

PATHOGENIC MICROORGANISMS ASSOCIATED WITH THE SOUTHERN PINE CONEWORM (LEPIDOPTERA: PYRALIDAE) ATTACKING LOBLOLLY PINE

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ABSTRACT

Larvae of the Southern pine coneworm, *Dioryctria amatella* (Hulst) (Lepidoptera: Pyralidae), were collected monthly during the growing seasons of 1996 and 1997 from loblolly pine, *Pinus taeda* L., seed orchards in Alabama, Florida, Georgia, South Carolina, and Virginia, and examined for pathogenic microorganisms. One fungus, *Beauveria bassiana* (Bals.) Vuill, a granulosis virus (Baculoviridae: Eubaculovirinae), and a protozoan (phylum Microspora) were found. Five larvae from three localities were infected with *B. bassiana*, 37 larvae from six localities were infected with the granulosis virus, and 69 larvae from 5 locations were infected with the microsporidian. Laboratory trials confirmed that *B. bassiana* and the granulosis virus caused coneworm mortality. *B. bassiana* isolates from all three locations were equally virulent to late instar larvae. Spores of the unidentified microsporidian are free, elongate oval, binucleate and contain 13-14 turns of an isofilar polar filament. The primary sites of infection were the Malpighian tubules and the silk glands. The microsporidian was found in 2 to 51% of larvae sampled. It caused 100% mortality in early instar larvae allowed to feed on artificial diet contaminated with 3×10^9 or 4.5×10^9 spores. More work is needed to determine the importance of these pathogens in regulating populations of southern pine coneworms or their potential utility in an IPM program.

Key Words: loblolly pine, *Pinus taeda*, *Dioryctria amatella*, pathogens, microsporidia, granulosis virus, *Beauveria bassiana*

RESUMEN

Larvas del gusano de las bellotas de pino sureño, *Dioryctria amatella* (Hulst) (Lepidoptera: Pyralidae) fueron recolectadas mensualmente durante las estaciones de crecimiento de 1996 y 1997 en huertos de semillas del pino, *Pinus taeda* L., en Alabama, Florida, Georgia, South Carolina, y Virginia, y fueron examinadas por microorganismos patógenos. Se encontraron un hongo, *Beauveria bassiana* (Bals.) Vuill, un virus granuloso (Baculoviridae: Eubaculovirinae), y un protozoario (Phylum Microspora). Cinco larvas de tres localidades fueron infectadas con *B. bassiana*, 37 larvas de seis localidades fueron infectadas con el virus granuloso, y 69 larvas de 5 localidades fueron infectadas con el microsporidio. Pruebas de laboratorio confirmaron que el *B. bassiana* y el virus granuloso causaron mortalidad en el gusano de las bellotas de pino. Los aislados de *B. bassiana* de las tres localidades fueron igualmente virulentos para las larvas en sus últimos estadios. Las esporas de un microsporidio no identificado son libres, alargadas, binucleadas, y contienen 13-14 vueltas de un filamento isofilar polar. Los sitios principales de infección fueron los túbulos de Malpighi y las glándulas de seda. El microsporidio fue encontrado en un porcentaje del 2 al 51% de las larvas muestreadas. Se causó el 100% de la mortalidad en las larvas en los primeros estadios que se permitieron alimentar de una dieta artificial contaminada con 3×10^9 o 4.5×10^9 de esporas. Se necesita hacer más trabajo para determinar la importancia de estos patógenos para regular la poblaciones de *Dioryctria amatella* o su uso potencial en un programa de Manejo Integrado de Plagas.

INTRODUCTION

Several coneworm species cause severe seed losses in loblolly pine (*Pinus taeda* L.) seed orchards. These include the southern pine coneworm, *Dioryctria amatella* (Hulst); the blister coneworm, *D. clarioralis* Walker; the webbing coneworm, *D. discusa* Heinrich; and the loblolly pine coneworm, *D. merkei* Mutuura and Monroe (Yates & Ebel 1975). *Dioryctria amatella* is the most serious of these pests.

Dioryctria amatella feeds on first and second-year cones, rust-infected conelets and terminals of southern pines (Coulson & Franklin 1970, Hedlin et al. 1980). It also attacks fusiform rust galls, *Cronartium quercuum* (Berkely) Miyabe ex Shirai F. sp. *fusiforme*, on stems and branches, and the cambium of tree wounds as alternate hosts necessary for overwintering (Neunzig et al. 1964, Coulson & Franklin 1970, Hedlin et al. 1980). The southern pine coneworm has up to four generations per year in the southern United States (Ebel 1965, Merkel & Fatzinger 1971, Coulson & Franklin 1970, Fatzinger 1981), but most of the damage is caused by progeny of adults that emerge in the spring (Chatelain & Goyer 1980).

Females lay eggs on or near second-year cones (Coulson & Franklin 1970). Once in a cone, larvae develop through five instars (Fatzinger 1970) that feed throughout the cones where they eventually pupate (Neunzig et al. 1964). *Dioryctria clarioralis*, *D. merkei* and *D. amatella* often occur in second year cones in the same orchard and several instars of the same species may be present in a single cone (Neunzig et al. 1964, Hanula et al. 1985).

Despite the importance of *D. amatella* and other *Dioryctria* species in limiting seed production, no pathogens have been reported from southern pine coneworms. However, there are records of pathogens associated with other *Dioryctria* species. These include a granulosis virus from *D. abietella* (D. and S.) (Zhimerikin & Gulii 1972) and a nuclear polyhedrosis virus from *D. pseudotsugella* Munroe (Martignoni & Iwai 1986) and *D. sylvestrella* (Kunimi 1993). The fungi *Beauveria bassiana* and *Hirsutella satumaensis* have been recovered from *D. splendidella* H. and L. and *D. sylvestrella*, (Humber & Hansen 2001) and *Metarhizium anisopliae* has been recovered from *D. sylvestrella* in Japan (Kunimi 1993). An unidentified microsporidium was reported from *D. splendidella* in Russia (Sprague 1977).

The objectives of this study were to identify pathogenic microorganisms present in immature stages of southern pine coneworms attacking loblolly pine, their prevalence in natural host populations, and their pathogenicity under laboratory conditions.

MATERIALS AND METHODS

Cones were collected monthly during the summer (July-September) from six seed orchards in 1996 and from seven seed orchards in 1997. In 1996, samples were collected from loblolly pine seed orchards in Escambia and Nassau Counties, Florida; Bibb and Toombs Co., Georgia; Rapides Parish, Louisiana and York Co., South Carolina. In 1997, samples were obtained from orchards in Choctaw Co., Alabama; Escambia Co., Florida; Toombs Co., Georgia; Rapides Parish, Louisiana; York and Dorchester Co., South Carolina; and Albemarle Co., Virginia. Cooperating seed orchard managers collected 50-150 cones per sample from trees scattered throughout their orchards. Samples included almost equal numbers of newly attacked cones (green), older infested cones (brown-green), and old infested cones that were almost dried (brown). The cones were cut open and all larvae and pupae removed. Larvae with external or internal parasitoids were noted along with cadavers. Larvae that appeared healthy were placed individually in small cups containing an artificial diet (Fedde 1982), held at room temperature until they completed development or died, and checked periodically for signs of disease. Those that died were dissected in Ringer's solution (Poinar & Thomas 1978) and examined for disease if no external symptoms were evident.

Larvae that appeared unhealthy, or were damaged while being extracted from cones, were dissected immediately in Ringer's solution. Larval tissues that appeared abnormal during dissection were prepared in wet mount slides and examined with a phase contrast microscope (100-1000 \times) for pathogens.

Suspected fungal pathogens were isolated either by culturing them from hyphae or spores scraped from the cuticle of cadavers, or by placing whole, surface sterilized cadavers on growth media. Surface sterilization was done by immersing them in a 5% solution of sodium hypochlorite (NaClO) followed by three rinses in sterile water (Poinar & Thomas 1978). After pure cultures were obtained, additional plates were prepared for use in experiments.

We used Sabouraud dextrose agar (SDA) with yeast extract for fungal isolation since it is effective for many entomogenous fungi and the acid reaction (pH 5.6) retards bacterial growth (Poinar & Thomas 1978). We added streptomycin sulfate (0.03g/l) to further inhibit bacterial growth. Cultures were held in a dark growth chamber at 20-25°C for 1-2 weeks for growth and sporulation. Fungi that sporulated in culture were stored for up to 1 month in a refrigerator at 5°C after which new isolates were prepared.

Preparations for microscopic examination were made by growing fungi on cellulose membranes placed on water agar (Alexopoulos &

Beneke 1962). Cellulose membranes (dialysis tubes, Fisher Scientific) were then removed and examined in wet mount preparations after 24 hours, and every 2-3 hours (for 12 hours) thereafter, for diagnostic characteristics (Samson et al. 1988).

Occasionally we were unable to determine a cause of death through dissection and light microscopy. In those cases, the larvae were ground up in Ringer's solution and a drop of the homogenate was placed on a carbon coated grid, stained for 5 minutes with 5% uranyl acetate, and examined by transmission electron microscopy for viral particles. Forty larvae were examined in this way.

Several experiments were conducted to determine the virulence of pathogens recovered from field-infected larvae under laboratory conditions. Coneworms for these experiments were obtained from a laboratory colony of *D. amatella*.

A fungus suspected of causing mortality was tested in one trial to insure that it was the causal agent. Thirty late instar *D. amatella* larvae were inoculated per inoculum density and we tested densities of 0, 6.0×10^4 , 1.0×10^5 and 1.6×10^5 spores/ μ l in sterile water. One 2 μ l droplet of each spore suspension was placed on the cuticle of the larvae which were then placed in individual cups of artificial diet and held in a growth chamber at $26^\circ\text{C} \pm 1^\circ\text{C}$ and $92\% \pm 3\%$ RH until they died or completely developed. Fungi were reisolated from cadavers which exhibited active fungal growth. Pure cultures were then examined and compared to the original cultures to confirm that the same fungus was present and the cause of mortality.

In a second trial, conducted under the same conditions, we compared strains of the same fungus isolated from *Dioryctria* spp. collected from three different localities at a single inoculum density of 4.0×10^4 spores/ μ l. We also compared mortality following inoculation on the cuticle or *per os*. *Per os* inoculations were done by placing 2 μ l droplets of 4.0×10^4 spores/ μ l in a 5% sucrose solution on the mouthparts of CO_2 anesthetized larvae. Cuticular inoculations were the same as before.

To test the granulosis virus, infected, field collected larvae were homogenized in a tissue grinder, diluted with a 5% sucrose solution, and 2l droplets of this inoculum were placed on the mouthparts of anesthetized test larvae. Treated larvae were observed daily and left to develop completely or die. Observations of shape and size of virus particles (virus body) and inclusion bodies through TEM were used to determine the type of virus (Smith 1967).

Microsporidia infections were diagnosed by dissection and light microscopic examination of fresh tissue or stained tissue smears. The latter consist of tissue smeared on slides, air-dried, fixed in methanol (5min), and stained with a 10% Giemsa stain solution (pH 7.4). Larvae anesthetized

with CO_2 were inoculated *per os* with 2 μ l of 1×10^4 microsporidia spores and dissected at 2 day intervals to find which tissues were the primary site of infection. Geimsa stained smears of various tissues were prepared and examined for infection. Since the primary site of infection appeared to be the silk glands, infected portions of that tissue were prepared for electron microscopy. For rapid initial fixation of fresh silk glands, infected tissue was submerged for one hour at 25°C in 2.5% (w/v) glutaraldehyde buffered with 0.1M Na-cacodylate (pH 7.5) to which 5% (w/v) sucrose and 0.5% (w/v) CaCl_2 were added. Tissues were post-fixed with 4% (w/v) OsO_4 in cacodylate buffer (pH 7.5) for 1 hour, dehydrated through an ethanol series and then tissues were submerged in propylene oxide twice for 20 min. Tissues were embedded in 812 Epon® plastic (Sabatini et al. 1963). Sections were post-stained with 2% (w/v) aqueous uranyl acetate followed by lead citrate (Harris 1997).

