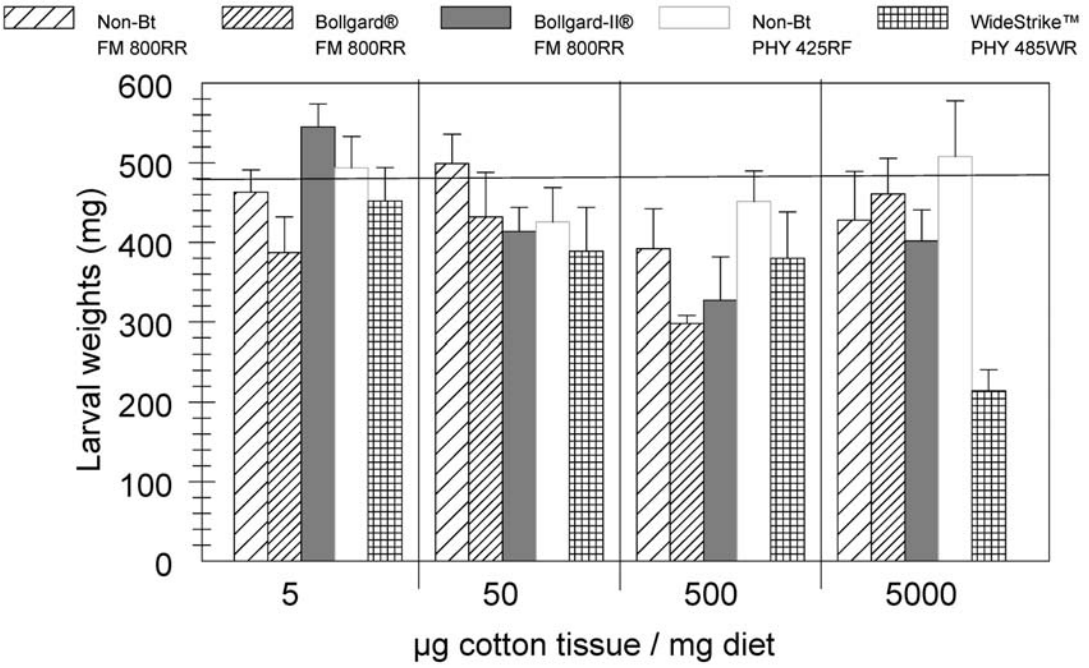

Fig. 7. Fall armyworm larval weights following 10 d of being reared on meridic diet incorporated with lyophilized cotton tissue containing non-Bt FM 800RR, Bollgard® (FM 800 BGRR), Bollgard-II® FM 800BGII, non-Bt PhytoGen 425RF, and WideStrike™ (PHY 485WRF) with the overall mean represented by the horizontal dashed line. Larvae were reared to 3rd instar before being placed on diet.

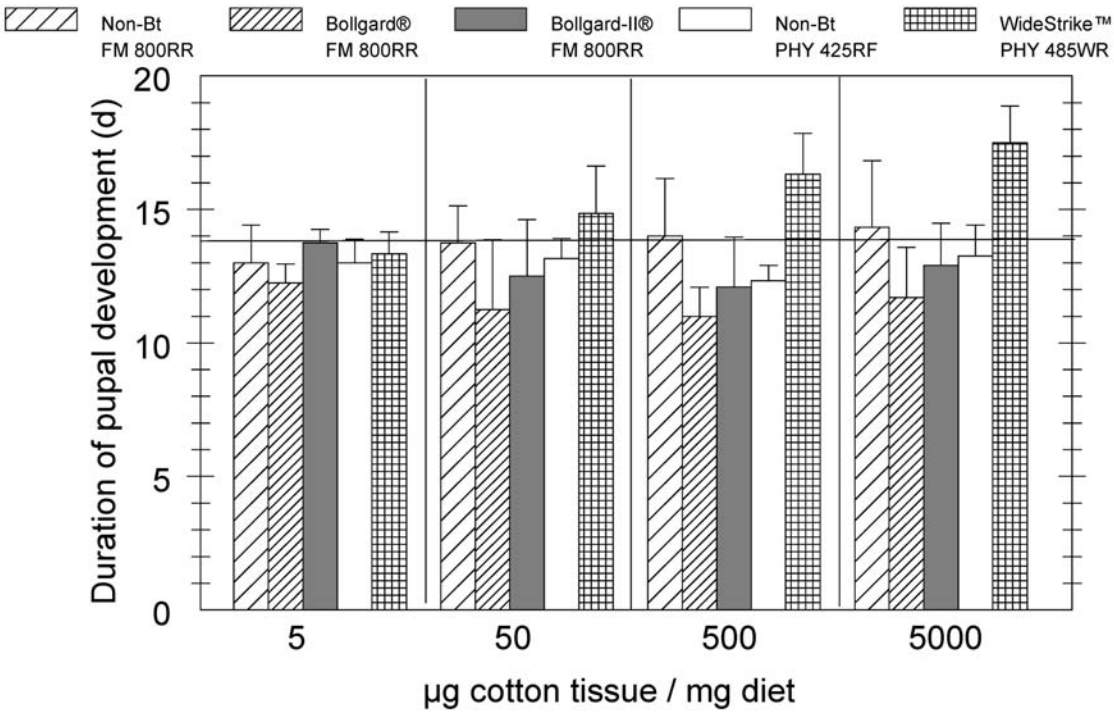

Fig. 8. Fall armyworm pupal duration in days for 3rd instar reared on meridic diet incorporated with lyophilized cotton tissue containing non-Bt FM 800RR, Bollgard® (FM 800BGRR), Bollgard-II® FM 800BGII, non-Bt PhytoGen 425RF, and WideStrike™ (PHY 485WRF) with the overall mean represented by the horizontal dashed line.

terms of larval weights, duration to pupation in d, and time in d to adulthood for the neonates. The effects of the traits and dosages of traits on FAW reared to 3rd instar before exposing them on the meridic diet was much less apparent than that of the neonates. Older larvae have been noted to be harder to kill with different Bt traits (Stewart et al. 2001) and this is good information to have, but infestations will more than likely originate from adults moving into the field, where young larvae feeding on leaf tissue should be exposed to older or younger cotton tissue depending upon the time of infestation.

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LABORATORY TOXICITY AND FIELD EFFICACY OF SELECTED INSECTICIDES AGAINST FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE)[1]

JARROD T. HARDKE[2], JOSHUA H. TEMPLE[2], B. ROGERS LEONARD[2] AND RYAN E. JACKSON[3]
[2]LSU AgCenter, Louisiana State University, Baton Rouge, LA

[3]USDA-ARS, Southern Insect Management Research Unit (SIMRU), Stoneville, MS

[1]Presented verbally at The Armyworm Symposium held in conjunction with the Entomological Society of America Southeastern Branch Meeting, March 6-10, 2010 in Atlanta.

ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is an occasional but often serious pest of several row crops in the southern U.S., including cotton, field corn, and grain sorghum. The objective of these studies was to generate baseline dose-mortality responses for fall armyworm larvae in laboratory bioassays, to confirm field efficacy against natural infestations, and to determine residual efficacy of selected insecticides. These studies evaluated 4 recently developed insecticides (chlorantraniliprole, cyantraniliprole, flubendiamide, and spinetoram) and 5 commercial standards (indoxacarb, lambda-cyhalothrin, methoxyfenozide, novaluron, and spinosad). In diet-incorporated assays, the LC_{50} values of chlorantranilprole and spinetoram were significantly lower than the LC_{50}'s of all other insecticides. The results of a field trial against a native fall armyworm infestation in grain sorghum indicated that chlorantraniliprole reduced the number of infested whorls below that in the non-treated control and the lambda-cyhalothrin- and methoxyfenozide-treated plots at 3 d after treatment (DAT). At 7 DAT, no insecticides significantly reduced the number of infested whorls below that in the non-treated plots. In residual efficacy studies, exposure of fall armyworm larvae to chlorantraniliprole- and cyantraniliprole-treated tissue resulted in significantly greater mortality compared to those exposed to non-treated tissue and lambda-cyhalothrin-, flubendiamide-, novaluron-, and methoxyfenozide-treated tissues at 7 DAT. In addition, chlorantraniliprole and cyantraniliprole were the only compounds that resulted in >40% mortality at 28 DAT. These results indicate that newer insecticides are equal to or more efficacious against fall armyworm than traditional insecticides.

Key Words: *Spodoptera frugiperda*, dose-mortality responses, chemical control, IPM

RESUMEN

El gusano cogolllero, *Spodoptera frugiperda* (J. E. Smith), es una plaga ocasional pero a menudo seria en varios cultivos de surcos en el sur de los Estados Unidos, incluyendo algodón, maíz de campo y sorgo de grano. El objectivo de estos estudios fue para generar una linea basal de respuestas a las dosis mortales para el gusano cogollero en bioensayos del laboratorio, para confirmar la eficacia en el campo contra infestaciones naturales, y para determinar la eficacia de residuos de insecticidas seleccionados. Estos estudios evaluaron 4 de los insecticidas recién desarrollados (chlorantraniliprole, cyantraniliprole, flubendiamide y spinetoram) y 5 productos comerciales estandares (indoxacarb, lambda-cyhalothrin, methoxyfenozide, novaluron, y spinosad). En ensayos de dietas incorporadas, los valores de CL_{50} de chlorantranilprole y spinetoram fueron significativamente más bajos que los CL_{50} de los otros insecticidas. Los resultados de las pruebas de campo contra una infestación nativa del gusano cogollero en sorgo de grano indicaron que chlorantraniliprole redujó el número de los cogollos infestados y fue mas bajo que en las parcelas de control no-tratadas y tratadas con lambda-cyhalothrin- y methoxyfenozide a los 3 dias despues del tratamiento (con sus siglas en inglés - DAT). A los 7 DAT, ninguno de los insecticidas redujeron significativamente el número de cogollos infestados más bajo que en las parcelas no-tratadas. En estudios de la eficacia de residuo, larvas de gusano cogollero expuestos al tejido tratado con chlorantraniliprole y cyantraniliprole resultaron en una mortalidad significativamente mas alta comparada con tejidos no tratados y tejidos tratados con lambda-cyhalothrin, flubendiamide, novaluron, y methoxyfenozide a los 7 DAT. Además, el chlorantraniliprole y cyantraniliprole fueron los unicos compuestos que resultaron en >40% mortalitdad a los 28 DAT. Estos resultados indican que los insecticidas más nuevos son iguales o más eficaces contra el gusano cogollero que los insecticidas tradicionales.

Translation provided by the authors.

The fall armyworm, *Spodoptera frugiperda* (J. E. Smith), is an occasional, but serious pest of cotton, *Gossypium hirsutum* (L.), field corn, *Zea mays* (L.), and grain sorghum, *Sorghum bicolor* (L.) Moench, across much of the mid-south and southeastern United States (Luginbill 1928; Buntin 1986; Meagher et al. 2004). Fall armyworm larvae feed on vegetative as well as reproductive structures in these crops (Buntin 1986; Adamczyk et al. 1997). The significance of this pest in crops has been related to the inconsistent performance of many insecticide strategies across a range of plant growth stages.

Ovipositional preference and larval behavior for this species within host plants greatly reduces susceptibility to many insecticides. Adults may deposit clusters of 10-500 eggs throughout the plant canopy, but often prefer to oviposit in the lower two-thirds of cotton plants or in the whorls of corn or sorghum. First instars can be observed in an aggregate near the site of the egg mass, however late instars aggressively disperse within and across adjacent plants (Ali et al. 1989, 1990). Control with insecticides in broad-leaved crops such as cotton can often be difficult due to a lack of sufficient deposition in the lower region of the cotton canopy. As larvae age, they feed inside fruiting structures, or deeper in the whorls of grass crops further reducing their exposure to insecticide applications (Morrill & Greene 1973; Young 1979; Martin et al. 1980; Pitre 1986). In addition, larvae become more tolerant to insecticides as larval age/size increases (Yu 1983; Mink & Luttrell 1989). This tolerance further compounds problems in effectively controlling fall armyworm, as infestations of this pest are typically not discovered until large larvae are common across crop fields.

The development of dose-mortality responses to insecticides is necessary to provide baseline data for future resistance monitoring efforts for pests (Cook et al. 2004). Insecticide resistance surveys exist for bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), but no such coordinated program currently exists for fall armyworm. In addition, several new insecticides have been developed in recent years which exhibit activity against Lepidopteran pests. In most instances, the most appropriate time in the life of an insecticide to establish baseline responses is prior to the widespread use of these products in crops.

Many of these compounds exhibit novel modes of action to which the insect has not yet been exposed. One such group of insecticides is the diamides and includes chlorantraniliprole, cyantraniliprole, and flubendiamide. These molecules are described as ryanodine receptor modulators and affect nerve and muscle action (IRAC Mode of Action Working Group 2009). Spinetoram is another new compound in the chemical class known as spinosyns. Spinosyns are nicotinic acetylcholine receptor allosteric activators that affect nerve action. The modes of action for diamides and spinosyns differ greatly from that of products currently recommended for control of fall armyworm in crops. Examples of registered products used against this pest are novaluron, a benzoylurea which inhibits chitin biosynthesis and acts as an insect growth regulator (IGR); methoxyfenozide, a diacylhydrazine that is an ecdysone receptor agonist also acting as an IGR; lambda-cyhalothrin, a pyrethroid which acts as a sodium channel modulator affecting nerve action; indoxacarb, a blocker of voltage dependent sodium channels in the nervous system; and spinosad, an older spinosyn with a similar mode of action to that of spinetoram. Newer compounds with novel modes of action have the potential to improve integrated pest management (IPM) and delay insect resistance in row crops in southern states by providing growers with additional tools to control fall armyworm.

The objective of these studies was to generate insecticide dose-mortality responses for fall armyworm larvae in diet-incorporation bioassays, confirm field efficacy against natural infestations, and determine residual properties in the field environment. These results will provide reference data for future insecticide susceptibility surveys and give support to IPM recommendations for the use of insecticides against field infestations of fall armyworm.

Materials and Methods

Laboratory Bioassays

Fall armyworms were obtained from a laboratory colony (LSU-FAW) maintained at the Louisiana State University Department of Entomology, Baton Rouge, LA. This colony was established in 2005 from multiple collections in cotton, and supplemented with additional samples from field corn during 2006 and 2008. Based on mitochondrial markers, the colony was validated as the corn strain of fall armyworm (Unpublished communication, R. Nagoshi, USDA-ARS, Gainesville, FL).

Larvae were fed a meridic semi-solid diet (Ward's Natural Science, Rochester, NY) prepared according to manufacturer's recommendations. Rearing conditions consisted of a 14:10 light-dark photoperiod, 23.9 to 29.4°C, and 80% relative humidity (Cook et al. 2004).

Insecticides used in the bioassay included chlorantraniliprole (Coragen 200 g/L Soluble Concentrate [SC], DuPont Crop Protection, Wilmington, DE), cyantraniliprole (HGW-86, 200 g/liter SC, DuPont Crop Protection, Wilmington, DE), flubendiamide (Belt 480 g/L SC, Bayer Crop Science, Research Triangle Park, NC), indoxacarb (Steward 150 g/L Emulsifiable Concentrate [EC],

DuPont Crop Protection, Wilmington, DE), lambda-cyhalothrin (Karate-Z 250 g/liter EC, Syngenta Crop Protection, Greensboro, NC), methoxyfenozide (Intrepid 240 g/L Flowable [F], Dow AgroSciences, Indianapolis, IN), novaluron (Diamond 100 g/liter EC, Makhteshim Agan of North America, Inc., Raleigh, NC), spinetoram (Radiant 120 g/L SC, Dow AgroSciences, Indianapolis, IN), and spinosad (Tracer 480 g/L SC, Dow AgroSciences, Indianapolis, IN). Formulated products were used to create all initial concentrations.

Procedures similar to Temple et al. (2009) were used for preparing diet-incorporated insecticide bioassays. Insecticides were dissolved in distilled water to create a stock solution of 100 µg/mL. Serial dilutions of desired concentrations were standardized to 30 mL for each insecticide: water mixture. The insecticide solution was mixed with meridic diet to yield 200 mL of a diet/insecticide mixture. This mixture of diet and insecticide solution was agitated for 30-45 s in a 2.0-L bowl with a hand mixer. Insecticide-treated diet was then placed in 30-mL plastic cups with approximately 7 mL of diet per cup. Insecticide concentrations in the diet ranged from 0.25 µg/mL to 30.0 µg/mL diet. The insecticide-treated diet was stored in a refrigerator and used within 7 d of preparation. Four to 7 replicates (30-105 larvae per dose) were used for each insecticide. Fall armyworms (L3 stage; 30-45 mg) were placed on insecticide-treated and non-treated (control) diet. Insect mortality was evaluated at 96 h after exposure (HAE). A larva was considered dead if it could not right itself after being placed on its dorsal surface. Data were corrected for control mortality (0-5%) (Abbott 1925) and analyzed by probit analysis with Polo-Plus (LeOra Software 2006) to obtain LC_{50} values. Non-overlapping confidence limits (95%) were used to indicate significant differences among insecticides. Values are reported as concentration of insecticide (µg)/mL diet.

Field Experiments

Insecticide screening studies on grain sorghum were conducted during 2009 at the LSU AgCenter Macon Ridge Research Station (Franklin Parish, LA). Plots were planted to sorghum var. Terral TV 1050 (Terral Seed, Inc., Lake Providence, LA) on 8 Jun 2009. Plots consisted of 8 rows on 1-m centers and 15.24 m long. Treatments were arranged in a randomized complete block design with 4 replications. Cultural practices recommended by the LSU AgCenter were used to maintain plots in a consistent manner within the trial.

The treatments included chlorantraniliprole, cyantraniliprole, flubendiamide, lambda-cyhalothrin, methoxyfenozide, novaluron, and a non-treated control. Insecticides were applied on 24 Jul 2009, with a high-clearance sprayer and a CO_2-charged spray system calibrated to deliver 89.78 L per ha through TX-8 hollow cone nozzles (Spraying Systems Company, Wheaton, IL). Pretreatment samples across the test area indicated that >50% of plant whorls were infested with 1 or more fall armyworm in several stages of larval development.

Treatment efficacy was determined 3 and 7 d after treatment (DAT). Within each plot, a single plant was randomly selected on 1 of the center rows (Rows 4 or 5). That plant and the next 9 consecutive plants were destructively sampled and examined for fall armyworm infested whorls. Number of infested whorls was calculated as percent infestation of plants within each plot. Data were analyzed by PROC GLM and means separated according to Tukey's Studentized Range Test (SAS Institute 2004).

Residual Efficacy in a Field Environment

Larvae were removed from the same colony (LSU-FAW) previously described for the laboratory experiments. At 0 (4 HAT), 7, 14, 21, and 28 DAT, sorghum leaf tissue (non-treated and insecticide-treated) was removed from plants in the previously described field trial. Plants were mapped for leaf collars at the time of treatment application to ensure that the leaves selected during all time periods of the study were present at the time insecticides were applied. Leaf tissue was harvested on each date from the uppermost fully-expanded leaf that was present at the time of treatment application. Leaves were immediately transported to the laboratory and dissected into tissue sections averaging 2.5 cm^2. Two second instars (3-4-d-old) were placed into each cell of a plastic bioassay tray (CD International, Pitman, NJ), each containing 3 pieces of leaf tissue. Thirty-two larvae were infested on each treatment at each infestation timing (8 larvae per replication). Larvae were evaluated for mortality 72 HAE on leaf tissue. Larval mortality was determined by methods previously described in the laboratory bioassays. Percent mortality data was analyzed and compared among treatments at each DAT interval according to methods described for the field trials.

Results and Discussion

Laboratory Bioassays

The LC_{50} values among insecticides ranged from 0.066 µg/mL for spinetoram to 5.27 µg/mL for lambda-cyhalothrin (Table 1). The newer insecticides, chlorantraniliprole, cyantraniliprole, flubendiamide, and spinetoram had LC_{50}'s ranging from 0.066 µg/mL to 0.93 µg/mL and were generally lower than those observed for the older traditional

TABLE 1. DOSE-MORTALITY RESPONSES OF FALL ARMYWORM (LSU-FAW) LARVAE IN DIET-INCORPORATED ASSAYS 96 H AFTER EXPOSURE.

Insecticide[1]	n[2]	LC_{50}3	95% C.L.[3,4]	Slope ± SE	χ^2[5]	df[6]
Diamide (28)						
Chlorantraniliprole	685	0.068	0.060-0.077	2.55 ± 0.23	2.92	6
Cyantraniliprole	310	0.118	0.097-0.141	2.88 ± 0.34	1.95	4
Flubendiamide	420	0.930	0.775-1.126	1.99 ± 0.18	2.87	4
Indoxacarb (22A)						
Indoxacarb	300	0.392	0.317-0.481	2.35 ± 0.25	4.09	5
Pyrethroid (3A)						
Lambda-cyhalothrin	210	5.270	4.028-6.797	2.02 ± 0.25	3.29	4
Diacylhydrazine (18)						
Methoxyfenozide	225	0.875	0.658-1.037	3.13 ± 0.62	2.33	3
Benzoylurea (15)						
Novaluron	270	0.166	0.112-0.220	1.74 ± 0.25	1.75	3
Spinosyn (5)						
Spinetoram	210	0.066	0.053-0.081	2.54 ± 0.36	2.43	4
Spinosad	210	0.557	0.382-0.879	2.21 ± 0.30	4.21	4

[1]IRAC Mode of Action Working Group 2009, http://www.irac-online.org/wp-content/uploads/2009/09/MoA-classification_v6.3.3_28july09.pdf
[2]Number of insects tested.
[3]µg/mL.
[4]Confidence Limits.
[5]Chi square values (no significant values).
[6]Degrees of freedom.

insecticides (indoxacarb, lambda-cyhalothrin, methoxyfenozide, novaluron, and spinosad) with LC_{50}'s ranging from 0.166 µg/mL to 5.27 µg/mL. Fall armyworm larvae were significantly less susceptible to lambda-cyhalothrin than all other insecticides. Spinetoram (0.066 µg/mL) and chlorantraniliprole (0.068 µg/mL) were significantly more toxic to fall armyworm than all other insecticides.

These results represent initial efforts to develop baseline data for new insecticides with reference data for several commercial products that are currently used against fall armyworm. Although the use of diet-incorporated bioassays may not provide the optimum measure of the toxicity for all compounds, the procedure appeared to perform well for those products that require ingestion. Evaluations for mortality at 96 HAE may not allow sufficient time to accurately gauge the maximum effectiveness of the IGR's, novaluron and methoxyfenozide, but did allow for comparisons among several chemistries. This standard methodology and baseline data should assist in monitoring for changes in susceptibility to these new insecticides as their use becomes widespread across multiple crops in the southern United States.

Several insecticides representing various classes of chemistry have been evaluated against fall armyworm in recent years with bioassays of meridic diet surface-treated with insecticides. Adamczyk et al. (1999) exposed third instar fall armyworms to insecticide-treated diet and developed LC_{50} values for methoxyfenozide (197.9 ppm, ppm = parts per million) and spinosad (4.4 ppm), both of which represent toxicity values significantly higher than those found in the current study. Cook et al. (2001) conducted a study similar to Adamczyk et al. (1999) using first instars on indoxacarb-treated diet (LC_{50} = 0.59 ppm). This value is similar to data presented herein, although for smaller larvae. Argentine et al. (2002) also exposed first instars to diet-overlay assays using chlorfenapyr (1.2 ppm), emamectin benzoate (0.0029 ppm), fipronil (2.4 ppm), and tebufenozide (0.95 ppm). Results from these studies suggest significant effects on insecticide toxicity are present between the surface-treated (diet-overlay) assays and diet incorporated assays.

Field Trials

Fall armyworm infested whorls in the insecticide-treated plots ranged from 10 to 45% at 3 DAT and from 2.5 to 40% at 7 DAT (Table 2). Chlorantraniliprole (10.0%), cyantraniliprole (12.5%), and novaluron (15.0%) significantly reduced fall armyworm infested whorls compared to that in the non-treated control (50.0%) and lambda-cyhalothrin-treated (45.0%) plots at 3 DAT. Chlorantraniliprole also significantly reduced infestations below that in the methoxyfenozide-treated (40.0%) plots. At 7 DAT, no significant treatment effect was detected compared to the non-treated control. However, the

TABLE 2. EFFICACY OF SELECTED INSECTICIDES AGAINST FALL ARMYWORM IN A GRAIN SORGHUM FIELD TRIAL.

Treatment	Rate per ha (kg AI)	Percent (± SE) fall armyworm-infested whorls	
		3 DAT[1]	7 DAT[1]
Chlorantraniliprole	0.101	10.0 c ± 5.8	2.5 b ± 2.5
Cyantraniliprole	0.098	12.5 bc ± 6.3	5.0 b ± 5.0
Flubendiamide	0.106	32.5 abc ± 7.5	10.0 b ± 7.1
Lambda-cyhalothrin	0.034	45.0 a ± 6.5	40.0 a ± 7.1
Methoxyfenozide	0.101	40.0 ab ± 9.1	22.5 ab ± 6.3
Novaluron	0.088	15.0 bc ± 6.5	2.5 b ± 2.5
Non-treated control	—	50.0 a ± 4.1	35.0 ab ± 2.9
df		6, 18	6, 18
F value		6.78	12.45
(*P* > F) ANOVA		0.0007	<0.0001

Means within columns followed by a common letter are not significantly different ($P \leq 0.05$ Tukey's Studentized Range Test).
[1]Days after treatment.

newer compounds (chlorantraniliprole, cyantraniliprole, and flubendiamide) reduced fall armyworm infestations by >2.5-fold below that in the non-treated control. The newer insecticides displayed efficacy equal to or greater than standard insecticides (indoxacarb, lambda-cyhalothrin, methoxyfenozide, novaluron, and spinosad) currently recommended for control of fall armyworm (Baldwin et al. 2010; Catchot 2010; Studebaker 2010). Although additional research with these insecticides is needed, the results presented in this study should aid producers in making fall armyworm management decisions. The poor control with lambda-cyhalothrin in this study was not surprising given that previous by Guillebeau & All (1990) evaluating a range of insecticides for control of fall armyworm in whorl-stage corn and sorghum showed considerable variability in the effectiveness of several pyrethroids.

Mink & Luttrell (1989) exposed fall armyworm larvae to insecticide-treated cotton tissue in the laboratory. Their findings indicated significant levels of mortality when larvae were directly exposed to organophosphate, carbamate, and pyrethroid-treated tissue. However, against natural infestations in a field environment, the performance of these insecticides on cotton may not be as consistent; especially if larvae are located low in the plant canopy and insecticide deposition is an issue.

Residual Efficacy Experiments

Fall armyworm mortality on all insecticide-treated tissue at 0 DAT (4 HAT) (90.6 to 100%) and at 7 DAT (28.1 to 96.9%) was significantly higher than that on non-treated control tissue (Table 3). Mortality on chlorantraniliprole

TABLE 3. RESIDUAL EFFICACY OF SELECTED INSECTICIDES AGAINST FALL ARMYWORM (LSU-FAW) LARVAE ON GRAIN SORGHUM TISSUE.

Insecticide	Rate per ha (kg AI)	% Mortality 72 HAE[1]				
		0 DAT[2]	7 DAT[2]	14 DAT[2]	21 DAT[2]	28 DAT[2]
Chlorantraniliprole	0.101	100.0 a	96.9 a	85.9 a	82.8 a	53.1 a
Cyantraniliprole	0.098	100.0 a	93.8 a	75.0 ab	75.0 a	43.8 ab
Flubendiamide	0.106	93.8 a	53.1 cd	26.6 c	9.4 bc	—[3]
Lambda-cyhalothrin	0.034	90.6 a	28.1 d	5.6 cd	6.3 bc	—[3]
Methoxyfenozide	0.101	92.2 a	89.1 ab	53.1 b	29.7 b	20.3 bc
Novaluron	0.088	92.2 a	65.6 bc	23.4 cd	14.1 bc	12.5 bc
Non-treated control	—	9.4 b	1.6 e	1.6 d	0.0 c	0.0 c
df		6, 38	6, 38	6, 39	6, 30	4, 20
F value		175.45	41.30	34.58	40.22	7.51
(*P* > F) ANOVA		<0.0001	<0.0001	<0.0001	<0.0001	0.0007

Means within columns followed by a common letter are not significantly different ($P \leq 0.05$ Tukey's Studentized Range Test).
[1]h after exposure.
[2]d after treatment.
[3]Not included in the analysis due to low sample number of larvae.

(96.9%) and cyantraniliprole-treated tissue (93.8%) significantly differed from that for all other treatments, except methoxyfenozide (89.1%) at 7 DAT. At 14 DAT, mortality on chlorantraniliprole (85.9%), cyantraniliprole (75.0%), flubendiamide (26.6%), and methoxyfenozide-treated tissue (53.1%) was significantly different from that of larvae on the non-treated tissue. In addition, chlorantraniliprole caused significantly higher mortality than all insecticides except cyantraniliprole. At 21 DAT, chlorantraniliprole (82.8%) and cyantraniliprole (75.0%) caused significantly higher mortality than the non-treated control (0.0%) and all other insecticide treatments (6.3 to 14.1%) except methoxyfenozide (29.7%). Only chlorantraniliprole (53.1%) and cyantraniliprole (43.8%) caused mortality significantly higher than that on the non-treated control (0%) at 28 DAT. These results suggest that the newer insecticides generally exhibited longer residual efficacy compared to that for several of the standard insecticides (lambda-cyhalothrin, methoxyfenozide, and novaluron) currently recommended for fall armyworm management.

Long (>21 DAT) residual efficacy provided by compounds may help to reduce insecticide application frequency necessary to achieve satisfactory control of persistent fall armyworm infestations. Additional research is necessary to determine the ecological effects of the persistent nature of these products in a row-crop ecosystem. Further fieldwork is also needed to compliment these laboratory studies to determine the most effective rates of compounds given their respective residual properties. Finally, research is needed to understand the most appropriate timing for applications of these insecticides in order to maximize their effectiveness in various cropping systems.

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LEAF GAS EXCHANGE AND GROWTH RESPONSES OF GREEN BUTTONWOOD AND SWINGLE CITRUMELO TO *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE) LARVAL FEEDING AND FLOODING

CLIFF G. MARTIN[1], CATHARINE MANNION[1], AND BRUCE SCHAFFER[1]
[1]University of Florida, Tropical Research and Education Center, 18905 S.W. 280 Street, Homestead, FL 33031

ABSTRACT

Effects of flooding and herbivory by *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) larvae on leaf gas exchange [net CO_2 assimilation (A), transpiration (E), and stomatal conductance (g_s)] and growth of green buttonwood (*Conocarpus erectus* L.) and Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.] trees were tested. Growth and survival of the larvae were also examined. For each plant species, there were 2 larval infestation treatments (infested and non-infested) and 2 flooding treatments (flooded and non-flooded). Beginning 6 d after larval infestation, plants were flooded in three 1-wk cycles each with 2 d of flooding followed by 5 d of non-flooding. For green buttonwood, E was higher for non-flooded than flooded plants on the third of 5 measurement dates and A and g_s were higher for non-flooded than flooded plants on the fifth (final) measurement date. For Swingle citrumelo, E and g_s were higher for non-infested than infested plants on the fifth (final) measurement date. Root dry weight of Swingle citrumelo was higher for flooded, infested than for non-flooded, infested plants and for non-flooded, non-infested than for non-flooded, infested plants. Larval survival rate, head capsule width, and root damage rating of Swingle citrumelo were lower for flooded than for non-flooded plants, whereas flooding did not affect larval survival or growth on green buttonwood. Thus, short-term cyclical flooding of three 2-d cycles may control *D. abbreviatus* larvae on Swingle citrumelo but did not control larval populations or reduce damage on green buttonwood.

Key Words: net CO_2 assimilation, transpiration, stomatal conductance

RESUMEN

Se evaluaron los efectos de inundación y herbivoria por larvas de *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae) sobre el intercambio de gases foliar (asimilación de CO_2, transpiración, y conductancia estomatica) y el crecimiento de buttonwood verde (*Conocarpus erectus* L.) y Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.]. Tambien, se evaluaron el crecimiento y supervivencia de larvas. Para cada especie de planta, hubo dos tratamientos de infestación larval (infestado y no infestado) y dos tratamientos de inundación (inundado y no inundado). Seis dias después de la infestación larval, las plantas fueron inundadas en tres ciclos de una semana. Cada ciclo tuvo 2 dias de inundación seguido por 5 dias de no inundación. En la tercera de las cinco fechas de registro, la transpiración en buttonwood verde fué más alta en plantas no-inundadas que en las inundadas mientras que la asimilación de CO_2 y conductancia estomatica fueron más altas en el quinto (ultimo) día de registro en plantas no-inundadas que en las inundadas. Para Swingle citrumelo, el quinto (ultimo) día de registro, la transpiración y la conductancia estomatica fué más alta en plantas no-infestadas que en las infestadas. El peso seco de las raices de Swingle citrumelo fue más alto en plantas inundadas e infestadas y en plantas no-inundadas, no-infestadas que en las no-inundadas e infestadas. La tasa supervivencia de las larvas, el ancho de sus cabezas, y la tasa de daño a la raíz de Swingle citrumelo fué más baja en plantas inundadas que en las que no fueron inundadas. Sin embargo, la inundación no afectó la supervivencia ni el crecimiento de larvas en buttonwood verde. En resumen, inundaciones cíclicas de tiempo corto con tres ciclos de 2 dias cada vez, puede controlar larvas de *D. abbreviatus* en Swingle citrumelo, pero no controla las poblaciónes larvales, ni reduce el daño, en buttonwood verde.

Translation provided by the authors.

Diaprepes abbreviatus L. (Coleoptera: Curculionidae: Entiminae) commonly called *Diaprepes* root weevil is a pest of sugarcane and citrus in its native Puerto Rico (Woodruff 1964). In Florida, it infests approximately 24,281 ha (60,000 ac) of citrus, and control costs and losses have exceeded $2,965 per ha ($1,200 per ac) (Stanley 1996). Agricultural losses due to the weevil in Florida have been estimated at $70 million annually (Weissling et al. 2004). There is a continued need for improved management strategies because *D. abbreviatus* has threatened the survival of several crop plants in the past (Simpson et al. 1996) and continues to be an economic pest for both citrus and the ornamental industry. This pest has a very large host range of at least 317 varieties, 280

species, 180 genera, and 68 families of plants (Simpson et al. 1996, 2000; Knapp et al. 2000; Mannion et al. 2003; Godfrey et al. 2006). In addition to damage caused by the pest, there are regulatory concerns of spreading the weevil into noninfested areas, which are particularly important to the ornamental plant industry because plants are shipped throughout the U.S. and abroad (Mannion and Glenn 2003).

Some plant species support only 1 stage of the insect: for example, *Ardisia crenata* Sims supports only larval feeding. However, many plants including green buttonwood (*Conocarpus erectus* L.) and citrus are affected by both larval and adult feeding (Simpson et al. 1996). Mannion et al. (2003) surveyed several ornamental plant nurseries in southern Florida and found that egg masses, feeding damage, and adult weevils were common on many woody ornamental plant species. Young weevil larvae feed on small roots, but as they grow may excavate deep grooves on larger roots and consume the outer bark and cambial layers (McCoy et al. 2002). Roots may be girdled causing severe root damage or death, which reduces the ability of the plants to take up nutrients, and often kills small citrus trees (Wolcott 1936, 1948; Quintela et al. 1998; McCoy et al. 2002).

Measurements of leaf gas exchange, including net CO_2 assimilation *(A)*, transpiration *(E)*, and stomatal conductance *(g_s)*, can help quantify insect damage to plants before visual symptoms appear. Insect herbivory can increase, decrease, or have no effect on leaf gas exchange (Andersen and Mizell 1987; Welter 1989; Schaffer and Mason 1990; Schaffer et al. 1997). How insect herbivory affects leaf gas exchange can vary with the type of feeding damage or guild (i.e., mesophyll feeders, phloem feeders, stem borers, root feeders, and direct leaf consumers) (Root 1973; Welter 1989). *Diaprepes abbreviatus* has 2 feeding guilds; larvae are in the root-feeder guild whereas adults are in the direct-leaf-consumer guild.

Agriculture in southern Florida is often in low-lying areas with high water tables which are prone to periodic flooding (Schaffer 1998). Flooding typically depletes soil oxygen which can inhibit root metabolism causing decreased plant growth and photosynthesis. Prolonged flooding can result in plant mortality (Schaffer et al. 1992; Kozlowski 1997). Green buttonwood is a popular ornamental tree or shrub in southern Florida and is native to the tidal swamps of central and southern Florida (Watkins and Sheehan 1975; Wunderlin 1998). Hence it tolerates flooding well, though it also thrives in non-flooded, moderately moist soils in which landscape plants are commonly found.

Previous research with *D. abbreviatus,* including interactions between larval infestation and soil flooding, soil type, or soil pH mainly focused on *Citrus* spp. or their intergeneric crosses with *Poncirus* spp. (Li et al. 2003, 2004, 2006, 2007). Swingle citrumelo [*Poncirus trifoliata* (L.) Raf. × *Citrus paradisi* Macf.] was used in several studies of *Diaprepes* and flooding interactions because it is a very common rootstock for commercial citrus trees in Florida (Auscitrus 2004; F. S. Davies, personal communication 2008). Unlike buttonwood, however, Swingle citrumelo has moderate to low flood tolerance (Auscitrus 2004). Only very young plants infested with neonates were evaluated in previous studies with citrus (Li et al. 2003, 2004, 2006, 2007). To the authors' knowledge, there is no published research on interactions between *D. abbreviatus* larval feeding and soil flooding with more mature larvae on larger citrus plants. Also, little information is available on effects of flooding on *D. abbreviatus* damage to woody ornamental plants.

Our primary objective was to investigate effects of cyclical (intermittent) soil flooding and herbivory by large (fourth to sixth instar) *D. abbreviatus* larvae and their interactions on green buttonwood and Swingle citrumelo trees. An additional objective was to compare effects of flooding on the survival and growth of *D. abbreviatus* larvae on green buttonwood with those on Swingle citrumelo, a trees species known to be sensitive to interactions between soil flooding and *D. abbreviatus* neonates (Li et al. 2003, 2006).

Materials and Methods

The experiment was conducted in fall 2008 in Homestead, Florida with green buttonwood and Swingle citrumelo plants in 11-liter plastic containers placed on ground cloth at an outdoor site exposed to full sun.

Plant Material

Green buttonwood and Swingle citrumelo trees (obtained from a commercial nursery) were approximately 2 yrs old and 1 yr old, respectively when treatments were initiated. Initial plant height 6 d before infestation was 122 ± 11 cm (mean ± SD) for green buttonwood and 132 ± 14 cm for Swingle citrumelo. In a previous study (Martin et al. 2010), no difference was observed between marl soil (typical in landscape plant nurseries in southern Florida) and standard potting medium for survival of *D. abbreviates* larvae in flooded or non-flooded conditions. In this study we used a standard medium, typical for potted ornamental plants in southern Florida ornamental plant nurseries, to avoid potential damage to root systems from repotting plants in marl soil. The potting medium for both plant species was Fafard mix 2 (70% Canadian peat, 20% perlite and 10% vermiculite).

Flooding Treatments

Plants of each species were flooded by submerging their 11-liter containers into 19-liter plastic buckets filled with tap water with water levels maintained at 10 cm above the soil surface. Control plants were not flooded. For each replication in each test, there were 2 flooded plants (1 infested and 1 non-infested) and 2 non-flooded plants (1 infested and the other non-infested). Flooded treatments were initially flooded 6-8 Nov 2008. Plants were flooded for 2 d followed by a 5-d drying period resulting in a 7-d cycle that was repeated 3 times. All plants (flooded and non-flooded) were irrigated throughout the experiment by overhead sprinkler for 30 min twice per day.

Larval Infestation

For each plant species, one-half of the plants in each flood treatment (flooded or non-flooded) were infested with *D. abbreviatus* larvae on 31 Oct 2008. Larvae were obtained from a rearing facility with the Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, FL (see http://www.doacs.state.fl.us/pi/methods/diaprepes.html for rearing procedures). At the time of infestation, larvae were about 28 d old with an average head capsule width of 1.15 ± 0.14 mm, hence they were fourth to sixth instar or late fifth instar on average (Quintela et al. 1998). Larvae were placed individually into each of 10-20 holes made in the soil that were 3-10 cm deep, 4-8 cm from the stem, and 3 cm apart with 20 total larvae per container. The holes were then covered with soil. All containers remained non-flooded for 6 d to allow larvae to become established.

Temperature and Soil Redox Potential

Air and soil temperatures were recorded at 1-h intervals throughout the experiment with 2 air sensors and 2 soil sensors (StowAway® Tidbit® temploggers, Onset Co., Pocasset, MA). Sensors were placed in the soil (soil temperature) or canopies (air temperature) of plants not included in the experiment but in the same potting media and container type, which were located next to the test plants. Air sensors were each placed in plant canopies 66-71 cm above the soil surface and soil sensors were placed at a soil depth of 6 cm, two-thirds the distance from the center to the outer edge of the pot.

To provide an indication of soil oxygen content, soil redox potential was measured with a metallic combination electrode (Accumet Model 13-620-115, Fisher Scientific, Pittsburgh, PA) attached to a portable volt/pH meter (Accumet model AP62, Fisher Scientific, Pittsburgh, PA). Soil redox potential was measured daily during each flood period for 2 flooded, infested plants and 2 flooded, non-infested plants. The 4 measurements were averaged to calculate the mean redox potential for flooded treatments of each plant species. Soil redox potential was measured by inserting the electrode into a polyvinyl chloride (PVC) pipe inserted into the soil 2 cm from the edge of the pot. In addition, soil pH was measured for all flooded plants 2 times per flood period with a pH electrode attached to the same portable volt/pH meter used for redox measurements. For each flood cycle, the first pH measurement was made on the same day that plants were flooded and the second was 2 d later on the day they were drained. An exception was for Swingle citrumelo plants during the first flood cycle, when pH was measured 1 d after plants were flooded, and again 1 d later when plants were drained.

Plant Data Collection

Leaf gas exchange (A, E, and g_s) was measured on 2 fully expanded, recently mature leaves or leaflets per plant with a CIRAS-2 portable gas analyzer (PP Systems, Amesbury, Massachusetts). Values of the 2 leaves or leaflets were averaged and the mean value per plant (replication) was used for statistical analyses. Leaf gas exchange was initially measured 2-3 d before infesting plants with larvae and then periodically throughout the experiment. On each measurement date, measurements of all 4 treatment combinations in each replication per plant species were made within 50 min of each other. During gas exchange measurements, the photosynthetic photon flux was maintained at 1,000 µmol photons m^{-2} s^{-1} with a halogen lamp attached to the leaf cuvette and the reference CO_2 concentration in the cuvette was kept constant at 375 µmol mol^{-1}. Swingle citrumelo has compound leaves with 3 leaflets per leaf, and the terminal leaflet is larger than lateral leaflets (Hutchison 1974; Wunderlin 1998). All leaf gas exchange measurements for Swingle citrumelo were made on the large terminal leaflets.

Plant height was measured from the soil surface to the apex of the highest plant part (leaf or branch), and stem diameter was measured 10 cm above the soil surface; for plants with multiple stems at this height, diameter of the largest stem was recorded. The first measurement of plant height and stem diameter was made before infestation and flooding, and the second measurement was after the final draining but before harvest. For plant height or stem diameter, final minus initial values were calculated to compare growth data among treatments. All plants were harvested 32-33 d after larval infestation, 26-27 d after initially flooding, and 10-11 d after the final draining. At harvest, stems were cut 2-3 cm above the surface of the potting medium. The roots were

removed from the potting medium and the medium was placed into bins and carefully inspected for larvae. The number of live and dead larvae was determined for each plant and preserved in separate vials of 75% ethanol. Head capsule widths were measured in a laboratory with a microscope micrometer. Roots, stems, and leaves were then oven-dried for 5 d at 75°C to a constant weight and dry weights were determined. Leaf dry weight included leaf blades and petioles for green buttonwood plants and leaflets, petiolules, and petioles for Swingle citrumelo plants. Root damage was evaluated for infested Swingle citrumelo plants using a visual rating system where 0 = no visible damage, 1 = minimal visible damage, 2 = moderate visible damage, and 3 = maximum visible damage. However, root damage was not rated for green buttonwood because there were no visible signs of root damage.

Experimental Design and Statistical Analysis

For each plant species, there were 2 larval infestation treatments (infested or not infested) and 2 flooding treatments (flooded or non-flooded) arranged in a 2 × 2 factorial design with 5 single-plant replications per treatment combination. A two-way factorial analysis of variance (ANOVA) was used to test for significant interactions between infestation and flooding treatments, separately for each sampling date and plant species for leaf gas exchange variables. For plant growth data (root, stem, leaf, and total dry weights, stem diameter, and plant height), a separate ANOVA was performed for plant species. For each variable or group of variables per plant species (*A, E,* g_s, stem diameter, plant height, or dry weights), if there were no significant statistical interactions for any 2-way ANOVA, data were pooled and non-paired t-tests were used to compare flooded with non-flooded and infested with non-infested treatments. For percentages of larvae surviving, proportional data based on ratios of live/total larvae were arcsine transformed prior to statistical analysis. All statistical analyses were performed with SAS Statistical Software Version 9.1 (SAS Institute, Cary, North Carolina).

Results

Temperature, Soil Redox Potential and Floodwater pH

During the treatment period, mean daily soil temperatures ranged from16.8°C to 27.7°C and air temperatures ranged from 13.0°C to 24.3°C (Fig. 1). Soil redox potential for green buttonwood during the first, second and final flood periods ranged from +193 mV to +162 mV, +597 mV to +166 mV and +508 mV to +153 mV, respectively. For Swingle citrumelo, the corresponding values ranged from +378 mV to +165 mV, +498 mV to +174 mV and +523 mV to +193 mV, respectively. For each plant species in every flood cycle, the highest redox potential occurred on day 1 (when flooded) and the lowest was on day 3 (when drained) except for green buttonwood flood cycle 1, in which the highest redox potential was on day 2 and the lowest was on day 3. The pH of the floodwater during the flood period was 7.2-7.8.

Leaf Gas Exchange

There were no significant statistical interactions between flooding and larval infestation for leaf gas exchange variables on any of the 5 measurement dates for either plant species. Hence, to test responses to flooding for all leaf gas exchange variables of each plant species, infestation treatments were pooled, and to test responses to larval infestation, flooding treatments were pooled.

For green buttonwood, A (t = -2.21, df = 18, P = 0.0403) and g_s (t = -2.70, df = 18, P = 0.0146) were significantly higher for non-flooded than flooded plants on the fifth (final) measurement date (Fig. 2a and c). However, there were no significant differences between infested and non-infested green

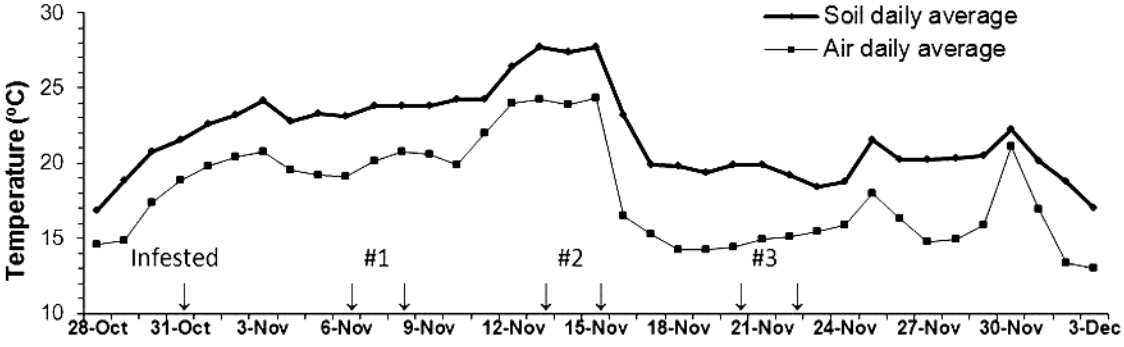

Fig. 1. Air and soil temperatures during the experiment. Each point is the average of 2 sensors. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows.

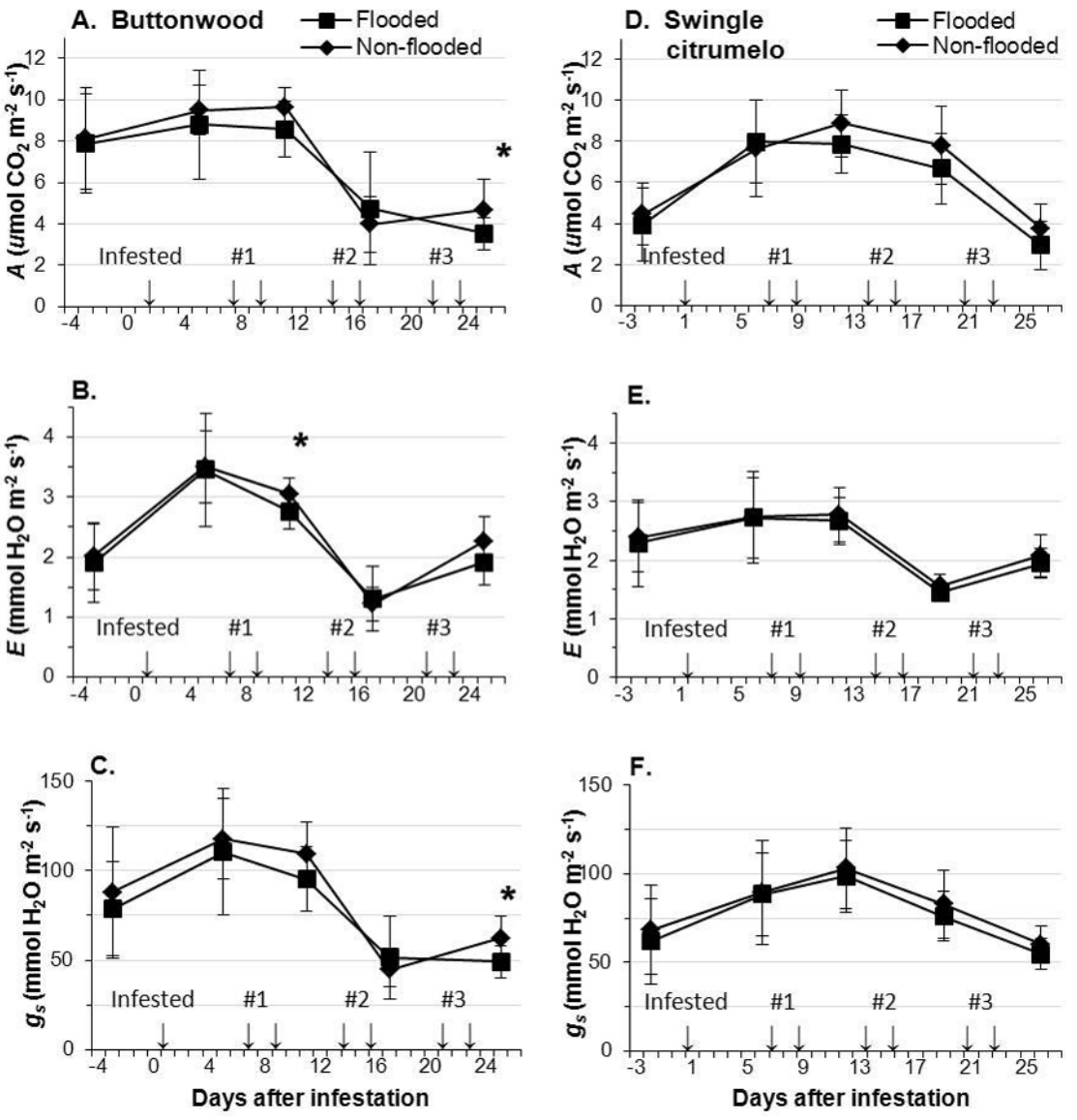

Fig. 2. Effects of flooding on A) net CO_2 assimilation (A), B) transpiration (E), and C) stomatal conductance (g_s) of green buttonwood trees and D) net CO_2 assimilation (A), E) transpiration (E), and F) stomatal conductance (g_s) of Swingle citrumelo trees. Symbols represent means ± SD. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle shown above the arrows. Asterisks indicate significant differences between treatments ($P \leq 0.05$) according to a non-paired t-test.

buttonwood plants for A or g_s (Fig. 3a and c). For green buttonwood, E was significantly higher for non-flooded than flooded plants on the third measurement date, or after infestation and the first flood cycle but before the second flood cycle (t = -2.24, df = 18, P = 0.0381) (Fig. 2b). There were no significant differences in E between infested and non-infested green buttonwood plants (Fig. 3b).

There were no significant differences in A between flooded and non-flooded or infested and non-infested Swingle citrumelo plants (Figs. 2d and 3d). For Swingle citrumelo, E (t = -2.64, df = 18, P = 0.0167) and g_s (t = -3.10, df = 18, P = 0.0061) were significantly higher for non-infested than infested plants on the fifth (final) measurement date (Fig. 3e and f). However, there were no other

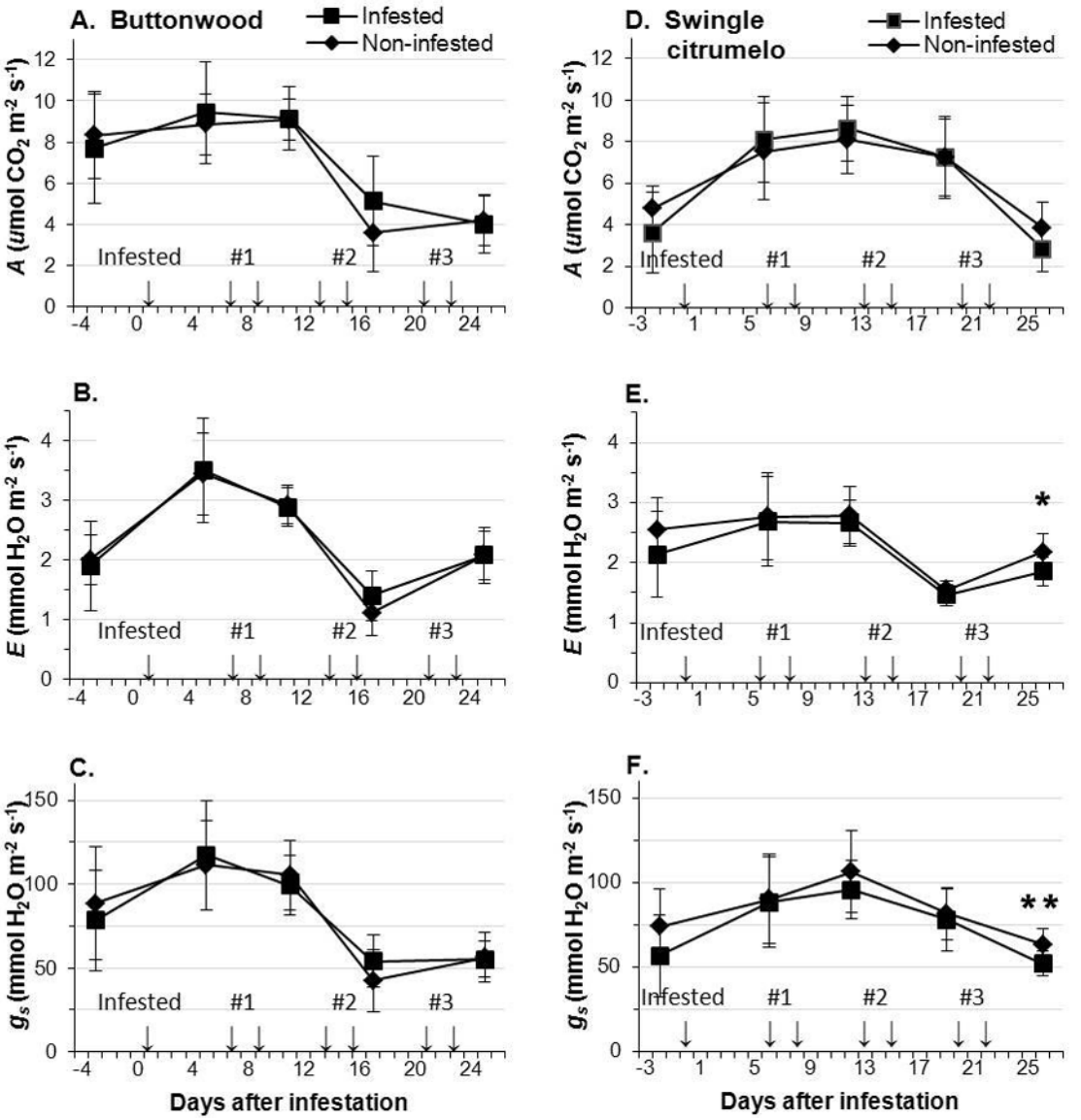

Fig. 3. Effects of larval infestation on A) net CO_2 assimilation (A), B) transpiration (E), and C) stomatal conductance (g_s) of green buttonwood trees and D) net CO_2 assimilation (A), E) transpiration (E), and F) stomatal conductance (g_s) of Swingle citrumelo trees. Symbols represent means ± SD. Successive flood cycles are denoted by pairs of arrows with the number of the flood cycle above the arrows. Asterisks indicate significant differences between treatments at * $P \leq 0.05$ or ** $P < 0.01$ according to a non-paired t-test.

significant differences in E or g_s between flooded and non-flooded or infested and non-infested Swingle citrumelo plants (Figs. 2e and f, 3e and f).

Plant Growth

There were no significant statistical interactions between larval infestation and flooding for stem diameter or plant height of either plant species. For tissue dry weights, the only significant flooding × larval infestation interaction was for root dry weight of Swingle citrumelo trees ($F = 4.87$; $df = 3$; $P = 0.0422$). Therefore, dry weights of Swingle citrumelo were not pooled for analysis, whereas for all other dry weight, stem diameter and plant height data were pooled for each plant species.

There were no significant effects of flooding or larval infestation on stem diameter or plant height for either green buttonwood or Swingle citrumelo (data not shown). There were no significant effects of larval infestation or flooding on root, stem, leaf, or total dry weights of green buttonwood (ranges for roots 49-103 g, stems 82-204 g, leaves 56-110 g, and total 193-407 g), or stem, leaf, or total dry weights of Swingle citrumelo (Table 1). However, root dry weight of Swingle citrumelo was significantly higher for flooded, infested than for non-flooded, infested plants (Table 1). Root dry weight of Swingle citrumelo was also significantly higher for non-flooded, non-infested than for non-flooded, infested plants (Table 1).

Larval Survival and Growth

For green buttonwood, there were no significant effects of flooding on percent larval survival or head capsule width of recovered larvae (Table 2). For Swingle citrumelo, however, percent survival and head capsule width were each significantly lower for flooded than non-flooded plants (Table 2). Root damage rating (Mean ± SD) for infested Swingle citrumelo was also significantly lower for flooded (0.2 ± 0.45) than for non-flooded (2 ± 1.2) plants (t = -3.09, df = 8, P = 0.0150).

Discussion

The average monthly soil temperatures during this study were 0.9 to 6.2°C below the ideal developmental temperature for *D. abbreviatus* and up to 2.2°C below the ideal survival temperatures for this weevil (Lapointe 2000). Lapointe (2000) found that the highest larval survival rates occurred at 22 and 26°C with lowest survival at 30°C and the highest developmental rate was at 26°C with slower rates at 22 and 30°C. Although larval development rates in the present study may have been slower than their maximum, larval survival rates were probably close to or slightly below their maximum levels.

Effects of flooding on physiology and growth of woody perennial plant species can vary among soil types and are partly based on rates of O_2 depletion in the soil and other factors such as soil pH (Schaffer et al. 1992). Soil redox potential provides an indication of oxygen content in the soil. Well-drained, well-oxygenated soils have redox

Table 1. Effects of flooding and *Diaprepes abbreviatus* larval infestation on dry weights of swingle citrumelo plants.

Tissue	Dry weight (g)		T	*df*[c]	*P*	Sig[d]
Infested[a]	Flooded	Non-flooded				
Roots	38 ± 2.0[b]	30 ± 5.1	3.16	8	0.0134	*
Stems	94 ± 18	84 ± 19	0.81	8	0.4425	NS
Leaves	15 ± 5.8	14 ± 3.2	0.17	8	0.8706	NS
Total	146 ± 24	129 ± 26	1.12	8	0.2970	NS
Non-infested	Flooded	Non-flooded				
Roots	39 ± 8.3	43 ± 5.7	-0.79	8	0.4513	NS
Stems	86 ± 21	93 ± 17	-0.59	8	0.5693	NS
Leaves	15 ± 5.2	13 ± 2.7	0.96	8	0.3669	NS
Total	141 ± 32	149 ± 24	-0.46	8	0.6604	NS
Flooded	Infested	Non-infested				
Roots	38 ± 2.0	39 ± 8.3	-0.32	4.48	0.7627	NS
Stems	94 ± 18	86 ± 21	0.62	8	0.5532	NS
Leaves	15 ± 5.8	15 ± 5.2	-0.20	8	0.8466	NS
Total	146 ± 24	141 ± 32	0.32	8	0.7589	NS
Non-flooded	Infested	Non-infested				
Roots	30 ± 5.1	43 ± 5.7	-3.65	8	0.0065	**
Stems	84 ± 19	93 ± 17	-0.79	8	0.4544	NS
Leaves	14 ± 3.2	13 ± 2.7	0.70	8	0.5008	NS
Total	129 ± 26	149 ± 24	-1.27	8	0.2412	NS

[a]Data were not pooled because there was a significant interaction between flooding and insect infestation ($P \leq 0.05$) with root dry weight based on a two-way analysis of variance (ANOVA).
[b]Mean ± SD in grams.
[c]Degrees of freedom. Variances were equal according to a test for equality of variances.
[d]Significance levels at * $P \leq 0.05$, ** $P < 0.01$ and NS (non-significant) were determined with a non-paired t-test; n = 5.

TABLE 2. EFFECTS OF FLOODING ON PERCENT SURVIVAL AND HEAD CAPSULE WIDTH OF *DIAPREPES ABBREVIATUS* LARVAE RECOVERED AT HARVEST.

Variable	Species	Treatments[a] Flooded	Non-flooded	T	*df*[b]	*P*	Sig[c]
Percent survival[d]							
	Buttonwood	44 ± 12	54 ± 20	-0.96	8	0.3649	NS
	Swingle	24 ± 7.4	42 ± 15	-2.44	8	0.0406	*
Head capsule width (mm)							
	Buttonwood	1.79 ± 0.10	1.89 ± 0.07	-1.89	8	0.0949	NS
	Swingle	1.76 ± 0.15	2.07 ± 0.06	-4.32	8	0.0025	**

[a]Mean ± SD.
[b]Degrees of freedom. All variances within each flooded vs. non-flooded pair were equal according to a test for equality of variances.
[c]Significance levels at * $P \leq 0.05$, ** $P < 0.01$ and NS (non-significant) were determined with a non-paired t-test; n=5.
[d]Percent survival data were Arcsine transformed before statistical analysis.

potentials of +300 mV or more, whereas flooded soils have redox potentials of +200 mV or less (Ponnamperuma 1972, 1984). All mean soil redox potentials for this experiment varied from 140 to 597 mV indicating the soil was either aerobic, or moderately hypoxic (low in oxygen). In addition, for both plant species, during all 3 flood cycles (except for green buttonwood flood cycle 1), the highest redox potential occurred on day 1 (when flooded) and the lowest was on day 3 (when drained). Hence, redox potential of cyclically flooded soil in this study indicated that while there was a decline in soil O_2 content during the flooding periods, the soil did not become very depleted of oxygen. This may have resulted from the short duration of the 3, 2-d cyclical flood periods each separated by 5 d without flooding. However, longer flooding durations are uncommon in ornamental plant nurseries in southern Florida (B. Schaffer, personal observations).

The duration of flooding and larval infestation periods in the present study were relatively short compared to previous studies, such as by Diaz (2005), where green buttonwood was exposed to 21-36 d of flooding followed by 90 d infestation. For green buttonwood plants in the present study, *E* was significantly higher for non-flooded than flooded plants on the third measurement date, and *A* and g_s were each significantly higher for non-flooded than flooded plants on the fifth (final) measurement date. However, there were no significant differences between flooded and non-flooded green buttonwood plants in stem diameter and no adventitious roots were observed on flooded plants. In 2 other studies with green buttonwood (Martin 2009), flooding for longer durations (23 d or 180 d) resulted in larger stem diameters of flooded compared to non-flooded plants. Also, plant adaptations to hypoxic soil conditions, such as development of adventitious roots and hypertrophic stem lenticels (Schaffer et al. 1992), were observed on flooded plants (Martin 2009). In both these previous studies with longer flood durations, there were almost no significant differences in *A* or g_s between flooded and non-flooded green buttonwoods (Martin 2009), whereas in the present study *A* and g_s were each significantly higher for non-flooded than flooded plants on the last of 5 measurements. For flooded green buttonwood, the reduction in leaf gas exchange without a growth or developmental response in the present study was presumably due to short flooding durations, which apparently were not long enough for buttonwood to exhibit statistically significant growth changes or morphological or anatomical adaptations to flooding.

There were no significant differences in leaf gas exchange between flooded and non-flooded Swingle citrumelo plants. Based on leaf gas exchange and plant growth in the present study, green buttonwood was more susceptible to flooding than Swingle citrumelo. This seems unusual because buttonwood is relatively flood tolerant (Watkins and Sheehan 1975; Wunderlin 1998; Martin 2009), whereas Swingle citrumelo is not particularly flood tolerant (Auscitrus 2004). Although plants in the present study were subjected to repeated flood cycles, durations of flooding and larval infestation were short compared to previous studies where Swingle citrumelo was exposed to 20-40 d of flooding followed by 40-56 d of larval infestation (Li et al. 2004, 2007). In addition, Syvertsen et al. (1983) found that when rough lemon *(Citrus jambhiri* Lush.) and sour orange *(C. aurantium* L.) seedlings were flooded for at least 3 wk, there was sloughing of fibrous roots and significant reduction in g_s, shoot growth, root conductivity, and leaf water potential. Thus, unlike in studies of longer flooding duration, we observed no significant effects of flooding on leaf gas exchange for Swingle citrumelo trees despite repeated flooding cycles. The only significant effect

of flooding on Swingle citrumelo growth was higher root dry weight under flooded, infested than under non-flooded, infested conditions; this may have resulted from flooded conditions preventing feeding larvae from decreasing root dry weight instead of flooding directly affecting plant growth. Although flooding durations in the present study were relative short, each intermittent flooding event was similar to the length of time that standing water is generally observed in southern Florida plant nurseries after heavy rains or tropical storms (B. Schaffer, personal observations). Durations of flooding under these conditions were apparently too short for buttonwood to develop flooding adaptations such as adventitious roots, and Swingle citrumelo may have not been flooded long enough to reduce its leaf gas exchange or growth.

Lapointe and Shapiro (1999) determined that optimal survival to pupation of *D. abbreviatus* in the laboratory occurred at 30-70% soil moisture, under which 60-65% of larvae survived to pupation (Lapointe and Shapiro 1999). The poorest survival of larvae occurred in low (20%) and in high (80%) soil moisture levels (Lapointe and Shapiro 1999). Our results support the observation that the poorest survival occurs under flooded conditions. Larval survival was significantly lower in cyclically flooded than in non-flooded soil with Swingle citrumelo. Similarly, Martin et al. (2010a) observed significantly lower *D. abbreviatus* larval survival rates on green buttonwood in flooded marl soil than in non-flooded marl soil and in flooded potting medium than in non-flooded potting medium. Also, significantly smaller head capsule widths were noted from flooded marl soil than from non-flooded marl or non-flooded potting medium (Martin et al. 2010a). Hence, the lowest survival rates of *D. abbreviatus* larvae would be expected under flooded conditions, whereas highest survival should be in non-flooded conditions without excessively low soil moisture (30-70%) (Lapointe and Shapiro 1999).

Nearly all root damage appeared to occur on roots larger than 2 mm diameter and involved gouging of the bark and presumably cambium of roots. Very few roots smaller than 2 mm in diameter showed evidence of larval damage, thus, larval damage was disproportionately biased towards the crown and larger diameter roots. A rating of 3 (maximum visible damage) corresponded to 10-15 percent removal of bark and cambium by larvae on roots at least 2 mm diameter with girdling more than half the crown circumference in at least 1 place, whereas minimum damage rating was 0 percent. Larval feeding by *D. abbreviatus* has been shown to reduce leaf gas exchange and growth in several woody ornamental plant species including green buttonwood (Diaz 2005; Diaz et al. 2006; Martin et al. 2009). In the present study, effects of flooding and insect damage on each plant species were presumably cumulative because most significant differences from flooding or insects were after the final flood cycle.

Previous results suggest that flooding plants in potting medium for at least 3 d would help control *D. abbreviatus* larvae (Martin et al. 2010a). Flooding is sometimes used in southern Florida sugarcane fields to control larvae of *Tomarus subtropicus* (Blatchley) (Coleoptera: Scarabaeidae) (Cherry 1984) and *Melanotus communis* (Gyllenhal) (Coleoptera: Elateridae) (Hall and Cherry 1993). Shapiro et al. (1997) found that mean mortality of flooded, unfed larvae of *D. abbreviatus* exceeded 90% by the third week after flooding at 24 and 27°C. Mortality may have been caused by drowning (suffocation) from a lack of oxygen and surplus carbon dioxide or by sepsis from a buildup of microbes in stagnant water and larval cadavers (Shapiro et al. 1997). Thus, flooding may be useful for controlling *D. abbreviatus* larvae infestations on green buttonwood, and flooding was recommended by Li et al. (2007) as a possible control method in citrus.

In the present study, cyclical flooding did not significantly affect larval growth (head capsule width) or survival on green buttonwood. However, Swingle citrumelo plants had significantly reduced larval growth and survival in flooded compared to non-flooded soil. Therefore, 3 periods of 2-d flooding with 5-d drying periods in-between, such as may occur in the field from heavy rain, may help control *D. abbreviatus* larvae without affecting leaf gas exchange or growth of trees on Swingle citrumelo rootstock, but these short-term flood periods would probably not benefit green buttonwoods. Additionally, root dry weight of Swingle citrumelo was significantly greater for flooded infested than for non-flooded infested plants, and when plants were not flooded, it was significantly greater for non-infested than infested plants. Reduced larval growth, survival, feeding, and root damage rating of flooded infested compared to non-flooded infested Swingle citrumelo plants may have allowed for the increased root dry weight of flooded infested plants. Thus, flooding may reduce effects of *D. abbreviatus* larval herbivory and damage to Swingle citrumelo plants.

Diaprepes abbreviatus larvae were exposed directly to flooding in the present study. However, Li et al. (2003, 2006, 2007) and Diaz (2005) drained plants before infestation with larvae. In the study by Diaz (2005), plants were drained 1 d before larval infestation so both stresses were not simultaneous. Overall, decreases in leaf gas exchange and plant dry weight observed by Diaz (2005) were attributed more to flooding than to larval infestation in green buttonwood.

Effects of flooding and *D. abbreviatus* larval infestation on plant growth and larval survival on

Swingle citrumelo and 1 other citrus rootstock were previously examined in a greenhouse (Li et al. 2003, 2006, 2007). Li et al. (2006) found that Swingle citrumelo plants flooded for at least 20 d were more stressed and more prone to *D. abbreviatus* larval feeding injury than non-flooded control plants. Their results suggested that avoidance of flooding and early control of *Diaprepes* larvae may help protect young plants. Similarly, Li et al. (2006) investigated the effects of flooding and soil type on *Diaprepes* larval survival and found that for plants previously flooded for 20 d, larval survival averaged 25% higher in sandy soil than in loam soil. Waterlogged soils are also typically denser than non-flooded soils (Saqib et al. 2004), which is a potential problem for survival of larvae in flooded soil (Li et al. 2006). Other factors such as soil type, compaction, bulk density, and soil water content may also influence larval survival and growth (Riis and Esbjerg 1998; Rogers et al. 2000; Li et al. 2007). Flooding may hence reduce larval survival while plants are flooded. However, depending on soil pH, flood-stressed plants may be more susceptible to *Diaprepes* larval feeding when un-flooded than non-stressed plants that were either never flooded or flood-tolerant and previously flooded. Hence, flooding may either increase or decrease larval survival rates based on soil moisture, pH, and plant health while soil is infested.

In summary, the following suggest that flooding reduced insect damage to Swingle citrumelo plants after three 2-d flood cycles: reduced larval growth, survival, root damage, and increased root dry weight of flooded, infested compared to non-flooded, infested plants; higher root dry weight for non-flooded, non-infested than for non-flooded, infested plants; and reduced E and g_s in infested compared to non-infested plants. However for green buttonwood plants, flooding seemed to have no effect on larval growth, survival, or insect damage. Thus, while cyclical flooding for three 2-d cycles may control *D. abbreviatus* larvae on Swingle citrumelo, short-term flooding seems unlikely to control larvae or reduce damage to green buttonwood.

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SEASON-LONG INSECTICIDE EFFICACY FOR HEMLOCK WOOLLY ADELGID, *ADELGES TSUGAE* (HEMIPTERA: ADELGIDAE), MANAGEMENT IN NURSERIES

STEVEN D. FRANK[1] AND ANTHONY LEBUDE[2]
[1]North Carolina State University, Campus Box 7613, Raleigh, NC 27695

[2]Mountain Horticultural Crops Research and Extension Center North Carolina State University, Mills River, NC 28759

ABSTRACT

Nursery growers and extension personnel have to rely on efficacy data from forest and landscape systems to manage hemlock woolly adelgid in nurseries. Considerable differences in tree size and culture and application logistics could make such data unsuitable. We evaluated 12 different insecticide formulations for short and long-term control of hemlock woolly adelgid in container grown Eastern hemlocks, *Tsuga canadensis*. All products provided control of first generation hemlock woolly adelgids, though efficacy of foliar applications of neonicotinoids dinotefuran, imidacloprid (Marathon® II), and acetamiprid and foliar or drench applications of spirotetramat acted the most quickly. Foliar and soil applications of neonicotinoids and spirotetramat also prevented reinfestation of second generation crawlers. In contrast, second generation hemlock woolly adelgids successfully colonized trees treated with the contact insecticides, horticultural oil and bifenthrin. Systemic insecticides provided season-long control of hemlock woolly adelgid when applied to foliage, which is the preferred method of application of nursery growers.

Key Words: insecticide efficacy, foliar application, drench, neonicotinoids, nursery, spirotetramat, soilless substrates, container-grown nursery trees

RESUMEN

Los productores de plantas en viveros y personal de extensión tienen que confiar en los datos de eficacia de los sistemas forestales y del campo para manejar el adélgido lanoso del abeto en los viveros. Diferencias considerables en el tamaño del árbol, y su cultura y su aplicación logística podrían hacer estos datos inadecuados. Se evaluaron 12 diferentes formulaciones de insecticidas para el control a corto y largo plazo de adélgido lanoso del abeto en recipientes con plantas de Falso Abeto del Canada, *Tsuga canadensis*. Todos los productos controlaron la primera generación del adélgido lanoso del abeto, aunque la eficacia de las aplicaciones foliares de dinotefuran neonicotinoides, imidacloprid (Maratón® II) acetamiprid y aplicaciones foliares o de empapar de spirotetramat actuaron más rápidamente. Aplicaciones foliares y de suelo de los neonicotinoides y spirotetramat también impidió la reinfestación de los rastreadores (1 estadio de la ninfa) de la segunda generación. Por el contrario, la segunda generación del adélgido lanoso del abeto, colonizaron los árboles tratados con los insecticidas de contacto, el aceite de la horticultura y la bifentrina. Insecticidas sistémicos provieron el control del adélgido lanoso del cicuta por toda la temporada cuando fueron aplicados al follaje, que es el método preferido de aplicación de los productores de plantas en viveros.

Hemlock woolly adelgid, *Adelges tsugae* (Annand), has devastated stands of Eastern hemlock, *Tsuga canadensis* L., and Carolina hemlock, *T. caroliniana* Engelmann, in 18 states from Maine to Georgia (USFS 2011a). Hemlock woolly adelgid has also become a major pest of hemlocks in ornamental landscapes and urban forests where hemlocks are planted as hedges, shrubs, and shade trees (McClure 1987; Quimby 1996; Raupp et al. 2008). Hemlock woolly adelgid feeding depletes trees of carbohydrates and other resources and rapidly reduces the health and aesthetic value of trees (McClure et al. 2001). Trees lose their characteristic dark green color that is valued in ornamental landscapes and instead become gray, pale-green, or yellow (McClure 1987). Infestation also causes bud mortality, needle loss, reduction of new growth, branch dieback, and tree death (McClure 1987; McClure et al. 2001).

Nurseries that produce hemlocks for ornamental landscapes are typically within the native range of hemlock forests. These hemlock trees are subject to a constant influx of hemlock woolly adelgid crawlers carried by wind or animals (McClure 1990). Growers from locations with active hemlock woolly adelgid infestations are prohibited from

selling plants to many states that have established quarantine laws (USFS 2011b). Even shipments within quarantine areas must be adelgid free to prevent rejection by customers or agriculture inspectors. Importantly, transportation of nursery stock is a primary mechanism of long-distance transport of hemlock woolly adelgid (USFS 2005).

Nursery growers and extension personnel rely on efficacy data derived from forest or ornamental landscapes in order to manage hemlock woolly adelgid in nurseries. For example, nursery growers primarily use horticultural oil, bifenthrin, acephate, and imidacloprid applied to tree foliage, which effectively control the hemlock woolly adelgid in forest and landscape trees (McClure 1987; Stewart & Horner 1994; Rhea 1996; McClure et al. 2001; Raupp et al. 2008). However, landscape and forest systems differ from nurseries in many ways. Landscape and forest trees are typically larger than trees in nurseries, which could affect insecticide coverage and distribution of systemic insecticides (Byrne et al. 2010). Nursery trees are grown in soilless substrates rather than mineral soil and receive consistent water and nutrients via irrigation that are not available to landscape or forest trees. Soil moisture and organic matter can affect systemic insecticide uptake and transport and thus efficacy (Rouchaud et al. 1996; Diaz and McLeod 2005; Lui et al. 2006). Therefore, differences in plant habit and culture could result in better or worse efficacy on container-grown nursery trees than would be predicted by research in landscapes or forests. Knowing the relative efficacy of foliar and soil insecticide applications will allow nursery growers to manage hemlock woolly adelgid in the most effective and economical way.

The objective of this study was to provide growers with necessary information to achieve optimal control of hemlock woolly adelgid with a single insecticide application. To achieve this we evaluated the efficacy of 6 different insecticides using foliar, drench, soil-applied granular, and tablet formulations for control of the first hemlock woolly adelgid generation in spring. We then evaluated whether the insecticides can prevent re-infestation by the second generation of hemlock woolly adelgid in summer. Finally we evaluated how treatments and hemlock woolly adelgid infestation affect plant growth. To date there are no published evaluations of insecticide efficacy for hemlock woolly adelgid in container nurseries.

Materials and Methods

This study was conducted at the North Carolina State University, Mountain Horticultural Crops Research & Extension Center (MHCREC) in Mills River, North Carolina. We purchased Eastern hemlock trees that were 134.5 ± 2 cm tall in #7 (26.5 L) containers from a local grower. The trees were free of hemlock woolly adelgids and had never received insecticide applications. For our study, trees were grown under 30% shade cloth on a gravel pad with drip irrigation. Trees were potted in 7 pine bark: 1 sand substrate amended with 2 lbs dolomitic limestone per cubic yard of substrate and 1 lb per cubic yard micronutrients (Micromax® Scott-Sierra Horticultural Products Co., Marysville, Ohio). Plants were top dressed with a controlled release fertilizer to receive 21g nitrogen per container (Osmocote®, 18-6-12, Scott-Sierra Horticultural Products Co., Marysville, Ohio). Insecticides employed in this study are displayed in Table 1.

Efficacy of insecticides targeting first generation hemlock woolly adelgids

On Apr 12 and Apr 20, 2010, we infested plants by cutting infested Eastern hemlock branches

Table 1. Insecticide treatments (alphabetical by active ingredient) applied to hemlock trees in 7 gallon containers to control the hemlock woolly adelgid.

Trade Name	Active Ingredient	Application Method	Rate	Manufacturer
Untreated Control	—	—	—	—
TriStar® 30SG	acetamiprid	foliar	8 oz/100 gal.	Cleary Chem. Corp
Talstar® F	bifenthrin	foliar	0.22 oz/ gal.	FMC Corp.
Safari® 2G	dinotefuran	granular	2.6 g/gal. of pot	Valent USA Corp.
Safari® 20 SG	dinotefuran	foliar	8 oz/100 gal.	Valent USA Corp.
Marathon® 1%G	imidacloprid	granular	5 g/gal. of pot	Bayer
CoreTect™	imidacloprid	tablet	5 tablets/pot	Bayer
Marathon® II	imidacloprid	foliar	1.7 oz/ 00 gal.	Bayer
Horticultural Oil	paraffinic oil	foliar		
Kontos™	spirotetramat	foliar	1.7 oz/100 gal.	Bayer
Kontos™	spirotetramat	foliar	3.4 oz/100 gal.	Bayer
Kontos™	spirotetramat	drench	0.05 ml/l of pot	Bayer
Kontos™	spirotetramat	drench	0.1 ml/l of pot	Bayer
Horticultural oil		foliar	44 ml/gal.	Southern Agric. Insecticides

from trees in nearby natural areas when ovisacs and crawlers were present (Montgomery et al. 2009). We secured the infested branches to experimental plants with zip ties for 1 week each time. On Apr 28, 2010 we collected 1 branch tip from each cardinal direction of each tree and counted the number of crawlers on the terminal 4cm. We assigned trees to 1 of 5 blocks based on initial crawler density. Within each block trees were randomly assigned to 1 of 13 treatments (Table 1).

We applied insecticides on Apr 29, 2010. Foliar treatments were applied using a CO_2 powered backpack sprayer fitted with a single Spraying Systems D2-33 full-cone nozzle at 60 psi delivering 12.5 gpa. All foliar applications, except horticultural oil, included an adjuvant, Dyne-Amic (23.6ml/gal.; Helena Chemical Company, Collierville, Tennessee). We applied drench formulations by mixing product with 1 liter of water and pouring the solution evenly over the substrate. Granular applications were spread evenly on surface of substrate. CoreTect™ (20% imidacloprid and 80% 12-9-4 fertilizer) tablets were inserted approximately 10cm below the substrate surface. Hemlock woolly adelgids were counted as described 1, 7, 14, 28, and 42 d after treatment.

Residual efficacy to prevent re-infestation by second generation hemlock woolly adelgid

In Jun 2010, ovisacs and second generation crawlers were present on natural hemlock stands near MHCREC. On Jun 23 and 31, 2010, we re-infested the experimental trees as described previously. At this time we also infested a second set of untreated, previously uninfested trees to measure the success of second generation infestation in the absence of insecticides. Hemlock woolly adelgids were counted as described on Jul 8 (70 DAT) and Jul 22 (84 DAT) then again on Oct 8 (154 DAT), 2010.

Effect of insecticides and hemlock woolly adelgid on plant growth

As a measure of overall plant growth, the height of each plant and 2 perpendicular width measurements were recorded before the trial on Apr 28 (0 DAT) and on Oct 8, 2010 (154 DAT). To evaluate plant growth more specifically, the length of current year's growth was measured on 5 randomly selected branch tips per plant (Montgomery et al. 2009).

Statistical analysis of first generation and second generation hemlock woolly adelgid abundance and plant growth was conducted with ANOVA using initial abundance as a blocking factor (Proc Mixed, SAS 9.1 2002). If the ANOVA was significant ($P < 0.05$) means were compared using Fisher's protected LSD (Proc Mixed, SAS 9.1 2002).

Results

Efficacy of insecticides targeting first generation hemlock woolly adelgids

All insecticides significantly reduced the abundance of first generation crawlers compared to untreated controls by 2 wk after treatment (Table 2). In general, foliar products reduced hemlock woolly adelgid abundance more quickly

Table 2. Mean (±SE) hemlock woolly adelgid abundance (in order of abundance) on 4 4-cm hemlock branch tips collected 0, 1, 7, 14, 28, and 42 days after treatment (DAT) with insecticides.