A trial was conducted to determine if the microsporidian caused mortality in *D. amatella* larvae allowed to feed on artificial diet contaminated with spores. Second instar larvae were placed in 1.5 ml microcentrifuge tubes containing a small quantity of artificial diet contaminated with 0, 5×10^2 , 2×10^3 , 3×10^3 or 4.5×10^3 spores obtained from infected tissues of field collected larvae. Fifty to 60 larvae were treated per dose. The centrifuge tubes were plugged with cotton and the larvae were allowed to feed for 7 d. After 7 d they were transferred to 30 ml capacity diet cups with fresh uncontaminated food. Larvae were monitored every other day until all of the control group pupated. Those that died were dissected and examined for the presence of microsporidian spores. At the end of the experiment all surviving larvae and pupae were examined for microsporidium infections.

Data from laboratory trials were analyzed using the SAS procedure FREQ to analyze contingency tables (SAS Institute Inc. 1987).

RESULTS

We examined 1626 coneworm larvae from six locations during the summers of 1996 and 1997. Of those, 306 (18.8%) were parasitized by other insects, 32 (2.4%) were infected with a granulosis virus, 5 (0.4%) were infected with the fungus *B. bassiana*, and 69 (5.2%) were infected with a microsporidium (Table 1).

The virus was identified as a granulosis type (Baculoviridae: Eubaculovirinae) based the presence of virions occluded individually in granules (Federici 1997). A total of 32 field collected larvae were infected with a granulosis virus at six locations during 1996 and 1997. In 1998, we found 5 larvae infected in a Baldwin Co., Georgia seed orchard. Prevalence ranged from 1 to 14% in field populations (Table 1). Nineteen of 30 late instar

TABLE 1. OCCURRENCE OF PATHOGENIC MICROORGANISMS IN SOUTHERN PINE CONEWORM POPULATIONS.

Sample location Counties and states	year	Occurrence of pathogens			
		No. of larvae examined	Microsporidia No. (%)	Granulosis virus* No. (%)	<i>B. bassiana</i> No. (%)
York, SC	1996	88	0	0	0
Escambia, FL	1996	106	7 (6.6)	1 (0.9)	0
Nassau, FL	1996	89	0	0	0
Bibb, GA	1996	89	0	0	0
Pineville, LA	1996	114	0	0	0
Toombs, GA	1996	410	10 (2.4)	0	0
Toombs, GA	1997	99	12 (12.1)	13 (13.1)	1 (1.0)
Choctaw, AL	1997	74	1 (1.3)	7 (9.5)	1 (1.4)
Escambia, FL	1997	116	17 (14.7)	4 (3.5)	3 (2.6)
York, SC	1997	35	18 (51.4)	5 (14.3)	0
Albemarle, VA	1997	45	0	0	0
Dorchester, SC	1997	34	0	0	0
Rapides, LA	1997	21	4 (19.1)	2 (9.5)	0

Note: Larvae parasitized by insects were not examined for diseases.

*In 1998, 5 additional larvae were found infected with virus in Baldwin CO., GA.

larvae inoculated *per os* with the virus in laboratory trials died, while all of the control group survived to the adult stage. Infected larvae exhibited a variety of symptoms including prolonged development, cessation of feeding, discoloration of the integument (light gray or brown) and a milky white appearance of the hemolymph. The latter was the most reliable diagnostic characteristic for determining infection.

The fungus *B. bassiana* was recovered from *Dioxyctria* spp. larvae at three locations, although prevalence was low (Table 1). In laboratory trials, *B. bassiana* (Florida isolate) caused approximately equal mortality at all inoculum densities tested (Table 2). In addition, *per os* inoculations did not increase mortality over cuticular inoculations with the Alabama isolate and we detected no differences in mortality among the three isolates we tested.

Sixty-nine field collected larvae from six locations were infected with a microsporidia (Table 1). Prevalence of this disease organism ranged from 2% in Lyons, Georgia to over 51% at Catawba, South Carolina.

Uninucleate and diplokaryotic vegetative stages were observed in Geimsa stained smears using light microscopy (1000 \times), but only diplokaryotic stages were detected with the electron microscope. Meronts and/or sporonts of the microsporidium were diplokaryotic (Fig. 1A). Spores of the microsporidium observed through light and electron microscopy, were oval, binucleate, contained a single coiled isofilar polar filament with 13-14 turns (Fig. 1B), and measured $5.87 \times 2.85 \mu\text{m}$ ($n = 20$) in fresh preparations. Spores were not enclosed in any type of sporoporous vesicle and they always occurred individually. Malpighian tubules and silk glands were

TABLE 2. MORTALITY OF LATE INSTAR SOUTHERN PINE CONEWORMS FOLLOWING APPLICATION OF VARIOUS DOSES AND ISOLATES OF *B. BASSIANA* APPLIED TO THE CUTICLE OR *PER OS* TO ESTABLISH THAT THE FUNGUS RECOVERED FROM CADAVERS WAS RESPONSIBLE FOR THE MORTALITY OBSERVED. LARVAE WERE HELD AT $26^{\circ}\text{C} \pm 1^{\circ}\text{C}$ AND $92\% \pm 3\%$ RH UNTIL DEATH OR COMPLETE DEVELOPMENT.

Isolate/inoculation	dose spores/ μl	N	No. dead	Percent mortality
Controls/cuticle	0	30	4	13.3 a
Controls/ <i>per os</i>	0	30	3	10.0 a
Florida/cuticle	6.00×10^4	30	20	66.7 b
	1.00×10^5	30	23	76.7 b
	1.65×10^6	30	22	73.3 b
Louisiana/cuticle	4.10×10^4	30	21	70.0 b
Alabama/cuticle	4.10×10^4	30	19	63.3 b
Alabama/ <i>per os</i>	4.10×10^4	30	20	66.6 b

Note: Percent mortality followed by the same letter are not significantly different (chi-square test, $p < 0.01$).

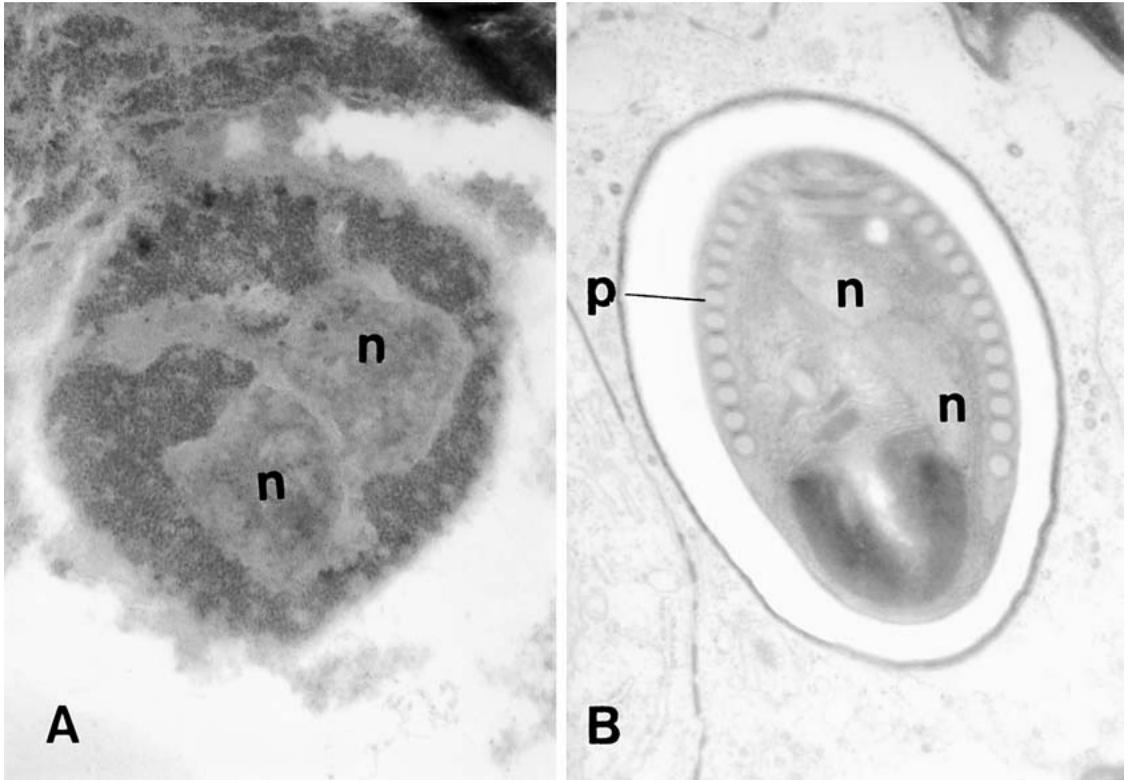


Fig. 1. Electron-micrographs of a microsporidian meront (A) from *Dioryctria amatella* silk gland containing two nuclei (n) in a diplokaryotic arrangement and a binucleate spore (B) showing the polar filament (p) with 13-14 turns. Magnification of A = 40,000 \times and B = 20,000 \times .

the primary sites of infection in lightly or newly infected individuals, but we were unable to determine which tissue was infected first. In advanced infections, the microsporidium could be found throughout the host's tissues including the fat body, midgut and epidermis. Larvae reared on artificial diet were successfully infected *per os*. Other means of infection or transmission are unknown.

In laboratory trials, the highest two doses (3×10^3 or 4.5×10^3 spores) caused 100% mortality in larvae exposed in the second instar. Only 45% of the control group survived to pupation but none was infected with the microsporidium.

DISCUSSION

We recovered three pathogenic microorganisms from widely separated populations of the southern pine coneworm. It is likely that these pathogens are found throughout the range of *D. amatella*.

The fungus, *B. bassiana*, is a common pathogen with an extensive insect host list (Tanada & Kaya 1993). Although we only isolated it from five larvae at three locations, laboratory trials showed

that it was capable of causing mortality. The low occurrence of *B. bassiana* in field populations may be due to the protected habitat of coneworms. Most cones occur near the tops of pine trees and once *D. amatella* larvae enter cones they rarely leave them, so they are protected from wind-borne pathogens. Vandenberg & Soper (1978) suggested that increased frequency of fungal diseases was due to greater host exposure, favorable physical conditions for fungal spores and the potential for spore accumulation in lower canopy areas. It may be that naturally occurring epizootics of fungal pathogens in coneworm populations are unlikely because larvae occupy protected microhabitats, the majority of loblolly pine cones occur in the tops of trees, and southern pine forests are exposed to frequent periods of hot, dry weather unfavorable to spore longevity outside the soil environment.

This is the first granulosis virus reported from *Dioryctria* spp. in North America, although one was reported from *D. abietella* in Siberia (Zhimerikin & Guli 1972). Baculoviridae are the most commonly observed viral infections in insects with 80% occurring in the Lepidoptera (Evans & Entwistle 1987) and all granulosis virus infec-

tions have been recorded from Lepidoptera (Federici 1997). It is unclear why the granulosis virus is more common than *B. bassiana* in the southern pine coneworm. It may be the virus occlusion bodies or capsules are more persistent in the environment than *B. bassiana* spores. In addition, under laboratory conditions the granulosis virus prolonged larval development while *B. bassiana* killed larvae within a few days. The extended development of viral infected individuals may allow greater viral reproduction (Tanada & Kaya 1993) or it may have increased the likelihood that we encountered infected individuals in our samples.

The microsporidian was the most prevalent pathogen we encountered with up to 51% of the larvae from the York Co., SC population infected with this protozoan. The spores, observed through light and electron microscopy, were oval, diplokaryotic, and had a long, flexible polar filament. Based on characteristics of the spores and the presence of uninucleate and binucleate stages consistent with the description of the type species *N. bombycis* (Sprague et al. 1992) we thought the microsporidian was a member of the genus *Nosema* (Nosematidida: Nosematidae). However, analysis of small subunit ribosomal DNA and comparison to an extensive database of other microsporidia suggests that the microsporidian we found is not closely related to other *Nosema* spp. and is not likely a member of that genus (C. R. Vossbrinck, personal communication). Further genetic analyses, and light and electron microscopy studies are underway to determine the identity and phylogenetic relationships of this species.