Treatment	App. Method	Mean (±SE)[1] hemlock woolly adelgid abundance					
		0 DAT	1 DAT	7 DAT	14 DAT	28 DAT	42 DAT
Untreated Control	—	7.9 ± 3.4	4.9±1.2 ab	5.7 ± 1.8	4.2 ± 1.7 a	4.8 ± 1.2 a	6.1 ± 2.4 a
Marathon 1%G	granular	6.4 ± 1.7	5.8 ± 1.8 a	0.8 ± 0.4	0.1 ± 0.1 b	0.3 ± 0.3 c	0.0 ± 0.0 b
Safari 2G	granular	6.0 ± 1.7	3.9 ± 1.3 abc	0.5 ± 0.5	0.2 ± 0.2 b	0.0 ± 0.0 c	0.0 ± 0.0 b
Hort. Oil	foliar	7.7 ± 2.5	3.6 ± 1.3 bc	1.2 ± 0.8	0.4 ± 0.2 b	0.0 ± 0.0 c	0.0 ± 0.0 b
CoreTect	tablet	6.1 ± 1.8	2.6 ± 0.8 cd	1.8 ± 1.4	0.8 ± 0.7 b	0.8 ± 0.4 bc	0.1 ± 0.1 b
Talstar	foliar	7.3 ± 2.2	0.7 ± 0.5 de	0.5 ± 0.4	0.3 ± 0.2 b	0.0 ± 0.0 c	0.2 ± 0.2 b
TriStar 30SG	foliar	6.1 ± 1.5	0.4 ± 0.1 e	2.1 ± 1.7	0.7 ± 0.7 b	2.6 ± 1.8 b	0.0 ± 0.0 b
Kontos (high rate)	drench	7.5 ± 2.7	0.3 ± 0.3 e	2.6 ± 1.1	1.7 ± 1.0 b	0.1 ± 0.1 c	0.0 ± 0.0 b
Marathon II	foliar	6.8 ± 2.0	0.2 ± 0.2 e	0.9 ± 0.6	0.3 ± 0.3 b	0.0 ± 0.0 c	0.3 ± 0.2 b
Kontos (low rate)	foliar	7.4 ± 2.6	0.1 ± 0.1 e	4.7 ± 2.7	1.4 ± 0.5 b	0.1 ± 0.1 c	0.1 ± 0.1 b
Kontos (low rate)	drench	8.4 ± 3.4	0.0 ± 0.0 e	2.0 ± 1.1	0.9 ± 0.3 b	1.7 ± 0.9 b	0.2 ± 0.1 b
Kontos (high rate)	foliar	7.8 ± 2.5	0.0 ± 0.0 e	2.1 ± 0.7	1.0 ± 0.4 b	0.3 ± 0.1 c	0.0 ± 0.0 b
Safari 20 SG	foliar	6.2 ± 1.6	0.0 ± 0.0 e	2.8 ± 1.7	1.2 ± 0.8 b	0.0 ± 0.0 c	0.0 ± 0.0 b
$F_{12,48}$; P		1.28; 0.259	8.95; <0.001	1.38; 0.209	2.58; 0.010	7.39; <0.001	9.37; <0.001

[1]Numbers followed by the same letter within a column are not significant at $P < 0.05$.

than drench, granular or tablet formulations. The exception to this was Kontos, which at high and low rate drench applications, reduced hemlock woolly adelgid abundance to levels similar to foliar applications by 24h after treatment (Table 2).

Residual efficacy to prevent re-infestation by second generation hemlock woolly adelgid

The second generation of hemlock woolly adelgids did not become as abundant on control trees as the first generation (Table 3). Two wk after reinfestation, hemlock woolly adelgid abundance was significantly greater on control trees than in all insecticide treatments except horticultural oil (Table 3). Hemlock woolly adelgid abundance decreased over the next 12 wk on all treatments. After summer aestivation, 14 wk after reinfestation and 22 wk after insecticide applications, only the control, horticultural oil, and Talstar treatments had hemlock woolly adelgid in our samples (Table 3).

Effect of insecticides and hemlock woolly adelgid on plant growth

There was no effect of any treatment on plant growth as measured by change in plant height ($F_{12,48}$ = 0.57; P = 0.856), change in plant width ($F_{12,48}$ = 0.52; P = 0.888), or tip growth ($F_{12,48}$ = 0.74; P = 0.702) (data not shown).

Discussion

Our research is the first published account of the speed and duration of insecticide efficacy for managing hemlock woolly adelgid in container-grown hemlock trees. In particular, we demonstrated that Kontos is a promising new insecticide for managing hemlock woolly adelgid in nursery stock that provides rapid, season-long efficacy. Our research also confirms the efficacy of imidacloprid and dinotefuran formulations that have been relied upon for hemlock woolly adelgid management in forest and landscape trees (Stewart & Horner 1994; Rhea 1996; McClure et al. 2001; Raupp et al. 2008).

Pyrethroid and organophosphate insecticides, such as bifenthrin and acephate, are among the insecticides most frequently used to manage hemlock woolly adelgid in nurseries (S. Frank, personal observation). Growers apply these products at least 2 times during the growing season to prevent hemlock woolly adelgid infestation of nursery stock that would otherwise make trees unsalable. These broad-spectrum insecticides kill natural enemies and other non-target organisms on contact and leave a toxic residual that lasts for weeks after application (Raupp et al. 2001). As a consequence, pyrethroid and organophosphate insecticide use can result in secondary outbreaks of mites (Hardman et al. 1988; Prischmann et al. 2005), scale (McClure 1977; Raupp et al. 2001), and other pests (DeBach and Rose 1977; Hardman et al. 1988). Imidacloprid is the other most commonly used insecticide to manage hemlock woolly adelgid in nurseries and other systems (S. Frank, personal observation). Although imidacloprid is less toxic to natural enemies it can still promote spider mite outbreaks (Raupp et al. 2004).

Table 3. Mean (±SE) hemlock woolly adelgid abundance on 4 4cm hemlock branch tips 14, 28, and 98 days after reinfestation (Dari) with second generation crawlers.

Treatment	App. Method	Mean (±SE)[1] hemlock woolly adelgid abundance		
		14 DARI (70 DAT)	28 DARI (84 DAT)	98 DARI (154 DAT)
Untreated control	—	2.0 ± 0.8 a	0.4 ± 0.2	0.2 ± 0.1
Kontos™ (high rate)	foliar	1.0 ± 0.4 ab	0.4 ± 0.4	0.0 ± 0.0
Horticultural Oil	foliar	0.8 ± 0.3 b	0.3 ± 0.2	0.8 ± 0.3
CoreTect	tablet	0.6 ± 0.2 bc	0.5 ± 0.3	0.0 ± 0.0
Kontos™ (high rate)	drench	0.6 ± 0.3 bc	0.0 ± 0.0	0.0 ± 0.0
Kontos™ (low rate)	foliar	0.6 ± 0.2 bc	0.5 ± 0.3	0.0 ± 0.0
Kontos (low rate)	drench	0.5 ± 0.4 bc	0.1 ± 0.1	0.0 ± 0.0
Marathon® 1%G	granular	0.4 ± 0.3 bc	0.0 ± 0.0	0.0 ± 0.0
Talstar® F	foliar	0.4 ± 0.1 bc	0.2 ± 0.1	0.2 ± 0.1
TriStar® 30SG	foliar	0.3 ± 0.1 bc	0.0 ± 0.0	0.0 ± 0.0
Marathon® II	foliar	0.3 ± 0.2 bc	0.2 ± 0.1	0.0 ± 0.0
Safari® G	granular	0.1 ± 0.1 c	0.0 ± 0.0	0.0 ± 0.0
Safari® 20 SG	foliar	0.1 ± 0.1 c	0.3 ± 0.2	0.0 ± 0.0
		$F_{12,50}$ = 2.37; P = 0.017	χ^2_{12} = 19.06 P = 0.0884	χ^2_{12} = 32.3 P < 0.001

[1]Numbers followed by the same letter within a column are not significant at $P < 0.05$.

Spider mites and soft and armored scale are also important pests of hemlocks in nurseries. Our research indicates that Kontos, Safari, TriStar, and horticultural oil are effective alternatives to pyrethroids, organophosphates, and imidacloprid in nursery production. Products such as Safari and TriStar effectively control many armored scales and could be used when elongate hemlock scale or other armored scales need to be managed in combination with hemlock woolly adelgid. Kontos and Horticultural oil are alternatives to neonicotinoids that growers could use particularly if mite outbreaks are common.

Growers prefer to make foliar rather than drench insecticide applications because they can make foliar applications rapidly with airblast or other spray equipment. As expected the granular, drench, and tablet formulations of imidacloprid and dinotefuran took longer to achieve control because they must move into the soil then be taken up by the plant before insects ingest them. Despite a brief delay of about 2 wk, granular and drench formulations reduced hemlock woolly adelgid abundance to near zero in the first generation and prevented reinfestation by the second generation. Surprisingly, drench applications of Kontos reduced hemlock woolly adelgid abundance 1 day after treatment to levels comparable to foliar applications.

In our experiment, manual reinfestation of trees with second generation crawlers simulated the natural reinfestation that trees would experience if grown in a nursery near natural hemlock woolly adelgid infestations because crawlers can be carried to new trees by wind or birds. Our infestation method did not achieve as high a population in the second generation as in the first but the new untreated trees were infested with 2 hemlock woolly adelgid per 4cm of branch. All insecticide treatments reduced second generation abundance even though they had been applied 12 weeks earlier. The trend 4 wk after reinfestation was for lowest hemlock woolly adelgid abundance on trees that received granular and drench formulations though Tristar also had no hemlock woolly adelgid at this time. Hemlock woolly adelgid abundance in all treatments declined by Oct when the insects came out of aestivation and began feeding again. At this time, the only treatments with hemlock woolly adelgids present were the control trees and trees treated with the contact insecticides, horticultural oil and Talstar, as opposed to systemic insecticides. This indicates systemic insecticides - neonicotinoids and Kontos - provide season-long control of hemlock woolly adelgid even if they are applied to foliage which is the most rapid and preferred method of growers.

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ACOUSTIC DETECTION OF ARTHROPOD INFESTATION OF GRAPE ROOTS: SCOUTING FOR GRAPE ROOT BORER (LEPIDOPTERA: SESIIDAE)

WILLIAM R. SANDERS[1], R. W. MANKIN[2], OSCAR E. LIBURD[1] AND LUKASZ L. STELINSKI[3]
[1]Entomology and Nematology Department, University of Florida970 Natural Area Drive, Gainesville, FL 32611

[2]United States Department of Agriculture, Agricultural Research Service Center for Medical, Agricultural, and Veterinary Entomology, 1700 SW 23rd Drive, Gainesville, FL 32608

[3]Entomology and Nematology Department, Citrus Research and Education Center, University of Florida, 700 Experiment Station Road, Lake Alfred, FL 33850

ABSTRACT

The grape root borer, *Vitacea polistiformis* Harris, is the principal pest of grapes (*Vitis* spp. L.) in Florida where chlorpyrifos is 1 of the few chemicals registered for its control. However, chlorpyrifos is not an ideal treatment because it is highly toxic to birds, fish, aquatic invertebrates, and honeybees. Also, the recommended timing of application conflicts with harvest dates. There is an effective cultural control method, known as mounding, but this method is currently cost prohibitive for commercial production and is not widely used. If mounding could be applied only to infested plants, the cost of this method would be reduced considerably. This study evaluated the potential of acoustics for detecting the larvae *in-situ*. Human listeners assessed likelihood of arthropod infestation for each site based on live acoustic samples as they were being recorded. Computer software later constructed acoustic indicators from these recordings that were used for computer assessment of infestation likelihood. After recording, the roots of sampled vines were excavated to determine infestation levels. Infestation likelihood predictions of both human listeners and computer software largely reflected infestation condition of tested sites. Consequently, acoustic methods could be developed as tools for growers to employ mounding only at sites most likely to be infested, and thus enable more cost-effective use of this cultural control tactic.

Key Words: IPM, monitoring, mounding, grape pest, *Vitis* spp.

RESUMEN

El barrenador de la raíz de la uva, *Vitacea polistiformis* Harris, es la principal plaga de la uva (*Vitis* spp. L.) en la Florida, donde clorpirifos es uno de los pocos productos químicos registrados para su control. Sin embargo, el clorpirifos no es un tratamiento ideal, ya que es altamente tóxico para aves, peces, invertebrados acuáticos y abejas. Además, el tiempo recomendado para la aplicación del producto esta en conflicto con la fecha de cosecha. Existe un método eficaz de control cultural, conocido como "el montonar" (agregando la tierra debajo de la vid después de que las larvas se empupan en el suelo, que impeden que los adultos emergen), pero actualmente este método es muy costoso para la producción comercial y no se utiliza ampliamente. Si se aplica el montonar sólo a las plantas infestadas, el costo de este método se reduciría considerablemente. Este estudio evaluó el potencial de la acústica para detectar las larvas *en-sitio*. Oyentes humanos evaluaron la probabilidad de infestación por artrópodos para cada sitio basado en las muestras acústicas en vivo, mientras que fueron grabadas. El programa de computadora más tarde construyeron indicadores acústicos de estas grabaciones que se utilizaron para la evaluación hecha por computadora de la probabilidad de infestación. Después de la grabación, las raíces de la vid muestreadas fueron excavados para determinar los niveles de infestación. Las predicciones de la probabilidad de infestación tanto por los oyentes humanos y por los programas de computadora en gran parte reflejó la condición de infestación de los sitios evaluados. En consecuencia, los métodos acústicos se podrían desarrollar como una herramienta para que los productores empleen el montonar sólo en los sitios de mayor probabilidad de estar infestados y por lo tanto permita un uso más rentable de esta táctica de control cultural.

In Florida, the amount of land devoted to grape (*Vitis* spp. L.) cultivation has steadily increased over the past several years and is now over 400 hectares (Weihman 2005). The number of registered Florida wineries has also increased from 13 to 17 in the past 4 years (FGGA 2009). In 2008, Florida was the fifth largest wine producer of all states in the U.S. with total production equaling 6.6 million liters (Hodgen 2008), and in 2009 Florida was the second largest consuming state (Anderson 2009).

The grape root borer, (GRB) *Vitacea polistiformis* Harris, is the key pest of grapes in Florida (Liburd & Seferina 2004) and Georgia (Weihman 2005) and an important pest in North Carolina (Pearson & Schal 1999) and South Carolina (Pollet 1975). As the Florida grape industry expands, the grape root borer will become a more serious threat to the industry.

Upon hatching, larvae immediately burrow into the soil where they bore into and feed upon grape

roots, reducing vine vigor and cold tolerance, increasing susceptibility to pathogens and drought, and hastening vine death (Pearson & Meyer 1996). A low economic injury level (EIL) has been established in Georgia, 0.074 larvae per vine (Dutcher & All 1979). One larva feeding at the root crown can cause as much as 47% decrease in yield. Entire vineyards have been destroyed in Florida, and the grape root borer was cited as the reason for cessation of grape production in South Carolina (Pollet 1975).

The organophosphate chlorpyrifos (Lorsban®) is currently 1 of the few registered chemicals for control of GRB. Chlorpyrifos is applied to the root area as a soil drench but is not ideal for control of GRB because it is toxic to birds, fish, aquatic invertebrates, and honeybees. It is also moderately toxic to pets and livestock and is suspected of being carcinogenic in humans (USCB 1996). Florida vineyards are relatively small, usually 1 to 4 ha, and are typically family owned and operated. Most grape growers live on site with their families so many are reluctant to use chlorpyrifos because of its potential safety and environmental hazards.

A practice known as mounding has shown some promise as an effective control alternative to pesticides. When larvae are ready to pupate, they usually migrate to within 5 cm of the soil surface to form their pupal cells. At this depth, pharate adults are easily able to emerge from the soil. However, placing a mound of soil around the base of the vine after larvae have begun to pupate forces pharate adults to travel farther before reaching the soil surface, and mortality increases with the distance traveled. Sarai (1969) found 100% mortality when mounds were 19 cm high. Once emergence begins to decline for the year, mounds must be removed so that mounding may be done the next year. Mounding is currently labor intensive, which makes the technique cost prohibitive for most growers. The cost of mounding would be greatly decreased if growers were able to determine whether or not a given plant is infested. This would eliminate the cost of unnecessarily mounding vines that are not infested.

This study evaluated the potential of acoustic detection as a means of determining the presence or absence of larvae in an individual grapevine's root system. This detection system will make the sustainable practice of mounding much more attractive to growers, decrease pesticide use, and its associated environmental impact. The acoustic detection method could be implemented wherever the grape root borer is a problem.

Materials and Methods

Acoustic Instruments, Signal Recording, and Soil Sampling Procedures

Acoustic records were collected from 28 root systems at a commercial vineyard near Lithia, Florida, and 8 root systems at a commercial vineyard near Florahome, Florida. Recordings were taken between April 28 and June 9, 2009. Air temperatures ranged between 29 and 35°C during the recordings. Two accelerometer amplifiers and a recorder (details of the instruments are described in Mankin et al. 2009) were set up in the storage bed of an electric cart and transported throughout the vineyard to vines exhibiting symptoms of infestation: wilting, yellowed or dead leaves, and reduced leaf area as compared with neighboring plants of the same variety. A 30 cm nail was inserted into the root system of the selected vine. The accelerometer was attached to the nail head by a magnet. One or more listeners took notes and monitored the signals from potential larval feeding and movement in the roots during a recording period of 3 min or longer. Within 1 to 2 h after recording, the vine was excavated and the contents of the root system were examined to obtain an independent verification of whether a site was uninfested or contained insects.

Listener Assessment of Infestation Likelihood

Subterranean larvae typically produce spectrally distinctive, 3 to 10 ms sound impulses during movement and feeding activities (Mankin et al. 2000, 2009). These sound impulses can be identified and recognized as insect-produced sounds by most listeners after 10 to 20 min practice with the accelerometer and headphones. In this experiment, there were 2 primary listeners and 5 occasional listeners.

Assessments were performed as in Mankin et al. (2007), where l_{ow} indicates detection of no valid, insect-produced sounds or only a few faint sounds during a recording period, m_{edium} indicates detection of sporadic or faint groups of valid sounds, and h_{igh} indicates detection of frequent, easily detectable groups of valid sounds. No attempt was made to distinguish between pest and non-pest species in the assessment. Comparisons between the distributions of assessed infestation likelihoods at infested and uninfested recording sites were performed using the NPAR1WAY procedure in SAS (SAS Institute 2004).

Digital Signal Processing and Classification

Recorded signals were band-pass filtered between 0.2 and 5 kHz to facilitate subsequent analysis, and visualized with audio playback using Raven 1.3 software (Charif et al. 2008). In initial screenings, we confirmed the presence of groups (trains) of discrete, 3 to 10 ms impulses separated by intervals <250 ms that had occurred frequently where insects were recovered in previous studies (Mankin et al. 2009). Trains containing 6 or more impulses were a focus of analysis because they often were identified as insect

sounds in playbacks of recordings from infested sites in this and previous studies (Mankin et al. 2009).

The impulses and impulse trains detected in the recordings were analyzed with customized software, DAVIS (Digitize, Analyze, and Visualize Insect Sounds, Mankin et al. 2000), which discarded long-duration, low frequency background noise (Mankin et al. 2007) and then compared the spectrum of a 512-point time-slice centered around the peak of each impulse against averaged spectra (spectral profiles) constructed as described in RESULTS.

The impulse sequences were screened to identify and characterize trains of impulses that listeners typically classify as separate, individual sounds. Each train was labeled according to the spectral profile matched by a plurality of its impulses. The beginning and ending times of impulse trains, their labels, and the number of impulses per train were stored in separate train-sequence spreadsheets for each recording.

RESULTS

The root systems of 25 (of 36 total) recording sites exhibited *V. polistiformis* larval damage, although only 1 live larva was recovered. Collectively, 27 root systems contained 1 or more invertebrates of various species (Tables 1 and 2). Among these were 41 Coleoptera (including 4 *Mycotrupes* (Coleoptera: Geotrupidae), 3 Tenebrionids, 1 Cerambycid, 4 *Phyllophaga* (Coleoptera: Scarabaeidae) larvae and 1 *Anomala* (Coleoptera: Scarabaeidae) larva) 1 Cetoniid larva, 6 *Lepisma saccharina* (L.) (Thysanura: Lepismatidae), and 3 burrowing roaches. Six sites contained *Solenopsis invicta* Buren (Hymenoptera: Formicidae) workers, and 3 had termite workers. Other organisms found in the root systems included 5 unidentified worms, 3 Diplopoda, 3 large spiders, and an earthworm. Only the *V. polistiformis* was to be targeted as a pest (see DISCUSSION), but for purposes of categorizing sites, we considered a site to be infested if the excavated root system contained 1 or more invertebrates capable of producing sounds.

Spectral Profiles

Two types of impulses that could be readily identified by their temporal patterns as insect-produced sounds (Mankin et al. 2000, 2009) appeared frequently in initial screenings of signals detected at recording sites where excavations verified infestation, and a third type appeared at only 9 recording sites. All 3 types of impulses stood out against the background noise because their short durations and distinctive spectral patterns (Mankin et al. 2000, 2007, 2009). Spectral profiles of these impulses, i.e., averaged measurements of their power spectra (Mankin et al. 2000), were calculated to assist in discriminating insect sounds from background noise (Fig. 1). A profile of 1 of the 2 most frequently occurring insect sound impulses, s_{highdB}, was constructed from a series of 128 consecutive impulses in a relatively noise-free recording that contained several sounds identified in previous studies (Mankin et al. 2009) to be indicative of insect burrowing activity. The second profile, s_{middB}, was constructed from a series of 94 consecutive impulses in a recording that

TABLE 1. NUMBERS OF INVERTEBRATES RECOVERED FROM ROOTS, LISTENER ASSESSMENTS, AND RATES OF S_{HIGHDB}, S_{MIDDB}, AND S_{LOWDB} TRAINS AND BURSTS AT SITES WHERE S_{LOWDB} BURSTS WERE DETECTED.

No. recovered					Rate (No./min) of					
beetle			other inv.[3]	Assessed infest. likelihood	s_{highdB}		s_{middB}		s_{lowdB}[4]	
Ants	larvae[1]	adult[2]			trains	bursts	trains	bursts	trains	bursts
0	0	1	4	m_{edium}	6.70	0.00	4.69	0.67	15.41	10.72
≥1	0	2	3	h_{igh}	8.31	2.77	2.77	2.77	2.77	2.77
0	0	2	0	h_{igh}	23.39	3.19	8.50	0.53	2.66	1.06
≥1	0	0	0	m_{edium}	10.55	0.00	14.90	0.62	9.93	3.10
0	3	2	1	h_{igh}	3.55	0.00	2.13	0.00	4.97	1.42
0	2	0	1[5]	h_{igh}	7.97	0.61	9.19	0.00	0.61	0.61
0	0	2	1	h_{igh}	0.00	0.00	0.00	0.00	3.20	1.07
0	1	3	8	m_{edium}	1.49	0.00	13.42	0.00	2.24	0.75
0	0	1	0	m_{edium}	2.70	0.00	4.32	0.00	1.08	0.54

[1]Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp.
[2]Including *Mycotrupes* sp.
[3]Other invertebrates included Lumbricid sp., Diplopoda sp., Blattella sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and large spider.
[4]Recording sites arranged in order of the rates of s_{lowdB} bursts
[5]One *V. polistiformis* larva was found in the root system at this recording site.

TABLE 2. NUMBERS OF INVERTEBRATES RECOVERED FROM ROOTS, LISTENER ASSESSMENTS, AND RATES OF s_{highdB} AND s_{middB} TRAINS AND BURSTS AT SITES WHERE s_{lowdB} BURSTS WERE NOT DETECTED.

No. recovered					Rate (No./min) of			
	beetle		other	Assessed infestation	shighdB		smiddB[4]	
Ants or Termites	larvae[1]	adults[2]	invert.[3]	likelihood	trains	bursts	trains	bursts
0	0	0	1	h_{igh}	61.82	28.17	10.96	0
≥1	0	0	1	h_{igh}	37.56	14.44	7.22	1.44
0	1	0	0	h_{igh}	13.38	10.03	1.34	0.67
≥1	2	0	1	h_{igh}	17.43	2.32	4.65	4.65
≥1	0	4	0	h_{igh}	3.32	0	17.43	5.81
≥1	0	0	0	m_{edium}	26.3	5.58	10.36	0
0	0	1	2	m_{edium}	3.16	0	8.2	3.16
0	0	1	0	h_{igh}	15.77	2.1	5.26	0
0	0	0	1	h_{igh}	9.71	0.75	11.95	0.75
0	0	1	0	l_{ow}	1.22	1.22	2.43	0
≥1	0	2	0	m_{edium}	7.64	0.69	1.39	0
0	0	1	0	m_{edium}	15.83	0.66	9.23	0
≥1	0	2	1	m_{edium}	0.55	0	3.3	0.55
0	1	1	0	m_{edium}	14.56	0	2.24	0
≥1	0	0	0	m_{edium}	3.6	0	8.99	0
0	0	0	0	l_{ow}	1.33	0	5.33	0
0	0	0	0	l_{ow}	0	0	5.48	0
0	0	0	0	l_{ow}	0.65	0	3.92	0
0	0	0	0	m_{edium}	1.84	0	1.84	0
0	2	0	0	m_{edium}	0.47	0	2.85	0
0	0	3	0	m_{edium}	0.47	0	1.41	0
0	0	0	0	l_{ow}	0.62	0	1.23	0
0	0	0	0	l_{ow}	0.79	0	0.79	0
0	0	0	2	m_{edium}	1.09	0	0	0
0	0	0	0	l_{ow}	0	0	0.52	0
0	0	0	0	l_{ow}	0	0	0	0
0	0	0	0	m_{edium}	0	0	0	0

[1]Including, *Phyllophaga* sp., *Anomala* sp., Tenebrionid sp., Cetoniid sp. Cerambycid sp.
[2]Including *Mycotrupes* sp.
[3]Including Lumbricid sp., Diplopoda sp., Mutillid sp. *Blattella* sp., *Lepisma saccharina* (L), *Nerthra stygica* Say, and a large spider.
[4]Recording sites arranged in order of summed rates of s_{highdB} and s_{middB} bursts.

contained several larval scraping sounds of slightly lower frequency. The third, less frequently occurring profile, s_{lowdB}, was constructed from a 0.1 s period containing 13 consecutive impulses of this distinctive type. The 3 types of impulses had similar temporal patterns but their spectral patterns diverged at frequencies above 2.6 kHz.

Various types of background noise also occurred frequently in all recordings, comprising about 80% of all sounds detected. Continuous noise could be discounted easily because insect sounds usually occur as brief impulse bursts (Mankin et al. 2009), but some low-frequency impulsive noise was discarded by matching it with 1 of 2 noise profiles. To exclude higher-frequency noise impulses, we constructed a noise profile, n_{highdB} (Fig. 1), as an average spectrum of impulses produced during a gust of light wind. A second noise profile, n_{lowdB} (Fig. 1), was constructed as an average spectrum of a 5 s period where impacts of water droplets from an irrigation hose were detected.

Insect Sound-Impulse Bursts

Although isolated s_{highdB}, s_{middB}, and s_{lowdB} impulses occurred frequently in the recordings, most of the signals that listeners interpreted as insect sounds appeared in bursts of more than 6 but less than 50 impulses of a given type, similar to bursts used successfully to construct indicators of insect infestation in other insect acoustic detection studies (Mankin et al. 2007, 2009). In analogy with such studies, we defined trains of type s_{highdB}, s_{middB}, and s_{lowdB} impulses to be a series of impulses of each type, separated by durations <0.25 s. Bursts of type s_{highdB}, s_{middB}, and s_{lowdB} were trains

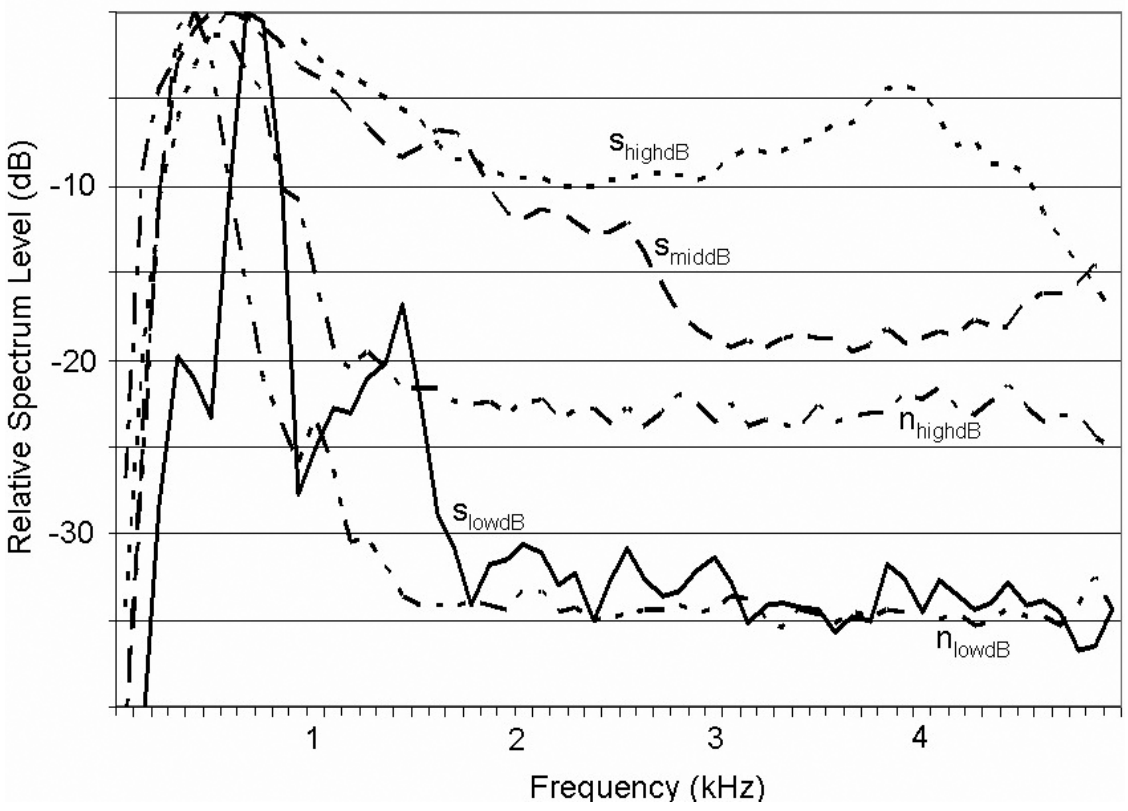

Fig. 1. Spectral profiles of insect-produced sound impulses, s_{highdB}, s_{middB}, and s_{lowdB}, compared with spectral profiles of wind-gust noise, n_{highdB}, and low-frequency background noise, n_{lowdB}, used in analyses to distinguish insect-produced sounds from background noise. The subscripts, highdB, middB, and lowdB, refer to the magnitudes of the relative spectrum levels of these profiles near 2.6 kHz, the midpoint of the 0.2-5 kHz range of frequencies analyzed. Spectrum level is relative to the maximum acceleration measured in the 0.2-5 kHz reference range.

of each impulse type that contained at least 7 but less than 50 impulses. We analyzed recordings from each of the 36 root systems using the DAVIS signal analysis system (Mankin et al. 2000). The DAVIS software calculated a power spectrum for each sound impulse with amplitude above a user-set threshold, and matched it against the spectra of the 3 signal and 2 noise profiles (Fig. 1) by calculating the least-squares difference between the impulse and profile signal levels at each spectrum frequency. The impulse was categorized according to the profile type for which the summed least-squares differences were smallest, unless that smallest least-squares sum exceeded a user set threshold, designating the impulse as uncategorized noise. The burst was categorized then according to the type of profile of its largest fraction of impulses. The rates of detection of trains and bursts in the 9 root systems that contained bursts of type s_{lowdB} are listed in Table 1. One site, assessed by listeners at h_{igh} likelihood of infestation, contained a *V. polistiformis* larva as well as 2 *Phyllophaga* larvae. Bursts of type s_{highdB} also were detected at this site. The rates of detection of trains and bursts in the other root systems are listed in Table 2. As in previous studies (Mankin et al. 2009), the rate of trains was correlated with, but not necessarily proportional to, the rate of bursts at each recording site. There were 14 root systems in which no bursts of any insect-sound profile type were detected. Five of these did contain insects but 9 were found to be uninfested when they were excavated.

Assessments of Infestation Likelihood

The listener assessments of infestation likelihood matched significantly with the presence or absence of insects in the root systems at the recording sites (Table 3). Only 1 infested site was ranked at l_{ow} likelihood of infestation, and all of the sites ranked at h_{igh} likelihood of infestation were infested.

To develop a computer assessment of infestation likelihood, we examined the rates of bursts of different types detected at different infested and

TABLE 3. LISTENER ASSESSMENTS OF RECORDING SITES DETERMINED BY EXCAVATION TO BE UNINFESTED OR INFESTED.

	No. sites	
Assessed likelihood	infested	uninfested
l_{ow}	7	1
m_{edium}	2	14
h_{igh}	0	12

$P = 0.0002$ that listener assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, $S = 61.5$, $Z = -4.09$).

uninfested sites, and constructed indicators of infestation likelihood as described in Mankin et al. (2007). Sites with rates of bursts of all 3 insect-sound profile types <0.5 / min were considered to have l_{ow} likelihood of infestation, whereas sites with rates of bursts of any insect-sound profile type >1.5/min were assessed at h_{igh} likelihood of infestation. Sites with intermediate rates were assessed at m_{edium} likelihood. Assessments of the results in Tables 1 and 2 based on these criteria are listed in Table 4. The computer assessments, like the listener assessments in Table 3, matched significantly with the presence or absence of insects in the root systems at the recording sites.

DISCUSSION

One of the goals of this acoustic detection study was to develop a method for detecting infestations of *V. polistiformis* within the root system, thereby decreasing the cost and labor of treatments such as mounding. Although it would be helpful to obtain more recordings from *V. polistiformis* larvae, the results of the study are sufficient to provide some insight into how detection might be accomplished. An important finding was that a vineyard contains a large variety of nontarget, sound-producing insects. The signals produced by such insects could easily confound the identification of a targeted pest unless the pest produces a distinctive, easily identifiable sound that distinguished it from nontarget insects.

A partial solution to this problem would be to include ambiguous signals as positive, i.e. count a false positive as a potential GRB larva. Considering the invertebrates and the burst rates in Table 1, for example, targeting all the sites that contained s_{highdB}, s_{middB}, and s_{lowdB} bursts would result in treatment of 9 out of 36 sites, only 1 of which actually contained a *V. polistiformis*. However, treating ¼ of the sites would be much less costly than treating all of them.

It is common for a vineyard to have approximately 735 vines per hectare. Assuming that the average price for unskilled farm labor is $20 per hour and that it takes 10 minutes to build a mound around a vine and 10 more minutes to remove the soil at the end of the season, the labor to treat 1 hectare would cost approximately $4900. If we assume that our findings of 25% infestation level apply to any vineyard, a farmer would spend approximately $1225 on mounding per hectare. It would therefore need to cost less than $3675 per hectare to acoustically sample all vines for the farmer to break even. It is estimated that the equipment would cost ~$3000 and last for 5 to 10 years. A farmer or scout could perform the assessment after a 15-20 minute training period.

Both human listeners and computer software were able to predict the presence or absence of infestation at statistically significant levels based upon spectral profile and temporal pattern analysis. However, human listeners were more likely to commit type I error whereas the computer was more likely to commit type II error. A type I error will cause the treatment of a vine when it is unnecessary, slightly raising the cost of treatment. However, a type II error will leave an infested site untreated, allowing emergence and reproduction. Without further refining of the spectral profiles or improvement of the software's analysis algorithm, it is recommended for a human listener to assess likelihood of infestation for pest management decisions.

TABLE 4. COMPUTER ASSESSMENT OF RECORDING SITES DETERMINED BY EXCAVATION TO BE UNINFESTED OR INFESTED.

	No. sites	
Assessed likelihood	infested	uninfested
l_{ow}	9	5
m_{edium}	0	9
h_{igh}	0	13

$P = 0.0005$ that computer assessment is independent of the absence or presence of infestation in the excavated roots (Wilcoxon two-sample exact test, $S = 67.5$, $Z = -3.83$).

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FLOWER THRIPS (THYSANOPTERA: THRIPIDAE) DISPERSAL FROM ALTERNATE HOSTS INTO SOUTHERN HIGHBUSH BLUEBERRY (ERICALES: ERICACEAE) PLANTINGS

ELENA M. RHODES AND OSCAR E. LIBURD
Department of Entomology and Nematology, University of Florida, Gainesville, FL 32611

ABSTRACT

Frankliniella bispinosa (Morgan) is the key pest of southern highbush blueberries (*Vaccinium corymbosum* L. × *V. darrowi* Camp) in Florida. Thrips feeding and oviposition injury to developing flowers can result in fruit scarring that renders the fruit unmarketable. Previous studies have shown that flower thrips can disperse into cultivated crops from surrounding host plants. Therefore, the objectives of this study were to identify alternate hosts of *F. bispinosa* adjacent to blueberry plantings and to determine if *F. bispinosa* emigrates into blueberry plantings from these hosts. Plant surveys conducted in Apr of 2007 and from Nov 2007 until Mar 2008 revealed several reproductive hosts of *F. bispinosa*, including: Carolina geranium (*Geranium carolinianum* L.), white clover (*Trifolium repens* L.), and wild radish (*Raphanus raphanistum* L.). In a subsequent study, we monitored thrips population development in a blueberry planting and in an adjacent white clover field during early spring in 2009 and 2010. Flower thrips populations in the white clover and blueberry planting developed at the same time with the highest numbers of thrips recorded from the center of the blueberry field in both years. Although white clover grows abundantly adjacent to blueberry plantings in the spring our findings indicate that clover does not appear to be a significant source for thrips inoculation of southern highbush blueberry plantings in Northern Florida.

Key Words: flower thrips, *Frankliniella bispinosa*, southern highbush blueberries, *Vaccinium corymbosum* × *V. darrowi*

RESUMEN

Frankliniella bispinosa (Morgan) es la plaga clave del arándano del sur un arbusto alto (*Vaccinium corymbosum* L. × *V. darrowi* Camp) en la Florida. La alimentación de los trips y el daño asociado con la oviposición a las flores en desarrollo pueden resultar en cicatrices en la fruta que hace que la fruta no se pueda vender. Estudios previos han demostrado que trips de las flores pueden dispersarse a los cultivos desde los hospederos de plantas en el arrededor. Por lo tanto, los objetivos de este estudio fueron identificar hospederos alternativos de *F. bispinosa* adyacentes a las plantaciones de arándanos y el determinar si *F. bispinosa* en estas plantaciones plantaciones emigra desde esos hospederos. Un sondeo de plantas realizado en abril del 2007 y desde noviembre del 2007 hasta marzo del 2008 reveló varios hospederos que pueden soportar la reproducción de *F. bispinosa*, entre ellos: el geranio de Carolina (*Geranio carolinianum* L.), trébol blanco (*Trifolium repens* L.) y el rábano silvestre (*Raphanus raphanistum* L.). En un estudio posterior, monitoreamos el desarrollo de la población de trips en una plantación de arándanos y en un campo de trébol blanco adyacentes durante el principio de la primavera del 2009 y del 2010. La poblacion de trips de flores en el trébol blanco y en la plantación de arándanos desarrollaron al mismo tiempo con el mayor número de trips registrada en el centro del campo de arándanos por ambos años. A pesar que el trébol blanco crece en abundancia al lado de los campos de arándanos sembrados en la primavera, nuestros resultados indican que el trébol no parece ser una fuente importante para la inoculación de trips en los campos de arándano en el norte de la Florida.

Blueberries are a high value crop in Florida. During 2009, 6.4 million kg (14.1 million lbs) of fresh market blueberries were harvested from 1,295 ha (3,200 acres) at an average of $11.89 per kg ($5.40 per lb) (USDA 2010). The development of southern highbush (SHB) blueberries (*Vaccinium corymbosum* L. × *V. darrowi* Camp) allows Florida growers to take advantage of the highly profitable early season market (Williamson & Lyrene 2004). Southern highbush blueberry plants begin flowering in late Jan or early February and usually set fruit by mid to late Mar. Flower thrips, primarily *Frankliniella* spp., are the key pest of SHB blueberries.

A complex of flower thrips species causes injury to SHB blueberries in Florida (Arévalo-Rodriguez 2006). *Frankliniella bispinosa* (Morgan) is the most common species, accounting for approximately 90% of the adult thrips collected from both traps and flowers (Arévalo & Liburd 2007).

Flower thrips feed and reproduce on all parts of developing blueberry flowers. The resulting injury is magnified into scars when the fruit form, which make the fruit unsalable on the fresh market (Arévalo-Rodriguez 2006).

Thrips emigrate into crops from other cultivated plants that flower earlier and from wild plant species that also serve as hosts (Chellemi et al. 1994; Toapanta et al. 1996). Chellemi et al. (1994) found that 31 of 37 plant species adjacent to tomato fields contained thrips. Eighty-seven percent of the adult thrips collected were *Frankliniella* spp. *Frankliniella tritici* (Fitch) was the most common species collected, but species composition varied over time. *Frankliniella bispinosa* was the second most common species collected followed by *F. occidentalis* (Pergande), and *F. fusca* (Hinds).

It is often difficult to determine the true host range of a particular thrips species because thrips will often alight and feed upon many plants on which they cannot reproduce (Mound 2005; Paini et al. 2007). For example, although *F. fusca*, *F. occidentalis*, and *F. tritici* are found on tomato plants in Florida and can cause injury, only *F. occidentalis* reproduces on the tomato plants (Salguero-Navas et al. 1994).

Thrips will also use wild plant hosts when crop hosts are not flowering. Paini et al. (2007) found that *F. bispinosa* used 2 plant species, *Ligustrum sinense* Lour. and *Lagerstroemia indica* L., as reproductive hosts from May to Aug in north Florida. Similarly, Cockfield et al. (2007) found that native vegetation surrounding apple orchards supported *F. occidentalis* populations when apple trees were not flowering.

In blueberries, thrips are monitored using sticky traps or by direct sampling of the flowers. Although white, yellow, and blue traps attract thrips (Liburd et al. 2009), white traps are the best to employ. Yellow traps attract a large number of other insects including beneficials and the dark coloring of the blue traps can make it difficult to observe the thrips that are present on them (Liburd et al. 2009).

Flowers can be sampled in several ways. The simplest method involves gently tapping the flowers and allowing the thrips to fall onto a white sheet below for counting. Flowers can also be collected in a vial or plastic bag and then examined in the laboratory. Arévalo & Liburd (2007) developed a "shake and rinse" method that is as accurate as dissecting flowers and much more efficient.

The objectives of this study were 2 fold. 1) To examine blueberry plantings and adjacent fields for alternate hosts of thrips. 2) To investigate thrips dispersal from these host plants into blueberry plantings. The hypothesis of this study is: flowering plants support and sustain *F. bispinosa* populations when blueberry plants are not flowering and thrips disperse into blueberry plantings from these flowering plants when blueberries begin to flower and cause economic damage.

Materials and Methods

Preliminary Plant Survey

In our initial survey, flower samples from 3 of the most common flowering plants found at the University of Florida Plant Science Research and Education Unit (PSREU) in Citra, Florida, were collected in Apr 2007. These plants included cutleaf evening primrose (*Oenothera laciniata* Hill), white clover (*Trifolium repens* L.), and wild radish (*Raphanus raphanistum* L.). Based on size relative to each other, 8 primrose flowers, 6 clover flowers, and 25 wild radish flowers were collected randomly and placed into vials containing 70% ethanol. Thrips adults and larvae were extracted from flowers using the "shake and rinse" method developed by Arévalo & Liburd (2007). In this method, each vial was shaken vigorously for 1 min and then the contents of the vial were emptied onto a metal screen (6.3 × 6.3-mm mesh) placed over a 300-ml white polyethylene jar. The flowers were gently opened, rinsed with water, and then the rinsate was examined under a dissecting microscope. The numbers of thrips and other arthropods present were recorded. The flowers left on the screen were emptied into another 300-ml polyethylene jar containing 10 ml of water. Once the lid was placed on the jar, the jar was shaken vigorously for 1 min as before. The rinse procedure was repeated as before except that the flowers were rinsed with 70% ethanol. If thrips were found in the second rinse water, the procedure was repeated for a third time (shaking the flowers in 70% ethanol and rinsing with water). Thrips adults were identified to species using a key developed for Florida SHB blueberries by Arévalo et al. (2006). Thrips that did not match the character descriptions in the key were sent to the Division of Plant Industry (DPI) in Gainesville, Florida for identification.

Plant Survey 2

In our second survey, the flowering plant species within a 0.52-ha (1.2 acre) blueberry planting and the surrounding area at the Citra PSREU site were flagged and sampled to determine whether or not they were suitable hosts for *F. bispinosa*. For the purposes of this study, a suitable host was defined as 1 in which *F. bispinosa* reproduces and is abundant. Plants were identified to genus and species (if possible).

Ten 27-m transects were taken from the blueberry planting and surrounding area described above. Two transects were on the border of the blueberry field and 8 were within the field (Fig. 1).

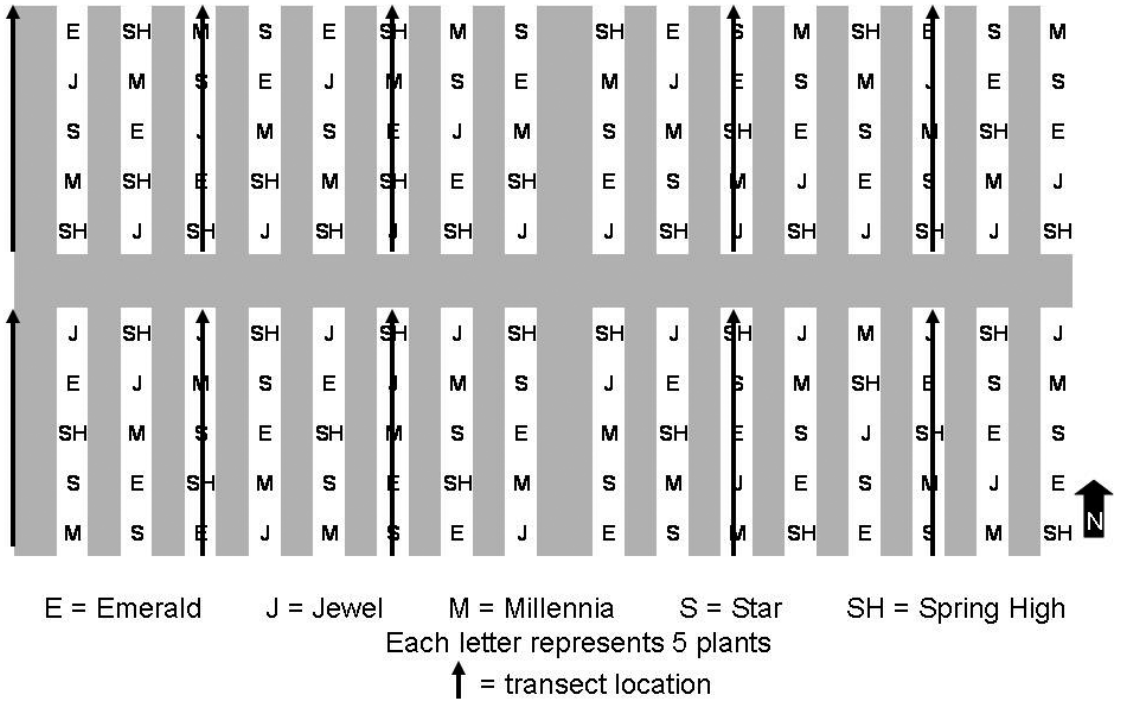

Fig. 1. Locations of transects (arrows) in blueberry planting. The letters indicate different southern highbush blueberry varieties (E = Emerald, J = Jewel, M = Millennia, S = Star, and SH = Spring High).

Flowering plants within a 0.6-m (2-ft) radius were sampled every 3-m (10-ft). The height and maximum width of the plants and percent coverage were measured. Plant samples were collected in small press and seal bags and brought back to the University of Florida Small Fruit and Vegetable IPM laboratory for identification.

Twenty flowers were collected from each plant species and placed in 50-ml plastic vials containing 70% ethanol. If less than 20 flowers were present, then all available flowers were collected. Samples were taken from the third week of Nov until the first week of Mar, during the first and third full week of each month. This time period encompasses the period 2 months before and during the blueberry flowering season. The samples were brought back to the laboratory at the University of Florida in Gainesville, FL. The "shake and rinse" method described above was used to collect the thrips from the flowers. Adults and larvae were counted and adults were identified to species as detailed previously.

Flower samples were also collected from an adjacent strawberry field to the west of the blueberry plots and from the blueberry bushes themselves. Ten strawberry flowers were collected from each of 4 rows. This was done once a month in Dec, Jan, and February. Twenty to 25 flowers were collected from SHB blueberries in each plot on each sample collection date.

Field Study

This study was conducted at a commercial blueberry farm in Windsor, Florida, during the spring of 2009 and 2010. This site was selected because white clover grows in the grassy areas adjacent to the blueberry bushes and our preliminary plant survey indicated that it is a reproductive host of *F. bispinosa* (see results section). All samples in the blueberry plot were collected from the same variety. In north Florida, white clover flowers from Dec through Jun. (Northfield et al. 2008). In contrast, southern highbush blueberries in north Florida flower from late Jan until early Mar. No insecticides were applied in either year during the course of the study.

The study area consisted of a field of white clover and part of a large blueberry planting that contained plants approximately 7 years old. In 2009 (Fig. 2a), 6 sampling sites within a 625-m^2 area of the clover and 12 sampling sites within a 2,400-m^2 area of the blueberry planting were selected. Four traps were placed in the corners of the clover sampling area and the other 2 were placed in the center, 8-m apart. The blueberry research area consisted of 3 rows of blueberries containing traps spaced 15 m, 30 m, 45 m, and 60 m from the clover with a buffer row between each replicate row. In 2010 (Fig. 2b), the setup was expanded to include 10 sampling sites in the clover

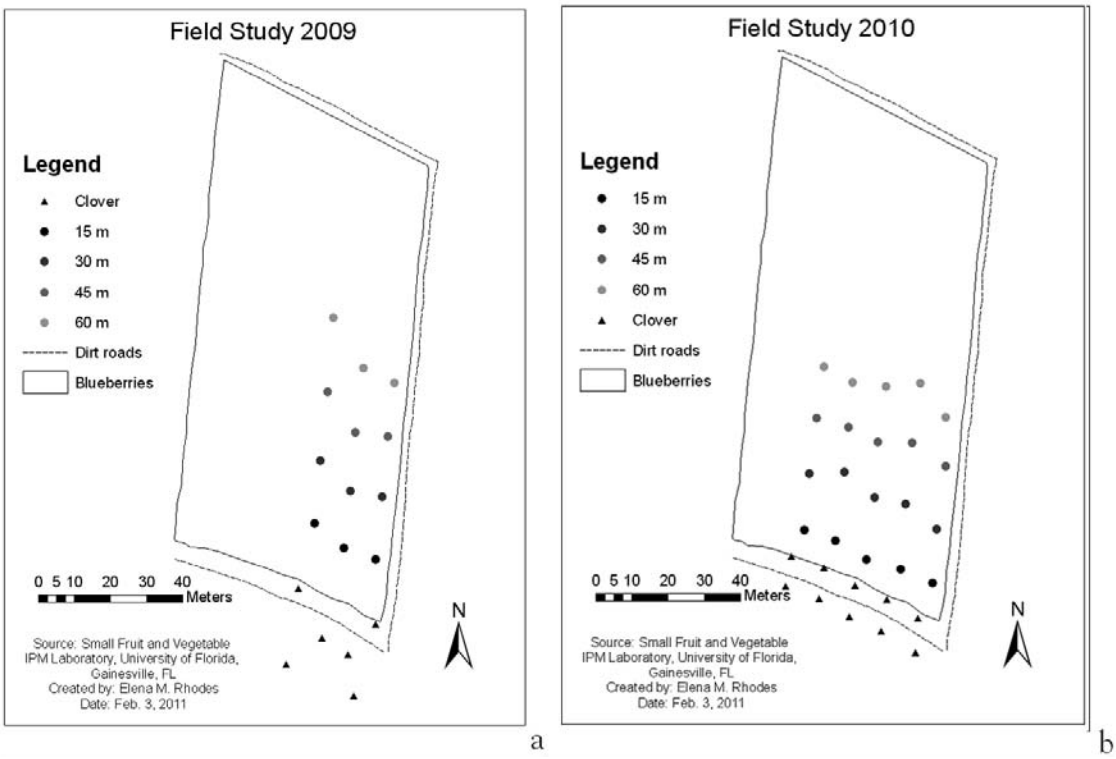

Fig. 2. Sampling point locations for the field study in a) 2009 and b) 2010. The block of blueberries continued to the west. Other blocks were located across the north and east dirt roads.

(660-m^2) and 5 rows (2,464-m^2) in the blueberry planting. All of the traps in the clover were spaced 10-m apart and hung so that the bottom of the trap was only a few centimeters from the tops of the clover plants. The traps in the blueberry rows were spaced as in 2009.

In 2009, white sticky traps with an 18 × 22 cm sticky surface (Great Lakes IPM, Vestaburg, MI) were set out every week and collected weekly for 5 weeks from Jan 31 to Mar 5 in the clover and blueberries. In 2010, traps were set out every week and collected weekly for 7 weeks from Feb 4 to Mar 25. When the traps were replaced, flower samples were collected from both the clover and blueberries adjacent to traps. Three to 5 clover flowers and 4 to 5 blueberry flower clusters (~20-25 flowers) per sample were collected each week.

Data analysis

The treatments included the 15 m, 30 m, 45 m, and 60 m samples in the blueberry planting in all data sets and the clover (0 m) only in the sticky trap data set. Clover and blueberry flowers differ too much in size and structure to allow for statistical comparison between them. Treatments were compared each week using a one-way analysis of variance (ANOVA) (SAS Institute 2002) and means were separated using the Least Significant Differences (LSD) test. Sticky trap data (x) were $\log_{10}$x transformed to meet the assumptions of the analysis. In 2009, the $\log_{10}$ (x +1) transformation was also used for thrips adults per flower (x), while thrips larvae per flower were transformed using the equation 1/√ (thrips per flower +1). For the 2010 flower sample data, transformation was not enough to cause the data to meet the ANOVA assumptions. Therefore, the nonparametric Friedman, Kendall-Babington Smith test (Hollander & Wolfe 1999) for general alternatives in a randomized complete block design was used to analyze the data.

RESULTS

Preliminary Plant Survey

In our initial survey, all of the adults collected were *F. bispinosa*. Forty seven percent of the thrips recorded were found in clover flowers consisting of 12 adults and 9 larvae whereas 49% of the thrips collected were from wild radish flowers consisting of 15 adults and 7 larvae. Only 4% of the thrips, 2 adults and no larvae, were collected from primrose flowers.

Plant Survey 2

Twelve different species of plants were found in the blueberry planting during our second survey (Table 1). Of these, 8 species flowered during the sampling period and thrips were found on 3 {Carolina geranium (*Geranium carolinianum* L.), hairy indigo (*Indigofera hirsuta* L.), and pusley (*Richardia* sp.)}. Thrips were also found in the blueberry and strawberry flowers.

Both adult and larval thrips were found in the Carolina geranium, pusley, strawberry, and blueberry flowers. The single adult found in the Carolina geranium was *F. bispinosa*. *Frankliniella fusca* and *Haplothrips graminis* Hood were found in the pusley (1 *F. fusca*, 18 *H. graminis*), and strawberry (4 *F. fusca*, 2 *H. graminis*) flowers. Most of the 60 adult thrips in the blueberry flowers were *Thrips* species, either *T. hawaiiensis* (Morgan) (24) or *T. pini* Karny (23). Seven *Frankliniella bispinosa*, three *Franklinothrips* sp., and 3 *H. graminis* were also present in the blueberry flowers. Three *H. graminis* adults were collected from the hairy indigo flowers, but no larvae were present.

Field Study 2009

Traps

On Feb 12, significantly more thrips per trap were collected at 45 m compared with the clover, at 15 m, and at 60 m (F = 3.92, df = 4, 17, P = 0.0267, Fig. 3a-b).

Flowers

No significant differences were found in thrips adults (all $F \leq 1.51$, df = 3, 11, $P \geq 0.29$) or larvae (all $F \leq 1.45$, df = 3, 11, $P \geq 0.30$) per blueberry flower among treatments on any sampling date. There were an average of 0.23 ± 0.08 thrips adults and 0.33 ± 0.12 thrips larvae per blueberry flower over the flowering season in the blueberry research area. Numbers of both adult and larval thrips increased as the season progressed.

A total of 65 thrips adults and 16 larvae were collected from the clover flowers during the blueberry flowering period. Larval numbers remained low throughout the flowering period, while adult numbers increased as the flowering period progressed.

Of all of the adult thrips sampled, 98% were identified as *F. bispinosa*. In the clover and 30 m samples respectively, all of the 65 and 50 thrips sampled were *F. bispinosa*. In the 15 m samples, 52 of the 54 thrips sampled were *F. bispinosa*. The remaining 2 were a *T. hawaiiensis* and a *T. pini*. In the 45 m samples, 60 of the 61 thrips sampled were *F. bispinosa*. The remaining thrips was a *Franklinothrips* sp. In the 60 m samples, 57 of the 59 thrips sampled were *F. bispinosa*. The remaining 2 were a *Franklinothrips* sp. and a *T. hawaiiensis*.

Field Study 2010

Traps

On Feb 11, there were significantly higher numbers of thrips per trap in the clover field compared with 15 and 60 m (F = 3.12, df = 4, 29, P = 0.0327, Fig. 4a-b). On Feb 25, there were significantly more thrips per trap at 30 and 45 m compared with the clover field (F = 2.89, df = 4, 29, P = 0.0429). On Mar 11, there were significantly higher numbers of thrips per trap at 30, 45, and 60 m compared with 15 m and the clover field (F = 5.95, df = 4, 29, P = 0.0017). On Mar 25, there were significantly higher numbers of thrips per trap at 45 m compared with all of the other treatments and at 60 m compared with 15 m and the clover field (F = 6.86, df = 4, 29, P = 0.0007).

TABLE 1. COMMON AND SCIENTIFIC NAMES OF THE PLANTS FOUND IN THE BLUEBERRY PLANTING, THE MONTHS WHEN THEY WERE FOUND, AND THE MONTHS WHEN THEY FLOWERED.

Common name	Scientific name	Months present	Months flowering
Carolina geranium	*Geranium carolinianum* L.	Nov-Mar	Feb and Mar
coffee senna[1]	*Senna occidentalis* L	Nov	none
hairy indigo	*Indigofera hirsuta* L	Nov and Dec	Nov
narrowleaf cudweed	*Gnaphalium falcatum* Lam.	Dec-Mar	Jan-Mar
oldfield toadflax	*Nuttallanthus canadensis* (L.)	Jan-Mar	Jan-Mar
pennywort (dollarweed)	*Hydrocotyle umbellata* L	Nov-Mar	none
pigweed[1]	*Amaranthus* sp.	Nov	none
pusley	*Richardia* sp.	Nov-Mar	Nov-Mar
red sorrel	*Rumex Acetosella* L.	Jan-Mar	none
spurge	*Euphorbia* sp	Nov	Nov
thistle	*Circium* spp.	Nov-Mar	Jan and Feb
wandering cudweed	*Gnaphalium pensylvanicum* Willdenow	Dec-Mar	Mar

[1]Identification is uncertain because flowers were not present.

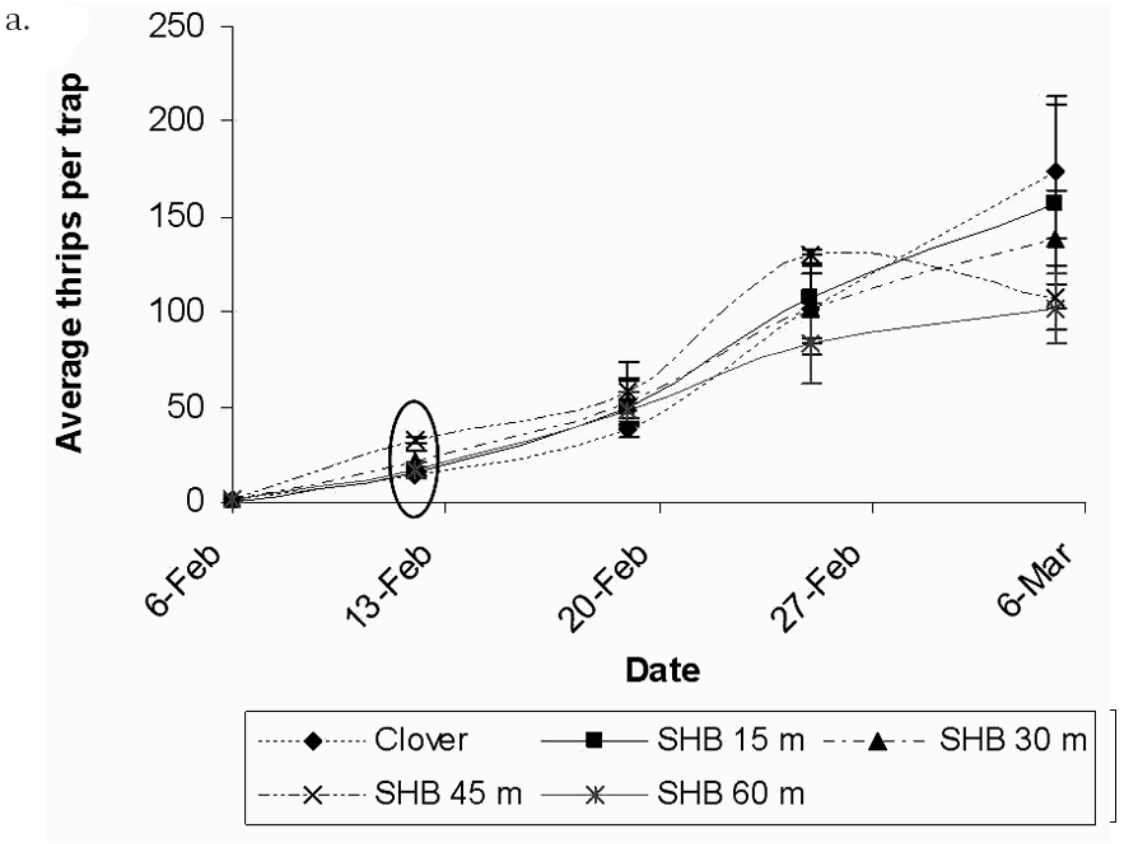

Fig. 3. a) Average thrips per trap in each treatment on each sampling date in 2009. Circled data indicate significant differences ($P \leq 0.05$). b) Average thrips per trap on Feb 12, 2009. Means with the same letter are not significantly different from each other at $P = 0.05$. Error bars indicate standard error of the mean. SHB = southern highbush.

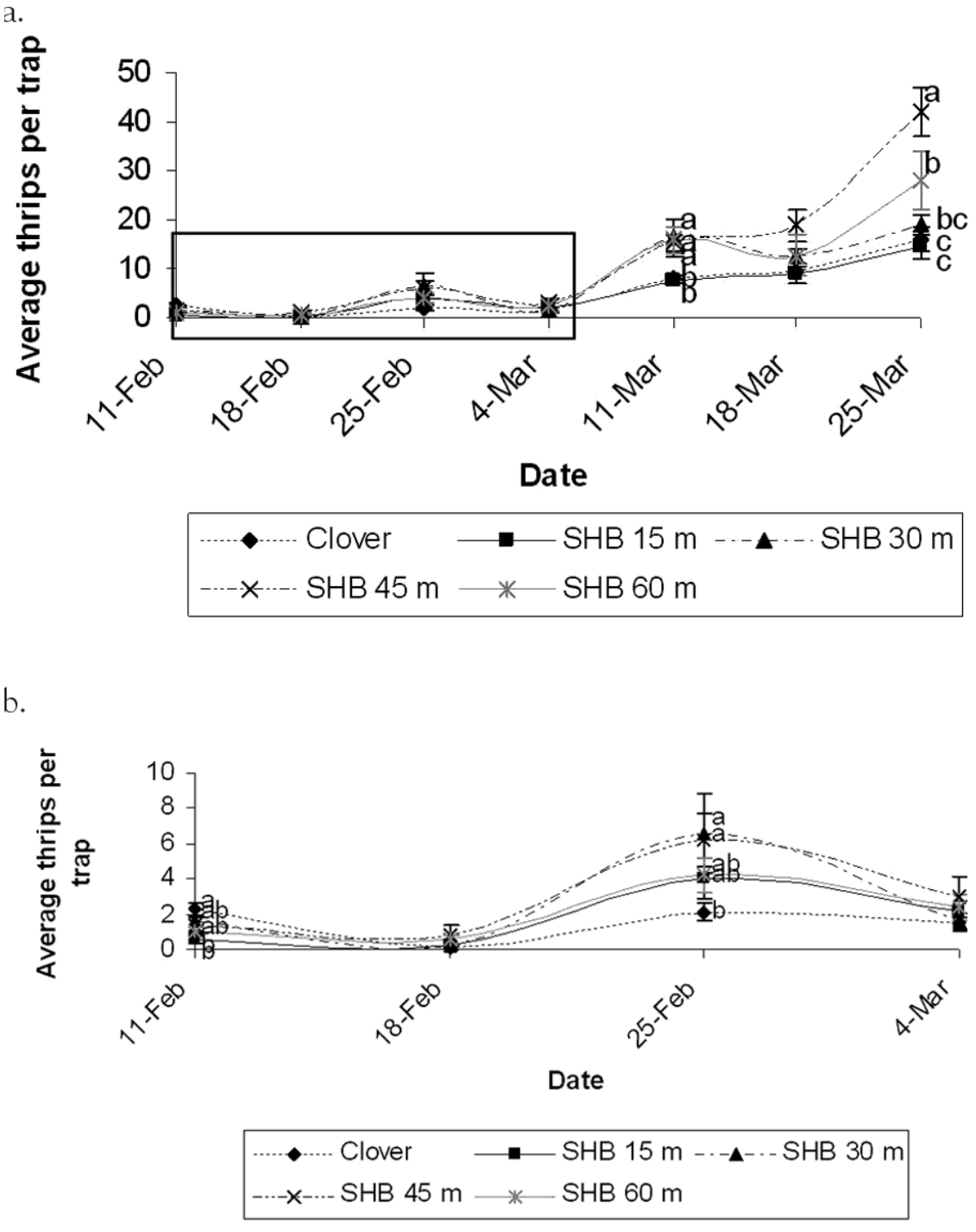

Fig. 4. Average thrips per trap a) throughout the flowering period and b) during the first 4 weeks of the flowering period (indicated by the box in a) in 2010. Treatments with the same letter are not significantly different from each other at P = 0.05. Error bars indicate standard error of the mean. SHB = southern highbush.

Flowers. There were no significant differences in thrips adults (all $S' \leq 6$, k, n = 5, 4, $P > 0.1$) or larvae (all $S' \leq 6.43$, k, n = 5, 4, $P \geq 0.09$) per blueberry flower on any sampling date. Average thrips adults per blueberry flower did not exceed 0.08 ± 0.04 during the sampling period and only a few adults were collected until Mar 4. A single thrips larva was collected on Feb 11 and another was collected on Feb 18. Thrips larvae were not collected again until Mar 18. Average thrips larvae per blueberry flower did not exceed 0.13 ± 0.06 larvae during the sampling period.

One, 3, and 8 thrips adults were present in the clover flowers on Feb 11, Mar 18, and Mar 25 respectively. In contrast, only a single larva was collected from the clover flowers on Feb 18.

As in 2009, most of the thrips (89%) collected during the blueberry flowering period in 2010 were *F. bispinosa*. Eight of the 12 adult thrips sampled from the clover were *F. bispinosa*. The remaining 4 were a single *F. fusca* and 3 specimens of an unknown species. In the 15 m samples, 14 out of 17 thrips were *F. bispinosa*. The other thrips sampled at 15 m were 2 *Franklinothrips* sp. and a single *Limothrips* sp. All of the 11, 7, and 19 thrips in the 30, 45, and 60 m samples respectively were *F. bispinosa*.

Discussion

Flower samples were collected from Carolina geranium, hairy indigo, narrowleaf cudweed (*Gnaphalium falcatum* Lam.), oldfield toadflax (*Nuttallanthus canadensis* (L.)), pusley, spurge (*Euphorbia* sp.), thistle (*Circium* spp.), white clover, and wild radish. However, it appears that only Carolina geranium, white clover, and wild radish are reproductive hosts of *F. bispinosa* due to the presence of immature stages. Northfield et al. (2008) also found that white clover and wild radish are reproductive hosts of *F. bispinosa*, especially in the spring (Apr-Jun). In contrast, Paini et al. (2007) found only adult *F. bispinosa* on wild radish (white clover was not sampled in this study). Carolina geranium was not sampled in either of these studies. Cutleaf evening primrose appears to be only a feeding host, since no larvae were found in the flowers.

Several other species of thrips were found on other plants that flowered during the sampling period. Hairy indigo had only *H. graminis* adults, which are predatory and may have been feeding on the large number of aphids also present in the flowers (data not shown). *Haplothrips graminis* adults were also frequently found in the pusley flowers. A single *F. fusca* adult was also found in the pusley flowers, as were a number of thrips larvae. Whether the *H. graminis* were feeding on the thrips larvae or other insects present in the flowers is not known. The same 2 species of adult thrips and a few thrips larvae were also found in the strawberry flowers.

The 2 thrips species, *T. hawaiiensis* and *T. pini*, were the dominant species collected from the blueberry flowers early during the survey. The blueberry flowering season at the Citra PSREU began during the last week of Jan. Most of the blueberry flowers collected during the survey were scattered, early blooms. A study conducted at the Citra PSREU following the survey showed that *F. bispinosa* quickly became the dominant species in the SHB blueberry flowers once the flowering season had begun (Liburd unpublished data).

Carolina geranium is a common weed in disturbed areas, agricultural land, and on roadsides (Hall et al. 1991). Since it does not begin to flower until February, it is unlikely that it is a source of *F. bispinosa* inoculation in blueberry fields. Similarly, wild radish is also an unlikely source of *F. bispinosa* in blueberries because it does not flower until early spring (Ferrell et al. 2005). In contrast, white clover is a cool-season plant (Newman et al. 2006) that flowers throughout the late fall, winter, and spring in north Florida. Therefore, it could serve as a source for thrips in blueberry plantings. If clover was a primary source of inoculation, initial thrips population (larvae and adults) would have been recorded in the clover followed by 15 m from the clover field, 30 m from the clover field, etc.

In 2009, the thrips population in the clover appeared to develop at the same time as the population in the blueberry planting. Two extreme cold events, 1 in late Jan and the second in early February (FAWN 2009), may have contributed to this population growth pattern. The cold may have reduced the thrips population in both the clover and blueberry flowers to very low levels, which then rebounded together. The difference in thrips per trap occurred on Feb 12, approximately 1 week after the second extreme cold event. The traps spaced 45 m from the clover, which had higher numbers of thrips per trap compared with those at 15 m, 60 m, and the clover, were in the center of the sampled blueberry block. It is possible that the thrips were better sheltered from the cold there.

Thrips numbers were low throughout the 2010 SHB blueberry flowering season. Thrips adults were collected from the blueberry flowers in low numbers throughout the flowering season, but the population did not begin to increase until Mar 11. In the clover flowers, a single adult unknown was collected on Feb 11. Thrips adults were not found in clover flowers again until Mar 18. Thrips larvae were not collected from blueberry flowers until Mar 18 and the only larvae collected from the clover flowers was found on Feb 18.

The flowering season itself began later than the average and was extended until the end of Mar. Both of these factors were most likely due to the extended extreme winter temperatures that occurred during Jan and February of 2010 (FAWN 2010).

Despite their low numbers, we recorded statistically significant differences in thrips per trap on Feb 11 and 25 and Mar 11 and 25. As in the previous year, thrips numbers were higher in the middle of the field. However, in 2010, they remained higher instead of equalizing as occurred in 2009.

In both years, there were significant differences in thrips per trap but not in thrips per flower. Rodriguez-Saona et al. (2010) found that sticky trap data were useful for predicting thrips' flight activity. Flower samples, in contrast, provide information on how many thrips are feeding and reproducing in the flowers. Arévalo-Rodriguez (2006) found a strong correlation (r = 0.7621) between thrips per flower and thrips per trap in rabbiteye blueberries. The presence of so few thrips per flower in both years may have masked any differences among the treatments.

From these studies, it would appear that clover is not a significant source of *F. bispinosa* inoculation in SHB blueberry fields. This is supported by Northfield et al. (2008) who found that *F. bispinosa* uses white clover as a reproductive host in the spring, particularly in Apr and May. Southern highbush blueberries in Florida flower from late Jan through early Mar. Most likely the flower thrips utilize the clover after the blueberry flowering season has ended.

Since they are found almost exclusively in flowers (Northfield et al. 2008), *F. bispinosa* may move from 1 or a few hosts to different hosts as they flower. *Frankliniella occidentalis* also exhibits this pattern of behavior in Washington apple orchards (Cockfield et al. 2007). Further research is needed to determine which plants are sources of *F. bispinosa* for SHB blueberry plantings and if controlling these plants could reduce flower thrips numbers in blueberry bushes.

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SUSCEPTIBILITY AND ACTIVITY OF GLUTATHIONE *S*-TRANSFERASES IN NINE FIELD POPULATIONS OF *PANONYCHUS CITRI* (ACARI: TETRANYCHIDAE) TO PYRIDABEN AND AZOCYCLOTIN

JIN-ZHI NIU[1], GUO-YING LIU[2], WEI DOU[1] AND JIN-JUN WANG[1]
[1]Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, P.R. China

[2]Sichuan Entry-Exit Inspection and Quarantine Bureau, Chengdu 610041, P.R. China

ABSTRACT

Nine field collected populations of *Panonychus citri* from Chinese citrus orchards were assayed for susceptibility to pyridaben and the alternative acaricide azocyclotin and activity of glutathione *S*-transferases (GSTs). The results showed that populations from Pujiang, Wanzhou, and Pengshan exhibited a low level of sensitivity to pyridaben, but demonstrated a high level of sensitivity to azocyclotin. The correlation coefficient between GSTs activities and the LC_{50} of pyridaben was $r = 0.93$ while the correlation coefficient between GSTs activities and the LC_{50} of azocyclotin was $r = 0.03$. The V_{max} value of CDNB (1-chloro-2, 4-dinitrobenzene) in populations from Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian exhibited a: 2.5-, 11.6-, 7.0-, 5.1-, and 6.4-fold increase in resistance, respectively, relative to the pyridaben susceptible population. In addition, azocyclotin was the most sensitive inhibitor of the GSTs compared with the EA (ethacrynic acid) and pyridaben, based on the values for I_{50}. The current study suggested that GSTs might be involved in resistance of *P. citri* to pyridaben and but not azocyclotin in the field.

Key Words: citrus red mite, glutathione *S*-transferases, pyridaben, azocyclotin, resistance

RESUMEN

Nueve poblaciones de *Panonychus citri* recolectadas en huertos de cítricos en China fueron analizadas por su susceptibilidad al piridaben y un acaricida alternativo la azociclotina y la actividad de *S*-transferasa de glutatión (STG). Los resultados mostraron que las poblaciones de *P. citri* en Pujiang, Wanzhou y Pengshan presentaron un bajo nivel de susceptibilidad al piridaben, pero demostró un alto nivel de susceptibilidad a la azociclotina. El coeficiente de correlación entre las actividades de STG y la CL_{50} de piridaben fue de $r = 0.93$, mientras que el coeficiente de correlación entre las actividades de STG y la CL_{50} de azociclotina fue de $r = 0.03$. El valor de V_{max} del CDNB (1-cloro-2, 4-dinitrobenceno) en las poblaciones de *P. citri* en Beibei, Jintang, Pengshan, Wanzhou y Zhongxian exhibió un aumento de: 2.5, 11.6, 7.0, 5.1 y 6.4 veces en resistencia, respectivamente, en relación con la población susceptible al piridaben. Además, azociclotina fue el inhibidor más sensible de la STG en comparación con el EA (ácido etacriníco) y piridaben, basado en los valores de I_{50}. El estudio actual sugiere que los STG podrían estar implicados en la resistencia de *P. citri* al piridaben, pero no a la azociclotina en el campo.

The citrus red mite, *Panonychus citri* (McGregor) (Acari: Tetranychidae), is a major pest worldwide (Gerson 2010). This mite can feed on 111 host plants and very commonly on citrus (Migeon & Dorkeld 2010). In southern China (Feng & Shi 2006) as well as in Japan (Furuhashi 1980), the populations have 2 infestation peaks every year, one in early summer (Jun-Jul), and the other in autumn (Oct-Nov), but maintain low density during late summer and winter. Failure to implement timely pest management adversely affects citrus harvest quantity and quality (weight, sugar content, and appearance) (Wang et al. 1999). Control of this mite depends largely on acaricide applications. Compared with insects, phytophagous mites including this species have biological/ecological traits which favor rapid development of resistance to acaricides, such as a short life cycle, abundant progeny, and arrhenotokous reproduction. For example, *Tetranychus urticae* and *P. ulmi* from the family Tetranychidae are among the 10 arthropod species, which were the first to develop pesticide resistance. Indeed *P. citri* is the third species that developed severe resistance from this family, and the control resistant mites has become exceedingly challenging (Van Leeuwen et al. 2010). According to the Arthropod Pesticide Resistance Database, 48 cases of resistance to acaricides by *P. citri* have been reported around the world, including 22 cases in China, 15 in Japan, 9 in USA, 1 in South Africa, and 1 in Georgia (Whalon et al. 2010). In USA, acaricide resistance of citrus red mite has been reported in California and Florida, and the acaricides include carbophe-

nothion, chlorfenson, demeton, dicofol, dioxathion, ethion, parathion, and tetradifon. In Japan, citrus red mite populations have developed resistance amitraz, benzoximate, binapacryl, chlorfenson, DDT, dicofol, dimethoate, fluoroacetate, oxydeprofos, phenkapton, and quinomethionate (Whalon et al. 2010). In addition, the citrus red mite has developed resistance to the recently developed acaricide, bifenzate, in Belgium, Japan, and Spain (Van Leeuwen et al. 2011). In China, the resistance status of citrus red mite is more severe compared to other counties, because Chinese citrus growers prefer to use acaricide sprays as the main means to control this mite (Lu et al. 2009). To control the mite population below the economic injury levels of 3-5 mites per leaf, weekly sprays are common during the peaks of heavy infestations (Ho 2000). Although acaricide spraying was an effective control measure in the past, its continued use in citrus orchards has disrupted natural biological systems and led to dramatic resurgences in mite populations. Resurgences are often a result of undesirable effects of acaricides on non-target organisms and the development of acaricide resistance (Zhao 2000). Increasing resistance levels to the most commonly used acaricides have led to multiple treatments, including overdoses; thereby raising serious environmental and human health concerns. Since acaricide resistance was first reported in 1979 in China, this mite has developed resistance to dicofol, pyrethroids, organotin miticide, hexythiazox, spirotetramat, amitraz, propargite, diafenthiuron, abamectin, and mitochondrial electron transport inhibitor (METI) acaricides (Hu et al. 2010; Huang 1979; Ran et al. 2009). These problems have highlighted the need to establish an efficient resistance management strategy based on all available information concerning the extent and nature of resistance.

The METI acaricides (i.e., tebufenpyrad, fenpyroximate, pyridaben, and fenazaquin), which are now widely used globally, were developed in the 1990s and inhibit complex I (NADH: ubiquinone oxidoreductase) of the mitochondrial respiratory pathway (Hollingworth & Ahammadsahib 1995). Pyridaben (2-*tert*-butyl-5-(4-*tert*-butyl-benzoylthio)-4-chloropyridazin-3(2H)-one) is a pyridazinone-derived acaricide that functions by binding its active site to a crucial co-enzyme in mitochondria (complex I at coenzyme site Q), and thus inhibits electron transport in phytophagous mites and insects (Denholm et al. 1998; Hirata et al. 1995). However, azocyclotin is an organotin miticide whose mode of action is to disrupt ATP formation by inhibiting oxidative phosphorylation (Van Leeuwen et al. 2010).

Pyridaben was introduced in China in 1992 and has been widely used in ornamentals and orchards to control mite pests resistant to conventional acaricides (Shi & Feng 2006). According to the Institute of the Control of Agrochemicals, Ministry of Agriculture, P. R. China (ICAMA), 346 commercial products of pyridaben were registered for use against *P. citri*. Compared to pyridaben, commercial products of other METI acaricides registered in China between 2008 and 2010 were 2 products containing fenazaquin and 44 products containing fenpyroximate, (ICAMA 2010). However, several field populations of *P. citri* have already developed high levels of pyridaben resistance in spite of its short term use. Recent investigation has demonstrated that the populations collected from the Chongqing municipality, Pinghe in Fujian Province, Linhai in Zhejiang province, and Yidu in Hubei province have expressed 163.3-, 266.5-, 417.9-, and 601.5-fold resistance to pyridaben, respectively (Hu et al. 2010). Judging by the present status, effort needs to be placed on resistance management to ensure future effective pest control. In addition, better understanding of mechanisms of resistance to pyridaben in *P. citri* is needed for logical selection of alternate acaricides for resistance management.

Glutathione *S*-transferases (GSTs, EC 2.5.1.18) belong to a supergene family of enzymes that are involved in phase II detoxification of xenobiotics, protection from oxidative damage, and intracellular transport of hormones, endogenous metabolites, and exogenous chemicals (Freitas et al. 2007; Huang et al. 1998). In insects, GSTs have been implicated as a major detoxification mechanism for several classes of insecticides i.e., organophosphates, pyrethroids, carbamates, and chlorinated hydrocarbons such as DDT (Lumjuan et al. 2005; Willoughby et al. 2007; Zhu et al. 2007). However, elevated levels of GSTs activity have been shown recently to be associated with the resistance of spider mite to acaricides, particularly in abamectin resistant populations of *T. urticae* (Konanz & Nauen 2004). Furthermore, experiments with synergists suggest that GSTs have a major role in pyridaben resistance in *P. citri* (Liu et al. 2010; Meng et al. 2000).

In the present study, 9 field collected populations of *P. citri* from China were investigated. Preliminary susceptibility screening of the commercially important acaricide pyridaben and a potential alternate acaricide, azocyclotin, was conducted. In addition, comparison analyses of the GSTs from the 9 populations were performed (including the activities, the kinetics, and an *in vitro* assay), in order to better understand possible mechanisms of pyridaben resistance in field populations of *P. citri*.

Materials and Methods

Mites

In 2010, *P. citri* were collected from citrus orchards in 9 locations in Sichuan Province and Chongqing municipality, China (Table 1). In each

TABLE 1. LOCATIONS, ORIGIN AND YEAR OF SAMPLING OF CHINESE *P. CITRI* POPULATIONS.

Population	Location	Origin	Collection date
Beibei	Chongqing municipality	*Citrus reticulate* Banco	4-16-2010
Jiangjin	Chongqing municipality	*Citrus reticulate* Banco	5-03-2010
Wanzhou	Chongqing municipality	*Citrus reticulate* Banco	5-11-2010
Zhongxian	Chongqing municipality	*Citrus reticulate* Banco	5-19-2010
Jintang	Sichuan Province	*Citrus reticulate* Banco	5-21-2010
Jianyang	Sichuan Province	*Citrus reticulate* Banco	5-14-2010
Pengshan	Sichuan Province	*Citrus reticulate* Banco	5-16-2010
Pujiang	Sichuan Province	*Citrus reticulate* Banco	5-29-2010
Meishan	Sichuan Province	*Citrus reticulate* Banco	4-11-2010

sample location, more than 2,000 mites with associated citrus leaves were collected from more than 10 citrus trees (Table 1). Fresh leaves, collected at random from *Citrus reticulata* Blanco mandarin orange trees with no prior pesticide exposure from orchards at the Citrus Research Institute, Chinese Academy of Agricultural Sciences, were used to rear the collected mites in the laboratory during the experiments. The leaves were replaced every 3 d. Sites were selected based on their importance as citrus production areas, and they received various levels of acaricide applications for the management of *P. citri* (Liu et al. 2010). The identity of *P. citri* was confirmed by J. J. Wang. Voucher specimens (50 for each population) were deposited in the insect collection of Southwest University, Chongqing, China.

Chemicals

Formulated acaricides used in this study were pyridaben 150 g L^{-1} EC (Saomanjing®) and azocyclotin 200 g L^{-1} SC (Sanzuoxi®), which were purchased from Jiangsu Kesheng Group Co., Jiangsu, China, and Jiangxi Huxing Chemical Co., Jiangxi, China, respectively. Bovine serum albumin (BSA), coomassie brilliant blue G-250, and 1-chloro-2, 4-dinitrobenzene (CDNB) were supplied by Shanghai Chem. Ltd., Shanghai, China. Reduced glutathione (GSH) and Ethacrynic acid (EA) were purchased from Sigma (St. Louis, Missouri, USA).

Bioassays

In general the bioassay procedures recommended by the Food and Agriculture Organization of the United Nations were followed (FAO 1980). Each field population was assayed by a slide-dip method with 7 treatment concentrations of each acaricide. Each acaricide was diluted with double-distilled water (ddH_2O) and various concentrations were tested until a satisfactory range (10-90% mortality) was ascertained. The control group was treated with ddH_2O alone. Each slide, containing 30-40 adult female individuals that were 3-5 d old, was dipped into the pesticide solution for 5 s. The slides were placed in an incubator at 28 ± 1°C, 75-80% relative humidity, with a photoperiod of light: dark, 14:10 h. Mortality was assessed after 24 h. Mites that did not move after stimulation by a camel hair brush were scored as dead. Each treatment (comprising 3 slides) was replicated 3 times on 3 different days. Mortality data was corrected by Abbott's Formula (Abbott 1925) and analyzed by probit analysis to determine the lethal concentrations (LC_{50}) (Raymond 1985).

GSTs Preparation

One hundred female adults from each population were homogenized in 4 mL ice-cold sodium phosphate buffer (0.02M; pH 7.3) and centrifuged at 5,000 g for 5 min at 4°C in a CF16RX refrigerated centrifuge (Hitachi Ltd, Tokyo, Japan). The pellets were discarded and the supernatant was again centrifuged at 4°C for 15 min at 17,500*g*. Finally, the supernatant was used as the enzyme source for GST activity assays. The protein contents of enzyme homogenates were determined according to the Bradford method with BSA as a standard (Bradford 1976). The measurement was performed with the A-5002 thermomax kinetic microplate reader (Tecan Ltd., Salzburg, Austria) at 595 nm.