Although the microsporidium can kill its host when they are treated with very high doses, it is unclear what effect this microsporidium has on hosts or host populations under natural conditions. Onstad & Maddox (1989) modeled the effects of *N. pyrausta* (Paillot) on the population dynamics of its pyralid host the European corn borer, *Ostrinia nubilalis* (Hübner). Their results suggest that *N. pyrausta* can regulate *O. nubilalis* populations well below the carrying capacity of its environment. The timing of infection is important in determining what effect a microsporidian has on its host (Onstad & Carruthers 1990). For example, Sajap & Lewis (1992) found that early instars of *O. nubilalis* infected with *N. pyrausta* formed abnormal pupae or adults. Infections in later instars resulted in reduced adult longevity and up to 50% reduction in fecundity. We found that the microsporidium caused mortality at high doses, but we ended our experiment at pupation so we are uncertain if the surviving infected individuals would have developed normally or experienced normal adult longevity. If the nosema-like microsporidian we found has similar effects on its pyralid host, *D. amatella*, then it may be an important factor limiting coneworm populations.

The potential utility of the pathogens found infecting *D. amatella* as biological control agents needs to be determined. The granulosis virus and *B. bassiana* kill their host quickly, and therefore might have potential as biopesticides. *Beauveria bassiana* has the added advantage of being easily cultured. The microsporidian may be important in regulating host populations but additional work is needed to determine its effect. In addition, these pathogens may be useful in biological control programs of other *Dioryctria* spp. Of the three pathogens reported here, only *B. bassiana* was recovered from coneworms in surveys of 5 populations in California and Oregon (Hanula, unpublished data).

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**DENSITY DEPENDENT PARASITISM AND HOST-KILLING
OF *LIRIOMYZA TRIFOLII* (DIPTERA: AGROMYZIDAE) BY
DIGLYPHUS INTERMEDIUS (HYMENOPTERA: EULOPHIDAE)**

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ABSTRACT

Liriomyza trifolii (Burgess) is an important leafmining pest of numerous ornamental and vegetable crops. The pest is attacked by many species of parasitoids which inflict heavy mortality in the absence of insecticides. The functional response, as well as parasitoid-induced mortality, of one major parasitoid, *Diglyphus intermedius* (Girault), was estimated over a range of densities of third instar *L. trifolii* at 25-27°C in the laboratory. The functional response of *D. intermedius* was given by the equation $Y = Kp \cdot n_1 \cdot (1 - \exp(-n_1/Kh))$ where Y, the rate of parasitism, is the number of hosts parasitized per day; Kp is a constant of 7.3908 hosts parasitized per parasitoid-day; n_1 and n_2 are the densities of leafminer larvae and parasitoid adults (numbers/cm² leaf area), respectively, and Kh is a constant of 0.0144 leafminer larvae/cm² leaf area. The relationship between host and parasitoid density and parasitoid-induced host mortality was given by $Z = Cp \cdot n_2 \cdot (1 - \exp(-n_1/Ch))$ where Z, the rate of parasitoid-induced mortality, is the number of leafminer larvae killed per day; Cp is a constant of 9.2064 hosts killed per parasitoid-day and Ch is a constant of 0.0165 leafminer larvae/cm² leaf area. The observed rate of parasitism at a particular parasitoid density was always lower than the observed rate of parasitoid-induced host mortality at that density. Lower leafminer larval densities resulted in increased multiple oviposition by *D. intermedius*. When eggs of the parasitoid were placed at increasing densities on leafminer larvae in artificial mines, the number of parasitoid eggs surviving to adulthood decreased while the number of individuals surviving per host tended to remain at about one.

Key Words: *Liriomyza*, *Diglyphus*, biological control, leafminer, parasitism, host-killing, functional response

RESUMEN

El minador de hojas, *Liriomyza trifolii* (Burgess) es una plaga importante de numerosas plantas ornamentales y de hortalizas. Varias especies de parasitoides atacan esta plaga infligiendo una mortalidad alta en la ausencia de insecticidas. Se estimaron la respuesta funcional y la mortalidad inducida por parasitoides, en el parasitoides principal, *Diglyphus intermedius* (Girault), en varias densidades del *L. trifolii* en el tercer estadio a 25-27°C en el laboratorio. La respuesta funcional de *D. intermedius* fué dada por la ecuación $Y = Kp \cdot n_1 \cdot (1 - \exp(-n_1/Kh))$ donde Y (la tasa de parasitismo) es el número de hospederos parasitados por día; Kp es un constante de 7.3908 hospederos parasitados por día-parasitoid; n_1 y n_2 son las densidades de las larvas del minador y los adultos de parasitoides (numeros/area cm² de la hoja), respectivamente, y Kh es una constante de 0.0144 larvas de minador /area cm² de la hoja. La relación entre la densidad del hospedero y la densidad del parasitoides y la mortalidad inducida por el parasitoides ha sido dada por $Z = Cp \cdot n_2 \cdot (1 - \exp(-n_1/Ch))$ donde Z (la tasa de mortalidad inducida por el parasitoides) es el número de larvas de minador matadas por día; Cp es una constante de 9.2064 hospederos matados por día-parasitoides y Ch es una constante de 0.0165 larvas de minador/area cm² de la hoja. La tasa de parasitismo observada en una densidad específica del parasitoides siempre fué más baja que la tasa de mortalidad observada inducida por el parasitoides de la misma densidad. Las densidades menores de larvas de minador resultaron en un aumento de oviposiciones múltiples por *D. intermedius*. Cuando los huevos del parasitoides fueron colocados en densidades crecientes sobre las larvas de minador en minas artificiales, el número de huevos parasitados que sobrevivieron hasta el estado adulto disminuyó mientras que el número de individuos que sobrevivieron por hospedero tendieron a permanecer aproximadamente uno por hospedero.

Liriomyza trifolii (Burgess) is an important pest of many ornamental and vegetable crops including tomato. Over 40 species of parasitoids have been recovered from *Liriomyza* spp. leafminers (Waterhouse & Norris 1987), including 20 in Florida (Schuster et al. 1991, Schuster & Wharton 1993) where, in the absence of insecticides, parasitism of the leafminer has ranged from about 65 to 75%. Applications of broad spectrum insecticides like methomyl have resulted in a decline in parasitism followed by an increase in leafminer density (Oatman & Kennedy 1976). Action thresholds for timing insecticide applications have been established for tomato (Pernezny et al. 1996, Schuster et al. 1996). While the extent of parasitism might be taken into account during the sampling process (Schuster et al. 1996), the relationship between leafminer and parasitoid density and subsequent parasitism and host death is not known.

Diglyphus intermedius (Girault) was one of the most abundant parasitoids found attacking *L. trifolii* on tomato in Florida (Schuster & Wharton 1993). The parasitoid is ectoparasitic and prefers third instar leafminers for oviposition. The lifetime fecundity (F) and the lifelong total number of hosts killed (Hm) were found to be functions of temperature (T) and were represented by $F = -196.11 + 42.65T - 1.1T^2$ and $Hm = 721.97 - 19.1T$, respectively (Patel & Schuster 1991). The temperature range used in these experiments was 15.6 to 31.1°C, which should be the limits for making interpretations and predictions. The peak 3 day moving averages of the number of eggs deposited and the number of hosts killed were highest at 23.3 and 26.7°C. The relationship between host and parasitoid densities was not addressed in these experiments. This relationship is often referred to as the functional response and can be either curvilinear (type 2 response) or sigmoid (type 3 response) (as summarized by Price 1997). The plateau of the type 2 response results from the limitation of prey handling at higher prey densities and is characteristic of invertebrate parasitism or predation. With the type 3 sigmoid response, the efficiency or rate of capture increases as the predator learns to find and recognize prey, with a resulting rapid increase in predation. Eventually, a plateau in the number of prey captured is reached as the limitation in prey handling is reached. The type 3 response is characteristic of vertebrate predation, although invertebrates can also demonstrate this response.

The leafminer-parasitoid interaction was a focus of the leafminer population dynamics model proposed by Smerage et al. (1980), which provides an excellent framework for the elucidation of the role of natural enemies in regulating leafminer populations. The model pertained to within-field populations of eggs, larvae, pupae and adults of leafminers and their parasitoids, broadly ex-

pressed as processes that contribute to the overall dynamics of the population. The rate of parasitism in the model was a function of host and parasitoid densities and the relationship between the rate of parasitism and host and parasitoid densities at constant temperature was hypothesized to be an exponential equation generating families of curves at different host and parasitoid densities.

The purpose of the present investigation was to obtain a mathematical description of the functional response of *D. intermedius* at various parasitoid densities when using *L. trifolii* as a host at a constant temperature.

MATERIALS AND METHODS

Several limitations were encountered in the experimental design. Ideally, observations on parasitoids should be made in a large arena which allows the parasitoids to move naturally in and out of the arena. This would enable the parasitoids to behave naturally and to have random access to hosts. Also, the likelihood of encountering the same host again would not be increased due to confinement to a restricted arena. Arena size would certainly be important if the period of observation was long and parasitoids were confined to a small region. Because of the small size of adult *D. intermedius*, it is not possible to monitor adult parasitoid densities in a large arena with confinement, nor is it possible to maintain uniformity in leafminer and parasitoid densities between observation plots and to maintain constant environmental conditions. To overcome these limitations, a compromise was made. Female *D. intermedius* were confined in 67 × 67 × 67 cm cages within a controlled environment room for 12 h observation periods. There were several advantages to using cages. *D. intermedius* density on a per cage basis was easy to record and manipulate, and other parasitoid species could be excluded. The relatively small cage size permitted them to be maintained in a controlled environment room.

Tomato plants, *Lycopersicon esculentum* Mill. cv Hayslip, used in the experiments were approximately 30 days post transplanting, 50 to 60 cm tall with 10 to 13 leaves, and were just beginning to flower. Plants of this size just fit into the above cages and allowed the maximum amount of leaf area possible on one plant confined within a cage. The plants selected had approximately 2,000 to 3,000 cm² leaf area, although on occasion some plants were smaller or larger. Three selected plants were placed in each of two 67 × 67 × 67 cm cages. The number of leafminer adults released into the cages ranged from as few as 10 to as many as 50 and the duration of exposure ranged from as little as one minute to as long as 4 h. By manipulating the number of adults and the exposure period, leafminer larval densities ranging from 0 to 0.06 larvae/cm² were obtained. The range then

was divided into six classes of equal size for experimentation. After exposure to leafminer adults, the plants were removed, examined for leafminer adults (which were removed, if present) and moved to cages of similar size in a temperature-controlled room at 25-27 °C and a photoperiod of 14:10 (L:D) h. After five d, the five plants most similar in height, quality and density of third instar leafminers were removed at 0800 h and placed individually into each of five 67 × 67 × 67 cm cages.

The parasitoid adults used for the experiments were collected from a laboratory colony maintained on *L. trifolii* on tomato (Patel 1987). Ten females and five males were confined in each of four, 150 × 15 mm Petri dishes. Each dish was provisioned daily with 40 to 60 third instar leafminers in tomato leaflets. On the fourth day, female parasitoids were isolated singly in 00 gelatin capsules and the males were discarded. Thus, the females were at the age of peak oviposition and peak host mortality inducement (Patel & Schuster 1991). One, two, three, four, or five parasitoid females were then released into each of the five cages holding the leafminer-infested plants. After 12 h (2000 h) the plants were removed from the cages, shaken to dislodge parasitoids and transferred to the laboratory where plant height and age, and the numbers of leaves and leaflets were recorded. The total leaf area, the area of leaflets containing leafmines and the area of leaflets containing parasitized leafminer larvae were measured (LI-3000, LiCor®, Lincoln, NE). The numbers of live and paralyzed (or dead) larvae were recorded in each of three categories: old leaves (usually the first three leaves which showed signs of yellowing), fully expanded leaves (the majority of leaves) and non-expanded leaves (usually the top three to four leaves, although occasionally there may have been several more in a small, tight bundle at the plant apex). Leafmines containing paralyzed (or dead) larvae were dissected and the number of parasitoid eggs deposited on each leafminer larva was recorded. Paralyzed or dead leafminer larvae on which no parasitoid eggs were deposited were categorized as parasitoid-induced mortality because in previous studies (Patel & Schuster 1991) no mortality of leafminer larvae was observed in the absence of parasitoid females.

The experiment was repeated four times. The assignment of parasitoid density to the cages was rotated so that the same cage had the same parasitoid density every fifth time. Because the plants varied in leaf area, and leafminer oviposition could not be made uniform on each plant, it was not possible to regulate leafminer density as uniformly as parasitoid density. The number of leafminer larvae on each plant, and, hence, in each cage, could have been kept the same by destroying some larvae on each plant; however, this would

not have been appropriate for two reasons. Parasitoids searching a greater leaf area with the same number of leafminers per cage would take longer than for the same number of leafminers on a smaller leaf area. Also, destroying leafminer larvae may elicit its own response from the parasitoid adults, if the adults cue in to host plant damage in locating leafminer larvae.