Determination of GSTs Activities and Kinetics

GSTs activity was determined with CDNB and GSH as substrates in 96-well microplates (Habig et al. 1974). The total reaction volume per well of a 96-well microplate was 300 µL. This consisted of 100 µL supernatant, 100 µL CDNB (containing 2% [v/v] ethanol) and 100 µL GSH in 0.05 M, pH 7.5 Tris-HCl, with a final concentration of 0.6 mM of CDNB and 6.0 mM and GSH. The non-enzymatic reaction of CDNB with GSH measured without homogenate served as the control. The change in absorbance was measured continuously for 5 min at 340 nm and 37°C in an A-5002 thermomax kinetic microplate reader

(Tecan Ltd., Salzburg, Austria). Changes in absorbance per minute were converted into nmol CDNB conjugated/min/mg protein based on the extinction coefficient of the resulting 2, 4-dinitrophenyl-glutathione (ε_{340nm} = 9.6 mM^{-1} cm^{-1}) (Habig et al. 1974).

The values of V_{max} and K_m of total GSTs from *P. citri* were determined for CDNB and GSH. The activity was recorded as a range of concentrations (0.02-0.6 mM for CDNB or 0.3-6.0 mM for GSH), while the concentrations of the other substrates were kept constant at 6.0 mM GSH or 0.6 mM CDNB. V_{max} and K_m values were calculated by the Michaelis-Menten equation in SPSS 10.0 for Windows (Rauch & Nauen 2004).

In vitro Assay

To evaluate the sensitivity of GSTs to different inhibitors, 3 chemicals (pyridaben, azocyclotin, and EA) were selected to assess *in vitro* inhibition against GSTs. Stock solutions of the inhibitors were diluted with 0.05 M, pH 8.0, Tris-HCl buffer. Twenty-five µL of the enzyme source and 25 µL inhibitor solutions, with an appropriate concentration range (i.e., 1.0-1,000 mg/L for EA, 0.48-384.70 mg/L for pyridaben, and 0.86-1,713.00 mg/L for azocyclotin (which were ascertained from various concentrations of each inhibitor to test the inhibition rate until concentrations causing 10-90% inhibition were delimited), were incubated for 5 min at 37°C and added to the CDNB/GSH substrate mixture as described above. Reactions without the inhibitor were included as controls. The median inhibition concentration (I_{50}) for each inhibitor was determined based on the log-concentration vs. probit (% inhibition) regression analysis. Three replications were conducted for each treatment above.

Statistical Analysis

All the data from the 9 field populations of *P. citri* were analyzed by analysis of variance (ANOVA), the means were separated by Duncan's Multiple Range Test or LSD Test for significance (P = 0.05) with SPSS 10.0 for Windows (SPSS 1999). Regression analysis was performed to calculate the GST kinetic parameters, LC_{50} values of different acaricides, and I_{50} values of different inhibitors. The significant level of resistance rate (RR) was ascertained by the confidence interval overlap of the LC_{50} values. The Chi-square goodness-of-fit test was used to determine the difference between the theoretic and the measured values of acaricide toxicity results. Correlation analyses were conducted to find the relationship between GSTs activity and LC_{50} values of each acaricide against the 9 populations of *P. citri*.

Results

Bioassays

Compared with the reference dose of each acaricide, the Jiangjin and Meishan populations were susceptible to pyridaben and azocyclotin, respectively. In addition, the population sampled from Jiangjin expressed the greatest sensitivity to pyridaben and thus was regarded as relatively susceptible to pyridaben compared to the other 8 populations. Comparison among resistance ratios (RR) with the relatively pyridaben-susceptible population indicated that all other 8 field collected populations had significant levels of resistance. Resistance ratios ranged from 2 (Jianyang population) to 140 (Pujiang population) (Table 2).

The Meishan population had the lowest LC_{50} value when treated with azocyclotin and was used as the relatively susceptible population to azocyclotin for comparison to the other 8 populations. Comparison of RR values to the relatively azocyclotin susceptible population suggested that only the Beibei, Jiangjin, Jintang, Wanzhou, and Zhongxian populations had significant levels of resistance. Resistance ratios ranged from 2 (Jianyang, Pengshan, Pujiang, and Wanzhou populations) to 36 (Beibei population) (Table 2).

Activity of GSTs

The Pujiang population exhibited 4.7-fold increased GST activity in comparison with the pyridaben susceptible population (Jiangjin population), and GST activity in the Beibei, Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations was increased 2.3-,1.9-, 1.8-, 2.1-, 2.2-, and 2.7-fold, respectively, in comparison with the Jiangjin population. GST activity in the Jianyang population was 0.9-fold lower than in the Jiangjin population (Fig. 1). In addition, the correlation coefficient between GST activity and LC_{50} of pyridaben was r = 0.93, while the correlation coefficient between GST activity and LC_{50} of azocyclotin was r = 0.03 (Fig. 2).

Kinetic Parameters of GSTs

The kinetic parameters of GSTs in *P. citri* were determined from Lineweaver-Burk plots and presented in Table 3. For the catalytic activity of GSTs toward GSH and CDNB as expressed by the V_{max} value, the Pujiang population had the highest V_{max} value and exhibited a 276.0-fold increased catalytic activity of GSH in comparison with the susceptible Jiangjin population. The catalytic activity of GSH in Jintang, Pengshan, and Zhongxian was increased (8.8-, 2.5-, and 19.0-fold, respectively) compared with the susceptible Jiangjin population. The catalytic activity of GSH in the Beibei, Jianyang, Meishan, and Wanzhou

TABLE 2. SUSCEPTIBILITIES OF 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI* TO PYRIDABEN AND AZOCYCLOTIN.

Acaricide	Population	*n*	χ^{2a}	Slope (±SE)	LC_{50} [95%CI] (mg liter^{-1})	RR [95%CI]
Pyridaben	Jiangjin	616	1.33	1.12 (±0.17)	0.2 [0.2; 0.3]	—
	Beibei	710	2.11	0.52 (±0.11)	2.0 [1.1; 3.1]	9 [6; 11]
	Jianyang	687	4.68	0.89 (±0.13)	0.5 [0.3; 0.6]	2 [2; 2]
	Jintang	485	3.71	0.61 (±0.13)	1.7 [1.0; 3.0]	8 [6; 10]
	Meishan	575	2.18	0.63 (±0.12)	1.1 [0.7; 1.4]	5 [4; 5]
	Pengshan	473	0.23	0.93 (±0.18)	2.6 [1.6; 4.0]	12 [9; 14]
	Pujiang	527	2.00	1.57 (±0.33)	30.9 [23.0; 55.1]	140[134; 192]
	Wanzhou	519	0.99	0.75 (±0.16)	5.3 [3.8; 7.8]	24 [22; 27]
	Zhongxian	670	2.08	1.22 (±0.17)	14.9 [11.7; 20.6]	68 [68; 71]
Azocyclotin	Meishan	470	5.48	0.31 (±0.08)	40 [21; 70]	—
	Beibei	566	2.06	0.63 (±0.10)	1463 [1063; 1656]	36 [30; 37]
	Jiangjin	515	5.29	1.00 (±0.15)	128 [72; 206]	3 [2; 5]
	Jintang	688	4.03	0.45 (±0.15)	304 [239; 337]	8 [7; 8]
	Jianyang	639	5.86	0.61 (±0.12)	88 [(50; 139)	2 [1; 3]
	Pengshan	507	5.50	0.71 (±0.12)	87 [53; 154]	2 [1; 3]
	Pujiang	519	3.76	0.80 (±0.13)	60 [32; 93]	2 [1; 2]
	Wanzhou	498	4.07	0.70 (±0.09)	104 [71; 157]	2 [2; 4]
	Zhongxian	621	3.39	0.46 (±0.10)	734 [683; 1115]	18 [17;25]

Note: *n* = number of mites; RR = resistance ratio; CI = confidence interval.
[a]Chi-squared goodness-of-fit test.

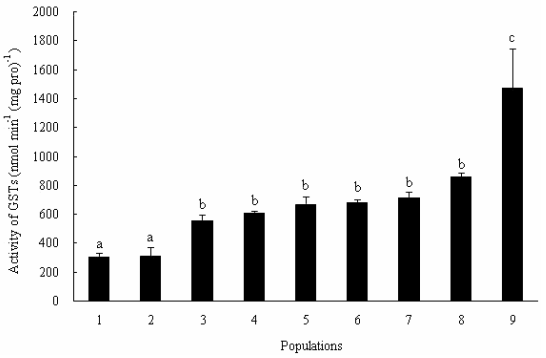

Fig. 1. Glutathione *S*-transferases activity of various populations of *P. citri*. 1, 2, 3, 4, 5, 6, 7, 8, 9 corresponding to Jianyang, Jiangjin, Meishan, Jintang, Pengshan, Wanzhou, Beibei, Zhongxian, Pujiang, respectively. Each value represents the mean of three determinations (n = 3). Error bars represent SE. Different letters indicate significant differences in ANOVA (LSD, $P < 0.05$).

populations decreased compared with the susceptible Jiangjin population. In addition, the catalytic activities of CDNB in the Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian populations were significantly higher than the pyridaben susceptible population, and exhibited 2.5-, 11.6-, 7.0-, 5.1-, and 6.4-fold increases, respectively. However, the catalytic activity of CDNB in the Jianyang, Meishan, and Pujiang populations was 0.7-, 0.1- and 0.3-fold lower, respectively, than that of the pyridaben susceptible population; but no significant difference of catalytic activity of CDNB was detected among these populations ($P < 0.05$) (Table 3).

All field collected populations had higher K_m values of GSH compared to the reference K_m value in the pyridaben susceptible population. Among these, the Pengshan population exhibited a 4.1-fold increase in K_m value. In addition, the K_m value of CDNB in the Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations suggested 2.3-, 1.3-, 10.2-, 5.4-, and 2.9-fold increases, respectively, compared with the pyridaben susceptible population. However, the Pujiang population was determined as having the smallest K_m value toward CDNB, while the Pengshan population was determined as having the largest K_m value toward CDNB among all the populations (Table 3).

In vitro assay

The median inhibition concentrations (I_{50}s) of the 3 inhibitors (EA, pyridaben, and azocyclotin) were calculated for GSTs from the 9 field collected populations of *P. citri* (Table 4). The statistical analysis indicated that azocyclotin was the most sensitive inhibitor of the GSTs (I_{50} values ranging from 0.00 to 0.52 mg liter^{-1}) compared with the I_{50} values of EA and pyridaben, which ranged between 0.03 to 3.60 and 0.03 to 28.94 mg liter^{-1}, respectively.

DISCUSSION

Panonychus citri presents a challenge to pest managers due to its inherent ability to develop re-

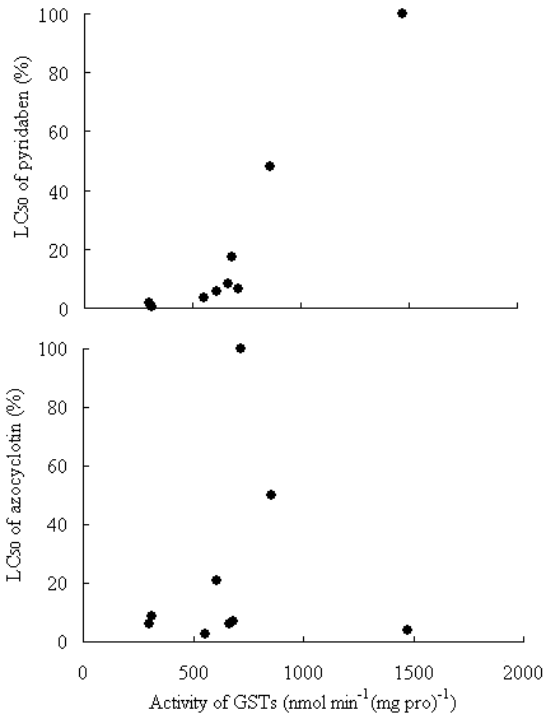

Fig. 2. Correlation analyses between glutathione *S*-transferases activity and LC_{50} of acaricides on various populations of *P. citri*. Percentage of LC_{50} of pyridaben and azocyclotin was calculated based on the highest LC_{50} value of pyridaben (Pujiang population) and LC_{50} value of azocyclotin (Beibei population), respectively.

sistance in a few generations. In recent years, some conventional control options for citrus red mite have become restricted, because the use of more toxic compounds, such as dicofol, has been strictly prohibited. Some novel acaricides, such as METIs and spirodiclofen, have been introduced recently. However, after several years of effective use in citrus, resistance problems have arisen. In some citrus growing areas, growers use these novel acaricides more than 5 times per growing season while the label guidelines specify only 2 applications per year (Hu et al. 2010). Development of resistance in citrus red mite can increase grower costs by more than two-fold and theses can mount further as resistance levels escalate. In the current study, the susceptibilities of 9 field collected populations of *P. citri* to the acaricides, pyridaben, and azocyclotin, were determined. Mites from the Pengshan, Pujiang, Wanzhou, and Zhongxian populations exhibited >10-fold resistance to pyridaben compared with the pyridaben-susceptible population collected in Jiangjin. However, with the exception of the Zhongxian population, the preceding populations also had the lowest resistance ratio (<5) for azocyclotin compared with the azocyclotin-susceptible population collected in Meishan. Therefore, azocyclotin may be an effective alternative to manage resistance to pyridaben. Previous studies with a field-collected population of *P. citri* selected with pyridaben for 12 generations showed that it had developed 35-fold resistance, while it still exhibited a low level of cross-resistance (4-fold) to azocyclotin (Meng et al. 2000). A similar low level of cross resistance to azocyclotin also was found in *T. urticae*. A pyridaben-resistant colony, maintained in the laboratory, was established by treatment with pyridaben for 20 generations (PR-20 population); and it was extremely resistant to pyridaben (resistance ratio = 240), but showed low levels of resistance (resistance ratio = 3.8) to azocyclotin (Kim et al. 2006). Pyridaben belongs to the METI-acaricides, and cross-resistance between METIs has been reported both in laboratory-selected populations and in field-collected populations of *T. urticae* (Kim et al. 2006; Stumpf & Nauen 2001). The responses of F_2 females from the reciprocal crosses of resistant and susceptible individuals suggest that resistance to pyridaben is under monogenic control (Van Pottelberge et al. 2009b). Monogenic resistance, which is considered more likely to spread within populations than polygenic resistance, tends to be more stable and is less easily managed (Roush & McKenzie 1987). Based on the different modes of action between pyridaben and azocyclotin, azocyclotin may be a good rotation acaricide for the management of pyridaben resistance.

The mechanisms of resistance to acaricides in mite species are reduced penetration, enhanced metabolism, and target site insensitivity. Of these factors, enhanced metabolic detoxification by GSTs, mixed function oxidases (MFOs), and/or esterases (ESTs) has been considered as a main mechanism of acaricide resistance of mites (Van Nieuwenhuyse et al. 2009; Van Pottelberge et al. 2009a). Experiments with synergists (DEM, PBO, and TPP inhibitors against GSTs, MFO, and EST, respectively) suggest that GSTs play a major role in pyridaben resistance in field collected pyridaben-resistant *P. citri* populations (Liu et al. 2010), while the same result was also reported in a laboratory pyridaben-selected population of *P. citri* (Meng et al. 2000). However, an experiment with synergists involving the PR-20 population of *T. urticae* (a field collected population that was further selected with pyridaben for 20 generations) revealed that MFO plays a major role in pyridaben resistance in this population (Kim et al. 2006). We suggest that the different results from the synergist experiments between *P. citri* and *T. urticae* might be caused by differences in interspecific and non-specialized synergistic activity (Young et al. 2005). In the present study, the activity of GSTs from 9 field collected citrus red mites increased as the pyridaben resistance lev-

TABLE 3. APPARENT KINETIC PARAMETERS OF GLUTATHIONE *S*-TRANSFERASES TOWARDS GSH AND CDNB IN THE 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI*.

	GSH		CDNB	
Population	V_{max}	K_m	V_{max}	K_m
Jiangjin	311 ± 11 a	87 ± 11 a	210 ± 10 a	58 ± 14 bc
Jianyang	264 ± 64 a	87 ± 2 a	153 ± 25 a	12 ± 2 a
Meishan	11 ± 0 a	328 ± 12 cd	16 ± 1 a	73 ± 10 c
Jintang	2744 ± 178 a	306 ± 15 c	2427 ± 36 f	134 ± 18 d
Beibei	194 ± 53 a	190 ± 59 b	527 ± 37 b	33 ± 2 ab
Pengshan	776 ± 148 a	359 ± 14 d	1484 ± 393 d	593 ± 56 f
Wanzhou	11 ± 1 a	343 ± 25 cd	1088 ± 26 c	316 ± 14 e
Zhongxian	5907 ± 276 a	318 ± 25 cd	1334 ± 280 cd	167 ± 11 d
Pujiang	85841 ± 10084 b	120 ± 7 a	68 ± 4 a	5 ± 0 a

Each value represents the mean (± SE). Mean values within the same column followed by different letters are significantly different in ANOVA (LSD, $P < 0.05$). V_{max}: nmolmin^{-1}(mg pro)$^{-1}$. K_m: μM.

els increased (Pujiang > Zhongxian > Wanzhou > Pengshan > Beibei > Jintang > Meishan > Jianyang > Jiangjin) with the exception of the Beibei and Jianyang populations. The analysis showed that the correlation coefficient between GSTs activities and the LC_{50} of pyridaben was $r = 0.93$, while the correlation between GSTs activities and the LC_{50} of azocyclotin was only $r = 0.03$ (Fig. 2). This could mean that in *P. citri* field populations GSTs were involved in resistance of to pyridaben but not to azocyclotin.

All the kinetic parameters (including V_{max} and K_m for both GSH and CDNB) in the Jintang, Pengshan, and Zhongxian populations increased significantly compared with the pyridaben susceptible population from Jiangjin. In addition, the catalytic activity of CDNB (presented by V_{max}) in the Beibei, Jintang, Pengshan, Wanzhou, and Zhongxian populations were found to be significantly greater than the pyridaben susceptible population (2.5-,11.6-, 7.0-, 5.1-, and 6.4-fold increase, respectively), indicating an over-expression (Konanz & Nauen 2004) or structural alteration (Wang et al. 2008) of GSTs in these populations. The K_m value of GSH in all field collected populations increased relative to the K_m value in the pyridaben susceptible population. In addition, the K_m values of CDNB in the Jintang, Meishan, Pengshan, Wanzhou, and Zhongxian populations was 2.3-, 1.3-, 10.2-, 5.4-, and 2.9-fold, respectively, greater than the K_m value of the pyridaben susceptible population.

Ethacrynic acid (EA) always presents a strong inhibitory effect on GSTs in many pests, i.e., *Blattella germanica* (L.) (Blattellidae) (Yu & Huang 2000), *T. urticae* (Konanz & Nauen 2004), and *Liposcelis paeta* (Pearman) (Liposcelididae) (Wu et al. 2009). In our current study, azocyclotin had the greatest inhibitory effect on GSTs compared with EA and pyridaben, indicating that azocyclotin could be used as an effective pesticide synergist for the control of pesticide resistance caused

TABLE 4. I_{50} VALUES OF *IN VITRO* INHIBITION OF GLUTATHIONE S-TRANSFERASES FROM 9 FIELD-COLLECTED POPULATIONS OF *P. CITRI*.

	I_{50}		
Population	EA	Pyridaben	Azocyclotin
Jiangjin	0.03 ± 0.00 a	3.21 ± 0.53 a	0.03 ± 0.00 a
Jianyang	0.37 ± 0.00 b	10.26 ± 0.00 b	0.52 ± 0.18 c
Meishan	0.83 ± 0.20 c	27.21 ± 2.38 e	0.32 ± 0.16 b
Jintang	3.60 ± 0.45 e	12.72 ± 4.41 c	0.00 ± 0.00 a
Beibei	1.16 ± 0.02 d	17.55 ± 0.74 cd	0.08 ± 0.01 a
Pengshan	0.06 ± 0.00 a	24.21 ± 2.52 de	0.02 ± 0.00 a
Wanzhou	0.98 ± 0.03 cd	28.94 ± 9.17 e	0.49 ± 0.00 c
Zhongxian	0.24 ± 0.00 ab	18.40 ± 3.28 bcd	0.01 ± 0.00 a
Pujiang	1.09 ± 0.02 cd	0.03 ± 0.01 a	0.00 ± 0.00 a

Inhibition measured as 50% inhibitory concentration (i.e., I_{50}) in mg liter^{-1}. Each value represents the mean (± SE). Mean values within the same column followed by different letters are significantly different in ANOVA (LSD, $P < 0.05$).

by GSTs, i.e., azocyclotin could block GST activity due to rapid depletion of GSH (Li et al. 2009). Nevertheless, the effectiveness of azocyclotin as a synergist requires further research.

In summary, the present study has provided some basic information on the toxicity of pyridaben and azocyclotin against *P. citri*, and certain characteristics of GST activity in 9 field populations of *P. citri* were described. In addition, our study illustrated that GSTs could be associated with varying susceptibility levels to pyridaben. This is only the first step in the biochemical differentiation of various *P. citri* field populations. The results of further studies will contribute to the understanding of the mechanisms of acaricide resistance in *P. citri*.

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A CONTRIBUTION TO THRIPS-PLANT ASSOCIATIONS RECORDS (INSECTA: THYSANOPTERA) IN COSTA RICA AND CENTRAL AMERICA

ALCIDES SÁNCHEZ-MONGE[1], AXEL RETANA-SALAZAR[1], STEVEN BRENES[2] AND RENÁN AGÜERO[2]
[1]Programa Universitario de biología Aplicada (PUA). Centro de Investigación en Estructuras Microscópicas, Universidad de Costa Rica

[2]Centro de Investigaciones en Protección de Cutlivos (CIPROC), Universidad de Costa Rica

ABSTRACT

Thrips are small, cosmopolitan insects directly or indirectly associated with plants. Records of these associations in the neotropics add greatly to better understanding of Thysanoptera, not the least because some thrips species are economically important in agriculture and amenity plantings. In this paper we report new plant associations of *Franklinothrips vespiformis* (Crawford), *Gastrothrips* sp. Hood, *Haplothrips gowdeyi* Franklin, *Leptothrips astutus* Johansen, *Leptothrips obesus* Johansen, *Liothrips* spp. Uzel, *Torvothrips martinezi* Johansen, *Arorathrips mexicanus* Crawford, *Caliothrips fasciapennis* (Hinds), *Caliothrips nanus* (Hood), *Caliothrips punctipennis* (Hood), *Echinothrips caribbeanus* Hood, *Echinothrips selaginellae* Mound, *Frankliniella cephalica* Crawford, *Frankliniella standleyana* Hood, *Hoodothripiella ignacio* Retana-Salazar, *Microcephalothrips abdominalis* (Crawford) and *Retanathrips silvestris* (Hood). Some records of the presence of thrips species are new for Costa Rica and Central America.

Key Words: amenity plantings, arvenses, banana, ecology, weeds, accomplice species, host

RESUMEN

Los thrips son pequeños insectos cosmopolitas asociados a las plantas directa o indirectamente. El conocimiento de asociaciones con plantas es información valiosa para un mejor conocimiento de este grupo, poco es conocido en las regiones neotropicales donde algunas especies son plagas agrícolas importantes. En este escrito se presentan nuevos registros de asociaciones con plantas para *Franklinothrips vespiformis* (Crawford), *Gastrothrips* sp. Hood, *Haplothrips gowdeyi* Franklin, *Leptothrips astutus* Johansen, *Leptothrips obesus* Johansen, *Liothrips* spp. Uzel, *Torvothrips martinezi* Johansen, *Arorathrips mexicanus* Crawford, *Caliothrips fasciapennis* (Hinds), *Caliothrips nanus* (Hood), *Caliothrips punctipennis* (Hood), *Echinothrips caribbeanus* Hood, *Echinothrips selaginellae* Mound, *Frankliniella cephalica* Crawford, *Frankliniella standleyana* Hood, *Hoodothripiella ignacio* Retana-Salazar, *Microcephalothrips abdominalis* (Crawford) y *Retanathrips silvestris* (Hood). Algunos reportes son nuevos para Costa Rica y para Centroamérica.

Translation provided by the authors.

With more than 2000 thrips species currently described in the Neotropics, this region has great diversity within the Thysanoptera (Mound 2002). Species from 1.0 mm or less to 10.0 mm long can be found just in Central America (Mound et al. 1993) where they can be collected from a wide array of habitats; forests, grasslands, desserts, crops and gardens (Soto-Rodríguez et al. 2009). Feeding habits vary among different taxa (Mound et al. 1993; Soto-Rodríguez et al. 2009) and, commonly, phytophagous species are considered of economic importance in various crops (Childers & Nakahara 2006; González et al. 2010a), especially in the tropics (Johansen & Mojica 2007). Phytophagous species cause economic damage by feeding directly on vulnerable plant species, by vectoring virus causing major crop losses (Jones 2005) or requiring the erection of quarantine barriers to their spread (Vierbergen et al. 2006; González et al. 2010b). On the other hand, other thrips species serve beneficially as pollinators and decomposers (Pinent et al. 2006), and some species have been suggested as biological control agents against various arthropod pests (Zegula et al. 2003) or weeds (Cock et al. 2000; Mound & Zapater 2003; Soto-Rodríguez et al. 2009). Weeds often are very important in agriculture because they compete with the crop, or they serve as hosts or accomplices of pests or pathogens including some thrips species (González et al. 2010b). Hernández-Ayar et al. (2009), for example, found that the number of thrips taxa present at a given site varied according to the type of plants that grow associated with the crop, in this case, Persian lime, *Citrus latifolia* (Tan.).

According to Mound (2002), the majority of thrips studies in the neotropics have been limited to insecticide trials or taxonomic studies. Therefore it is especially important to conduct plant-association surveys in order to understand the role of thrips species in the ecosystems, to ascertain which plant species aid and abet various species of thrips pests (González et al. 2010b), and to assess the impact of different thrips species on populations of other organisms within crops, amenity plantings or noncultivated areas. In this paper we present the findings of Neotropical and cosmopolitan thrips species collected from weeds from banana farms and neighboring areas such as paddocks and roadsides.

Materials and Methods

Implementation of Project CONICIT FV 24-07, UCR 813-A8-506, involved the monthly collection of foliar samples (leaves, stems and flowers in few cases) of several weed species in Limón, Costa Rica throughout 2008 and 2009. Weed samples were collected in plastic bags and sealed with adhesive tape to prevent the escape of captured specimens. Plants samples were identified *in situ* and their identities were verified by Steven Brenes of the Weed Laboratory of the University of Costa Rica. Most of the samples were collected within banana farms, other samples were obtained from neighboring areas and pasture fields.

To extract the thrips specimens, each weed sample was placed in a container filled with boiling water. After approximately 3 minutes, the water was poured through a 212 mesh sieve. The plant sample was washed twice in the container and the water was poured through the sieve. Specimens on the sieve were transferred to a petri plate, after which the thrips were stored in 70% ethanol in labeled eppendorf tubes for further identification. The thrips specimens were mounted on microscope slides and were identified by Axel Retana-Salazar of the Centro de Investigación en Estructuras Microscópicas (CIEMIC), University of Costa Rica, according to the keys provided by Johansen (1980, 1987); Mound et al. (1993); Mound & Marullo (1996); Soto-Rodríguez & Retana-Salazar (2003); Retana-Salazar (2007) and the Official Collection of Thysanoptera of the University of Costa Rica, CIEMIC.

Results and Discussion

Locations and dates of sampling for thrips reported in this project are elaborated in Table 1. In total 829 plant samples were collected and examined. Three thrips families, 13 genera and 19 species were identified in this research, and these19 species were involved in 45 different thrips-plant associations (Table 2).

Most of the thrips specimens were found on samples collected from outside the banana farms, and this distribution is consistent with other arthropod taxa observed during this research (Fig. 1). Weed samples belonged to 70 different

Table 1. Collection of thrips on weeds in Limón Province, Costa Rica in 2008-2009: locations, site descriptions and dates of collection.

Location code	Date of sampling	Location name	Detail
1	3-IV-2008	La Teresa Banana Farm	Cariari, Limón.
2	3-IV-2008	Junior Jiménez Paddock	Guácimo, Limón.
3	12-IV-2008	Est. Exp. Diamantes, INTA (Paddock) 1	Guápiles, Limón.
4	7-V-2008	Agrícola 2 Banana Farm	Cariari, Limón.
5	8-V-2008	Roadside to Guácimo	Guácimo, Limón.
6	8-V-2008	San Diego Pineapple Farm (nearby)	Guácimo, Limón.
7	27-V-2008	Bonanza Campo Cinco Banana Farm	Cariari, Limón.
8	28-V-2008	Est. Exp. Diamantes, INTA (Paddock) 2	Guápiles, Limón.
9	6-VIII-2008	Rio Palmas Hotel surrounding forest	Guácimo, Limón
10	4-IX-2008	San Pablo Banana Farm	Matina, Limón.
11	4-IX-2008	28 Millas, CORBANA facilities	Matina, Limón.
12	9-X-2008	Calinda Banana Farm	Guácimo, Limón.
13	10-X-2008	6 years Organic Banana Farm, EARTH	EARTH University, Guácimo, Limón
14	11-II-2009	Támesis Banana Farm	Cariari, Limón.
15	11-II-2009	Valquirias Banana Farm	Cariari, Limón.
16	12-III-2009	Bananos Dora Banana Farm	Siquirres, Limón
17	13-III-2009	Ecoturismo Banana Farm	Siquirres, Limón
18	8-VII-2009	La Estrella Banana Farm	Siquirres, Limón
19	16-IX-2009	Verde Azul Banana Farm	Siquirres, Limón

TABLE 2. ASSOCIATIONS OF THYSANOPTERAN FAMILIES AND SPECIES WITH FAMILIES AND SPECIES OF PLANTS AT THE LOCATIONS SAMPLED IN LIMÓN PROVINCE, COSTA RICA IN 2008-2009.

Thrips species/weed species	Host Botanical Family	Location Code[1]
AEOLOTHRIPIDAE		
Franklinothrips vespiformis		
Synedrella nodiflora L.	Asteraceae	1
PHLAEOTHRIPIDAE		
Idolothripinae		
Gastrothrips sp.		
Solanum nigrum L.	Solanaceae	17
Phlaeothripinae		
Haplothrips gowdeyi		
Digitaria setigera Roth ex Roem. et Schult.	Poaceae	18
Eleusine indica L.	Poaceae	10, 19
Emilia sonchifolia L.	Asteraceae	17
Spermacoce assurgens Ruiz & Pavón	Rubiaceae	8, 9
Leptothrips astutus		
Stachytarpheta jamaicensis L.	Verbenaceae	4
Synedrella nodiflora L.	Asteraceae	1, 5, 6, 9
Leptothrips obesus		
Lantana trifolia L.	Verbenaceae	2, 8
Liothrips sp.1		
Synedrella nodiflora L.	Asteraceae	5, 7, 8, 19
Liothrips sp.2		
Gouania polygama Jacq.	Rhamnaceae	3, 8, 13
Torvothrips martinezi		
Sida ulmifolia Mill.	Malvaceae	8
THRIPIDAE		
Arorathrips mexicanus		
Drymaria cordata L.	Caryophyllaceae	2, 6, 10, 17
Eleusine indica L.	Poaceae	2, 7, 10, 16
Caliothrips faciapennis		
Scleria melaleuca Rchb.f. ex. Schtdl.Cham.	Cyperaceae	2, 3, 6, 8, 15, 17
C*aliothrips nanus*		
Gouania polygama Jacq.	Rhamnaceae	3, 11,13
Caliothrips punctipennis		
Eleusine indica L.	Poaceae	4, 10, 12, 16, 18
Echinothrips caribbeanus		
Alternanthera sessilis L.	Amaranthaceae	10
Cyathula prostrata L.	Amaranthaceae	15, 17
Drymaria cordata L.	Caryophyllaceae	2, 8, 15
Eleusine indica L.	Poaceae	4, 16
Emilia sonchifolia L.	Asteraceae	17
Laportea aestuans L.	Urticaceae	1, 3, 12, 13, 14, 15, 16
Ludwigia decurrens Walt.	Onagraceae	16
Melothria pendula L.	Cucurbitaceae	1, 17
Mikania micrantha Kunth ex H.B.K	Asteraceae	15
Oxalis barrelieri L.	Oxalidaceae	7
Phenax sonneratii Poir.	Urticaceae	17
Philodendron hederaceum (Jacq.) Schott	Araceae	12, 17
Rivina humilis L.	Phytolacaceae	16, 17

[1]Each Location Code is defined in Table 1.

TABLE 2. (CONTINUED) ASSOCIATIONS OF THYSANOPTERAN FAMILIES AND SPECIES WITH FAMILIES AND SPECIES OF PLANTS AT THE LOCATIONS SAMPLED IN LIMÓN PROVINCE, COSTA RICA IN 2008-2009.

Thrips species/weed species	Host Botanical Family	Location Code[1]
Solanum nigrum L.	Solanaceae	17
Spermacoce assurgens Ruiz & Pavón	Rubiaceae	15
Synedrella nodiflora L.	Asteraceae	10, 19
Echinothrips selaginellae		
Alternanthera sessilis L.	Amaranthaceae	10, 14, 15
Laportea aestuans L.	Urticaceae	12, 19
Scleria melaleuca Rchb.f. ex. Schtdl.Cham.	Cyperaceae	12, 17
Frankliniella cephalica		
Drymaria cordata L.	Caryophyllaceae	2, 11
Frankliniella standleyana		
Conostegia subcrustulata Beurl.	Melastomataceae	6
Mikania micrantha Kunth ex H.B.K	Asteraceae	7, 14, 15
Hoodothripiella ignacio		
Spermacoce latifolia Aubl.	Rubiaceae	2, 7, 8, 9
Microcephalothrips abdominalis		
Wedelia trilobata L.	Asteraceae	10, 11
Retanathrips silvestris		
Alternanthera sessilis L.	Amaranthaceae	16
Spermacoce latifolia Aubl.	Rubiaceae	7, 9
Spermacoce assurgens Ruiz & Pavón	Rubiaceae	8, 16
Synedrella nodiflora L.	Asteraceae	5, 6, 7, 8, 9

[1]Each Location Code is defined in Table 1.

species and the thrips specimens were found on 17 of 28 botanical families represented at the sampling sites. The highest number of thrips species (Fig. 2) was found on members of the Asteraceae. *Echinothrips caribbeanus* Hood was found on 16 plant species (Fig. 3), the most for any thrips species herein. Twelve thrips species were narrowly specific in their plant preferences; each being found on a single plant species (Fig. 3). Information of other locations and weed species sampled are detailed in Sánchez-Monge (2010).

AEOLOTHRIPIDAE

Franklinothrips Back 1912

Franklinothrips vespiformis (Crawford 1909)

F. vespiformis is a known predator of mites, white flies and other insects in Central and South America (Arakaki *et al.* 2001) as well as the Southwestern USA (Johansen 1983). *F. vespiformis* has been recorded previously from grasses, weeds and crops species in several Latin-American countries (Johansen 1976, 1983; Mound & Reynaud 2005). In Costa Rica *F. vespiformis* was reported on *Ricinus* sp. leaves (Mound & Marullo 1996) and associated with crops (Soto-Rodríguez *et al.* 2009). Our finding of *F. vespiformis* on *Synedrella nodiflora* (Asteraceae) (Table 2) is new information on its biology. We also found Homoptera immatures, predatory and phytophagous mites and some nematodes in this weed sample, some of them could be suitable prey for *F. vespiformis*, since its predatory behavior on small insects, eggs and other larvae has been documented (Johansen 1976; Mound & Reynaud 2005). The small number of *F. vespiformis* isolated from the weed sample is consistent with solitary predator behavior described for predator thrips species (Johansen & Mojica-Guzmán 1996).

PHLAEOTHRIPIDAE

Idolothripinae

Gastrothrips Hood 1912

Gastrothrips sp.

The specimen collected on black nightshade, *Solanum nigrum* L., (Table 2) has the major characters of the genus according to Mound & Marullo (1996), however the tube is not constricted at the apex, as is usually the case in New World species (Mound & Marullo 1996). Since *Gastrothrips* is a fungal spore feeding genus, there is not a direct

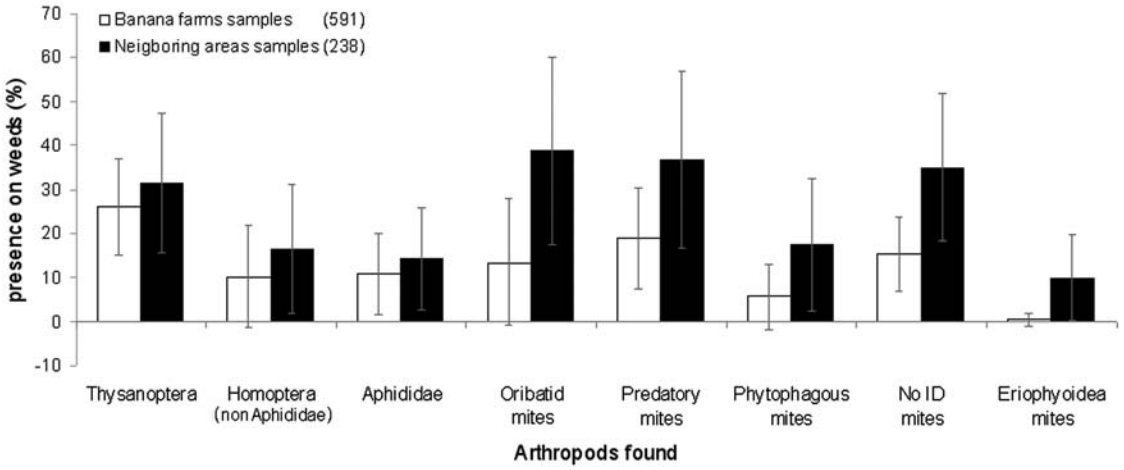

Fig. 1. Average percentages of various insect and mite taxa found in weed samples from 19 banana farms and 12 neighboring areas in Limón Province, Costa Rica in 2008-2009. Each vertical error bar represents the standard error of the mean percentage of the indicated taxon present either on banana farms or surrounding areas. The numbers of samples taken at each of these 2 types of sites are shown in parentheses.

host relationship of *Gastrothrips* sp. with *S. nigrum*; however, the plant species on which this thrips subfamily is found are always recorded (Sakimura & Bianchi 1977), and, indeed, some species in the Idolothripinae can also be found on dead leaves on hanging broken branches (Hoddle et al. 2004). It is interesting to point out that this specimen was found on just 1 of the 70 plant species sampled, and samples of the weeds surrounding in this location did not have any other specimens of *Gastrothrips*. A few Thysanoptera larvae were isolated from a *S. nigrum* sample at location 9 (Table 1), but we could not define their identity because diagnostic information on larval taxonomy is inadequate.

Phlaeothripinae

Haplothrips Amyot & Serville 1843

Haplothrips gowdeyi Franklin 1908

H. gowdeyi is a very common species in the Caribbean area (Mound & Marullo 1996), and it has been reported on pineapple, *Ananas comosus* (L.) Merr., species of *Aster* and *Bidens* (Asteraceae), *Salvia* (Lamiaceae), *Althaea* (Malvaceae)

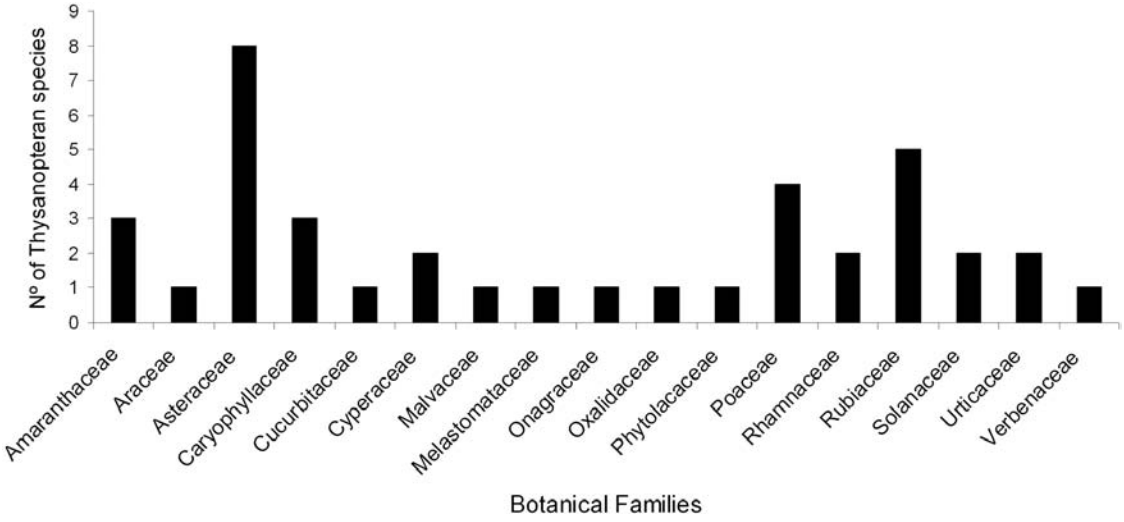

Fig. 2. Number or thrips species (Insecta: Thysanoptera) found on plant species belonging to each of the 17 botanical families sampled on 19 banana farms and 12 neighboring areas in Limón Province, Costa Rica in 2008-2009.

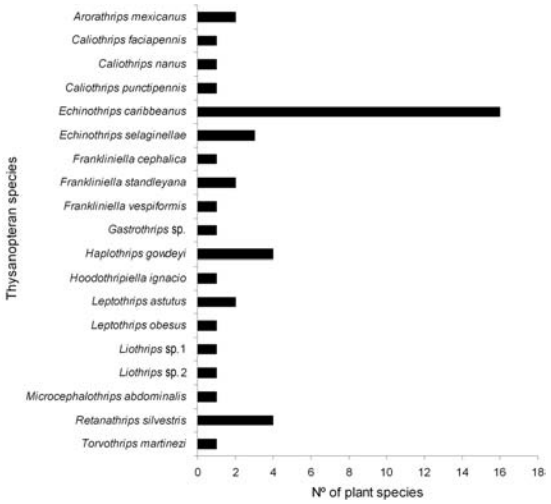

Fig. 3. Number of plant species on which each of the 19 Thysanopteran species was found while collecting samples from 70 species of plants belonging to 17 botanical families on banana farms and surrounding areas in Limón Province, Costa Rica in 2008-2009. The 19 thrips species represent 13 genera belonging to the Aeolothripidae, Phlaeothripidae and Thripidae.

and others in Costa Rica (Soto-Rodríguez *et al.* 2009), sugarcane leaves in South Africa (Way, 2008) and common pigweed, *Amaranthus hybridus* L., in Florida (Childers & Nakahara 2006). Herein (Table 2) we report new associations with Asteraceae (*Emilia sonchifolia* (L.) DC, lilac tasselflower), Rubiaceae (*Spermacoce assurgens* Ruiz & Pav., woodland false buttonweed) and Poaceae (*Digitaria setigera* Roth ex Roem. & Schult., East Indian crabgrass; and *Eleusine indica* (L.) Gaertn., Indian goosegrass). On these same samples we found some Thysanoptera larvae but we could not determine their identity.

Leptothrips Hood 1909

Leptothrips astutus Johansen 1978

L. astutus, a predatory thrips species, was found on several plant species in several botanical families (Johansen 1987) but our finding on *Stachytarpheta jamaicensis* (L.) Vahl, worryvine (Verbenaceae), is a first for this species (Table 2). This weed was sampled once and diverse organisms were isolated from it, i.e., Homoptera, Aphididae, nematodes and predatory mites. On *Synedrella nodiflora* (L.) Gaertn., nodeweed (Asteraceae), it was common to find Homoptera and Thysanoptera instars, aphids, oribatid and predatory mites among others that could be used as prey by this species (Johansen & Mojica-Guzmán 1996). This weed species was sampled 22 times. All *S. nodiflora* samples from areas neighboring banana farms and 62.5% of samples from within banana farms contained *L. astutus* adults, and some also contained unidentified thrips larvae.

Leptothrips obesus Johansen 1987

L. obesus was reported on *Verbesina greenmanii* Urb. (Asteraceae), and it was listed as the unique species of this genus for Mexico (Johansen 1987). This is the first report of this species for Central America, as well as the first report on *Lantana trifolia* L. and within the Verbenaceae family (Table 2). This weed was sampled twice in 2 different locations (paddocks), both of them had *L. obesus* specimens and predatory, phytophagous and unidentified mites that could become suitable prey for *L. obesus*. Homopterans were also represented in both samples and eriophyoid mites in 1 sample (Sánchez-Monge 2010).

Liothrips Uzel 1895

Liothrips spp.

Even though *Liothrips* is the largest genus within the Thysanoptera (Mound & Morris 2007), and even though some *Liothrips* species have been proposed as biocontrol agents of weeds (Cock *et al.* 2000; Mound & Pereyra 2008; Soto-Rodríguez *et al.* 2009), little is known about *Liothrips* hosts and accomplices in Central America, since most neotropical species are reported from Brazil (Mound & Pereyra 2008). Herein we report *Synedrella nodiflora* (Asteraceae) and *Gouania polygama* (Jacq.) Urb., liane savon (Rhamnaceae), as possible new hosts for *Liothrips* species (Table 2); several adults were found in most samples of these weeds and they were found most frequently outside of banana farms (Table 2). We also found some Thysanoptera larvae on these plant species, but we could not identify them due to the lack of larval keys to genera in current literature.

Torvothrips Johansen 1977

Torvothrips martinezi Johansen 1980

According to the key provided by Johansen (1980), the specimen we collected corresponds to *T. martinezi*; nevertheless, some characters do not fit with the species description, which lacks data on associated plant species. We found *T. martinezi* on *Sida ulmifolia* Mill. *Torvothrips* is Mexican in origin (Johansen 1982), and other species of this genus, i.e., *T. tremendous* (Johansen) and *T. kosztarabi* (Johansen), are associated with galls in *Quercus* spp. (Johansen 1982, Kosztarab 1982). The genus *Torvothrips* includes only parasitoid species within galls of the coccids, *Olliffiella* spp. (Kermisidae) (Johansen & Mojica-Guzmán 1996), but it is interesting that this taxon was not

found in any other sample during this research; not even in other samples collected at the same location. This is also the first record of *T. martinezi* for Costa Rica and Central America.

THRIPIDAE

Arorathrips Bhatti 1990

Arorathrips mexicanus Crawford 1909

A. mexicanus is widely distributed in neotropics where it is commonly associated with grasslands (Mound & Marullo 1996; Schuber et al. 2008), and it has been also reported from sugarcane leaves in South Africa (Way, 2008). We found *A. mexicanus* on 63% of *Drymaria cordata* L. (Caryophyllaceae) samples, and, other than on *Eleusine indica*, this thrips species has not been found on any monocotyledonous weed during this research (Table 2).

Caliothrips Daniel 1904

Caliothrips fasciapennis (Hinds 1902)

According to Mound & Marullo (1996), *C. faciapennis* has been collected from grasslands in North America, i.e., from Massachusetts and Illinois to California, Florida and Texas and as far as Mexico. Our report on *Scleria melaleuca* Rchb.f. ex. Schtdl. Cham. (Cyperaceae), a common weed on Neotropical grasslands (Gómez-Gómez *et al.* 2008), is a first of this thrips on a plant species in the Cyperaceae, and the first report of this thrips species for Central America. We found *C. fasciapennis* on all the samples from paddocks, a few specimens were isolated from 2 banana farm samples and 1 from another neighboring area (Table 2). Some thrips larvae were found on this weed species but their identities were not determined.

Caliothrips nanus (Hood 1927)

C. nanus is easy to recognize by the 2 dark stout grooved setae near the forked vein in the forewing, this species is known from Trinidad and West Indies (Wilson 1975), and has been reported from Panama by Mound & Marullo (1996). It was collected from *Parkinsonia aculeate* L., Jerusalem thorn (Fabaceae), in Trinidad, *Mucuna* (Fabaceae) leaves in Panama and from *Gliricidia sepium* (Jacq.) Kunth ex Walp., quickstick (Fabaceae), and *Ipomoea* (Convolvulaceae) leaves in Costa Rica. Although the specimens were isolated from few samples, all samples correspond to the same weed species: *Gouania polygama* (Rhamnaceae).

Caliothrips punctipennis (Hood 1912)

Apparently *C. punctipennis* is a grass feeder (Sakimura 1991), and it was previously reported in Mexico and Texas (Mound & Marullo 1996). Recent literature reports its presence in avocado trees in Mexico (Johansen & Mojica 2007). This is the first report (Table 2) on the grass *Eleusine indica* and the first report for Costa Rica and Central America.

Echinothrips Moulton 1911

Echinothrips caribbeanus Hood 1955

E. caribbeanus was collected in Panama and has been reported on at least 3 botanical families, i.e., Capparidaceae, Menispermaceae and Cucurbitaceae (Mound & Marullo 1996). The hosts reported in this paper (Table 2) are new records at the species and family level, except for Cucurbitaceae. Its occurrence on *Laportea aestuans* L. (Urticaceae) is remarkable since *E. caribbeanus* was present at 7 different locations, most of them banana farms (Table 1). *E. caribbeanus* was also particularly common at location 17, Ecoturismo Banana Farm (Table 2).

Echinothrips selaginellae Mound 1994

E. selaginella was collected on *Selaginella eurynota* A. Braun, spikemoss (Selaginellaceae) (Mound et al. 1994; Mound & Marullo 1996), and it is known only from Costa Rica. Our report on *Alternanthera sessilis* (Amaranthaceae), *Laportea aestuans* (Urticaceae) and *Scleria melaleuca* (Cyperaceae) are new association records for this species (Table 2), implying that this thrips might not has a strict monophagous habit, as it was asserted by Mound (2002). Unfortunately, we did not find any thrips larvae on these weeds species; but it is important to point out that *E. selaginellae* was present only on these weeds throughout 2 years of sampling, involving 70 weed species and 829 samples.

Frankliniella Karny 1909

Frankliniella cephalica Crawford 1910

F. cephalica is widely distributed in the Caribbean and it has been collected in Costa Rica from different locations and altitudes (Mound & Marullo 1996). This species has been reported on several hosts species and botanical families (Masís & Madrigal 1994) including mangroves (Frantz & Mellinger 1990). Herein we present the first report of *F. cephalica* on *Drymaria cordata* L. (Caryophyllaceae).

Frankliniella standleyana Hood 1935

F. standleyana was reported from *Conostegia subcrustulata* (Beurl.) Triana (Melastomataceae) flowers (Mound & Marullo 1996), but our finding is the first record for this species on *Mikania mi-*

crantha Kunth ex H.B.K and its botanical family (Asteraceae). Some unidentified Thysanoptera larvae were associated to *M. micrantha* at locations 7, 17 and 19 (Table 1), however, this Asteraceae was the only weed (other than *C. subcrustulata*) on which we found *F. standleyana*.

Hoodothripiella Retana-Salazar 2007

Hoodothripiella ignacio Retana-Salazar 2007

H. ignacio was found previously in several areas in Costa Rica; but the relevant plant species for these samples were not determined because they were collected with Malaise Traps (Retana-Salazar 2007). The presence of *H. ignacio* on *Spermacoce latifolia* Aubl. (Rubiaceae) is the first report on this weed species and its botanical family; this is important biological data on this thrips species. Interestingly, *H. ignacio* was found more frequently at locations outside the banana farms (Table 2) and it was not found on related weed species (*Spermacoce assurgens* or *S. capitata* Ruiz & Pav.).

Microcephalothrips Bagnall 1926

Microcephalothrips abdominalis (Crawford 1910)

M. abdominalis is a pest in ornamentals (Vierbergen et al. 2006), and an important vector of the Tobacco Streak Virus in Tobacco (Greber et al. 1991). Previously *M. abdominalis* was reported on *Ageratum conizoides* Lam., goat weed (Compositae-Eupatorieae) (Mound & Marullo 1996), Chrysanthemum and *Bidens pilosa* L. (Asteraceae) (Childers & Nakahara 2006). *M. Abdominalis*, is commonly associated with various Asteraceae genera (Childers & Nakahara 2006; Pirec 2007), but this is the first report of *M. abdominalis* on *Wedelia trilobata* L. (Asteraceae). *M. abdominalis* was sampled twice on *Wedelia trilobata* L.; whereas it was not found on any of the other 69 weed species sampled.

Retanathrips Mound & Nickle 2009

Retanathrips silvestris (Hood 1935)

Several specimens of *R. silvestris* were collected from 4 different plant species belonging to 3 botanical families (Table 2). All of them are new records for this taxon since the description of the *Retanathrips* species was based on few specimens and the associated plant species were not reported in this original work. Mound & Marullo (1996) considered that this species probably lives on the leaves of forest trees, but our reports suggests that *R. silvestris* is common on some weed species, especially *Synedrella nodiflora* (Asteraceae) (Table 2), on which specimens were found in 4 different locations and 1 banana farm (Table 1). The infrequent collection of this species may be result of incorrect searching and sampling procedures.

Few studies have focused on the diversity of Thysanoptera on plant species, whether beneficial or harmful, or on weeds associated with crops. As a matter of fact, the interaction of weeds and arthropods has been largely ignored in surveys of agricultural landscapes (Bàrberi et al. 2010). Most literature on Thysanoptera treats only taxonomy, pest species, control of pest populations and other practical topics (Mound 2005). Commonly, data on biology or ecology are not detailed in descriptions of species (Monteiro 2001). Consequently, the lack of such information, at best, results in sketchy and partial knowledge of the habits and behavior of Thysanopteran species. Childers & Nakahara (2006) found thrips species to be associated with weed cover, which varied seasonally. Moreover, Hernández-Ayar et al. (2009) found that the diversity of Thysanoptera was different according to the sample location and the vegetation at each site; that the number of captured thrips species was higher in locations with weed cover than where a crop was associated with a limited number of weeds; and that the number of thrips species was lower at locations with the crop and only 1 other plant species used as a cover. Such findings are predictable because diversity of substrates serves to maintain populations of different arthropod species; and through plant species diversity the number of possible ecological associations is increased. This principle has been applied in several agricultural landscapes and crops for increasing the diversity of insects and the presence of natural enemies for pests (Schellhorn & Sork, 1997).

According to Mound (2005), a thrips' host is commonly defined as a plant species on which a thrips species can successfully maintain a population; thus all life stages of a species of thrips must be able thrive on a plant species in order for it to be designated a host of the thrips species. This definition excludes plant species, which fail to meet this stringent definition, but which, nevertheless, still aid and abet the thrips species. Such accomplice plant species include those which may occasionally allow small thrips populations to establish and multiply fleetingly, and those on which adult thrips feed and acquire or transmit viruses, yet which fail to support the establishment of reproducing and multigenerational populations of certain thrips species. Clearly it is insufficient to rigidly classify plant species as either hosts or non-hosts of thrips species, because some plant species serve importantly as accomplice species.

Surveys like that of Hernández-Ayar et al. (2009) and the results obtained in this paper (Fig. 1) point out the effect that a crop or farm has on various arthropod populations. Moreover, these 2

studies have elucidated the diversity of direct and indirect associations between specific thrips species and specific plant species (Figs. 2 and 3). Further surveys on abundance and diversity of Thysanoptera on weeds are needed to clarify the relationships of these insects and their environments in the tropics, their impacts on plant and arthropod populations, and their population dynamics in cultivated and non cultivated areas.

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A NEW METHOD FOR SHORT-TERM REARING OF CITRUS PSYLLIDS (HEMIPTERA: PYSLLIDAE) AND FOR COLLECTING THEIR HONEYDEW EXCRETIONS

EL-DESOUKY AMMAR AND DAVID G. HALL
Subtropical Insects Research Unit, USDA-ARS, Fort Pierce, FL. 34945

Supplemental material online at http://www.fcla.edu/FlaEnt/fe942.htm#InfoLink1

The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), is an economically important pest of citrus in the United States, Asia and other parts of the world, as a vector of huanglongbing (HLB) or citrus greening, which is considered one of the world's most serious diseases of citrus (Gottwald 2010). Additionally, large populations of ACP can damage plants directly through feeding and excretion activities; ACP feeds on citrus phloem tissues and produces copious amounts of honeydew excretions (Brlansky & Rogers 2007). ACP adults can feed on mature citrus leaves, but nymphs must have young tender flush to survive. For biological and pathogen-vector relation studies on ACP (e.g., Wenninger & Hall 2007; Pelz-Stelinski et al. 2010) rearing of single or small groups of psyllid nymphs or adults on whole citrus plants takes considerable space, time and other resources. Here, we describe a new, simpler method for short-term rearing of ACP using detached mature citrus leaves for adults and detached young terminal shoots for nymphs (Fig. 1).

ACP adults and nymphs were reared singly or in small groups (5-10 per tube) in clear plastic (polypropylene) 50-ml conical centrifuge tubes (3 cm wide and 11.5 cm long; Fisher Scientific, Pittsburg, PA). Young psyllid adults (approximately 1-wk-old) were reared on detached, mature, medium-size leaves of sweet orange (*Citrus sinensis* (L.) Osbeck var. Ridge Pineapple). Leaf sizes used for adults ranged between 3-4 cm in width and 6-8 cm in length with petioles about 2-3 cm long. The petioles of these leaves were cut diagonally with a sharp razor blade, and each inserted in a small (0.3 or 0.5 mL) microfuge tube filled with water or a piece of moistened cotton wool (Figs. 1A and 1E). A piece of Parafilm membrane was wrapped around the top of this tube and the petiole to keep insects from drowning or contact with water. The detached leaf and microfuge tube were then inserted into the rearing tube, and the psyllid adults were added to the latter. The rearing tube was covered with a screw cap that had been finely perforated by a hot needle for ventilation. For better ventilation, however, wider holes can be cut in the plastic cap and a piece of fine mesh screen placed under the screw cap to prevent escape of the psyllids. The tubes were kept in an environmental chamber at 25°C and 14 h light per day.

The clear plastic wall of the rearing tubes allowed close observation and photography of the enclosed psyllids during their various activities either by the naked eye or through a stereomicroscope with minimal disturbance (Figs. 1B-1E). For example, adults were observed feeding for long periods in their normal feeding posture (Fig. 1B) mainly on the midrib or other veins on either side of the detached leaves. They were also observed excreting honeydew droplets regularly (Fig. 1E), and occasionally laying eggs (Fig. 1B inset), although they normally prefer younger leaves for laying eggs (Brlansky & Rogers 2007). Survival of young adults under the above conditions on detached mature leaves, that were changed to fresh ones weekly, was 89, 80 and 75% after 2, 3, and 4 weeks, respectively (n = 130 adults). It was later observed, however, that mature leaves can stay fresh in the rearing tubes at least for 2 weeks.

Young ACP nymphs were reared on younger citrus leaves using the above described rearing tubes under similar conditions. We followed the survival and adult emergence of young (2nd-3rd-instar) nymphs for 1 week on the following 3 types (leaf age/size) of sweet orange leaves (Fig. 1A): (A) partially expanded young leaves (2-3 terminal leaves on a young flush shoot); (B) fully expanded tender leaves; and (C) mature mid-size leaves similar to those used for rearing adults. After gently placing a group of young nymphs on each of these three types of leaves (10 nymphs/tube), the tubes were kept horizontally for a few h to overnight to allow the young nymphs to settle and start feeding. The overall proportion of nymphs that survived for 1 week in these tubes was 77.8% in treatment A (youngest leaves), 61.1% in treatment B (young leaves), and 56.7% in treatment C (mature leaves). Chi square (χ^2) analysis indicated that survival of nymphs was significantly higher in treatment A than in treatments B or C (Table 1). The proportion of nymphs that turned into adults during 1 week of rearing was significantly higher in Treatments A and B (55.7-67.3%) compared to that in treatment C (25.5%) (Table 1). Survival and development of nymphs in treatment A are comparable to those of ACP nymphs reared on whole citrus seedlings at 25°C (Liu & Tsai 2000). Nymphs were observed

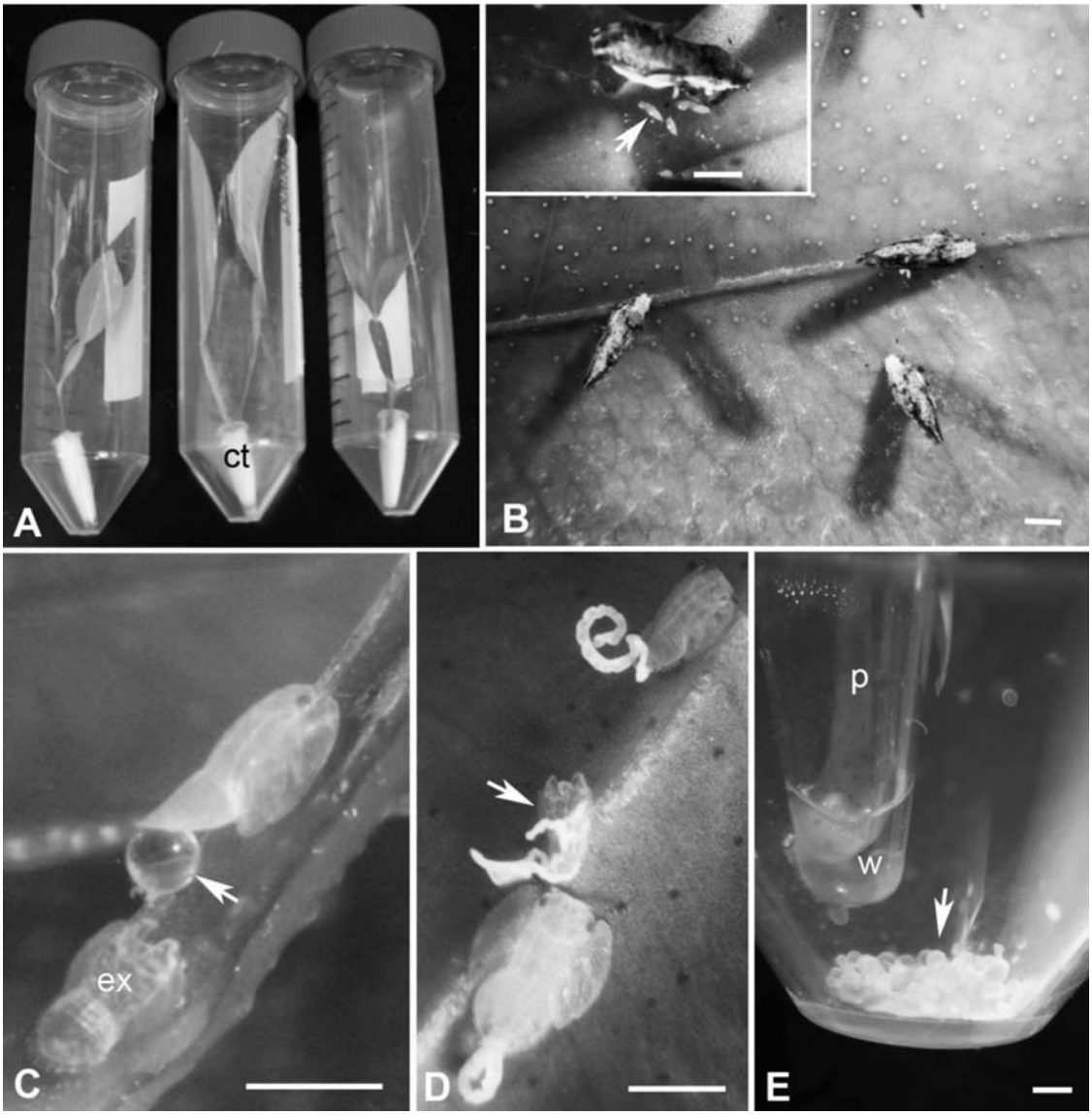

Fig. 1. A. Conical polypropylene 50-mL tubes used for rearing psyllids on citrus leaves of various ages (from left to right): a. young leaves on a flush shoot, b. fully expanded tender leaves, and c. mature mid-size leaves; mature leaves were used for rearing adults but the young leaves were more suitable for rearing young nymphs. The cut end of each leaf petiole/shoot was placed in a small microfuge tube filled with water or a piece of moistened cotton (ct). B. ACP adults in their normal feeding posture, feeding on the midrib or other veins. The inset shows an adult female and several eggs (arrow). C. Fifth instar nymph excreting a large droplet of honeydew (arrow), with an empty skin (exuvium) located behind it (ex). D. Third and fourth instars (upper and lower nymphs, respectively) excreting tubular-shaped material; arrow indicates an exuvium with tubular-shaped excretions still attached. E. Honeydew excretion droplets (arrow) accumulating in the conical bottom of the rearing tube in which 5 adults were kept for 5 d. Other abbreviations: p, petiole; w, water in the bottom of the microfuge tube. All scale bars = 1 mm.

feeding regularly for long periods, molting and excreting large amounts of honeydew in droplet or tubular forms especially on the youngest or younger leaves tested (Figs. 1C and 1D).

When the rearing tubes were kept vertical, most of the ACP honeydew excretion droplets fell down from the leaves and accumulated in the conical bottom of the rearing tubes (Fig. 1E). This can be a convenient and efficient way to collect the psyllid excretions for various studies on feeding behavior/chemistry (Hall et al. 2010). We believe that this new rearing method allows closer obser-

TABLE 1. SURVIVAL AND ADULT EMERGENCE OF YOUNG (2ND-3RD-INSTAR) NYMPHS OF *D. CITRI* FOR 1 WEEK IN REARING TUBES WITH DETACHED SWEET ORANGE LEAVES OF VARIOUS AGES.

		Trial 1		Trial 2		Trial 3		Overall**	
Attribute	Leaf Age*	No.	%	No.	%	No.	%	No.	%
Survival	A	24/30	80.0	24/30	80.0	22/30	73.3	70/90	77.8 a
	B	16/30	53.3	14/30	46.7	25/30	83.3	55/90	61.1 b
	C	27/30	90.0	11/30	36.7	19/30	63.3	51/90	56.7 bc
Adult emergence	A	18/24	75.0	17/24	70.8	4/22	18.2	39/70	55.7 a
	B	13/16	81.2	7/14	50.0	17/25	68.0	37/55	67.3 a
	C	12/27	44.4	0/11	00.0	2/19	10.6	12/51	23.5 b

*Leaf age designation: (A) Partially expanded young leaves on a young flush shoot; (B) Fully expanded young leaf; and (C) Mature mid-size leaf (Fig. 1A).

**χ^2 analysis conducted on overall proportions: For each attribute, percentages followed by different letters are significantly different ($P < 0.001$-0.003).

vation of psyllids, and can save time, space and other resources in various studies on the biology and management of ACP and probably other citrus psyllids. It can be particularly valuable for studying psyllid behavior, HLB pathogen-vector interactions, and for bioassay of biological or chemical agents against citrus psyllids.

We thank Kathy Moulton and Monty Watson for technical assistance. This article reports the results of research only. Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. Funds for this research were provided by the Florida Citrus Research and Development Foundation.

SUMMARY

We developed a new simple method for short-term rearing of the Asian citrus psyllid (ACP) using detached citrus leaves in 50-mL conical polypropylene tubes. Survival of young adults was 89, 80, and 75% after 2, 3, and 4 weeks, respectively, on detached mature leaves that were changed weekly. Survival and adult emergence of 2nd to 3rd-instar nymphs were significantly higher when reared on younger leaves compared to those reared on mature leaves. Honeydew excretion droplets of ACP accumulated and may be easily collected from the conical bottom of the rearing tubes. This new method allows closer observation and photography of psyllid nymphs and adults with minimal disturbance, and it can save time, space and other resources in various studies on the biology, behavior, management and pathogen-vector interactions of ACP and probably other citrus psyllids.

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Fig. 1 from El-Desouky and David G. Hall 2011. A New method for short-term rearing of citrus psyllids and for collecting their honeydew excretions. Florida Entomol. Vol 94 (2).

Fig. 1. A. Conical polypropylene 50-mL tubes used for rearing psyllids on citrus leaves of various ages (from left to right): a. young leaves on a flush shoot, b. fully expanded tender leaves, and c. mature mid-size leaves; mature leaves were used for rearing adults but the young leaves were more suitable for rearing young nymphs. The cut end of each leaf petiole/shoot was placed in a small microfuge tube filled with water or a piece of moistened cotton (ct). B. ACP adults in their normal feeding posture, feeding on the midrib or other veins. The inset shows an adult female and several eggs (arrow). C. Fifth instar excreting a large droplet of honeydew (arrow), with an empty skin (exuvium) located behind it (ex). D. Third and fourth instars (upper and lower nymphs, respectively) excreting tubular-shaped material; arrow indicates an exuvium with tubular-shaped excretions still attached. E. Honeydew excretion droplets (arrow) accumulating in the conical bottom of the rearing tube in which 5 adults were kept for 5 days. Other abbreviations: p, petiole; w, water in the bottom of the microfuge tube. All scale bars = 1 mm.

FIRST REPORT OF *GEOICA UTRICULARIA* (HEMIPTERA: APHIDIDAE) POPULATION ON PARASITIC BROOMRAPE *OROBANCHE FOETIDA*

SONIA BOUKHRIS-BOUHACHEM[1], S. BEN YOUSSEF[2] AND M. KHARRAT[3]
[1]Plant Protection Laboratory, National Institute of Agricultural Research of Tunisia

[2]Animal and Forage Laboratory, National Institute of Agricultural Research of Tunisia

[3] Field Crop Laboratory, National Institute of Agricultural Research of Tunisia INRAT, Rue Hédi Karray, 2049, Tunisia

Geoica utricularia (Passerini) is known as a species that alternates between galls on *Pistacia* and the roots of Gramineae, and occasionally Cyperaceae (Blackman & Eastop 1985). Its distribution has been recorded as Morocco, Europe, the Middle East, Central Asia and North America (Blackman & Eastop 1985). In 2009, we observed severe attacks of *G. utriculariae* on wheat and barley in Manouba and Cap-Bon regions in Northeast Tunisia. Previously, *G. utriculariae* had been captured in suction traps in the same region (Boukhris-Bouhachem et al. 2007).

Very few insects have been reported to feed on *Orobanche* (Orobanchaceae). The case of *Phytomyza orobanchia* Kalt. (Diptera: Agromyzidae) has been reported (Linke 1990; Klein & Kroschel 2002), and Zermane et al. (2001) found *Smicronyx cyaneus* Gyll. (Coleoptera: Curculionidae) on *Orobanche foetida* Poir. in Tunisia. Holman (2009) reported *Smynthurodes betae* WestWood (Hemiptera: Aphididae) on 3 Orobanche species (*O. aegyptiaca, O. crenata* and *O. variegata*).

Populations of aphids were found on the subterranean parts, and feeding on spikes of two broomrape plants, *Orobanche foetida,* attached to faba bean (*Vicia faba* L.) in May 2010 in a field located at Ariana (36°47' 57'N, 10°10'32'E). Samples of aphids were collected and prepared for microscopic examination in Canada balsam. They were identified using the Remaudière & Seco Fernandez (1990) and Blackman & Eastop (2000) keys, and also submitted for confirmation to Jon H. Martin at the Natural History Museum in London, UK. Aphid specimens in the colony were white cream, lightly dusted with wax, and broadly oval (Fig. 1). The aphids were identified as *G. utricularia* (Fig. 2) (determination confirmed by J. H. Martin) and compared favorably with specimens in the museum collection from Morocco and elsewhere. Voucher specimens were deposited at the British museum.

Fig. 1. Apterous viviparae *Geoica utricularia*.

Fig. 2. Colony of *G. utricularia* feeding on *O. foetida*.

To our knowledge, no associations of this aphid species have been reported previously on *Orobanche foetida,* so even if the species identification is suspect due to the unusual host range, it represents a host range expansion for the genus. Broomrape infestations are increasing in different regions of Tunisia (Kharrat et al. 2004). Heretofore, the association between *G. utriculariae* and *Orobanche* has not been reported, and it is quite curious because the aphids normally feed on Gramineae except when forming galls on *Pistaca*. A supplementary study will be conducted to determine if aphids can reduce seed production of *Orobanche*.

Summary

A new association between aphids (Aphididae) and *Orobanche foetida* was observed in Tunisia. The aphids were identified as *Geoica utricularia* (Passerini). The feeding of *G. utricularia* on *Orobanche* is illustrated.

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FIRST REPORT OF *CHRYSOMYA MEGACEPHALA* (DIPTERA: CALLIPHORIDAE) IN NORTHWESTERN ARGENTINA

María S. Olea[1], María J. Dantur Juri[1] and Néstor Centeno[2]

[1]"Dr. Abraham Willink" Higher Entomology Institute, Faculty of Natural Sciences and Miguel Lillo Institute, Tucumán National University, Miguel Lillo 205, San Miguel de Tucumán, T4000JFE, Tucumán, Argentina

[2]Studies and Research Center, Quilmes National University, Sáenz Peña 352, Bernal, B1876BXD, Buenos Aires, Argentina

The Calliphoridae family comprises around 150 genera and more than 1000 species distributed world wide (Hennig 1973; Pont 1980; Shewell 1987). *Chrysomya* Robineau-Desvoidy (Diptera: Calliphoridae) is an especially important genus because its species are reported as invaders in South America (Guimaraes et al. 1978), and they are involved in the transmis-

Table 1. Calliphoridae species collected in Tucumán, northwestern Argentina, Oct 2009-Jul 2010.

Species	Collections	Date (Months)	Sites
Phaenicia cluvia	43	Oct	Jardín Botánico
	9	Nov	Jardín Botánico
	1	Dec	Jardín Botánico
	52	Mar	Jardín Botánico
	8	Nov	Nueva Esperanza
	7	Dec	Nueva Esperanza
	18	Jan	Nueva Esperanza
	6	Mar	Nueva Esperanza
	26	Apr	Jardín Botánico
	4	Apr	Taficillo
Phaenicia sericata	11	Oct	Jardín Botánico
	1	Nov	Jardín Botánico
	2	Dec	Jardín Botánico
	30	Jan	Jardín Botánico
	1	Jan	Nueva Esperanza
Phaenicia eximia	4	Oct	Jardín Botánico
	3	Oct	Taficillo
	1	Dec	Taficillo
Phaenicia peruviana	2	Oct	Taficillo
	2	Dec	Taficillo
	3	Jan	Taficillo
	22	Mar	Taficillo
	3	Apr	Taficillo
Sarconesiopsis magellanica	2	Oct	Taficillo
Chrysomya chloropyga	2	Nov	Jardín Botánico
	1	Mar	Nueva Esperanza
Chrysomya albiceps	2	Nov	Nueva Esperanza
	1	Jan	Jardín Botánico
	1	Jan	Taficillo
	1	Mar	Nueva Esperanza
	4	Mar	Taficillo
Chrysomya megacephala	1	Nov	Taficillo
	3	Mar	Jardín Botánico
Paralucilia pseudolyrcea	2	Mar	Taficillo

sion of enteric bacteria, protozoa and helminths (Greenberg 1973). These species can act as dispersers of disease because their special feeding habits, which include human food products and human or animal faeces (Bohart & Gressit 1951; Zumpt 1965). In subtropical and tropical Africa and Asia the old world screwworm, *Chrysomya bezziana* Villeneuve is an obligate parasite of mammals (Sutherst et al. 1989).

García (1959) reported seven species of Calliphoridae in Argentina, and Mariluis (1982) reported new species for the country, increasing to 12 the species included in the Calliphorinae, Chrysomyinae, and Toxotarsinae subfamilies. Later, Mariluis & Schnack (2002) cited 25 species for the country and Mariluis & Mulieri (2003) recorded 13 species in the Tucuman province, Northwestern Argentina, including *Calliphora nigribasis* Macquart, *Calliphora vicina* Robineau-Desvoidy, *Phaenicia cluvia* (Walker), *Phaenicia peruviana* (Robineau-Desvoidy), *Phaenicia sericata* (Meigen), *Cochliomyia macellaria* (Fabricius), *Compsomyiops fulvicrura* (Robineau-Desvoidy), *Compsomyiops verena* (Walker), *Chrysomya albiceps* (Wiedemann), *Chrysomya chloropyga* (Wiedemann), *Paralucilia pseudolyrcea* (Mello), *Sarconesia chlorogaster* (Wiedemann), and *Sarconesiopsis magellanica* (Le Guillou) (Mariluis & Mulieri 2003). *Chrysomya megacephala* (Fabricius) was reported in Argentina for Misiones, Santa Fé, and Buenos Aires provinces (northeast and center of the country) (Mariluis & Mulieri 2003).

The present study updates the distribution of *Chrysomya megacephala* for Argentina. The new records extend westward by approximately 500 km the known geographic distribution of the species, being the first report of the species in the Northwestern region of the country.

We collected adult calliphorids with Ferreira traps (Guimarães et al. 1983) from 1 Oct 2009 through 30 Jul 2010 in different locations of the Tucuman province, i.e., Jardín Botánico Miguel Lillo (26°49.8'S; 65°13.3'W) (Capital department), Nueva Esperanza (26°42.6'S; 65°15.9'W), and El Taficillo (26°41.3'S; 65°16.8'W) (Tafí Viejo department). The locations were placed according to relationship with anthropic activities, and Jardín Botánico represents the major degree of association with man, decreasing in Nueva Esperanza (with corn and citrus crops) and in El Taficillo (native rainforest). Traps were hung from tree branches at a height of 1.0 m. The collected specimens were taken to the laboratory and identified with the key of Mariluis & Schnack (2002). Voucher specimens were deposited in the collection of the Miguel Lillo Foundation Institute (Instituto-Fundación Miguel Lillo-IMLA).

The presence and abundance of *C. megacephala* adults and of the others calliphorid species is reported in Table 1.

Summary

Chrysomya megacephala is reported by the first time to Tucumán province, Northwestern Argentina. Eight other calliphorid species were collected in the same locations. The voucher specimens were deposited in the collection of the Miguel Lillo Foundation Institute (Instituto-Fundación Miguel Lillo-IMLA).

Acknowledgments

We thank Marcos Foguet for permission to place the traps in the Nueva Esperanza location.

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NATURAL HOST PLANTS AND NATIVE PARASITOIDS ASSOCIATED WITH *ANASTREPHA PULCHRA* AND OTHER *ANASTREPHA* SPECIES (DIPTERA: TEPHRITIDAE) IN CENTRAL AMAZON, BRAZIL

Beatriz Ronchi-Teles[1], Vivian Siqueira Dutra[1], Alexandra Priscilla Tregue Costa[1], Elen De Lima Aguiar-Menezes[2], Aline Cristina Araujo Mesquita[1] and Janisete Gomes Silva[3]
[1]Coordenação de Pesquisas em Entomologia, Instituto Nacional de Pesquisas da Amazônia, Caixa Postal 478, 69011-970 Manaus, Amazonas, Brazil

[2]Departamento de Entomologia e Fitopatologia, Universidade Federal Rural do Rio de Janeiro, Caixa Postal 74538, 23890-000 - Seropedica, Rio de Janeiro, Brazil

[3]Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Rodovia Ilhéus-Itabuna km 16, 45650-000 - Ilhéus, Bahia, Brazil

The Brazilian Amazon harbors a high diversity of fruit flies in the genus *Anastrepha* Schiner (Diptera: Tephritidae) with 53 of the 103 described species reported in Brazil, and 12 *Anastrepha* species occur exclusively here (Zucchi et al. 1996; Silva & Ronchi-Teles 2000; Zucchi 2008).

In the Central Amazon, most fruit fly studies have been carried out in agroforestry systems (Silva et al. 1996; Zucchi et al. 1996) or with traps (Ronchi-Teles & Silva 2005), and the only study on fruit flies in an unperturbed forest area focused on parasitoids (Costa et al. 2009). Thus, the information on host/fruit fly/parasitoid associations is still limited.

In this study, we documented fruit fly-host associations for *Anastrepha* species for both the Central Amazon and Brazil, and identified the braconid larval-pupal parasitoids associated with *Anastrepha*.

Our study site was located in a 30 km^2 area of the Reserva Florestal Adolpho Ducke (RFAD) of the Instituto Nacional de Pesquisas da Amazônia (INPA), located northeast of Manaus (02°53'S and 59°59'W) in the state of Amazonas, Brazil. The area is primary forest of about 100 km^2. The mean annual temperature is 26.5°C, with a monthly mean maximum of 38.6°C (Dec) and minimum of 18.2°C (Jul) and mean annual relative humidity of 82% (Araújo 1970). Samples of ripe or ripening fallen fruit were collected randomly at the ground level under the canopies every 2 weeks from Oct 2002 to Jun 2003, from Mar to May 2009, and from Mar to May 2010. The fruits were collected inside the forest in an area of about 30 km^2 where all tree species had already been identified by botanists from Instituto Nacional de Pesquisas da Amazônia (INPA). The collected fruits were counted, weighed, and placed in plastic containers with a layer of vermiculite and covered with voile cloth until larvae emerged and pupated. All pupae obtained were placed in 30-mL plastic containers with a layer of vermiculite at the bottom and covered with voile cloth until adults emerged. Voucher specimens were deposited at the Coleção de Invertebrados of INPA.

We collected a total of 63.7 kg of fruit from 50 plant species in 18 families. A total of 1,398 fruits weighing 19.7 kg from 13 species in 7 families were infested by fruit flies, with a total of 880 puparia recovered (Table 1). We report for the first time field infestations under natural conditions by *Anastrepha pulchra* Stone on *Mouriri collocarpa* Ducke (Melastomataceae), a native tree species, and its associated parasitoid *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) in Brazil. *Anastrepha pulchra* has been reported in Panama, Venezuela, and Brazil (Amazon) (Norrbom 2002).

We also report 2 new hosts for *Anastrepha atrigona* Hendel: *Strychnos jobertiana* Baillon (Loganiaceae) and *Pouteria durlandii* (Standley) Baehni (Sapotaceae). Three hymenopteran parasitoid species, *Opius bellus* Gahan, *Opius* sp. (Braconidae), and *Aganaspis pelleranoi* (Brèthes) (Figitidae), are associated with *A. atrigona* for the first time. *Anastrepha atrigona* has been reported only in Venezuela, Guyana, Surinam, and Brazil (state of Amazonas) to date (Norrbom et al. 1999; Zucchi 2008).

We found 1 new host for *Anastrepha bahiensis* Lima, *Helicostylis scabra* (Macbride) Cornelis Christiaan Berg (Moraceae). *Anastrepha bahiensis* has been found from Mexico to Brazil (several states) (Norrbom et al. 1999; Zucchi 2008). Four other described species, *Anastrepha bondari* Lima, *Anastrepha coronilli* Carrejo & González, *Anastrepha obliqua* Macquart, and *Anastrepha striata* Schiner were found in this study and have already been reported for the hosts listed on Table 1 and numerous other hosts in previous studies (Norrbom 2002; Zucchi 2007, 2008) (Table 1). Four presumed new species of *Anastrepha,* yet to be described, were reared from single species of Anonaceae and Bignoniaceae, and 2 species of Sapotaceae, respectively (Table 1).

Three species of braconids (*D. areolatus*, *Opius* sp., and *O. bellus*) and 2 species of figitids (*Aganaspis nordlanderi* Wharton and *A. pelleranoi*) were associated with *Anastrepha* species. This is the first report of *A. nordlanderi* parasitizing

TABLE 1. *ANASTREPHA* SPECIES AND ASSOCIATED PARASITOIDS COLLECTED IN CENTRAL AMAZON, BRAZIL.

Plant family	Plant species	Hosts	Number of fruit	Sample weight (kg)	Number of pupae	*Anastrepha* species (n)	Parasitoid species (n)
Anonaceae	Anonaceae (unidentified)	Native	3	0.317	10	8 *Anastrepha* sp. 1	0
Bignoniaceae	*Clytostoma* sp.	Native	2	0.096	2	1 *Anastrepha* sp. 3 1 *Anastrepha* sp. 4	0
Loganiaceae	*Strychnos jobertiana* Baill. ▲	Native	56	1.970	17	12 *A. atrigona*	0
Melastomataceae	*Bellucia grossularioides* (L.) Triana	Native	758	5.029	68	59 *A. coronilli*	7 *D. areolatus* 1 *A. nordlanderi* ■
Melastomataceae	*Mouriri collocarpa* Ducke ▲	Native	13	0.153	108	59 *A. pulchra*	18 *D. areolatus* ■
Moraceae	*Helicostylis scabra* (Macbr.) C. C.Berg. ◆	Native	92	0.978	290	218 *A. bahiensis*	48 *D. areolatus*
Moraceae	*Helicostylis tomentosa* (Planch. & Endl.) Rusby	Native	239	2.794	275	162 *A. bahiensis*	24 *D. areolatus*
Moraceae	*Naucleopsis* sp.	Native	21	0.803	9	8 *A. bondari*	0
Myrtaceae	*Eugenia patrisii* Vahl.	Native	15	0.083	16	14 *A. obliqua*	1 *A. pelleranoi*
Myrtaceae	*Psidium guajava* L.	Native	12	0.486	5	3 *A. striata*	0
Sapotaceae	*Chrysophyllum prieurii* A.DC.	Native	55	2.970	12	6 *Anastrepha* sp. 4	0
Sapotaceae	*Pouteria durlandii* (Standl.) Baehni ▲	Native	19	0.567	66	29 *A. atrigona*	10 *Opius bellus* ■ 2 *Opius* sp. ■ 2 *A. pelleranoi* ■
Sapotaceae	*Pouteria williamii* (Aubrév. & Pellegrin) T.D. Penn.	Native	8	0.358	2	1 *Anastrepha* sp. 2	0

▲First host record
◆New host record
■New parasitoid record

Anastrepha coronilli. The braconids and figitids reported in this study previously were found associated with other *Anastrepha* species (Canal & Zucchi 2000; Guimarães et al. 2000; Ovruski et al. 2000)

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Summary

A natural host (*Mouriri collocarpa*) and a parasitoid (*Doryctobracon areolatus*) for *Anastrepha pulchra* are reported for the first time in Brazil. We report new hosts for *Anastrepha atrigona* and *Anastrepha bahiensis* in the Brazilian Amazon. Parasititoids attacking *A. atrigona, Anastrepha coronilli*, and *A. pulchra* are reported.