To study the survival of parasitoid larvae to adulthood as influenced by the initial density of parasitoid eggs per third instar leafminer, parasitized third instar leafminers and associated parasitoid eggs were dissected from real leafmines obtained from the *D. intermedius* colony and were placed in artificial mines (Patel & Schuster 1983). The artificial mines consisted of a piece of construction paper with a 7 mm hole and a piece of filter paper, both equal in dimensions to a microscope slide, sandwiched between a glass microscope slide and a cover slip. Parasitoid eggs, less than 24 h old, were dissected from leafmines containing parasitized leafminer larvae and placed next to the larvae in the artificial mines at densities of one, two, three, or four eggs per larva. Each artificial mine was sealed with sticky tape and kept in a 100 × 15 mm Petri dish containing a water moistened filter paper to provide relatively high humidity in order to prevent desiccation of the leafminer larva and the parasitoid egg(s). The number of host larvae utilized was 12 with one egg per larva, six with two eggs per larva, four with three eggs per larva, and three with four eggs per larva. Thus, there were 12 eggs at each egg density. The Petri dishes were maintained at the same conditions as the leafminer per parasitoid density study and the filter paper in each Petri dish was moistened daily until no more parasitoid adults emerged. The experiment was replicated five times and the number of parasitoids emerging from each mine was recorded.

The NLIN procedure (SAS Institute 1982) was utilized to determine the parameters for the hypothesized exponential equations describing the relationships between leafminer larval density and the rate of parasitism and the rate of parasitoid-induced mortality at various parasitoid densities. Chi square tests were performed to determine if there was any effect of altering either leafminer or parasitoid density on the numbers of parasitoid eggs on paralyzed host larvae. The ANOVA procedure (SAS Institute 1982) was utilized to determine if any relationship existed between the initial total number of parasitoid eggs placed at each egg density and the total number of individuals reaching the adult stage at each egg density in the artificial mines.

RESULTS

The exponential equation obtained using the nonlinear regression to express the relationship

between host and parasitoid density and parasitism was

$$Y = Kp \cdot n_2 \cdot (1 - \exp(-n_1/Kh)) \quad (r^2 = 0.79)$$

where Y, the rate of parasitism, is the number of hosts parasitized per day. Kp is a constant of 7.3908 hosts parasitized per parasitoid-day and n_1 and n_2 are the densities of leafminer larvae and parasitoid adults (numbers/cm² leaf area), respectively. Kh is a constant of 0.0144 leafminer larvae/cm² leaf area. The exponential equation expressing the relationship between host and parasitoid density and parasitoid-induced host mortality was

$$Z = Cp \cdot n_2 \cdot (1 - \exp(-n_1/Ch)) \quad (r^2 = 0.78)$$

where Z, the rate of parasitoid-induced mortality, is the number of leafminer larvae killed per day. Cp is a constant of 9.2064 hosts killed per parasitoid-day and Ch is a constant of 0.0165 leafminer larvae/cm² leaf area.

The hypothesized relationship between host density, parasitoid density and the rate of parasitism utilizing the above equation is depicted in Fig. 1 and the hypothesized relationship between the rate of parasitoid-induced mortality and host and parasitoid density is depicted in Fig. 2. The observed rate of parasitism at a particular parasitoid density was always lower than the observed rate of parasitoid-induced mortality. These results confirmed previous findings that *D. intermedius* killed more hosts than it parasitized (Patel & Schuster 1991); however, the proportion of hosts killed that were also parasitized was not the same at all leafminer densities. In the 0-0.0099 host larvae/cm² range, 24 of 38 observations (63%) had a 100% parasitization rate while only 5 of 19 (26%) and 3 of 33 (10%) observations had 100% parasitization of the killed hosts when leafminer larval densities ranged from 0.01 - 0.0199 and 0.02 - 0.06 larva/cm² leaf area, respectively.

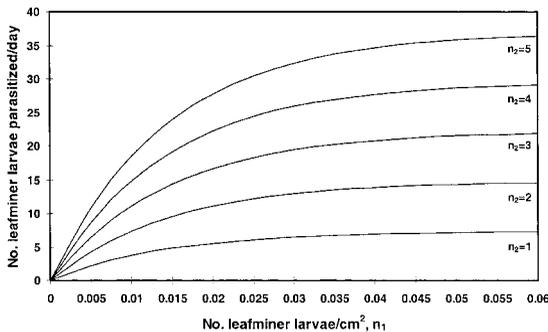


Fig. 1. The relationship between density of *Liriomyza trifolii* larvae (n_1) and parasitism rate at different densities of the parasitoid *Diglyphus intermedius* (n_2 , number of females/plant).

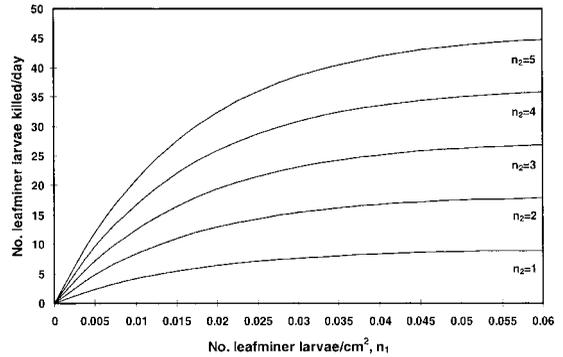


Fig. 2. The relationship between density of *Liriomyza trifolii* larvae (n_1) and rate of parasitoid-induced mortality at different densities (n_2) of the parasitoid *Diglyphus intermedius* (n_2 , number of females/plant).

D. intermedius did not always deposit a single egg per host as was assumed by Smerage et al. (1980) (Table 1). A χ^2 test of the data in Table 1 suggested a significant relationship between the number of parasitoid eggs per host and host density ($\chi^2 = 67.62$; $df = 25$; $P < 0.001$), while grouping the data according to parasitoid density suggested no significant relationship between the number of parasitoid eggs and parasitoid density ($\chi^2 = 15.6$; $df = 20$; $P > 0.70$). Increasing the number of artificially established parasitoid eggs per host larva from one to four resulted in decreasing numbers of individuals (of 12) surviving to adulthood from about 10 to three (Table 2). The difference in the number of parasitoids surviving per leafminer larva was significantly different between initial egg densities of one and three eggs per leafminer larva; however, there was little difference biologically in the numbers surviving per larva. Regardless of the initial density of parasitoid eggs per leafminer larva, generally one parasitoid survived per leafminer larva.

DISCUSSION

The results of this investigation clearly demonstrate that both parasitism and parasitoid-induced mortality of *L. trifolii* by *D. intermedius* are representative of the type 2 functional response. This suggests that handling time becomes a limitation to parasitization and host-killing as host density increases. The curvilinear description of the functional response further suggests that increased experience of *D. intermedius* with its host did not result in an increased rate of host discovery or decreased rate of host handling, i.e. learning. However, the female parasitoids used in these experiments already had been exposed to leafminer larvae for four days when the experiments were initiated. It is possible that naive females would exhibit a type 3 functional response. Even if this were so, the effect would be expected to di-

TABLE 1. FREQUENCY DISTRIBUTION OF THE NUMBERS OF LARVAE OF THE LEAFMINER, *LIRIOMYZA TRIFOLII*, KILLED BY THE PARASITOID, *DIGLYPHUS INTERMEDIUS*, IN CLASSES OF DIFFERENT EGG DENSITIES OF THE PARASITOID AT DIFFERENT DENSITIES OF LEAFMINER LARVAE.

Leafminer larvae/cm ² leaf area	No. parasitoid eggs/killed leafminer host larva						Total
	0	1	2	3	4	4+	
0.00 - 0.0099	19	108	60	19	11	16	233
0.01 - 0.0199	42	189	74	31	8	6	350
0.02 - 0.0299	49	163	50	11	10	3	286
0.03 - 0.0399	45	180	46	10	2	2	285
0.04 - 0.0499	43	135	43	9	1	0	231
0.05 - 0.0600	16	40	13	5	0	1	75
0.00 - 0.0600	214	815	286	85	32	28	1460

minish within at least four days, if not sooner. The functional response was studied in a room maintained at 25-27°C, using four-day-old parasitoids. These conditions of temperature and parasitoid age are ideal for estimating the maximum rate of parasitism. Both parasitoid age and temperature affect the rate of parasitism (Patel & Schuster 1991). Altering temperature and utilizing parasitoid females younger or older than four days old, unless naive females behave differently, would likely affect the observed rates of parasitism but not the exponential nature of the relationship between host and parasitoid density.

The term host-feeding was not used in this study to describe parasitoid-induced mortality because, as was shown in a previous study (Patel & Schuster 1991), *D. intermedius* kills more hosts than it parasitizes and because killed larvae can be used for oviposition or host-feeding or can be rejected, as was the case for *D. begini* (Heinz & Parrella 1989). In this latter study, the proportion of stung hosts in each category varied depending upon the host size distribution which the parasitoids were provided. When a large host size distribution (third instars) was encountered, 35% of the larvae were killed without oviposition (20% for host-feeding), while when a small host size distribution (late second and early third instars) was encountered, 62% were killed (18% for host feeding). Thus, the proportion of larvae used for host-

feeding remained fairly constant, while the proportion rejected increased as the frequency of small larvae increased. In a later study, Heinz & Parrella (1990a) observed that *D. begini* killed 1.3 *L. trifolii* larvae for every larva used for oviposition (about 23%) and made no distinction as to whether the excess larvae were used for host-feeding. Minkenberg (1989) determined that the proportion of *L. bryoniae* (Kaltenbach) larvae attacked by *Diglyphus isaea* (Walker) and used for host-feeding varied from 15 to 40%; however, he considered any stung host without oviposition to have been fed upon. In the present study, all *L. trifolii* larvae were large (third instars); nevertheless, the number of larvae killed per day always exceeded the number of larvae parasitized. About 15% of the larvae killed in the experiment were not used for oviposition (Table 1), which is lower than the total host-kill observed by Heinz & Parrella (1989) and Minkenberg (1989) but is about the same as the total host-kill observed by Heinz & Parrella (1990a) and about the same as the percentage used for host feeding observed by Heinz & Parrella (1989). The differences could be due to the larger arena used in the present study (67 × 67 × 67 cm cages) compared to the smaller arenas used by Heinz & Parrella (1989) (9 cm diam Petri dishes and 11.5 cm diam by 13.2 cm high cylindrical cages) and Minkenberg (1989) (7.5 cm diam by 61 cm high cylindrical cages). The cage size used

TABLE 2. SURVIVAL OF EGGS OF THE PARASITOID, *DIGLYPHUS INTERMEDIUS*, TO ADULTHOOD WHEN PLACED AT DIFFERENT DENSITIES OF LARVAE OF THE LEAFMINER, *LIRIOMYZA TRIFOLII*.

Initial egg density/host larva (12 eggs at each density)	Mean no. eggs surviving to adulthood	Survivorship of eggs/larva
1	9.6 a	0.80 b
2	6.6 b	1.10 ab
3	6.0 b	1.35 a
4	3.0 c	0.98 ab

Means in a column followed by the same letter are not significantly different at the $P = 0.05$ level, Duncan's multiple range tests.

by Heinz & Parrella (1990a) (50 × 50 × 50 cm) was similar to the cage size in the present study. Heinz & Parrella (1990a) also believed that the lower percentage of host-killing that they observed relative to other studies was due to the use of a larger cage. In the present study, the proportion of larvae killed in the absence of oviposition increased from 8 to 20% as the host density increased (Table 1). It is clear from the present study with *D. intermedius* and with the above studies with other species of *Diglyphus* that more hosts are killed than are needed for oviposition, that at least some are used for host-feeding, and that some may be rejected. While host-feeding can provide parasitoid females with necessary nutrients for egg development, the benefit of host rejection is less apparent, particularly in light of the energy and time expended in the process. Perhaps host rejection is a mechanism for managing the density of leafminer larvae on individual leaflets, thus ensuring that a leaflet containing parasitized larvae will not be lost to the leafmining of surviving, non-parasitized larvae on the same leaflet. Excessive leafmining can cause desiccation, necrosis and abscission of tomato leaflets, thus potentially resulting in reduced survival of parasitoid larvae.