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LARINUS MINUTUS (COLEOPTERA: CURCULIONIDAE), A BIOLOGICAL CONTROL AGENT OF SPOTTED KNAPWEED (*CENTAUREA STOEBE* SSP. *MICRANTHOS*), ESTABLISHED IN NORTHERN ARKANSAS

C. R. MINTEER, T. J. KRING, J. SHEN AND R. N. WIEDENMANN
Department of Entomology, University of Arkansas, Fayetteville, AR 72701 USA

Larinus minutus Gyllenhal (Coleoptera: Curculionidae) is a univoltine weevil that feeds on the seeds of spotted and diffuse knapweeds *Centaurea stoebe* ssp. *micranthos* (Gugler) Hayek and *C. diffusa* Lamarck. After emerging from overwintering sites in the leaf litter, adult weevils begin feeding on the vegetative portions of the plants. Adults, however, prefer to feed on flowers when they are available and development of beetle ovaries is dependent upon flower feeding (Groppe 1990). Females oviposit on newly opened flower heads (capitula). Two or 3 eggs can occur in each flower head, but only 1 larva usually develops in smaller capitula. Multiple larvae can survive in large spotted knapweed capitula (Groppe 1990). Under laboratory conditions (25°C), eggs hatch in 3-4 d (Groppe 1990). Larval development takes approximately 4 weeks and larvae go through 3 instars. Larvae feed on knapweed seeds and pupate in the capitula, making a cocoon out of the seed head material (Kashefi & Sobhian 1998). Larvae can destroy up to 100% of the seeds in a capitulum (Kashefi & Sobhian 1998). In the Western United States, adult weevils emerge in late Sep and feed on plants until winter, when the adults overwinter in leaf litter and emerge in the following Jun (Jordan 1995).

Larinus minutus was first released into the United States in 1991 with collections from Greece and Romania (Story 2002). Although 12 other natural enemy species were introduced into the Western United States and Canada to control spotted and diffuse knapweeds, only recently has adequate suppression of some populations been seen (Myers 2004; Smith 2004). Myers (2007) suggested that knapweed populations did not significantly decline until the establishment of *L. minutus*. Populations of *L. minutus* have been established in Washington, Wyoming, Oregon, Montana, Minnesota, Colorado, and Indiana (Lang et al. 1996; Story 2002).

No natural enemies of spotted knapweed have been released in Arkansas until the inception of this study. However, we found *Urophora quadrifasciata* (Meigen) (Diptera: Tephritidae) established throughout the range of spotted knapweed in the state in a survey in 2006 for knapweed natural enemies. This seedhead galling fly was introduced from Russia into Canada in 1980 and has since been redistributed or spread on its own to several states in the northeastern and northwestern United States (Story 2002). Duguma (2008) found that *U. quadrifasciata* reduced the number of seeds produced by spotted knapweed by 44% late in the season (Aug), at a time when plants are more environmentally stressed. However, the fly did not significantly reduce the number of seeds produced earlier in the season, a time when knapweed is most robust (Duguma 2008). Thus, it is likely that *U. quadrifasciata* alone will not significantly suppress knapweed populations in Arkansas, or stop its spread further into the southern United States.

To increase the level of control beyond that provided by *U. quadrifasciata*, we have introduced *L. minutus* in Arkansas. Collections of *L. minutus* were made from diffuse knapweed infestations in Colorado Springs, CO, for the Arkansas releases. A total of 8 releases were made during 2008 and 2009 (Fig. 1). Two releases were made in 2008 with 400 weevils released at the University of Arkansas Agriculture Experiment Station Farm, and 300 weevils released approximately 5 km south of the station. After the releases, sites were visually surveyed for surviving weevils each week. Weevils were observed for the remainder of the growing season in 2008. Six additional releases were made in 2009 in Washington County, Arkansas, with 600 to 700 weevils per release. Release sites in 2009 were of sufficient distance away from the release sites in 2008 that we believe the weevils had not spread to the new release sites. After releases in 2009, all sites were visually surveyed for adult weevils for the remainder of the growing season in 2009. *Larinus minutus* were found at all release sites (2008 and 2009) except one—the 2008- site just south of the University of Arkansas Agriculture Experiment Station.

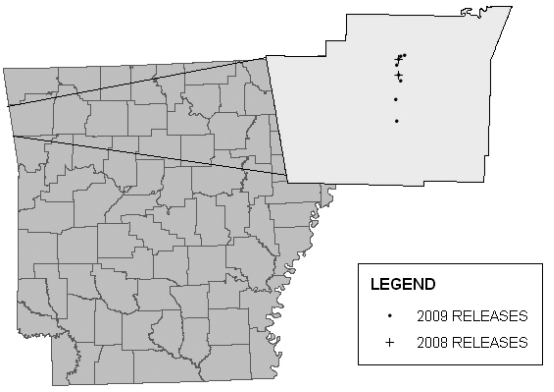

Fig. 1. Locations of releases of *Larinus minutus* in Washington County, Arkansas during 2008 and 2009.

Beginning in mid-Apr 2010, emerging adult weevils were collected weekly by sweep-netting at all Arkansas release sites, once plants had bolted, but before flowers were present. *Larinus minutus* were first found on May 5, 2010. Weekly sweeps yielded *L. minutus* at each of the 2008- and 2009-release sites, including the 2008-site at which no weevils were found during 2009. We believe that low population levels the first year after release prohibited us from collecting weevils at the south Fayetteville site. Finding weevils at all sites demonstrated establishment at the 2008-release sites and successful overwintering of adults at the 2009-release sites. Thus, *L. minutus* joins *U. quadrifasciata* as an established natural enemy of spotted knapweed in northern Arkansas.

Additional releases in northern Arkansas of ~17,000 *L. minutus* were made in 2010 with weevils collected from Colorado Springs, CO.

We thank Jerry Michels and his crew from Texas A&M AgriLife for help in locating and collecting weevils in Colorado.

Summary

Larinus minutus, a biological control agent of spotted knapweed, has been established at 2 sites in Washington County, Arkansas. The weevils were collected in Colorado and introduced into Arkansas at 2 sites in 2008 and 6 additional site in 2009. No *L. minutus* weevil was recorded in Arkansas prior to these releases.

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DISTRIBUTION OF *EUMICROSOMA BENEFICA* (HYMENOPTERA: SCELIONIDAE) IN SOUTHERN CHINCH BUG (HEMIPTERA: BLISSIDAE) POPULATIONS

RON CHERRY
Everglades Research and Education Center, 3200 E. Palm Beach Road, Belle Glade, FL 33430

St. Augustinegrass, *Stenotaphrum secundatum* (Walter) Kuntze, is a widely used turfgrass in tropical and subtropical climatic regions (Sauer 1972). It is a commonly grown residential turfgrass species in the southern United States, covering 400,000 ha and accounting for 85% of the sod industry in Florida with an estimated value of $262 million (Haydu et al. 2005). The southern chinch bug, *Blissus insularis* Barber (Hemiptera: Blissidae), is the most serious insect pest of St. Augustinegrass (Crocker 1993). Management of this insect has been challenged by its development of resistance to several key insecticides (Reinert & Portier 1983; Cherry & Nagata 2005, 2007) and overcoming host plant resistance (Busey & Center 1987; Rangasamy et al. 2006).

The egg parasite *Eumicrosoma benefica* Gahan is the only known parasite of southern chinch bugs in Florida. Reinert (1972) first noted the parasite on southern chinch bugs in Florida and noted various aspects of its biology. In a later study of the natural enemy complex of Florida chinch bugs, Reinert (1978) again noted that *E. benefica* was the only parasite found. These previous studies provided information on the biology of the parasite, but did not provide a large scale survey of the overall distribution or parasitism rates of the parasite in chinch bug populations in Florida. McColloch & Yuasa (1915) reported the *E. benefica* was an important factor in the control of the chinch bug *B. leucopterus* (Say) in Kansas. More recently, Wright & Danielson (1992) reported that the wasp parasitized large numbers of eggs of *B. leucopterus* in Nebraska. Because of the potential importance of *E. benefica*, the objective of this study was to better understand the impact of the parasite on southern chinch bug populations in Florida.

Parasite samples were obtained from 3 contiguous counties (Palm Beach, Martin, and St. Lucie) located in the heavily urbanized southeastern coast of Florida. St. Augustinegrass is commonly used here and the chinch bugs have year round activity (Reinert 1972) because of the climate. Samples were taken for a 1-year period starting Jan 2009. Each 2 months, 1 different infestation was sampled in each county. Chinch bug infestations were located by driving in urban areas and looking for chinch bug damage (i.e., dead or yellow patches of St. Augustinegrass). The presence of chinch bugs (adults and nymphs) was then verified by visual examination for the insects in the grass. Chinch bug eggs are often found in crevices at the grass node or hidden between overlapping grass sheaths on St. Augustinegrass stolons (Nagata & Cherry 1999). These stolons are horizontally growing stems that root at nodes and vary in length e.g., similar to strawberry runners. Ten 50-cm St. Augustinegrass stolons were cut and bagged at each location. These clippings were taken to a laboratory where they were cut by hand with scissors and washed through a series of sieves with eggs being caught in the smallest sieve (U.S.A. Standard Testing Sieve #325, 45 micrometer opening) made by Fisher Scientific Company, U.S.A. A microscope was used to find eggs in the sieve which were identified as southern chinch bug eggs based on Vittum et al. (1999). Eggs were placed in vials (1 egg/vial) on a small piece of moistened paper and stored at 25°C. Eggs were examined microscopically each 2-3 d for chinch bug or parasite emergence for 30 d. McColloch (1914) reported that the average length of life cycle of *E. benefica* ranged from 10 to 28 d depending on temperature. Parasites emerging were stored in alcohol for later species and sex identification. Sex identification of *E. benefica* was determined based on descriptions of Gahan (1914).

An average 54.8 ± 15.8 SD eggs were collected per sample from the 18 samples (3 counties × 6 sampling times). Of these eggs, 57% were viable yielding chinch bugs or parasite emergence. Besides natural mortality, invariably some mortality was caused in processing the grass to obtain the eggs although this latter mortality is unknown. *Eumicrosoma benefica* was the only parasite found emerging from the eggs. The parasite was found in all 3 counties. This parasite was first reported in Florida in 1972 by Reinert (1972), although its overall distribution in the state is currently unknown. The parasite was found in samples in all time periods showing year round activity. These results are consistent with Reinert (1972), who observed *E. benefica* throughout the year in southern Florida.

In this study, the parasite was found at all chinch bug infestations sampled showing a widespread distribution and close association with southern chinch bugs. This is consistent with McColloch & Yuasa (1914), who noted that the parasite was found every place that eggs of the chinch bug *B. leucopterus* (Say) were found. The mean % parasitism at all locations was 20.2 ± 17.5 SD and ranged from 2 to 48%. Not using eggs, Reinert (1972) used adult *E. benefica* and

nymphal and adult *B. insularis* from one area to determine a parasite/host ratio. Interestingly, his parasite/host ratio was 27.7%, approximating the 20.2% parasitism rate I found from egg emergence. McColloch & Yuasa (1914) reported an average 16% parasitism rate of the parasite in *B. leucopterus*. More recently, Wright & Danielson (1992) reported 47.2% parasitism of *B. leucopterus* by *E. benefica* in wheat fields in Nebraska. However, at least in southern Florida, the parasites are active year round. Hence, the low parasitism rate in this study is somewhat deceptive since it represents an instant in time and not the larger cumulative mortality to chinch bugs over time. The topic of low parasitism by *E. benefica* underestimating chinch bug mortality was discussed much earlier by McColloch & Yuasa (1914), who concluded that the parasite was an important factor in *B. leucopterus* control (McColloch & Yuasa 1915).

In this study, an average of 62.0 ± 43.1 SD percent of parasites were females in the populations sampled. McColloch & Yuasa (1914, 1915) concluded that females of *E. benefica* exceeded males in both field and laboratory tests. Reinert (1978) reported that 42.8% of the parasites were females in his field samples in Florida. In Nebraska, 64% of the wasps were female (Wright & Danielson 1992). Data from this study and the previous studies indicate that both sexes will normally be present under varying ratios.

Summary

The parasitic wasp *Eumicrosoma benefica* has year round activity and was found at all southern chinch bug infestations that were sampled in southern Florida. This parasite is an important, if not the most important, biological control agent in reducing southern chinch bug populations in southern Florida.

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MULTIPLE DETECTIONS OF TWO EXOTIC AUGER BEETLES OF THE GENUS *SINOXYLON* (COLEOPTERA: BOSTRICHIDAE) IN GEORGIA, USA

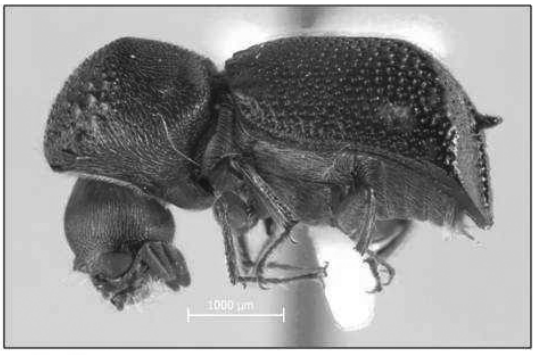
TERRY PRICE[1], KAYLA A. BROWNELL[2], MARK RAINES[1], CECIL L. SMITH[3] AND KAMAL J. K. GANDHI[2]
[1]Georgia Forestry Commission, 1508 Hwy. 25 South, Waynesboro, GA 30830, U.S.A.
E-mail: tprice@gfc.state.ga.us; mraines@gfc.state.ga.us

[2]Daniel B. Warnell School of Forestry and Natural Resources, The University of Georgia, Athens, GA 30602, U.S.A.
E-mail: brownellk@warnell.uga.edu; kgandhi@warnell.uga.edu

[3]Georgia Museum of Natural History, The University of Georgia, Athens, GA 30602, U.S.A.
E-mail: clsmith@uga.edu

False powderpost or auger beetles (Coleoptera: Bostrichidae) are important pests of agricultural and forestry products colonizing living plants, lumber, and finished wood products. Seventy three species of bostrichid beetles are present in North America, with an additional 34 exotic species intercepted at ports-of-entry with varying degrees of frequency (Ivie 2002; Haack 2006). Bostrichid beetles have frequently been found on crates, dunnage, and pallets (collectively termed "solid wood packing material") (SWPM) arriving from other countries (Haack 2006). In particular, members of the genus *Sinoxylon* Duftschmid, commonly known as auger beetles, have been transported on SWPM from the Old World tropics to other parts of the world. Between 1985 and 2000, *Sinoxylon* species accounted for 32% of total interceptions of bostrichid beetles, and half of the total number of bostrichid beetle species intercepted in 16 U.S. states (Haack 2006). Of the 50 or so described species of *Sinoxylon*, at least 2 species, *S. unidentatum* (F.) (synonym: *conigerum* Gerstaecker) (Borowski 2007) and *S. ceratoniae* (Linnaeus), are thought to be established in Florida and California, respectively (Peck & Thomas 1998; Ivie 2002).

We report multiple collections of *Sinoxylon*, including *S. anale* Lesne and *S. unidentatum* from various storage facilities and ports-of-entry in Georgia, USA (Fig. 1 A, B). Twenty one adults of *S. anale* (Fig. 1 A) were collected on 15 May 2004 emerging from wooden pallets holding peanuts from India in a storage facility in Albany, Dougherty County, Georgia. In addition, 12 adults of *S. anale* were collected on 22 Jul 2010 from SWPM originating from India and intercepted at a port-of-entry in Fulton County, Georgia. Two adults of *S. unidentatum* (Fig. 1 B) were collected on 3 Oct 1996 emerging from SWPM originating from India in Laurens County, Georgia. All specimens are deposited in the Georgia Museum of Natural History, University of Georgia in Athens.



(A)

(B)

Fig. 1. Lateral views of the adults of *Sinoxylon anale* Lesne (A) and *Sinoxylon unidentatum* (F.) (B). Note the differences in elytral declivity between these 2 species.

Sinoxylon anale, endemic to the Oriental Region, is one of the most commonly found bostrichid species in imported material around the world. This species has been reportedly introduced to Venezuela (Joly et al. 1994), Brazil (Teixeira et al. 2002), Israel (Argaman 1987), Australia (Stanaway et al. 2001), Poland (Sliwa 1971, Skalski 1971), and Ukraine (Gumovsky 2010). In North America, *S. anale* has been intercepted in New York, Detroit, Philadelphia, San Francisco, Miami, Florida, and Columbus (Fisher 1950; Teixeira et al. 2002). *Sinoxylon anale* is one of the most destructive woodboring beetles in India (Fisher 1950), and is quarantined in Hawaii, Brazil, Argentina, Uruguay, and Paraguay. It is

polyphagous, colonizing >70 deciduous woody plant species and a wide variety of products such as lumber, logs, stored wood, and plant seeds (Lesne 1906; Beeson & Bhatia 1937, Sittichaya et al. 2009). In Israel, an infestation by *S. anale* resulted in mortality of the ornamental tree species, *Delonix regia* (Bojer ex Hook.) Raf., which were subsequently burned, but *S. anale* still became established (Argaman 1987). *Sinoxylon unidentatum* is also of oriental origin, polyphagous, and has been introduced to all major continents including North America (Fisher 1950, Filho et al. 2006). Recently, *S. unidentatum* was found infesting wood pallets used to import tea to Italy from Sri Lanka (Savoldelli & Regalin 2009), and it was found for the first time in Colombia in imported furniture from India (cited as S. *conigerum*) (Quiroz-Gamboa & Sepúlveda-Cano 2008).

Our records for *S. anale* and *S. unidentatum* in Georgia indicate that SWPMs are the most common source of these exotic beetles. Similarly, *S. anale* was intercepted in wooden crates of manhole covers from India in Escambia County in Florida (Halbert 1996). We, therefore recommend a greater emphasis be placed on inspecting and treating SWPM originating from the Old World to reduce the introductions of exotic bostrichid beetles. It is unclear whether either of these bostrichid beetle species has become currently established in Georgia. However, our results indicate multiple introductions spanning >14 years and hence, a high potential for establishment of these 2 bostrichid beetle species over time.

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Summary

Two exotic bostrichid beetle species, *Sinoxylon anale* and *S. unidentatum*, were collected on multiple occasions over 14 years from solid wood packing materials (SWPM) originating from India that were either stored in warehouses or intercepted at ports-of-entry in Georgia, USA.

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AN IMPROVED METHOD FOR EXTRACTION AND PURIFICATION OF TERMITE ENDO-β-1,4-GLUCANASE FROM FTA® CARDS

NURMASTINI SUFINA BUJANG[1,*], NIGEL A. HARRISON[2] AND NAN-YAO SU[1]

[1]Department of Entomology and Nematology, Fort Lauderdale Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, 3205 College Avenue, Fort Lauderdale, FL 33314-7719, USA

[2]Department of Plant Pathology, Fort Lauderdale Research and Education Center, University of Florida, Institute of Food and Agricultural Sciences, 3205 College Avenue, Fort Lauderdale, FL 33314-7719, USA

Supplemental material online at http://www.fcla.edu/FlaEnt/fe942.htm#InfoLink2

FTA® technology is an approach designed to expedite and simplify the collection, preservation, storage, shipment, and recovery of nucleic acid from the storage matrix (Whatman Inc. 2002a). FTA® cards have been used and evaluated for DNA archiving of various biological sources (Moscoso et al. 2004; Harvey 2005). However, this method is expensive ($1.14/sample) and preparations can be elaborate, thus pushing researchers to devise alternative and cheaper methods for DNA preservation and storage (Owens & Szalanski 2005). Furthermore, in some cases, DNA retrieval from the FTA® cards is complex and requires highly selective conditions (Hide et al. 2003; Adams et al. 2008). Hence, some researchers have had to modify the protocols to retrieve DNA from the FTA® cards in their studies (Silva et al. 2004; Smith & Burgoyne 2004).

For termites, we have failed to obtain successful amplifications from the nucleic acid processed using any of the manufacturer-recommended procedures. Thus, we have developed a new combination of methods for DNA recovery from FTA® cards.

In our study, the gene of interest was a cellulase of termite origin in the glycoside hydrolase 9 superfamily. These endo-β-1,4-glucanases are produced by the termite itself to digest cellulose. Seven species of termites from the family Termitidae were analyzed. *Nasutitermes corniger* (Motschulsky) was obtained from a laboratory culture collected from Dania Beach, Florida, USA. *Nasutitermes* sp., *Macrotermes gilvus* (Hagen), *M. carbonarius* (Hagen), *Microcerotermes crassus* Snyder, *Microtermes pakistanicus* Ashmead and *Odontotermes* sp. were all fresh specimens collected at Universiti Sains Malaysia, Pulau Pinang, Malaysia.

After collection, termite workers were rinsed with 85% EtOH to remove any surface debris and then allowed to air-dry for several minutes. Nucleic acid from the head capsules of 10 termite workers was released and preserved on the FTA® Plantsaver Card (Whatman Inc., Newton, MA) according to the manufacturer's protocol. Only termite heads were used to avoid sample contamination by DNA of resident gut microorganisms.

Using a sterile blade and forceps to prevent contamination, we excised a 1-cm^2 piece containing dried nucleic acid extract from the FTA® card sample area and placed it in a 1.5-mL microcentrifuge tube containing 300 µL TE (10 mM Tris-HCl, 1 mM EDTA, pH 8) as elution buffer. The tube was then vortexed for 20 s before storage in a refrigerator at 4°C for 1 h. Each hydrated strip of FTA® card was squeezed with sterile forceps to release as much nucleic acid as possible into the elution buffer before the strip was discarded. The eluate was then purified with the Wizard® DNA Clean-Up System (Promega Corp., Madison, WI) following the manufacturer's procedure. As a negative control, a similar strip of FTA® card containing no sample was processed in the same manner to ascertain that the FTA® card alone did not yield positive results.

PCR was performed with specifically-designed endo-β-1,4-glucanase primers NTf3 (Tokuda et al. 1999) and NTr7 (Tokuda et al. 2004). Amplifications were conducted in 50 µL final reaction volumes, each containing standard PCR Buffer with 1.5 mM $MgCl_2$ (Innis & Gelfand 1990), 2 µL DNA template, 50 ng of each primer, 125 µM of each dNTP and 1 U Econo*Taq* DNA polymerase (Lucigen Corp., Middleton, WI). Apart from the standard positive control DNA (NC-Q) extracted with DNeasy® Blood & Tissue Kit, an additional positive control (1 µL NC-Q + 1 µL FTA® negative control) was performed to dismiss the possibility of inhibition solely by FTA® cards. The primers used for PCR are available as supplemental information. The temperature profile for the first cycle was 94°C for 2 min, 52°C for 2 min, and 72°C for 3 min. For the remaining 44 cycles, the temperature profile was 94°C for 1 min, 52°C for 2 min, and 72°C for 3 min.

The endo-β-1,4-glucanase form 7 species of higher termites was successfully amplified from the DNA retrieved from the FTA® card, as determined by electrophoresis of PCR products through 1% agarose gels and visualization of products by

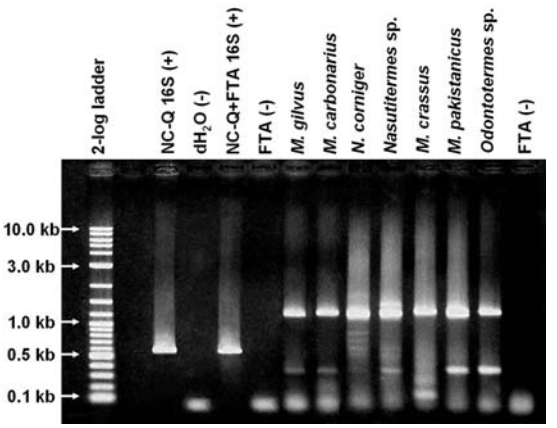

Fig. 1. Visualization of endo-β-1,4-glucanase amplification from 7 termitid species with 1% agarose gel electrophoresis of DNA retrieved by our recovery method.

UV transillumination after staining with ethidium bromide (Fig. 1). Aliquots (1 μL) of purified PCR products were quantified by comparison with serial dilutions of uncut lambda DNA (Promega) in 1% agarose gels (Fig. 2) and sequenced.

Our DNA retrieval method offers a few advantages over the processing method recommended by the manufacturer. For example, the latter's standard process necessitates the use of FTA® Purification Reagent (Whatman Inc., Newton, MA; $232.18), TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8) and Harris Micro Punches ($312.88) (or Harris Uni-Core Punches; $95.84). Our processing requires none of these, only the Wizard® DNA Clean-Up System ($1.38/prep) instead. The genomic DNA elution by a room temperature pH method (Whatman Inc. 2002b) is similar in concept to our method, but requires previous washing with the FTA® Purification Reagent, and the added use of 2 different solutions; namely 0.1 N NaOH, 0.3 mM EDTA (pH 13), and 0.1 M Tris-HCl (pH 7). In addition, sample disc size is a critical factor because insufficient DNA affects amplification while excessive DNA may cause inhibition. However, despite these considerations, neither the 1.2-mm, 2.0-mm nor the 3.0-mm diameter discs with the manufacturer's method has ever produced successful amplification of endo-β-1,4-glucanase in our experience.

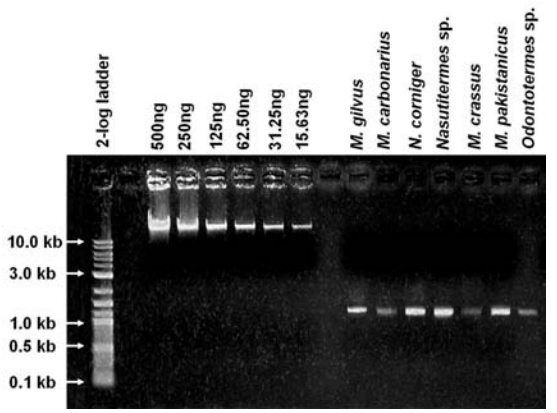

Fig. 2. Yield comparison of aliquots (1 μL) of endo-β-1,4-glucanase sequences amplified by PCR from DNAs of 7 termitid species retrieved from FTA® cards by our recovery protocol.

Summary

In conclusion, we report an improved method for extraction and purification of termite nucleic acid from the FTA® Plantsaver Card (Whatman), which involves elution with TE buffer (pH 8) and purification with Wizard® DNA Clean-Up System (Promega). Our DNA recovery protocol requires less material, equipment, preparation, and manipulation, and reduces processing time, costs and chances for contamination compared with the manufacturer-recommended FTA® purification protocol. Most important, in relation to our study, it has been consistently effective in obtaining termite nucleic acid permitting further DNA analysis when all other methods have failed.

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WHATMAN, INC. 2002a. FTA® protocols: collect, transport, archive and access nucleic acids - all at room temperature; WB120047. Copyright Whatman Inc.

WHATMAN, INC. 2002b. Whatman Application Note: Eluting Genomic DNA from FTA® Cards Using Room Temperature pH Treatment. Copyright Whatman Inc.

Table 1: Primers used in the study by NURMASTINI SUFINA BUJANG, NIGEL A. HARRISON AND NAN-YAO SU entitled AN IMPROVED METHOD FOR EXTRACTION AND PURIFICATION OF TERMITE ENDO-β-1,4-GLUCANASE FROM FTA® CARDS. Florida Entomol. Vol 94, No. 2.

Primer name	Direction	Gene	Sequence (5' to 3')	References
16br	Forward	16S	CGCCTGTTTAACAAAAACAT	
16ar	Reverse	16S	CCGGTCTGAACTCAGATCACGT	
NTf3	Forward	EG	GGCCGGCGAAACAGCCGCCGCCCTCGCTG	Tokuda et al. 1999
NTr7	Reverse	EG	GGCCAGTAGAACCTGTACACCGG	Tokuda et al. 2004

TOKUDA, G., LO, N., WATANABE, H., SLAYTOR, M., MATSUMOTO, T., AND NODA, H. 1999. Metazoan cellulase genes from termites: intron/exon structures and sites of expression. Biochim. Biophys. Acta. 1447: 146-159.

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FLIGHT ACTIVITY OF STINK BUG (HEMIPTERA: PENTATOMIDAE) PESTS OF FLORIDA RICE

RON CHERRY AND ALVIN WILSON
University of Florida, Everglades Research and Education Center, 3200 East Palm Beach Road, Belle Glade, FL 33430

Although many different insects can be found in rice fields in Florida, stink bugs are currently considered the most important pest. Jones & Cherry (1986) reported that the rice stink bug, *Oebalus pugnax* (F.), was the dominant species comprising >95% of the total stink bug population. Cherry et al. (1998) reported that the stink bug, *Oebalus ypsilongriseus* (DeGeer) was widespread in Florida rice fields. This was the first report of this species being found in commercial rice fields in the United States. Cherry and Nuessly (2010) reported that the stink bug, *Oebalus insularis* (Stal) is now widespread in Florida rice fields. This was the first report of this species being found in commercial rice fields in the United States.

Two other stink bug species which attack Florida rice are *Euschistus ictericus* (L.) and the southern green stink bug, *Nezara viridula* (L.) (Genung et al. 1979). Previous to this study, these 5 stink bug species were all observed by the author in light trap samples taken at the Everglades Research and Education Center, Palm Beach County, Florida. This center is located near the center of the rice producing area in southern Florida. The center itself consists of a mosaic of rice, sugarcane, vegetable, turf, and biofuel plots. Nothing is known on the seasonal flight of these 5 rice pests in southern Florida. Hence, the objective of this study was to determine the seasonal flight activity of these 5 stink bug species which attack Florida rice.

Flight activity of stink bugs was measured with a large, walk-in black light trap. This trap measured 2 m × 2 m × 2.5 m high and was made of wood with screened sides. On the top was a 15 watt black light with a funnel through which insects fell into the trap below. The trap was located on the Everglades Research and Education Center at Belle Glade, Florida in an area composed of mixed vegetation (various crops, grasses, weeds, trees). The trap was used twice weekly at 3-4-d intervals from Jan 1, 2008 to Jan 1, 2010. After collection by vacuuming, stink bugs were frozen for later taxonomic identification and sex determination. Samples from within each month for both years were pooled. Thereafter a Least Significant Difference Test (SAS 2010) was conducted to compare mean monthly catches for each stink bug species. Fifty adults of each species were randomly selected from samples and dissected to determine sex ratios. These ratios were tested by Chi-square analysis for each species to determine if the ratios were significantly different from an expected 1 to 1 sex ratio.

Adults of both sexes were caught in light trap samples in all 5 species. Chi-square analysis showed that there was no significant deviation from the expected 1 to 1 sex ratio in any of the 5 species. The largest Chi-square value found was 1.62 among the species, this not being significant at alpha = 0.05 (1 *df*).

Monthly catches of stink bugs in the black light are shown in Table 1. Flight activity of all 5 species was remarkably in synchrony, being unimodal with greatest catches in Jul in all spe-

TABLE 1 RICE STINK BUGS CAUGHT[1] IN A BLACKLIGHT TRAP TWICE WEEKLY FROM 1 JAN 2008 THROUGH 1 JAN 2010.

			Oebalus		
Month	*E. ictericus*	*N. viridula*	*insularis*	*pugnax*	*ypsilongriseus*
Jan	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b	0 ± 0 b
Feb	0 ± 0 b	0.2 ± 0.6 c	0 ± 0 c	0.7 ± 2.5 b	0 ± 0 b
Mar	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b	0 ± 0 b
Apr	0 ± 0 b	0 ± 0 c	0 ± 0 c	0 ± 0 b	0 ± 0 b
May	0 ± 0 b	0.8 ± 1.1 c	0 ± 0 c	1.9 ± 5.3 b	0 ± 0 b
Jun	0.6 ± 1.2 b	4.9 ± 7.3 c	0.2 ± 0.8 c	4.2 ± 9.0 b	0 ± 0 b
Jul	16.7 ± 14.5 a	22.3 ± 16.3 a	8.6 ± 9.3 a	233.0 ± 346.9 a	2.3 ± 5.2 a
Aug	3.4 ± 6.0 b	12.5 ± 25.7 b	3.8 ± 6.6 b	15.9 ± 26.5 b	1.9 ± 4.0 a
Sep	0.6 ± 1.2 b	2.3 ± 3.0 c	0.7 ± 1.6 c	3.4 ± 8.5 b	0 ± 0 b
Oct	0.1 ± 0.3 b	1.9 ± 3.2 c	0 ± 0 c	0.1 ± 0.4 b	0.3 ± 0.8 b
Nov	0 ± 0 b	0.7 ± 1.1 c	0 ± 0 c	0 ± 0 b	0 ± 0 b
Dec	0 ± 0 b	0.6 ± 1.1 c	0 ± 0 c	0.1 ± 0.4 b	0 ± 0 b

[1]Means ± SD. Means in a column followed by the same letter are not significantly different (alpha = 0.05) using the Least Significant Difference Test (SAS 2010).

cies. Reasons for this flight activity are not known. It is difficult to associate this flight activity with a particular food source. Any association is prevented by the polyphagous feeding habits of the stink bugs which result in a broad range of host plants. Numerous crops such as rice, sorghum, wheat, barley, oats, rye, corn, and soybean (McPherson & McPherson 2000) and numerous weeds (Cherry & Bennett 2005) are known to be hosts for 1 or more of the 5 stink bug species. However, it should be noted that this flight activity is in synchrony with the time of greatest rice heading (grain filling) in Florida. This heading occurs during Jul and Aug in the main crop (first crop) of rice and all 5 species attack rice near or at rice heading (Genung et al. 1979; McPherson & McPherson 2000). Although there is rice heading later in the 1 ratoon crop in Florida, not all growers keep their rice in production to grow this crop and heading in ratoon rice is more variable within the crop itself.

Timing of planting date has shown positive results in keeping stink bugs away from crops at their most vulnerable time (McPherson & McPherson 2000). Litsinger (1994) gives examples of planting dates being used to reduce insect damage of numerous rice pests including stink bugs. Our data suggest that planting date could be used to move rice heading from this period of greatest flight activity, thus reducing stink bug populations in the rice. However, moving rice planting dates may pose other problems for rice growers. Planting rice too early may result in frost damage, and rice in Florida may alternate with other crops such as corn and sugarcane grown at different times on the same land. Hence, rice planting and harvesting are frequently timed in conjunction with these other crops. Our data suggest that planting date may be used to avoid stink bug damage in rice, but movement of planting date is also contingent upon other grower considerations. Last, thanks are given to Dr. Joe Eger for help in taxonomy.

Summary

A 2-year light trap study was conducted to determine the seasonal flight activity of 5 species of stink bugs which attack Florida rice. Flight activity of all 5 species was remarkably in synchrony, being unimodal with greatest catches in Jul in all the species. These data suggest that planting date can be used to reduce stink bug damage in Florida rice.

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FIRST RECORDS OF BOTH SUBSPECIES OF *BRACHIACANTHA QUADRIPUNCTATA* (COLEOPTERA: COCCINELLIDAE) IN MISSISSIPPI, U.S.A.

LOUIS S. HESLER[1] AND PAUL K. LAGO[2]
[1]USDA-ARS, North Central Agricultural Research Laboratory, 2923 Medary Ave., Brookings, SD 57006

[2]Department of Biology, University of Mississippi, University, MS 38677

Supplemental material online at http://www.fcla.edu/FlaEnt/fe942.htm#InfoLink3

Brachiacantha Dejean 1837 (Coleoptera: Coccinellidae: Hyperaspini) is a New World genus with approximately 50 species and subspecies distributed from Canada to Argentina (Leng 1911; Gordon 1985). *Brachiacantha quadripunctata* (Melsheimer) is distributed over the eastern United States with 2 allopatric subspecies that have distinct elytral color patterns (Leng 1911; Gordon 1985). The elytron of *B. quadripunctata quadripunctata* (Melsheimer) has a basal spot and an apical spot in females, and an additional humeral spot that is often confluent with the basal spot in males (Fig. 1). The elytron of *B. quadripunctata flavifrons* Mulsant has 1 marginal spot in addition to the basal and apical spots (Fig. 2). *Brachiacantha quadripunctata quadripunctata* has a northern distribution from Kansas to Massachusetts, and south into Arkansas and Tennessee, whereas *B. quadripunctata flavifrons* is distributed in the southeastern U.S. from North Carolina to northern Florida and westward to southern Alabama (Gordon 1985). Neither subspecies has been recorded from Mississippi (Gordon 1985). Leng (1911) stated that the distribution of *B. quadripunctata* includes Mississippi and Louisiana, but he provided no records of this species for those states. However, in reviewing curated beetles, we discovered specimens of both *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons* from Mississippi, and here report their first records from the state.

New records of adult beetles were obtained from specimens in insect collections at the University of Mississippi (UMIC) and Mississippi Entomological Museum (MEM), Mississippi State University. We failed to find any *B. quadripunctata quadripunctata* or *B. quadripunctata flavifrons* from Mississippi in the collection database

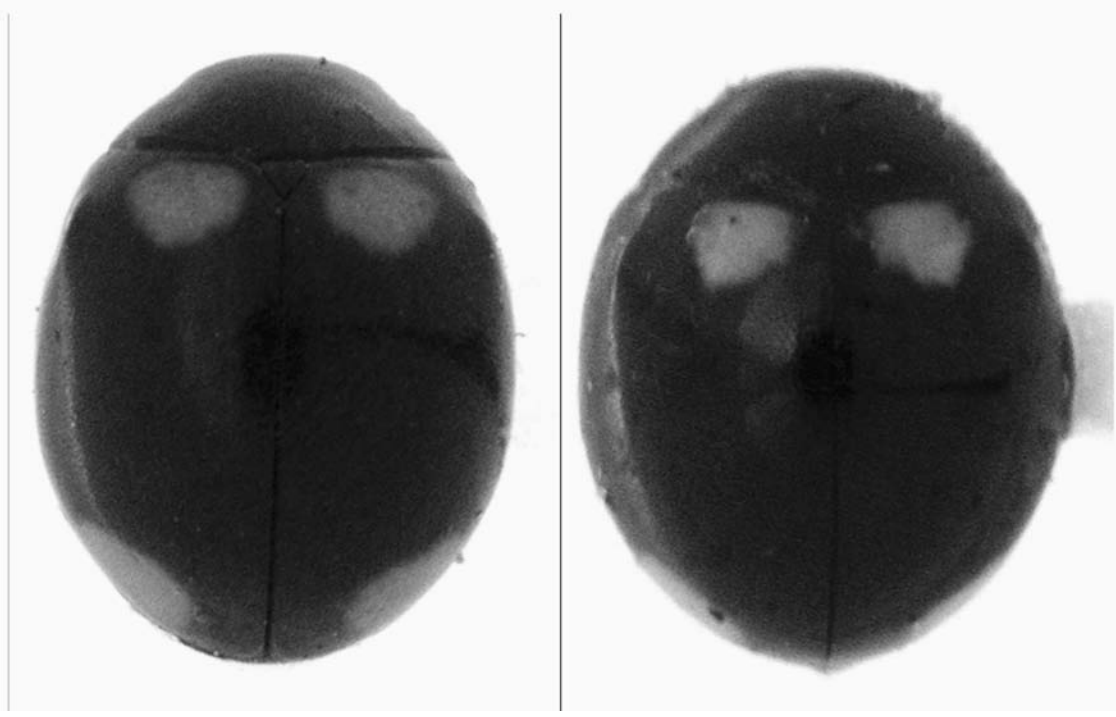

Fig. 1. Habitus views of a female (left) and a male *Brachiacantha quadripunctata quadripunctata*.

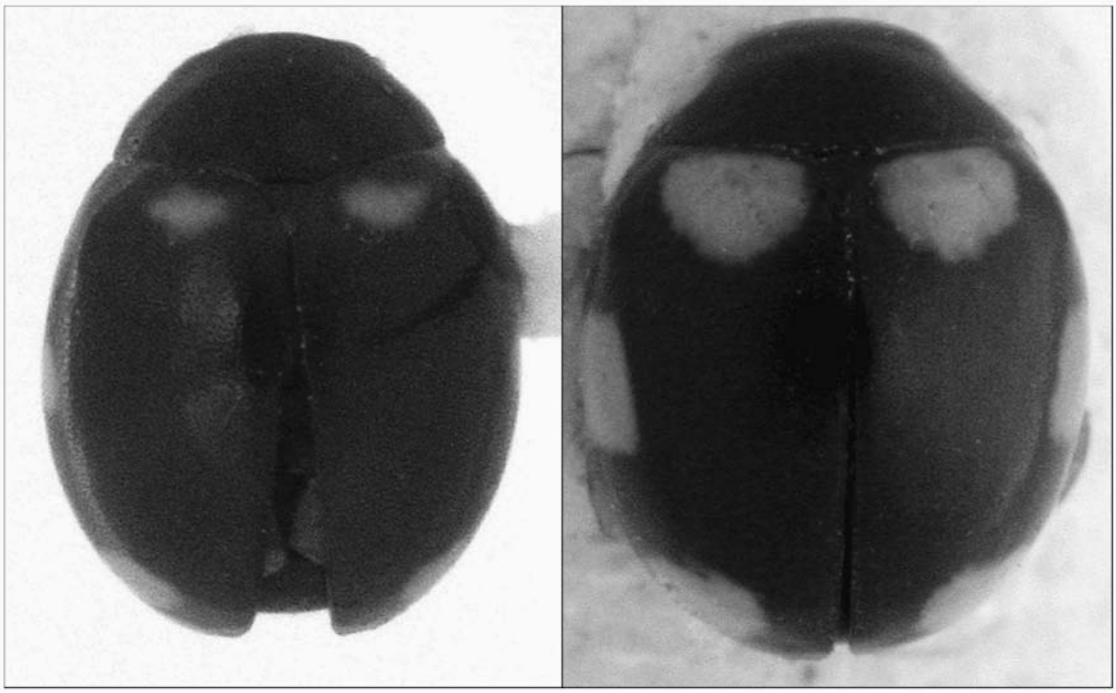

Fig. 2. Habitus views of a female (left) and a male *Brachiacantha quadripunctata flavifrons*.

at Louisiana State University (http://collection.silverbiology.com/lsam/collection/). In all, the following 15 specimens were recorded.