Little is known regarding the ability of *Diglyphus* species to locate infested leaflets relative to non-infested leaflets; however, other species of parasitoids attacking *Liriomyza* spp. have been shown to have discriminatory behaviors. In flight tunnel and olfactometer studies, Pettitt et al. (1992) demonstrated that females of *Opius dissitus* Musebeck responded preferentially to olfactory cues emanating from foliage infested with larvae of *L. sativae* Blanchard. A greater proportion of *Dacnusa sibirica* Telenga flew upwind in no-choice flight tunnel experiments when leafminer infested plants were placed upwind (Dicke & Minkenberg 1991). When given a choice, female *D. begini* landed on leaves mined by *L. trifolii* more than on leaves not mined (Heinz & Parrella 1990a). Although the present cage experiments were not designed specifically to study host-finding behavior, some deductions regarding the effect of spatial heterogeneity of host larvae on parasitism rate can be made. In 68 of 79 observations, the leaflet with the greatest number of leafmines was encountered by parasitoid adults as indicated by at least one leafminer larva being parasitized on that leaflet. In another four observations, the parasitoids similarly found and oviposited on larvae in the leaflet with the next highest leafminer density. In the remaining seven observations, the maximum number of leafminer larvae on the leaflets was four. These observations suggest that either the parasitoids were more attracted to leaflets that were more heavily mined or that, once landing on a leaflet, the parasitoids were more likely to encounter a host if the host density

on that leaflet was higher. Which behavior, or maybe both, that was exhibited cannot be determined because the area of each leaflet and the distance between individual leafminer larvae were not measured.

Heinz & Parrella (1990b) observed rates of superparasitism of 0 and 3.1% when *D. begini* was released in greenhouses for control of *L. trifolii*. These rates are much lower than the 35% superparasitism observed in the present study (Table 1). This much higher rate of superparasitism could have resulted from the confinement of *D. intermedius* in cages. Parrella et al. (1989) observed that superparasitism of *L. trifolii* by *D. begini* would occur in the rearing method they developed but that the extent was not known. They further observed that, if two parasitoid eggs were deposited adjacent to a third instar leafminer, two adults would be produced, although they would be smaller. In the present study, only one parasitoid generally completed development per leafminer larva, regardless of the initial parasitoid egg number per host (Table 2). Furthermore, the number of eggs surviving to adulthood declined as the initial parasitoid egg numbers per host increased. Thus, superparasitism by *D. intermedius* represents a waste of resources and an impediment to parasitoid population increase and, ultimately, to biological control of *L. trifolii*.

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DAMAGE BY INFESTATIONS OF TEXAS CITRUS MITE (ACARI: TETRANYCHIDAE) AND ITS EFFECT ON THE LIFE OF 'VALENCIA' LEAVES IN AN IRRIGATED CITRUS GROVE

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ABSTRACT

Studies were conducted during 1996-1999 to evaluate damage to citrus leaves by the Texas citrus mite, *Eutetranychus banksi* (McGregor), and its impact on leaf longevity in irrigated citrus. Natural mite infestations were followed in a citrus orchard ('Rhode Red Valencia') under irrigation management, and damage to leaves and leaf abscission were assessed periodically. The number of feeding stipples per cm² on the upper leaf surface was used as an index of feeding damage. A variable 'mite-days' [average number of mites per leaf multiplied by the number of days of infestation] was used to characterize infestation densities over time. Increases in average numbers of stipples per cm² per leaf (Y) across different mite-day values (X) were described by the equation $Y = 44.08 + 0.59X$ ($r^2 = 0.57$). A model including temperature was marginally better. The final mean density of feeding stipples on infested leaves for the 1996, 1998 and 1999 evaluation periods averaged 327, 134 and 873 per cm², respectively, with an overall mean of 470. Leaf life from the date of full expansion until abscission averaged 443, 387 and 380 days for the respective periods, with an overall average of 399 days. The observed life of the leaves was typical to what has been observed in Florida citrus. Overall, no significant negative relationship was found between leaf life and mite damage. The study indicated that damage by Texas citrus mites to 'Valencia' citrus leaves promoted little or no premature leaf abscission in irrigated trees.

Key Words: Citrus red mite, *Panonychus citri*, leaf abscission, damage assessment, Florida citrus

RESUMEN

Estos estudios fueron conducidos durante 1996-1999 para evaluar el daño en las hojas de cítricos causado por el ácaro téjano de cítricos, *Eutetranychus banksi* (McGregor), y su impacto sobre la longevidad de las hojas de cítricos en huertos irrigados. Las infestaciones naturales de los ácaros fueron observadas en un huerto de cítricos de la variedad 'Rhode Red Valencia' bajo el sistema de irrigación, y se evaluaron periódicamente el daño y la desprendimiento de las hojas. El número de picaduras por cm² sobre la superficie superior de la hoja fué usado como un índice del daño causado por la alimentación. Una variable 'días-de ácaros' [el promedio del número de ácaros por hoja multiplicado por el número de días de infestación] fué usada para caracterizar la densidad de la infestación sobre el tiempo. El aumento en el número promedio de las picaduras por cm² por hoja (Y) a través de diferentes valores de "días-de ácaros" (X) fué descrito por la ecuación $Y = 44.08 + 0.59X$ ($r^2 = 0.57$). Un modelo incluyendo la temperatura fué ligeramente mejor. El promedio final de la densidad de las picaduras en las hojas infestadas en los años 1996, 1997 y 1999 fué 327, 134 y 873 por cm², respectivamente, con un promedio total de 470. La vida de la hoja desde la expansión completa hasta el desprendimiento duró un promedio de 443, 387 y 380 días para los períodos respectivos, con un promedio total de 399 días. La longevidad de las hojas observadas fué típica de lo que fué observado en los cítricos de Florida. Sobre todo, no se encontró una relación negativa significativa entre la longevidad de la hoja y el daño hecho por los ácaros. El estudio indicó que el daño causado por el ácaro téjano de cítricos a las hojas de cítricos 'Valencia' promovió poco o nada el desprendimiento prematura de las hojas en arboles irrigados.

Of the four spider mite species (Acari: Tetranychidae) reported to infest Florida citrus, the Texas citrus mite (*Eutetranychus banksi* (McGregor)) and the citrus red mite (*Panonychus citri* (McGregor)) are the most prevalent and important spider mite pests (Childers 1994). The Texas citrus mite lives and (presumably) feeds almost exclusively on the upper leaf surface (Childers et al. 1991) while the citrus red mite

may live and (presumably) feed on either leaf surface (Muma 1961, Jones & Parrella 1984). Feeding by these spider mites on the upper leaf surface results in small, whitish or light-colored stipples within the palisade leaf layer where cytoplasmic contents including chlorophyll are removed (Albrigo et al. 1981). When leaves are heavily damaged by spider mites, mesophyll collapse may occur and leaves may abscise prema-

turely, particularly during dry, windy conditions (Browning et al. 1995, Hare & Youngman 1987). Whether mite damage and/or premature leaf abscission promotes economic losses in Florida citrus has never been documented but considered probable.

The stipple damage associated with spider mite injury to the upper surface of leaves serves as an index of the intensity of mite damage. Individual stipples are small, ranging from around 0.04 to 0.52mm in diameter (mean 0.172mm, SEM 0.025; 'Valencia' leaves; damage by *E. banksi* and/or *P. citri*) (Hall, unpublished). Variation in the size of individual stipples may be a function of how long a mite feeds and the developmental stage of a mite as well as other factors including leaf age and leaf tissue characteristics. Whether or not each individual stipple is always the result of a single feeding wound is not known. Individual stipples sometimes are so close to each other that they coalesce, and as the density of stipples increases, incidences of coalescence increase. Spider mite damage to a leaf may not be apparent to the naked eye until stipple densities exceed densities of 100 to 150 stipples per cm². In rating damage by the naked eye, visual damage ratings of faint, light, moderate and heavy damage may generally be associated with stipple densities of around 200, 400, 1,000 and 1,800 stipples per cm², respectively.

Little is known regarding the quantitative relationship between Texas citrus mite or citrus red mite infestation densities over time and resulting amount of leaf stippling damage to Florida citrus, information which could be helpful in establishing management guidelines. Economic thresholds for the citrus red mite in California during the 1980s (2 to 4 adult female mites per leaf depending on the time of year and density of predatory mites) were based largely on preventing excessive stipple damage in the absence of more appropriate information on the relationship between damage and economic losses (Pehrson et al. 1984, Hare & Youngman 1987, Hare et al. 1990). Whether these thresholds are appropriate for preventing excessive damage by infestations of Texas citrus mites or citrus red mites in Florida citrus is not known. Although premature leaf abscission may be a major concern with damage by these mites, particularly if trees with damaged leaves are subjected to adverse environmental conditions (e.g., drought and hot windy weather), quantitative data are lacking on the relationship between mite damage and premature leaf abscission in Florida citrus.

Presented here are the results of quantitative assessments of (1) the relationship between Texas citrus mite infestations over time and resulting damage to 'Valencia' citrus leaves and (2) the influence of Texas citrus mite damage on leaf abscission in 'Valencia' in irrigated citrus.

MATERIALS AND METHODS

Four cohorts of 100 flush citrus leaves ('Rhode Red Valencia') were studied during 1996-1999 at a well-managed, irrigated orange grove on a flatwoods (sandy spodosol) soil in Hendry County, Florida. The full-expansion dates for leaves of the four cohorts were approximately 1 September 1996 (trees 4.3 years old); 1 October 1997 (trees 5.4 years old); 1 October 1998 (trees 6.0 years old); and 1 March 1999 (trees 6.5 years old). The trees were planted on two-row beds with a tree spacing of 3.7m and row spacing of 7.6m. For each cohort, 50 newly-expanded flush leaves were tagged along the bed side of one row of trees and 50 were tagged along the bed side of the adjacent row along the same bed (one or two tagged leaves per tree; in cases where two were tagged per tree, these were 0.6 to 0.9m apart) All tagged leaves were 0.6 to 1.8m above the ground and near the outside of the canopy. Leaves of the 1999 cohort were tagged along a bed next to the bed where the 1998 cohort of leaves was tagged. The length of each leaf from the base (excluding the petiole) to the tip and the width at the widest point of each leaf were measured (cm). Leaf area (one surface) in cm² (Y) was estimated from leaf length (χ_1) and width (χ_2) using the following equation: $Y = 1.88 + 0.195(\chi_1)^2 + 0.487(\chi_2)^2$, $r^2 = 0.94$, $n = 100$ (Hall, unpublished).

Leaves were examined weekly to identify spider mites present on the upper leaf surface. For each species present, the number of spider mites (excluding eggs) was counted. Within each cohort of leaves, mite damage to 20 leaves was limited by periodically wiping mites off with a soft damp cloth or by misting them with either a 5% petroleum oil (FC-435-66) in water solution or a fenbutatin-oxide 50W treatment (0.6 g per 500ml water). For each cohort of leaves, numbers of mites per leaf were studied for 3 to 4 months, after which weekly mite counts were discontinued and all leaves were individually treated with the fenbutatin-oxide treatment to eliminate mites. Thereafter until abscission, each leaf was periodically treated with either the oil or fenbutatin-oxide treatment to prevent further mite infestations.

In addition to counting mites on the upper surface of leaves, damage by mites to the upper leaf surface was quantified weekly on 52, 40 and 50 infested leaves for the 1997, 1998 and 1999 cohorts. Early during the development of mite infestations on the leaves of each cohort, few infested leaves were available for damage evaluations. When numbers of infested leaves increased to more than 20, we split the leaves into two groups and evaluated them biweekly, one group evaluated one week and the second group the following week. When the number of infested leaves exceeded 40, we split the leaves into three groups and evalu-

ated damage to each group every three weeks, one group per week. The average density of stipples per cm² across the upper leaf surface was used as the measure of mite damage. Damage by mites to individual leaves was assessed beginning on the first day mites were observed on the leaves and continued until all leaves of a cohort were treated to eliminate mites. After all leaves had been treated with fenbutatin-oxide to eliminate mites, a final estimate of the average stipple density was made for every leaf within each cohort. For the 1996 cohort of leaves, the average density of stipples on each leaf was estimated only after appreciable damage had occurred to leaves and mites had been controlled. For all cohorts, estimates of average stipple densities per leaf were made by counting the number of stipples per cm² at each of 10 sites uniformly spaced across the upper surface of each leaf. To count stipples, an Edmond Scientific comparator (12X transparent base magnifier with 27mm contact reticule adapter ring, Kellner-type/AR coated lens, reticule with 1-cm² grid of 100 squares, Edmond Optics, Barrington, NJ USA) was placed against or just above the leaf surface. The leaf and magnifier were held so that as much light as possible illuminated the leaf surface being examined. All stipples within the grid were counted when there were less than approximately 100 stipples within the grid; however, when there were more than approximately 100 stipples within the grid, the number of stipples within a sub-sample of ten squares of the grid was counted and multiplied by ten to estimate the total number per cm². At high stipple densities per cm² (e.g., 1,500 or more), stipples sometimes coalesced, making it difficult to estimate the actual number present. In this case, the number of individual stipples constituting an area of coalesced stipples had to be estimated based on the average diameter of surrounding or nearby individual stipples. Leaves with dust or sooty mold were gently cleaned using a soft cloth dampened with water before counting stipples (the entire surface when no mites were present or 10 spots each approximately 1-cm² in size if mites were present). For final stipple counts, the entire upper surface of each leaf was gently cleaned with water, after which the magnifier was placed against the wet leaf surface.

Records were maintained for each leaf of each cohort on the incidence of injury by citrus leaf-miner (*Phyllocnistis citrella* Stainton) and citrus rust mites (*Phyllocoptruta oleivora* (Ashmead) and *Aculops pelekassi* (Keifer)), infection by greasy spot (*Mycosphaerella citri* Whiteside), nutritional disorders, mesophyll collapse, freeze damage and hail injury. The percentage surface area infected by greasy spot was estimated for 1998 leaves on 28 May 1999 and for 1999 leaves on 17 February 2000. Air temperature, rainfall, evaporation and wind data during the study period were obtained

from the Corp of Engineer's Moore Haven Lock 1 weather station about 4.8 km north of the study sites. Exceptional environmental events during the study were noted.

Relationship Between Mite Density and Damage

The quantitative relationship between spider mite densities per leaf (upper surface) and resulting damage to the upper surface of leaves was investigated by comparing the average mite density on a leaf over a period of time to the increase in average stipple density over the same period of time (data from cohorts 1997, 1998 and 1999). For each infestation period on each leaf, the average number of mites per leaf was calculated by averaging the numbers of mites observed on different observation dates during the infestation period. The duration of mite infestation (days) was determined by the number of days between the first and last observation dates during the infestation period. An infestation density/duration variable 'mite days' (see Allen 1976, Yang et al. 1995) was calculated for each infestation on each leaf: 'mite days' = (average number of mites per leaf) * (number of days). The resulting damage caused by mites feeding during each infestation period on each leaf was estimated by subtracting the mean number of stipples per cm² present at the beginning of the period from the mean number present at the end of the period. A linear regression analysis was conducted between the increase in mean stipple densities per cm² per leaf and 'mite days' for leaves of each cohort and over all three cohorts. Correlation analyses were conducted between the following variables: increases in stipple densities; 'mite days'; leaf area; mean, maximum and minimum daily air temperatures; daily rainfall; daily evaporation; and daily wind. Stepwise regression analyses were then conducted using variables significantly correlated ($P \leq 0.05$) with increases in damage to select a multiple regression model for predicting damage.

Relationship Between Mite Damage and Leaf Abscission

The leaves of three cohorts (1996, 1998 and 1999) were examined every 2 to 5 weeks (mean 20.7 days, SEM 2.1 days) after mite infestations were controlled to determine when the leaves abscised. The abscission date was estimated using the mid date between the date abscission was discovered and the date a leaf was last observed on a tree. 'Leaf life' was approximated as the period of time from the date of full expansion until the abscission date. 'Life after attack' by mites was approximated as the period of time between a leaf's mean infestation date (weighted on infestation densities across successive infestation dates) and its abscission date.

To determine if spider mite damage promoted premature abscission, linear regression analyses were conducted between 'leaf life' and damage (average number of stipples per cm²); and between 'life after attack' and damage (average number of stipples per cm²). Analyses on 'life after attack' were restricted to leaves on which mites were observed on at least three successive sample dates. Leaves with disorders such as damage by other arthropod pests, nutritional problems, freeze damage and hail injury were excluded from all analyses. Correlation analyses were conducted to investigate the relationship between 'leaf life' and each of the following variables: average number of stipples per cm²; mean surface area with greasy spot infection; mean, maximum and minimum daily air temperature; daily wind; daily rainfall; daily evaporation; and interaction effects between mite damage and each of the other independent variables. For each date on which leaf abscissions were discovered (i.e., an abscission event had occurred), the percentage of abscised leaves within each cohort was calculated. Correlation analyses were then conducted to investigate the relationship between percentage leaf drop and the aforementioned variables. For all correlation analyses, air temperature, wind, rain and evaporation data were averaged over a period of within 40 days prior to discovering leaf abscission. For variables which were significantly correlated ($Pr > |r| \leq 0.05$) with 'leaf life' and 'life after attack', stepwise regression analyses were conducted to select regression models for predicting the life of leaves damaged by spider mites.

RESULTS AND DISCUSSION

Both Texas citrus mites and citrus red mites were observed on leaves during this study, with Texas citrus mites being the predominant species (Table 1). Spider mites densities were greater on the 1996 and 1999 leaf cohorts (e.g., means of 18.8 and 21.9 Texas citrus mites per leaf, respectively) than on the 1997 and 1998 cohorts (e.g., means of 5.1 and 4.0 Texas citrus mites per leaf, respectively) (Table 1). Among the three fall flush cohorts, mite densities on leaves during the first several months after leaf expansion were greater during 1996 than either 1997 or 1998 and, based on correlation analyses (not presented), these infestation density differences were attributed to less rainfall during these months in 1996 based on rainfall at the weather station. This is consistent with Pratt & Thompson (1953), who previously reported a negative relationship between rainfall and citrus spider mite levels. During each of the four flushes investigated in this study, spider mite infestations generally began to develop within 1 to 3 months after leaves had fully expanded (Fig. 1). Leaves of the 1998 cohort, which were present on trees and being monitored when

TABLE 1. POPULATION DENSITIES OF TEXAS CITRUS AND CITRUS RED MITES OBSERVED DURING THE STUDY.

Cohort	Number of leaves	Sample period	Total number of leaf samples	Spider mite species observed	Number of leaf samples mites observed	Mean (SEM) number mites per leaf sample	Maximum number mites per leaf sample
Sep 1996	100	Sep '96-Jan '97	933	Texas mites	363	18.8(1.4)	241
				Red mites	22	1.4(0.2)	4
				Both mites	55	20.7(3.4)	122
Oct 1997	100	Oct '97-Mar'98	2088	Texas mites	88	5.1(0.8)	41
				Red mites	30	1.6(0.2)	4
				Both mites	7	8.6(2.4)	18
Oct 1998	100	Oct '98-Jan '99	1779	Texas mites	177	4.0(0.4)	43
				Red mites	51	1.7(0.2)	9
				Both mites	38	7.4(1.3)	43
Mar 1999	100	Mar '99-Apr '99	786	Texas mites	335	21.9(1.7)	236
				Red mites	26	4.2(2.0)	52
				Both mites	73	24.2(4.3)	237

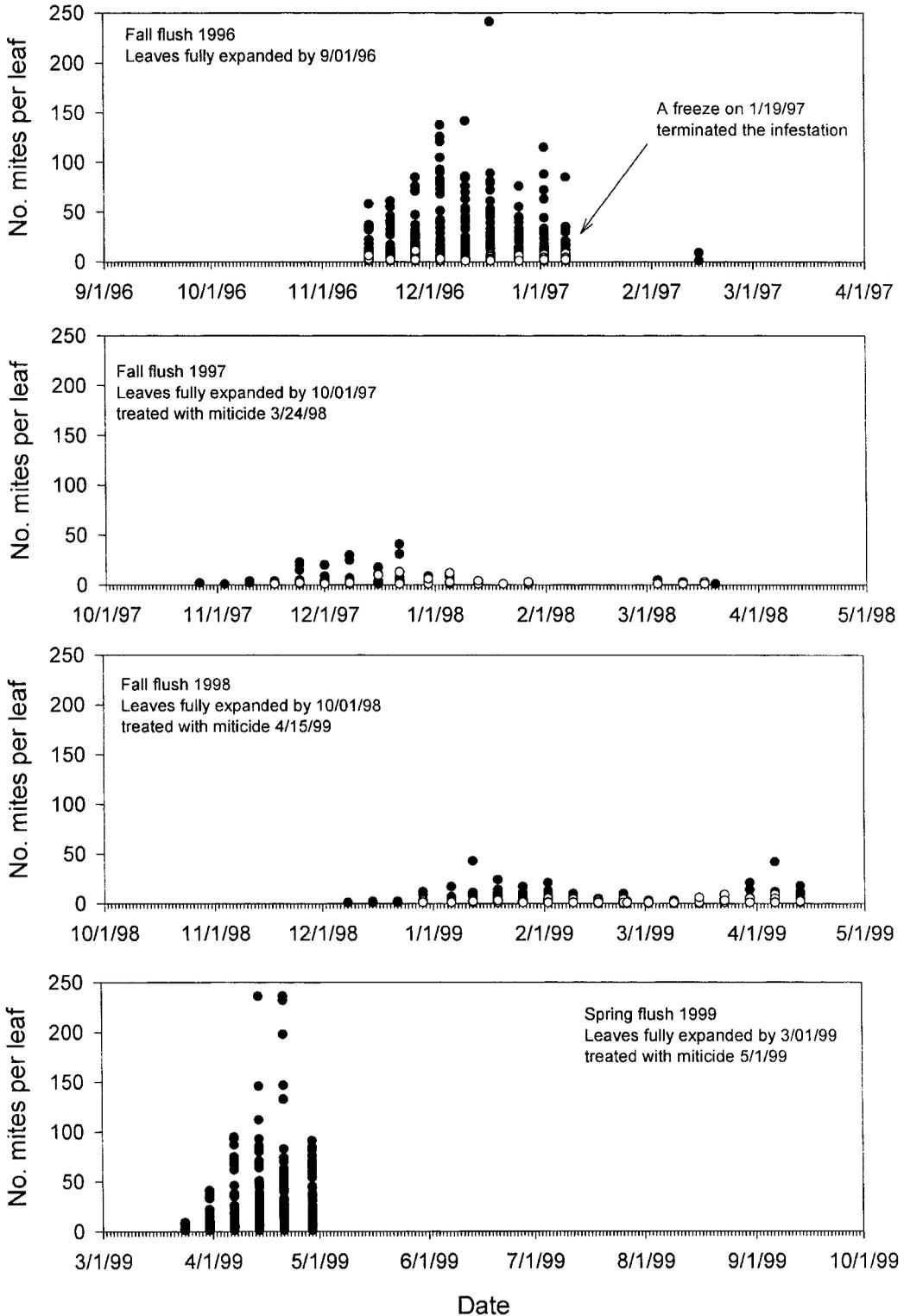


Fig. 1. Spider mite infestations (densities per leaf and dates) observed during the study (Texas citrus mite densities solid data points, citrus red mite densities open data points).

research was initiated on the 1999 cohort, were infested by lower densities of spider mites than the leaves of the 1999 cohort during March and April 1999. The reason mites were more abundant on the younger leaves was not known but could have been related to factors such as microclimate, leaf nutrition or biological control.

Relationship Between Mite Density and Damage

Since only a few citrus red mites were observed during this study, analyses on the quantitative relationship between mite infestations and resulting damage were restricted to leaves known to be infested solely by Texas citrus mites. Data for a total of 131 individual leaf infestations of Texas citrus mites were subjected to analyses comparing leaf infestation densities/durations to resulting damage, with data for 58, 34 and 39 individual infestations from the 1997, 1998 and 1999 cohorts, respectively. Overall, these infestations averaged 21.9 days in duration (SEM 0.5 days) at a mean density of 7.8 Texas citrus mites per leaf (SEM 1.3 mites).