Brachiacantha quadripunctata quadripunctata. MEM: Choctaw County, 9 mi. NE Ackerman, 25-VI-1971, J. E. Leggett; Oktibbeha County, 4.3 SW Starkville, 9-III-1976, W. H. Cross; Winston County, near Noxapater, 1-III-1977, W. H. Cross; Oktibbeha County, Craig Springs, 5-III-1982, W. H. Cross; Oktibbeha County, Adaton, 15-III-1982, W. H. Cross; Noxubee County, Noxubee Wild. Ref., 8-III-1986, A. Tonhasca; Choctaw County, Jeff Busby Park, 4-IX-1992, R. L. Brown [2 adults]; Oktibbeha County, Starkville, Dorman Lake, 25-IV-1994, D. M. Pollock; Oktibbeha County, Starkville, Dorman Lake, 12-V-1995, D. M. Pollock; Winston County, Tombigbee National Forest, 33°0'20"N, 89°03'55"W, 10-V-1999, T. L. Schiefer; Choctaw County, Natchez Trace, mi. 199.4, 34°29'04"N, 89°11'52"W, 18-III-2004, T. L. Schiefer; Itawamba County, Natchez Trace, mi. 280.8, 34°26'41"N, 88°30'46"W, 13-III-2008, T. L. Schiefer.

UMIC: Grenada County, 6 mi. S. Grenada, 20-III-1992, M. S. Caterino.

Brachiacantha quadripunctata flavifrons. MEM: Choctaw County, 9 mi. NE Ackerman, 17-VI-1971, J. E. Leggett; Choctaw County, 9 mi. NE Ackerman, 25-VI-1971, J. E. Leggett.

Our discovery of specimens of *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons* from Mississippi establishes state records of these subspecies, and these records extend their known geographic distributions about 150 km, respectively, southward and westward. Moreover, their co-occurrence in Choctaw County in Jun 1971 shows that these subspecies are sympatric and synchronic in this part of their range, and contrast with previous collections records that have shown that *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons* are allopatric (Gordon 1985).

The presence of both subspecies of *B. quadripunctata* suggests that each elytral color phenotype is generally favored in Mississippi. However, additional studies are needed to determine whether distinct phenotypes are maintained possibly under genetic introgression between the 2 subspecies, or if phenotypes are maintained because introgression is limited or perhaps absent. Other studies are needed to survey for the 2 subspecies in other areas in which their ranges could potentially overlap (i.e., northern Alabama, northern Georgia, and eastern Tennessee).

Little is known about the bionomics of *Brachiacantha* species and factors that determine their abundance (Gordon 1985; Majka & Robinson 2009). For instance, there is only very limited information regarding the larvae of *Brachiacantha*, but in the few known instances they have been associated with root-feeding coccids and aphids found in ant nests (Leng 1911; Gordon 1985).

Wheeler (1911) found larvae of *B. quadripunctata quadripunctata* in nests of the ant, *Lasius flavus* (F.) (as *Lasius umbratus* var. *aphidicola*), provisioned with root coccids and root aphids, but we are unaware of any reports for *B. quadripunctata flavifrons*. Similarly, published accounts on the ecology of adult *Brachiacantha* are generally lacking (Acorn 2007; Majka & Robinson 2009). Thus, additional studies are needed to understand factors that may allow the subspecies of *B. quadripunctata* to overlap in distribution in Mississippi but drive allopatry between them in other areas.

Faunal lists may increase from additional collecting, curation and examination of previously collected material, and geographic range expansion of species (Fauske et al. 2003; McCorquodale & Bondrup-Nielsen 2004). In the present study, discovery of specimens of *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons* dating to 1971 came from the examination of previously collected material. This discovery reinforces the argument that ongoing curation and periodic review of collections is important in maintaining accurate regional lists of species for comparisons across geographic regions, and for developing hypotheses about changes in faunal distributions over time (Brodman et al. 2002; McCorquodale & Bondrup-Nielsen 2004; Hesler & Kieckhefer 2008).

Summary

The first records of the lady beetles *Brachiacantha quadripunctata quadripunctata* (Melsheimer) and *B. quadripunctata flavifrons* Mulsant from Mississippi and their occurrence in sympatry are reported following a review of previously collected material. The new records also extend the known geographic distribution of *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons* about 150 km, respectively, southward and westward. Our findings support the argument that ongoing curation and periodic review of collections is critical for maintaining accurate regional faunal lists, in developing hypotheses about the geographic distributions of species, and to track changes in both through time.

Acknowledgments

Matthew Brust, Kent Fothergill, and Lauren Hesler reviewed drafts of this paper. Terry Schiefer loaned specimens from the Mississippi Entomological Museum, Mississippi State University. Terry Molengraaf photographed specimens of *B. quadripunctata quadripunctata* and *B. quadripunctata flavifrons*. This work was supported in part by a grant from the National Science Foundation to L.S.H.

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Supplemental Material

Louis S. Hesler and Paul K. Lago 2011. First Records of both Subspecies of Brachiacantha Quadripunctata (Coleoptera: Coccinellidae) in Mississippi, U.S.A. Florida Entomol. 94(2): 361-363.

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Fig. 1. Habitus views of a female (left) and a male *Brachiacantha quadripunctata quadripunctata*.

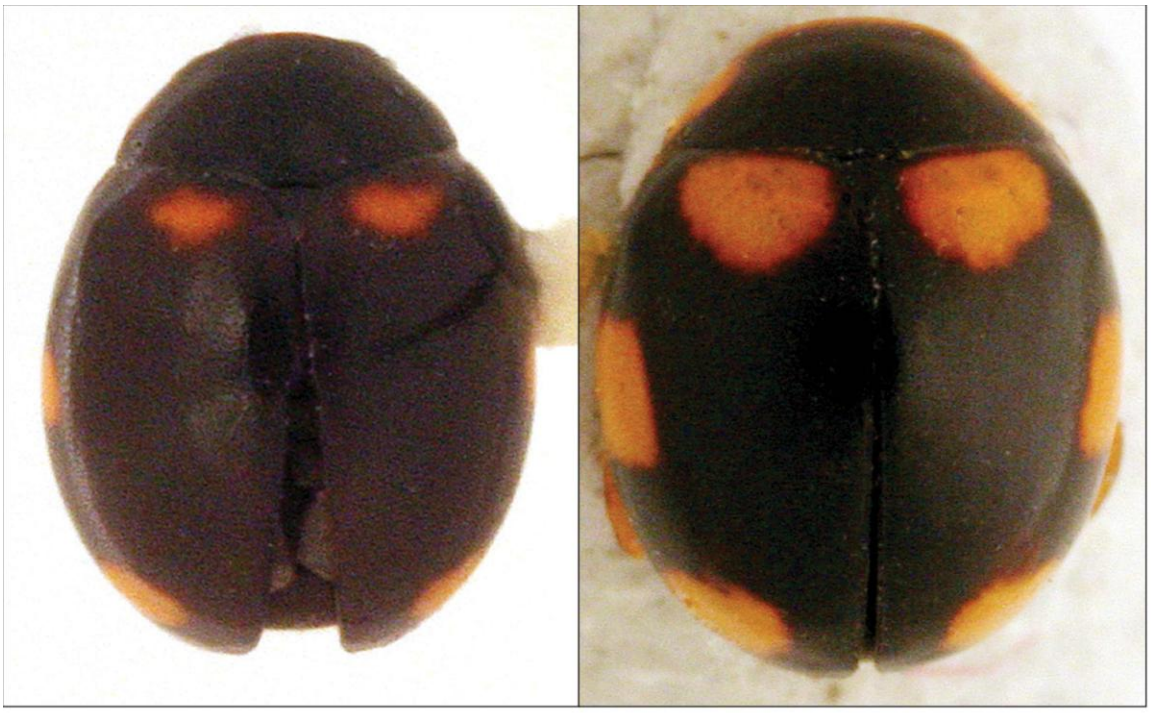

Fig. 2. Habitus views of a female (left) and a male *Brachiacantha quadripunctata flavifrons*.

NEW RECORDS ON THE GEOGRAPHICAL DISTRIBUTION OF SOUTH AMERICAN SHARPSHOOTERS (CICADELLIDAE: CICADELLINAE: PROCONIINI) AND THEIR POTENTIAL AS VECTORS OF *XYLELLA FASTIDIOSA*

GIMENA DELLAPÉ[1], GUILLERMO A. LOGARZO[2], EDUARDO G. VIRLA[3] AND SUSANA L. PARADELL[1]
[1]División Entomología. Facultad de Ciencias Naturales y Museo, UNLP, Paseo del Bosque s/n[o] (B1900FWA), La Plata, Buenos Aires, Argentina
E-mail: gimenadellape@fcnym.unlp.edu.ar

[2]USDA-ARS South American Biological Control Laboratory, Bolivar 1559 (1686), Hurlingham, Buenos Aires, Argentina

[3]PROIMI-Biotecnología, División de Control Biológico, Av. Belgrano y Pje Caseros, (4000), San Miguel de Tucumán, Tucumán, Argentina

The Proconiini comprises 422 species distributed in the continental Americas, the highest biodiversity is found in the Neotropical Region (Wilson et al. 2009). Members of the tribe Proconiini have been identified as vectors of many diseases caused by the bacteria *Xylella fastidiosa* Wells et al. 1978, which occurs only in the xylem of plants (Marucci et al. 2002).

Strains of *X. fastidiosa* cause diseases such as "Pierce's Disease" (PD) in grape (*Vitis vinífera* L.), "Phony Peach Disease" (PPD), "Coffee Leaf Scorch" (CLS), "Oleander Leaf Scorch" (OLS), and "Citrus Variegated Chlorosis" (CVC) among others. These incurable maladies produce substantial economic losses in a diverse variety of crops (Hernandez-Martinez et al. 2006).

In South America the major threat is CVC which has spread rapidly throughout Brazil (Lopes 1996). *X. fastidiosa* is also present in United States, México, Venezuela, Brazil, Paraguay, Uruguay, Argentina (Redak et al. 2004), and Costa Rica (Aguilar et al. 2005). However, CVC is not yet reported from the USA although it has the potential to threaten orange (*Citrus* × *sinensis* (L.) Osbeck) production in the Americas if a suitable vector is available (Damsteegt et al. 2006).

Diseases caused by *X. fastidiosa* have attained great importance worldwide as insect vectors of this pathogen have demonstrated an ability to spread, as happened with *Homalodisca vitripennis* (Germar), which invaded many islands in the Pacific Ocean (Pilkington et al. 2005). Pathogen acquisition and transmission by sharpshooters occurs because these insects feed exclusively on xylem fluids (Young 1968).

Despite this obvious importance, there are few studies from South America that have identified Proconiini species that can transmit *X. fastidiosa*. Moreover, there is no basic information on biology, geographic distributions, phenology, natural enemies or host plant associations for many South American Proconiini species. To address this shortcoming, work presented here provides new distributional records for thirteen South American Proconiini sharpshooters that may be potential vectors of *X. fastidiosa*.

The examined material is deposited in the following entomological collections of Argentina: Instituto Miguel Lillo (IMLA); Museo de Ciencias Naturales de La Plata (MLP) and Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (MACN). Specific identification and distributional data were compiled from Young (1968), Marucci et al. (2002) and Wilson et al. (2009).

Examined Material

Acrogonia citrina Marucci & Cavichioli, PARAGUAY: Carumbé, III-1965, Golbach Leg., 1♂, 1♀, without information on host plant and collecting method (IMLA).

Acrogonia flavoscutellata (Signoret), ECUADOR: Santo Domingo, IV-1958, Weyrauch Leg., 1♀, without information on host plant and collecting method (IMLA).

Dechacona missionum (Berg), URUGUAY: 1♂, without date, no information on host plant and collecting method (MACN).

Diestostemma huallagana Young, BOLIVIA: 1♂ without date and locality (MACN).

Molomea consolida Schröder, ECUADOR: El Puyo, IV-1958, 1♂. PERÚ: Tingo María, 1♂; Chanchamayo, II-1939, 1♂ (IMLA). BOLIVIA: Santa Cruz, I-1958, Wygodzinsky Leg.; 1♂ (IMLA), 1♀ (MACN), without information on host plant and collecting method.

Molomea personata (Signoret), PERÚ: Fundo Génova, IV-2002, Logarzo-Varone Legs., 8♀♀, on papaw; V-2002, Logarzo Leg., 2♀♀, on grasses, by sweeping (IMLA).

Ochrostacta diadema (Burmeister), PARAGUAY: Itagua, I-1957, Montes Leg., 3♀♀, without information on host plant and collecting method (MLP).

Oncometopia rubescens Fowler, PERÚ: Fundo Génova, IV-2002, Logarzo Leg., 14♂♂, 16♀♀, on grasses and papaw; V-2002, Logarzo Leg., 16♂♂,

4♀♀, by sweeping (IMLA). PARAGUAY: Carumbé, San Pedro, I-1971, Golbach Leg., 1♀, without collecting method (IMLA).

Proconia fusca Melichar, BOLIVIA: 9♂♂, 4♀♀, without information on host plant and collecting method (MACN).

Tapajosa doeringi (Berg), PERÚ: Cuzco, Machu Picchu, II-1952, Monrós Leg., 1♂, without information on host plant and collecting method (IMLA).

Tapajosa rubromarginata (Signoret), PARAGUAY: Caaguazú, XII-2000, Logarzo Leg., 1♂, on weeds (MLP).

Tapajosa similis (Melichar), BRAZIL: I-1948, Cuezzo Leg., 1♂, without information on host plant and collecting method (IMLA).

Tretogonia callifera Melichar, PARAGUAY: Carumbé, 3♂♂, 2♀♀; Caaguazú, I-1965, 2♂♂, 3♀♀, without information on host plant and collecting method (IMLA).

In Table 1, we summarize the species recorded for Central and South America, the presence of *X. fastidiosa* and the new distribution data for the species listed here. Sharpshooters are known to occur in 24 of the 37 Central and S. America countries. No data are available for some islands associated with Central America and the Caribbean. This lack of information about the Proconiini in these countries is probably due to a deficiency in surveys and collections and not because of the absence of representatives of these insects in those territories.

Most studies investigating the transmission of *Xylella* have been conducted in the USA. In the Neotropics, the majority of studies have been made in Brazil (Redak et al. 2004). Some South America countries, such as Perú, Bolivia, Colombia, and Ecuador have more than 50 sharpshooter species capable of vectoring *X. fastidiosa*, but no reference to occurrence of this bacterium in those countries is available.

Most South American countries are at risk from *X. fastidiosa* because the bacterium has a wide host range and may be transported accidently to new areas via infected plant species. There are strong epidemiological relationships between the presence of Proconiini sharpshooters and incidence of the bacterium. Resulting diseases can take months or years to develop significant symptoms, or infections may remain asymptomatic and undetected while acting as reservoirs from which continued bacterial transmission can occur (Hopkins 1989).

We thank the museum curators who provided access to the specimens used from their entomological collections.

TABLE 1. KNOWN AND NEW DISTRIBUTION RECORDS FOR 13 SPECIES OF PROCONIINI COLLECTED IN CENTRAL AND SOUTH AMERICA.

	Acrogonia citrina	*Acrogonia flavoscutellata*	*Dechacona missionum*	*Diestostemma huallagana*	*Molomea consolida*	*Molomea personata*	*Ochrostacta diadema*	*Oncometopia rubescens*	*Proconia fusca*	*Tapajosa doeringi*	*Tapajosa rubromarginata*	*Tapajosa similis*	*Tretogonia callifera*	*Presence of X. fastidiosa*	*Total specimens*
Argentina			*		*		*			*	*	*		*	38
Bolivia			*	X	X				X				*		58
Brazil	*	*	*	*	*	*	*	*			*	X	*	*	142
British Guiana		*													18
Colombia		*						*					*		65
Costa Rica								*						*	49
Ecuador		X			X			*							68
El Salvador		*													12
French Guiana		*											*		25
México														*	80
Panama		*						*							30
Paraguay	X		*		*		X	X			X		X	*	24
Perú			*	*	X	X		X	*	X			*		95
Suriname													*		14
Uruguay			X				*								8
Venezuela		*						*						*	47

*Data from Young 1968, Takiya 2008, and Wilson et al. 2009.
X—Present work.

SUMMARY

Xylella fastidiosa is endemic to the Americas, it causes economically important diseases in a variety of different crops, and is transmitted by xylem-feeding sharpshooters. This paper provides new geographic records for Proconiini sharpshooters in South America which helps to better understand their distribution. To develop these new records, we examined material from 3 of the main entomological collections held in Argentina. As a result, 5 species are cited for the first time from Paraguay; 4 for Perú; 3 for Bolivia; 2 for Ecuador; and 1 each for Uruguay and Brazil. Some of the species could be vectors of *X. fastidiosa* because congeners of the species studied here are known to transmit this bacterium.

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FASTER THAN A FLASH: THE FASTEST VISUAL STARTLE REFLEX RESPONSE IS FOUND IN A LONG-LEGGED FLY, *CONDYLOSTYLUS* SP. (DOLICHOPODIDAE)

ANDREI SOURAKOV
McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, University of Florida, Gainesville, FL 32611
E-mail: asourakov@flmnh.ufl.edu

Supplemental material online at http://www.fcla.edu/FlaEnt/fe942.htm#InfoLink4

Recently, fast reflex responses of skipper butterflies (Hesperiidae) to the photographic flash were reported and were found to be among the fastest ever recorded (<17 ms)—comparable to the fastest reflexes of the vertebrates (Sourakov 2009). Using a similar photographic technique, but a faster and more precise camera, even faster response times were found in *Condylostylus* flies (Diptera: Dolichopodidae). This new record reported here undoubtedly constitutes the fastest reflex response of a member of the animal kingdom ever recorded.

The observations were made in the Natural Teaching Laboratory, a forested area on the University of Florida campus, Gainesville, Florida. A *Condylostylus* sp. was photographed with a Canon EOS camera the built-in flash at a shutter speed of 1/200 s = 5 ms. Photographs in Fig. 1 were taken with an interval of ca. 20 s within a 4-min period. The fly, startled by the flash, was able to take flight 9 out of 10 times before the image was taken. As a result, the fly's image was repeatedly captured in flight (Fig. 1). The fly's visual startle reflex caused by the flash had a latent period of less than 5 ms and, perhaps, only 2 ms. Some habituation can occur as evidenced when the camera was fired consecutively 4 times: the fly did not react to the fourth flash and hence the fly remained in a resting position (Fig. 1D). However, the fly quickly recovered and continued reacting to the flash 20 s later. No other species of insects photographed during the same time in the same location with the same equipment, have exhibited similar behavior, which leads to conclusion that this extraordinary reaction time is particular to the long-legged flies.

While earlier studies considered startle reflexes to be particular to mammals, Hoy (1989) stated that "all behaviors that have survival value are likely to be found in all animals facing similar problems." For many insects, a quick escape by crawling or flying is the primary mode of defense. When moths react to the ultrasound produced by bats (Order Chiroptera), this evading behavior can be classified as an acoustic startle response (Hoy et al. 1989). The response latencies in noctuid moths (*Feltia* spp., *Leucania* spp., *Amathes normaniana* Grote, *Agrotis ypsilon* Rottemberg, *Ochropleura plecta* L. and *Euxoa obeliscoides* Gueneé) are very short, on the order of tens of milliseconds (Roeder 1967). Mechano-receptive hairs on tail appendages of a cockroach, *Periplaneta americana* L., detect the change in air pressure caused by a fast approaching object, and can trigger an escape response in less than 50 ms (Camhi & Tom 1978). House flies, *Musca domestica* L., have a similar reaction time of 30-50 ms to a visual threat (Holmqvist 1994). The startle reflex of *Condylostylus* fly most certainly constitutes the fastest in insects, as it is 3-10 times faster than the previously reported reflex response times.

Considering a great variety of insect species (estimates range between 2 and 30 million species) it is not surprising that the fastest escape response time should be found in that particular group of animals. However, many insects rely on camouflage, living in shelters, thick exoskeleton with sharp spines, venomous bites, and toxic substances for defense. Defense by fast escape response can be costly to maintain, as it assumes a very high metabolic rate as well as constant alertness, which for a cold-blooded animal can be problematic. Skipper butterflies and dolychopodid flies—as different as they are—both have a habit of perching openly on the upper surfaces of leaves in sunny areas of forests. This allows them to attract mates and repel competitors by territorial behavior, but it also makes them obvious prey for birds.

Capturing these insects can be very difficult, whether for the entomologist with a net or for a bird, because of their rapid escape responses. The *Condylostylus* flies and some of the very fast species of skipper butterflies possess bright metallic colors. Why would animals that are not chemically defended advertise themselves so openly to potential predators? The explanation may lie (as suggested by Daniel Janzen, pers. comm.) in the ability to escape repeatedly from predators, and thereby instill in the memories of predators the learned reflex of avoiding this particular prey. Just like yellow-and-black coloration signals the poisonous nature of their certain prey to birds, the bright metallic colors probably became a sign of fast escape response. In nature, these energy-saving signals are beneficial to both prey and predators; and hence these related

Fig. 1. The response to a visual startle reflex caused by a photographic flash exhibited by a long-legged fly, *Condylostylus* (Dolichopodidae). All photographs except (D) show the fly jumping in the air when the flash was fired with 20 second intervals. (D) shows that habituation had occurred on the fourth firing of the flash, but the reflex response resumed 20 seconds later. Reflex response time is <5 milliseconds. Photos by A. Sourakov.

behaviors tend to co-evolve in a number of species. The fact that the bright coloration occurs in a variety of fast-flying insects supports this hypothesis. Metallic, vibrant colors, similar to those of dolichopodid flies, are also found in other fast-flying insects, such as hairstreak butterflies (Lepidoptera: Lycaenidae: Theclinae), orchid bees (Hymenoptera: Apidae: Euglossinae), cuckoo wasps (Hymenoptera: Chrysididae), and some fast flying skippers (e.g., *Astraptes* (Lepidoptera: Hesperiidae)). Other metallically colored insects with fast escape responses include leaf beetles (Coleoptera: Chrysomelidae), which do not have fast flight, but have either fast hopping escape responses or fast "play-dead" responses. It would be very interesting to test if a mimicry complex exists among metallically colored insects, where mimics take advantage of fast escape responses developed by models and capitalize on their success without having to invest in the development of their own fast escape mechanism.

Summary

An extremely fast escape response time of less than 5 milliseconds was found in a long-legged fly of the genus *Condylostylus* (Dolichopodidae). This response to a visual startle reflex caused by a photographic flash was recorded repeatedly on camera and the synchronized shutter speed made it possi-

ble to measure the reflex time. Habituation was also observed in these trials. This newly recorded reflex is 3 times faster than any other previously reported.

On 30 March 2011, an opportunity arose to measure the speed of reaction of another *Condylostylus* sp. in the same location using the same technique and equipment, and the results were very similar to those reported here.

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Fig. 1. The response to a visual startle reflex caused by a photographic flash exhibited by a long-legged fly, *Condylostylus* (Dolichopodidae). All photographs except (D) show the fly jumping in the air when the flash was fired with 20 second intervals. (D) shows that habituation had occurred on the fourth firing of the flash, but the reflex response resumed 20 seconds later. Reflex response time is < 5 milliseconds. Photos by A. Sourakov.

Fig. 2. On 30 March 2011, an opportunity arose to measure the speed of reaction of another *Condylostylus* sp. in the same location using the same technique and equipment, and results were very similar to those reported here (see text and Fig. 1 for details). Photos by A. Sourakov.

FIRST REPORT OF *RAOIELLA INDICA* (ACARI: TENUIPALPIDAE) IN COLOMBIA

DANIEL CARRILLO[1]*, DENISE NAVIA[2], FRANCISCO FERRAGUT[3] AND JORGE E. PEÑA[1]
[1]University of Florida, Department of Entomology and Nematology, Tropical Research and Education Center, Homestead, FL 33031

[2]Lab de Quarentena Vegetal, Embrapa Recursos Genéticos e Biotecnologia, Parque Estação Biológica, final W5 Norte, CP 02372, 70770-900 Brasília, DF, Brasil

[3]Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera, s/n 46022, Valencia, Spain

*Author for correspondence; E-mail: dancar@ufl.edu

Raoiella indica Hirst (Acari: Tenuipalpidae), the red palm mite, is a phytophagous mite that recently invaded the Western Hemisphere. This mite was first detected in Martinique (Flechtmann & Etienne 2004) and it rapidly spread to multiple islands of the Caribbean [St. Lucia and Dominica (Kane et al. 2005), Guadeloupe and Saint Martin (Etienne & Flechtmann 2006), Puerto Rico and Culebra Island (Rodrigues et al. 2007), and Cuba (de la Torre et al. 2010) among other islands]. In 2007, the mite was found in West Palm Beach, Florida (FDACS 2007), and in the state of Sucre, Venezuela (Vásquez et al. 2008), and more recently, reported in the northern state of Roraima in Brazil (Marsaro Jr. et al. 2009), and Isla Mujeres and Cancun, Mexico (NAPPO 2009).

In January 2010, high populations of *R. indica* were found attacking coconut (*Cocos nucifera* L.), banana (*Musa acuminata* Colla) and heliconia (*Heliconia* sp.) plants in the Tayrona National Park located in the Colombian Caribbean littoral, near the city of Santa Marta, Magdalena. The presence of multigenerational colonies and exuvia was confirmed in 18 coconut palms, 4 heliconias and multiple banana plants located near the coast in the northern part of the park (11°18'44"N 73°56'04"W). In further surveys *R. indica* infestations were detected in commercial coconut and banana groves in June 2010 at Los Naranjos, Magdalena (11°17'49"N -73°53'49"W), approximately 6 km East of the Tayrona Park along the coast. In this locality the predatory mite *Amblyseius largoensis* Muma (Acari: Phytoseiidae) was found showing a conspicuous red coloration of the alimentary tract indicating recent feeding on *R. indica*. Previous studies indicated that populations of *A. largoensis* increased in numbers after the arrival of *R. indica* to Florida and some areas in the Neotropics (Peña et al. 2009; Carrillo et al. 2010).

Raoiella indica and *A. largoensis* specimens were collected (70% ethanol) and subsequently slide mounted, identified, and deposited in the collections of the Laboratory of Plant Quarantine reference collection, Embrapa Genetic Resources and Biotechnology, Brasilia, Brazil, and the Laboratory of Acarology from the Instituto Agroforestal Mediterráneo, Universidad Politécnica of Valencia, Spain.

The experienced negative effects of *R. indica* on coconut production in the Caribbean, where yield reduction has been estimated in over 50% at some locations (CARDI 2010), indicate the importance of adopting regulatory and other control measures in areas of recent invasion. The establishment of chemical practices needed to allow movement of host plant material, and continuous surveying (pre and post-invasion) using sentinel sites, have been adopted to prevent *R. indica*'s rapid dissemination in Florida (Roda et al. 2008). An integrated approach combining all available control tactics should be adopted and natural enemies identified for managing this species (Peña et al. 2009; Carrillo et al. 2010). In addition, studies are needed to determine the potential host plant range of *R. indica* in Colombia and the rest of the Neotropical region. Strict sanitary measures and other management tactics should be implemented to minimize the damage caused by *R. indica* in Colombia and other countries in South and Central America.

SUMMARY

In January 2010, high populations of *Raoiella indica* were reported for the first time in Colombia attacking coconut, banana, and heliconia plants in the Tayrona National Park. The predatory mite, *Amblyseius largoensis*, was found associated with *R. indica* in Los Naranjos, Magdalena. Strict sanitary strategies and other management tactics should be implemented to minimize the damage caused by *R. indica* in the Americas.

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NEW RECORDS OF THRIPS FROM MESOAMERICA AND COMMENTS REGARDING SPECIFIC CHARACTERS (TUBULIFERA: PHLAEOTHRIPIDAE)

RITA DINORA VALENZUELA-GARCÍA[1], AXEL P. RETANA-SALAZAR[2], OSWALDO GARCÍA-MARTÍNEZ[1] AND CARLOS CARVAJAL CAZOLA[3]

[1]Departamento de Parasitologia, Universidad Autónoma Agraria Antonio Narro, Calzada Antonio Narro 1923, Buenavista, Saltillo, Coahuila, México, C.P. 25315

[2]Programa Universitario de Biología Aplicada (PUA), Centro de Investigación en Estructuras Microscópicas (CIEMIC), Ciudad de la Investigación, Universidad de Costa Rica 2060

[3]Unidad Academica de Agricultura, Universidad Autónoma de Nayarit, Xalisco, Nayarit, México, Tepic-Compostela Km 9

Several genera in the Tubulifera are monotypic, and in some cases species were described based on a sole original holotype (Mound, 1976). This scenario complicates the study of morphological variation in species, and in several cases, intraspecific variation may be greater than variation between species (Retana-Salazar & Mound 1994). Reports of taxa from new localities and descriptions of specific character variation are fundamental in the study of these groups. Modest publications of this sort advance the construction of a more complete landscape of morphological variation on an interspecific and intraspecific level.

A new locality (Nayarit, Mexico) for two known species is reported in this paper. Comments about variations of characters are included.

Phlaeothripinae

Eurythrips Hinds 1902

Eurythrips is a New World genus, particularly common in the Neotropical region. The most recent revision of this genus placed in synonymy 16 of the 54 species included in this group (Mound 1976). The latest work concerning the thrips of Central and South America listed 37 species for this region (Mound & Marullo 1996). Several of these species are not well known and in some cases they are only known from the type material.

Eurythrips longilabris Watson

Eurythrips longilabris Watson 1921

Eurythrips harti Hood 1925

Material: Holotype female, USA, Florida State Collection of Arthropods (FSCA) and material from Texas (USNM) (Holotype of *E. harti*).

New Record. Collected on weeds, Santiago Ixcuintla County, Nayarit, México. 1 specimen.

Comments. Mound (1976), after of examination of the holotypes, considered both *longilabris* and *harti* species as synonymous; he considered that the morphological differences were not enough to consider these as two different species. Indeed, Mound (1976) showed that main differences are in color details and in the postocular setae. Each of these setae is acuminate in the *longilabris* holotype but softly pointed in the *harti* holotype. The color pattern of this specimen collected in Mexico is congruent with the most comprehensive key published to date (Mound, 1976). The postocular setae in the material collected in Mexico are similar to those of the *longilabris* holotype described by Mound (1976), that is to say, an acuminate shaped apex is present.

Idolothripinae

Ethirothrips Karny 1925

This is complex genus. Mound and Palmer (1983) considered 7 genera as synonyms of *Ethirothrips*. There are considerable differences between several species included in this genus. Mound and Marullo (1996) considered that some of these species are widely distributed and variable in color as well as in structure. *Ethirothrips* is a common member of the fauna of thrips associated with dead branches and leaf-litter in Brazil (Monteiro 2002).

Ethirothrips firmus Hood 1952

Material: Identified solely from a type series (5 specimens) collected in Sao Paulo, Brazil.

New Record. Collected on weeds, Santiago Country, Nayarit, Mexico. 1 specimen

Comments. Although described as a species of *Gastrothrips*, it is currently considered to be included in *Ethirothrips*. There are no reports of this species found anywhere else but at its original type locality. The structural variation in the species of this genus (Mound & Marullo 1996) presents a complex scenario for the study of this group. New material collected in México is congruent with all the morphological details described in the key presented by Mound and Marullo (1996). The presence of this species only in Brazil and Mexico suggests that Mound's hypothesis regarding distribution, related to human activity, holds true.

These two species have become identified through descriptions based on a single holotype, in the first case, and from a single type series in the second case. The new material presented allows new localities to be defined and the variability of morphological characters used in species definition to be explored further.

Summary

Two Mexican species, apparently absent in other places in Mesoamerica are briefly described and recorded in this paper. It is very important to note that *Eurythrips longilabris* is recognized solely from the holotype collected in Florida, USA.

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The protection of livestock and companion animals from pestiferous arthropods is of utmost concern to commercial agriculture, hobby farmers, veterinarians and pet owners. Veterinary Entomology: Livestock and Companion Animals is a concisely written and well-organized book that many readers will find extremely useful. This book has its origin in an earlier publication, with considerable updating and expansion into the companion animal area. Although this text will find its most use in college classrooms for teaching undergraduate veterinary or livestock entomology courses, it will be a valuable reference for anyone who regularly deals with these injurious pests in an extension capacity.

The book is organized into 15 chapters that include 2 introductory chapters: Importance of Arthropods, Principles of Arthropod Management, followed by 7 chapters demarking the primary groups of pests (Diptera, Myiasis-causing Diptera, Lice, Fleas, Mites, Ticks and Other Arthropod Groups of Veterinary Importance). Following the description of the individual pest players, the book takes the reader though the often-specialized pest management steps used in the agricultural and companion animal groups. These chapters use the following structure: Cattle, Swine, Sheep and Goat, Poultry, Equine and Pet. A selection of references and an index complete the book.

Chapter 1 provides a brief, but well summarized, overview of the types of parasitism, damage caused to animals by arthropods, and the use of economic injury levels. In Chapter 2, the reader is introduced to the unique methods of pest surveillance, types of pest management and an overview of the insecticides and application techniques used in veterinary entomology. This is particularly important as many products that can be used on other agricultural crops cannot be used on animals and many application techniques are specialized for animals (ear tags, pour-ons, and boluses).

To many of the non-entomologist readers, the array of insects found on livestock and pets is often overwhelming; requiring about two-thirds of this book to present. Additionally, this book predominantly covers the pests of the US and Canada, and as such, not all pests are present in all areas of these countries. Chapters 3, 4, 5, 6, 7, 8, and 9 present both the pests and their biological control agents to the reader in a repetitive format, first by introducing the geographic range, the importance, hosts or habitat, description and finally their biology/behavior. In following this approach, the reader can readily find the information that they require and easily move from the pest management chapters at the end of the book back into the arthropod-description chapter of interest. Each chapter is fronted by an overall description of the arthropod order (or sub-set, in the case of Chapter 4, Myiasis) followed by a very useful table outlining the taxonomic relationships of the specimens to be discussed. The distinction of pest status is often in the perception of the person being attacked or annoyed, or in the particular behavior or presence of a fly at a given time or place. Such perceptions are recognized and addressed throughout these chapters.

Chapters 3 and 4 cover the Diptera. Within Chapter 3, the material is divided into the biting and the non-biting flies. Although this presentation has little to do with their taxonomic placement, it does serve as an important structure for the reader to follow. Some of these flies are considered the most important arthropod pests of respective veterinary animals, and as such, their placement and detailed description is appropriate. In Chapter 4 the flies that cause myiasis (infestation of living vertebrates by dipteran larvae) is addressed. The flies discussed herein are some of the most fascinating and bizarre found in the animal kingdom. Students and the general public invariably are both intrigued and repulsed by these highly evolved parasites. The material in this chapter is introduced by providing a classification of the types of myiasis (dermal, gastrointestinal, etc. and obligatory/facultative/accidental). Within this chapter, images of the adult and immature flies as well as images of the parasitism sites on animals are presented. Here, in particular, color images would have made these images much more useful.

Each of the pest management chapters (10, 11, 12, 13, 14 and 15) follows a standard design, making locating information easy. Besides the geographic location, the livestock management system employed by the farmer or rancher will dictate which pests are likely to be problematic. As such, each system will in some respect, create their own problems, requiring specialized solutions. With respect to the filth flies that commonly plague cattle, swine, poultry and horses, the first step in successful pest management is to recognize the livestock management system used: type of confinement or pasture (cattle and horses). Because filth flies are among the most important pests of livestock, they have received considerable attention from research and extension professionals resulting in diverse pest management options. Therefore, the portions of chapters dealing with filth fly management are presented in greatest detail and are appropriate for much of the US and Canada. In each pest management program described, the following categories are used or sum-

marized: surveillance, cultural/mechanical control, biological control and chemical control. For several of the other pests, the control options are much less diverse and therefore, their control section is condensed.

Chapters 14 and 15 deal with equine and pet pest management. These topics are not generally found in similar books and it is refreshing to see them addressed here. In particular, the information provided on flea management is of particular interest as veterinarians took over much of the flea control market in the 1990s when topical applications were made available. However, we may be seeing a return to a truly integrated program as fleas continue to develop insecticide resistance to these materials.

The book contains over 200 illustrations, including a four-page color plate. Many of the illustrations are exceedingly helpful; however, the book would have been aided greatly by the addition of more color images. The use of line drawings is of particular note, especially for some of the mite species from which it is notoriously difficult to obtain quality images. Beyond simply including images of the arthropods, the book contains many line drawings of life cycles that help to illustrate the importance of the use of pest biology when planning control tactics. Furthermore, the use of images of environmental conditions, animal management practices and pest management tactics greatly aids in the readers understanding of these often unique monitoring and control tactics.

Although this book provides an excellent overview of the arthropods to be expected in these commodities, the relationship of these pests to the disease-causing pathogens that many are vectors of is not strong. Many of the potential pathogens are touched upon, but some very important opportunities were missed, particularly in relation to the mosquito- and tick-borne pathogens. A chapter on the relationship of these vectors and their associated pathogens should be considered in a subsequent edition. Additionally, although this book presents a very good overview of the principles of pest management, the techniques will continue to evolve. The inclusion of information on where to locate additional information would be helpful (such as University Cooperative Extension programs or eXtension), perhaps at the end of each management chapter or at the end of the introductory chapter, with an inclusion in the index.

P. E. Kaufman
Entomology & Nematology Dept.
University of Florida
Gainesville, FL 32611-0620
E-mail: pkaufman@ufl.edu

JULIO, CARLOS AGUILAR, 2010. Methods for Catching Beetles. Naturalia Scientific Collection, Montevideo-Asuncion, 303 pp., 16 color pls., 139 figs., 14.8 × 21 cm. Soft cover, $76.

Beetles are the most speciose animal group; and they are found in virtually all habitats on Earth. *Methods for Catching Beetles* is a comprehensive general sourcebook about where and how to collect members of this diverse group. The book makes a compelling case in its Introduction about the value of scientific collecting to taxonomy and conservation. It then goes on to provide a broad overview of collection methods in general, and also presents tailored information on collecting specific families of beetles. It is particularly suited for those new at collecting beetles, but will also benefit veteran, field-oriented coleopterists who may be switching to study different families of beetles or to collect beetles in unfamiliar habitats. The book is intended to facilitate teaching of entomology, and it could realistically serve as a supplemental text in a taxonomic survey course, a collection methods course, or a special topics class on beetles. The relatively small size of the book makes it amenable to carry in the field, but it best serves readers as a good desk reference for devising an effective collecting trip.

Individual chapters are devoted to the basics of planning a collecting trip, a survey of environments, an overview of collection methods and traps, and a summary of killing and preservation methods. An especially strong feature is the chapter titled "Where do they live . . . ," which devotes nearly 100 pages on characteristics of individual beetle families—and often subfamilies and genera—and how to collect them. Information in the book is based primarily on the author's own extensive experience and that of 10 additional contributing experts, and the content is supported by a plethora of references current through year 2010 (pp. 264-303). A warm, helpful tone of the book is set by first-person language in the Introduction and at various other points, and by a smattering of personal tips and direct quotes of the main author's colleagues on collecting beetles. The book also benefits from 16 exquisite color plates, 139 black-and-white figures, and a glossary.

Catching Beetles is strictly a survey of qualitative methods for catching beetles. Hence, it lacks content on quantitatively assessing catches by calculating statistics and indices, analysis and comparison of collection methods and catches over time and among places, or any discussion of topics such as absolute versus relative sampling methods.

The book has a few shortcomings such as illogical subject organization. For instance, a section titled "Traps" (pp. 130-149) discusses general concepts (e.g. active vs. passive trapping) and several specific types of traps. However, some types of traps are described and discussed earlier in the book, e.g. bottle traps (pp. 70-71), light traps (pp. 89-95), and traps for xylophagous beetles (pp. 101-105). Another subsection titled "CAR-CATCHER" describes the design and use of vehicle-mounted traps, but it is inexplicably inserted about midway in the chapter on Environments. A chapter on "Killing Storing, Preserving and Rearing" contains no information on rearing, but tips on rearing beetles are given earlier in the book (e.g. rearing larvae in wood, pp. 106-108). More information could have been presented about collecting stored-product beetles, and an index would have been useful.

Better placement and use of definitions is often needed, particularly with regard to environments. For instance, lentic and lotic habitats are discussed one page before being defined, and then their individual definitions are quirkily repeated one and three pages later, respectively. In another instance under "Lakes and Ponds," several definitions are presented for pond, but lake is never defined. Moreover, no definitions of any of these terms appear in the glossary.

Stylistic flaws and deficient English-language editing reduce the book's readability. Small font also diminishes readability of the section on beetle families, and font size is inconsistent in a few sections. Text within a few figures is indecipherable.

References are beset by formatting errors, inconsistencies, and miswordings. Mistakes often occur in alphabetical and chronological sequencing of references. Several times references are alphabetized by an author's first rather than last name, although these references are often cited that way in the text. Wikipedia and other websites are referenced authoritatively a few times instead of relying on original references to support some points.

Nonetheless, the book's strengths far outweigh its shortcomings. It will prove a thorough, useful resource to inform readers and aid them in devising study methods to enhance knowledge of the Coleoptera.

Louis S. Hesler
North Central Agriculture Research Laboratory
USDA, Agricultural Research Service
Brookings, SD 57006
E-mail: louis.hesler@ars.usda.gov

JUNE 2011
FLORIDA ENTOMOLOGICAL SOCIETY
CORPORATE AND SUSTAINING MEMBERS

CORPORATE

Atlantic Turf and
Ornamental Consulting
Attn: Scott Ferguson
7145 58th Avenue
Vero Beach, FL 32967

Bayer Crop Science
Craig Shelton
Lake Placid, FL 33852

Dow Agrosciences
Attn: Ellen Thoms
7257 NW 4th Blvd. #20
Gainesville, FL 32607

SUSTAINING

Bayer Crop Science
Attn: John H. Paige
328 Indian Lilac Rd.
Vero Beach, FL 32963

Best Termite & Pest Control
Attn: Frank A. Mongiovi
8120 N. Armenia Ave.
Tampa, FL 33604

Dow Agrosciences
Attn: Joe Eger
2606 S. Dundee Blvd.
Tampa, FL 33629

E.O. Painter Printing Co.
Attn: Dick Johnston
P.O. Box 877
DeLeon Springs, FL 32130

Mark B. Sivic
16744 W. Brighton Dr.
Loxahatchee, FL 33470

Syngenta Crop Protection
Attn: Nancy Rechiegl
11601 Erie Road
Parrish, FL 34219

Taylor Pest Management
Attn: James Taylor
851 NE Jensen Beach Blvd.
Jensen Beach, FL 34957

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