A statistically significant relationship was found between infestations ('mite days') of Texas citrus mites and resulting increases in densities of stipples per cm^2 for each of the 1997, 1998 and 1999 cohorts. Over all 3 cohorts, increases in the mean density of stipples per cm^2 per leaf (Y) were related to 'mite days' (X) by the equation $Y = 44.08 + 0.59 X$ ($r^2 = 0.57$, $F = 168.8$, $\text{Pr} > F = 0.0001$, $\text{df} 130$, slope SEM = 0.045) (Fig. 2). The intercept parameter 44.08 (significantly greater than zero, $t = 2.7$, $\text{Pr} > t 0.008$) reflected the presence of stipples which could not be attributed directly to mites observed on leaves. This may have been a result of mites moving from leaf to leaf or being subjected to mortality factors prior to leaf observations.

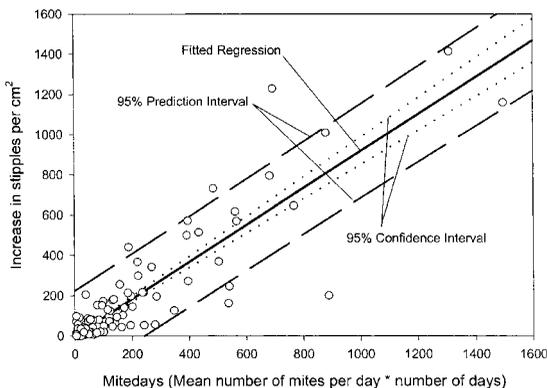


Fig. 2. Relationship between increases in feeding damage (mean stipple density per cm^2 per leaf) and mite days (mean number mites per leaf times the number of days of infestation) on leaves infested by Texas citrus mites, data from 1997-1999 combined.

Stepwise regressions indicated that increases in the mean density of stipples per cm^2 per leaf (Y) were best described by a multiple regression model based on 'mite days' (X_1) and maximum daily air temperatures at the weather station (X_2): $Y = -414.6 + 0.516X_1 + 17.9X_2$, $r^2 = 0.60$, $F = 94.4$, $\text{Pr} > F = 0.0001$, $\text{df} 130$. Texas citrus mites therefore caused more damage as temperature increased. The correlations between observed and estimated increases in damage were similar with respect to the multiple regression model ($R = 0.77$, $\text{Pr} > |R| = 0.0001$) and the simple model based only on 'mite days' ($r = 0.75$, $\text{Pr} > |r| = 0.0001$). A strong statistical relationship existed between estimates from the simple model (Y) and the multiple regression model (X): $Y = 6.89 + 0.95X$, $r^2 = 0.95$, $F = 2,512.8$, $\text{Pr} > F = 0.0001$, $\text{df} 130$. Based on the slope 0.95, the inclusion of maximum daily temperatures only marginally improve estimates across the temperatures observed during our study. Over all three cohorts, leaf area and increases in mite damage were negatively correlated, but regression analyses indicated leaf area was not a significant variable in predicting damage. The leaves studied were fairly uniform in size, with leaf area averaging 40.5 cm^2 (SEM = 1.2, $n = 127$). It remained probable that a given density of mites would cause more damage over a given period of time to small leaves than to large leaves.

Relationship Between Mite Damage and Leaf Abscission

Leaf disorders observed during the study included damage by citrus rust mites (species not identified), leaf miners and some other leaf-feeding insects, greasy spot disease, freeze damage, and hail injury. Although little damage by rust mites occurred during the study, two leaves of the 1996 cohort and one leaf of the 1999 cohort were dropped from leaf life assessments due to rust mite injury. Two leaves of the 1998 cohort were dropped from life assessments due to nutritional problems (yellowing), and 15 leaves of this cohort were dropped from life assessments due to damage by leaf-feeding insects. Among leaves within the 1996 cohort, 62 were damaged by a freeze on 19 January 1997 (temperatures as low as around -5°C for several hours at the weather station, probably colder at the study site); 48 of these leaves abscised within several days following the freeze and 14 others were rendered unfit for further research, leaving 36 leaves for life assessments. Among these 36 leaves, 13 had suffered considerable spider mite damage (e.g., averages in excess of 1,000 stipples per cm^2) before the freeze, suggesting that a freeze will not necessarily promote immediate abscission of leaves with mite damage. Among leaves of the 1998 and 1999 cohorts, 37 and 20 leaves, respectively, were ren-

dered unfit for leaf life assessments due to hail damage suffered on 28 May 1999. Twelve leaves of the 1999 cohort abscised or were hedged off before a final estimate of spider mite damage was made and were thus not available for leaf life assessments. Fifty-one of the 67 remaining leaves of the 1999 cohort were selected for leaf life assessment studies. None of the leaves studied developed any signs of mesophyll collapse. In spite of two standard summer treatments of copper and petroleum oil used for greasy spot control each summer, low infection levels of greasy spot developed on at least some leaves in each cohort. Usually, only one to several small infection sites could be found on any leaf with greasy spot. All leaves with greasy spot were therefore retained for leaf life assessments.

Data on spider mite infestations and damage, greasy spot infections, and leaf longevity are presented in Table 2. The life of all leaves studied, from full expansion to the abscission date, is depicted in Fig. 3. A total of only four leaves studied were known to have been infested solely by citrus red mites (Table 2). Among 45 leaves known to have been infested by both Texas citrus and citrus red mites, averages of 5.7 (SEM = 1.2) Texas citrus mites and 0.9 (SEM = 0.3) citrus red mites per leaf were observed. Data from leaves with citrus red mites were retained for all analyses, however, since so few citrus red mites were observed, conclusions from the data regarding the influence of mite damage on leaf abscission may only be applicable to Texas citrus mites.

An average leaf life of 399 days was estimated across all leaves ($n = 133$), with averages of 443, 387 and 380 days for the 1996, 1998 and 1999 cohorts, respectively (Table 2). The maximum life expectancy of the leaves appeared to be 18 to 20 months (Fig. 3). An average density of 470 stipples per cm^2 per leaf was estimated across all leaves studied, with averages of 327, 134, and 873 stipples per cm^2 per leaf for the 1996, 1998 and 1999 cohorts, respectively. Within the 1996 cohort, leaf life (Y) decreased as mite damage (X) increased ($Y = 489.7 - 0.144X$; $F = 9.47$, $\text{Pr} > F = 0.0041$; $df = 35$), but the relationship between leaf life and damage was statistically weak (e.g., $r^2 = 0.22$) (Fig. 4). No negative relationship was found between leaf life and spider mite damage among leaves of either the 1998 or 1999 cohorts (Fig. 4). Statistical analyses on the combined data from the three cohorts indicated no significant relationship between leaf life and mite damage. The 1996 cohort of leaves was subjected to a hard freeze during January 1997 and, although the leaves followed until abscission did not exhibit any signs of freeze damage, this adverse temperature event may have contributed to the shortened life of leaves with mite damage. Other unknown factors may have contributed to the reduced life of these leaves with mite damage. Al-

though the life of leaves within the 1996 cohort tended to shorten as mite damage increased, the average life of these leaves was longer than the life of leaves of the other two cohorts.

In addition to being subjected to a hard freeze on January 19, 1997, the 1996 cohort of leaves was subjected to near-freezing (1 to 4°C at the weather station) air temperatures for a short period of time one day during April 1997 (no leaf drop occurred during this month), on one day during December 1997 (20% of this cohorts leaves dropped during December), and on several days during January, February and March 1998 (some leaves of this cohort dropped during January and February of 1998, and 25% dropped during March 1998). The 1998 cohort of leaves was subjected to near-freezes on several days during December 1998, February 1999, March 1999, and January 2000 (none of the Fall 1998 cohort leaves dropped during any of these 4 months). The 1999 cohort of leaves was subjected to near-freezes on several days during March 1999 and January 2000 (none of this cohort's leaves dropped during March 1999 and few dropped during January 2000). Overall, near-freezing temperatures for short periods of time did not appear to promote premature abscission of leaves whether they were damaged by mites or not.

The 1998 and 1999 cohorts of leaves were subjected to two notable wind events, the first event during October 1999 associated with Hurricane Irene on 15 -16 October (23.7 and 25.5 mph average daily wind speeds, respectively, at the weather station) and the second event on one day during January 2000 (16.7 mph average daily wind speed at the station). These wind events did not result in an immediate drop of any leaves.

A total of 40 leaf abscission events (21, 11 and 8 for the 1996, 1998 and 1999 cohorts, respectively) were subjected to correlation analyses between leaf life (days after fully expanded to the abscission date), damage (stipple density per leaf) and environmental variables. Only 5 events could be subjected to correlation analyses with greasy spot infections for 1999 data because three leaves of this cohort abscised before greasy spot ratings were made. Analyses over all data indicated that leaf longevity was not correlated with the amount of mite damage to a leaf (Table 3). A significant negative correlation (i.e., $\text{Pr} > |r| \leq 0.05$) was found between leaf life and mite damage for the 1996 cohort of leaves ($r = -0.59$, $\text{Pr} > |r| = 0.01$) but not for either the 1998 or 1999 cohorts nor over all 3 cohorts combined. A significant negative correlation was found between leaf life and incidence of greasy spot. No significant correlations were found between leaf longevity and any of the environmental variables. Analyses on 'leaf life after attack' indicated that, for the 1996 cohort, life after damage decreased as mite damage increased (Fig. 5). However, the relationship be-

TABLE 2. SPIDER MITE DAMAGE AND GREASY-SPOT INFECTIONS ON LEAVES MONITORED UNTIL ABSCISSION.

Cohort	Mite species observed on leaves	Number of leaves	Mean number (SEM) mites per leaf ^a	Mean (SEM) no. stipples per cm ²	Percent leaves with greasy-spot	Mean (SEM) pct leaf area infected by greasy-spot	Mean (SEM) leaf life (days)
Sep 1996	Texas mites	19	11.0(3.5)	421.7(129.9)	26.3	— ^b	414.4(34.8)
	Citrus red mites	2	0.3(0.1)	8.2(5.6)	50.0	— ^b	445.0(9.0)
	Both mites	11	8.7(3.1) ^c	326.7(85.5)	27.3	— ^b	457.1(45.0)
	Neither mite	4	—	35.6(24.9)	50.0	— ^b	535.5(24.1)
	Over all	36	8.5(2.2)	326.8(76.1)	30.6	— ^b	442.6(23.5)
Oct 1998	Texas mites	11	0.7(0.3)	139.5(46.2)	90.0	1.2 (0.7)	352.6(34.6)
	Citrus red mites	2	0.1(0.04)	116.0(35.5)	50.0	0.3 (0.2)	259.0(0.0)
	Both mites	15	1.0(0.4) ^d	214.6(50.6)	92.9	0.9 (0.4)	392.7(31.2)
	Neither mite	18	—	66.4(22.9)	100.0	2.7 (0.7)	416.2(25.0)
	Over all	46	0.5(.02)	134.4(23.2)	93.2	1.6 (0.4)	386.5(16.8)
Mar 1999	Texas mites	31	12.2(2.0)	869.8(136.9)	4.2	0.1 (0.1)	373.3(7.3)
	Citrus red mites	0	—	—	—	—	—
	Both mites	19	9.9(2.0) ^e	922.8(155.7)	16.4	0.4(0.3)	391.9(12.3)
	Neither mite	1	—	4.1 (-)	0.0	—	336.0(-)
	Over all	51	11.1(1.5)	872.6(102.0)	9.5	0.2 (0.1)	379.5(6.5)
Overall	Texas mites	61	9.7(1.6)	598.6(88.4)	28.3	0.4 (0.2)	382.3(13.1)
	Citrus red mites	4	0.2(0.1)	62.1(28.1)	50.0	0.3 (0.3)	352.0(53.8)
	Both mites	45	6.7(1.3) ^f	541.0(87.6)	44.2	0.7 (0.2)	408.1(16.1)
	Neither mite	23	—	58.3(18.6)	90.0	2.7 (0.4)	433.4(22.4)
	Over all	133	6.7(0.9)	469.5(52.9)	45.9	1.1 (0.2)	399.0(9.2)

^aMean number per leaf over all dates mites were observed on a leaf.^bGreasy spot infection area on leaves was not estimated, but percentage surface area infected was low.^cAverages of 12.2 and 0.6 Texas and citrus red mites, respectively, observed per leaf.^dAverages of 0.7 and 0.4 Texas and citrus red mites, respectively, observed per leaf.^eAverages of 8.3 and 1.6 Texas and citrus red mites, respectively, observed per leaf.^fAverages of 5.7 and 0.9 Texas and citrus red mites, respectively, observed per leaf.

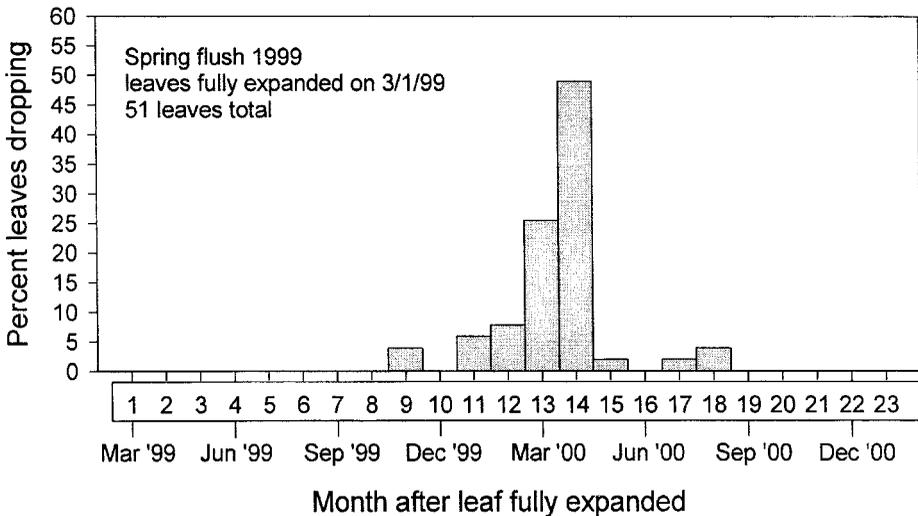
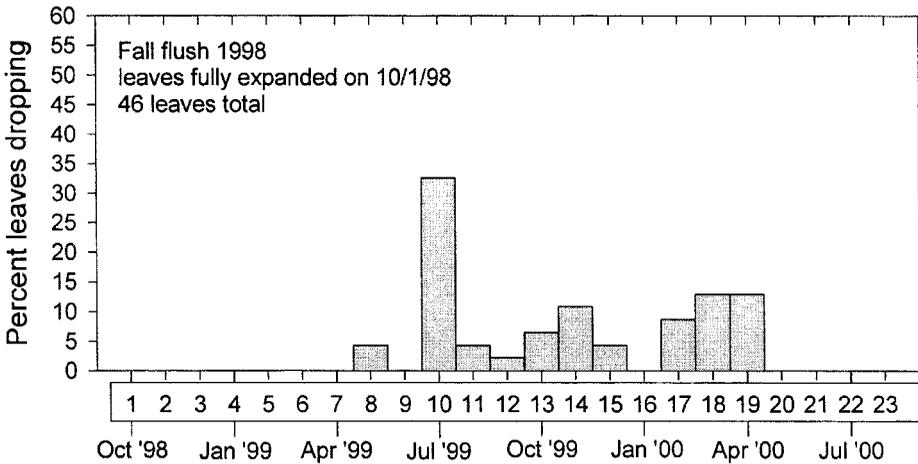
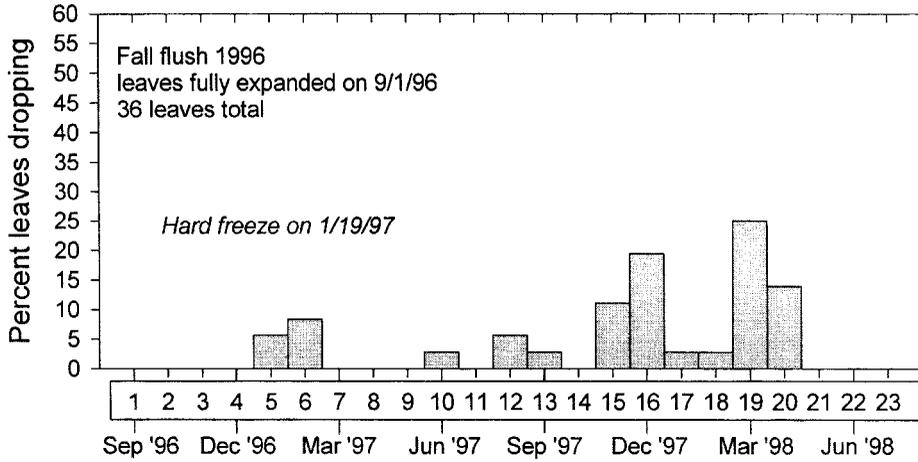


Fig. 3. Percentage abscission of citrus leaves over time.

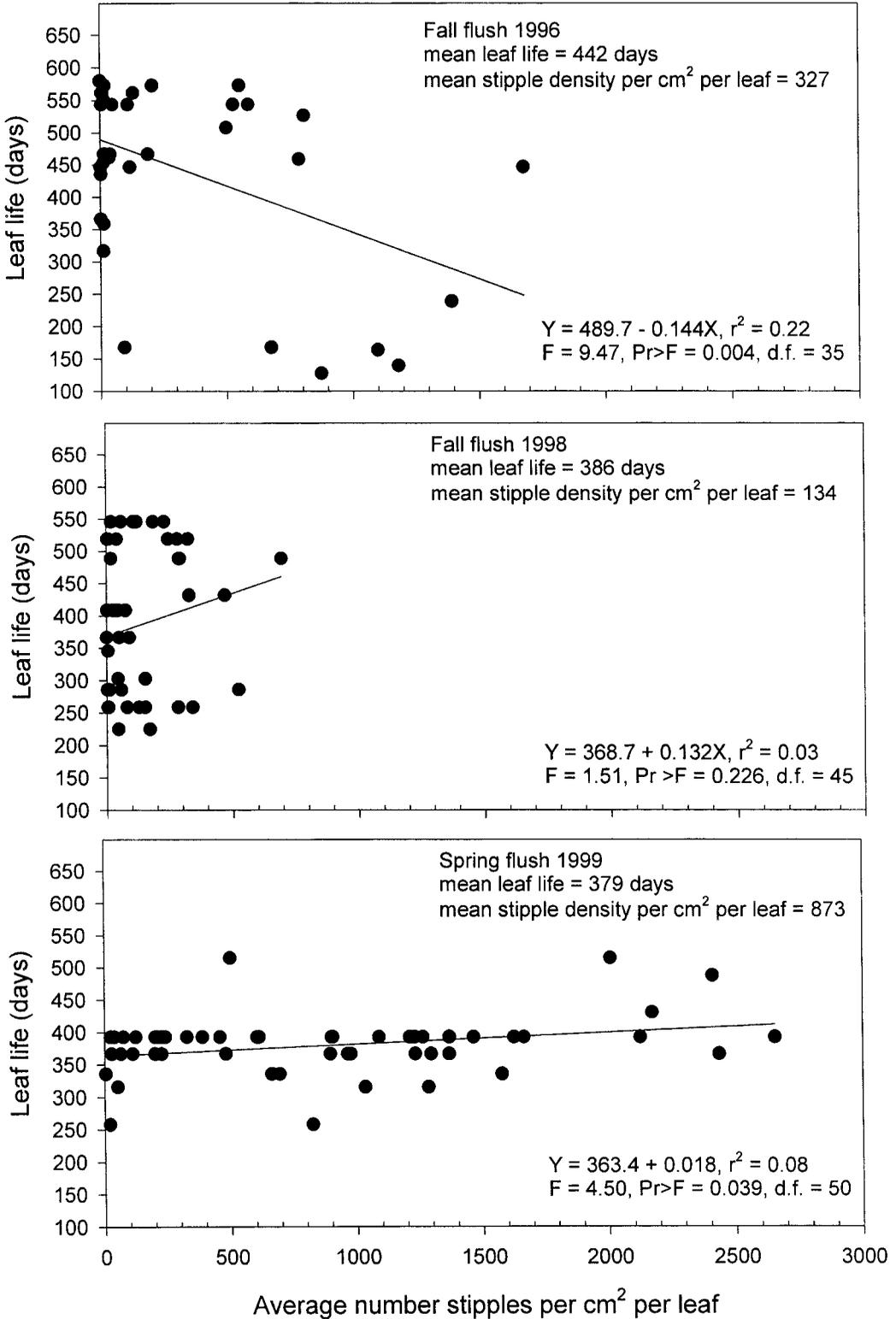


Fig. 4. Relationship between leaf life (days from full flush expansion until abscission) and spider mite damage (mean number of stipples per cm²).

TABLE 3. PEARSON CORRELATION COEFFICIENTS FOR THE RELATIONSHIP BETWEEN LEAF LIFE (DAYS FROM FULL EXPANSION TO DROP) AND SPIDER MITE DAMAGE TO LEAVES (MEAN NUMBER OF STIPPLES PER CM²), INCIDENCE OF GREASY SPOT, AIR TEMPERATURE AND WIND. FOR ENVIRONMENTAL VARIABLES, DATA FROM WITHIN 40 DAYS PRIOR TO DISCOVERING LEAF DROP WERE AVERAGED FOR THE CORRELATION ANALYSES.

Variable	Correlation between leaf life and the indicated variable	
	r	Pr > r
Mean number (#) stipples per cm ²	-0.13 ^a	0.42
Mean percent surface area per leaf with greasy spot	-0.58 ^b	0.02
Mean daily air temperature (°C)	-0.15 ^a	0.37
Mean minimum daily air temperature (°C)	-0.08 ^a	0.63
Mean maximum daily air temperature (°C)	-0.22 ^a	0.17
Mean daily wind (k)	0.21 ^a	0.19
Mean daily evaporation (cm)	0.03 ^a	0.88
Mean daily rainfall (cm)	0.07 ^a	0.66
Mean # stipples per cm ² X mean pct area per leaf with greasy spot	-0.34 ^b	0.20
Mean # stipples per cm ² X mean daily air temperature (°C)	-0.06 ^a	0.71
Mean # stipples per cm ² X mean minimum daily air temperature (°C)	-0.02 ^a	0.91
Mean # stipples per cm ² X mean maximum daily air temperature (°C)	-0.08 ^a	0.61
Mean # stipples per cm ² X mean daily wind (k)	0.04 ^a	0.81
Mean # stipples per cm ² X mean daily evaporation (cm)	-0.02 ^a	0.92
Mean # stipples per cm ² X mean daily rainfall (cm)	0.10 ^a	0.56

^an = 40.

^bn = 16.

tween damage and 'life after attack' for the 1996 leaves was weak ($r^2 = 0.16$), and no significant negative relationship between these variables was found among leaves of either the 1998 or 1999 cohorts. Further, leaves of the 1996 cohort stayed on trees longer after being damaged by mites than did leaves of the other two cohorts.

The longevity of leaves damaged by infestations of the Texas citrus mite, or by infestations of Texas citrus mites in combination with low levels of citrus red mites, was similar regardless of the amount of damage by mites (Fig. 6). The research indicated that, over all leaves evaluated in this study, Texas citrus mite damage promoted little or no premature abscission of citrus leaves. Also, damage resulting from low levels of citrus red mites in combination with infestations of Texas citrus mites did not decrease the longevity of citrus leaves during this study. No conclusions could be made from this study about the effect of extensive citrus red mite injury on leaf longevity. Thompson et al. (1954) observed mesophyll collapse in June following large outbreaks of the citrus red mite in April and May and reported that heavy leaf drop by citrus trees during late winter may sometimes be promoted in Florida by citrus red mite infestations (no data presented). Some scions may be more sensitive to damage by Texas citrus mites than 'Valencia' (e.g., 'Sunburst' mandarin, see Albrigo et al. 1987), and premature leaf abscission associated with mite injury may be more likely to occur in these scions even in an irrigated orchard.

Infestations of Texas citrus mites occur primarily on the upper surface of leaves and, consequently, feeding injury by these mites may occur primarily on this leaf surface. The Texas citrus mite could be a more important pest if it fed on the lower leaf surface. McCoy (1976) found that injury by citrus rust mites (species not indicated) to the lower surface of leaves promoted more mesophyll collapse and leaf drop than damage by the mite to the upper leaf surface. For this same reason, the citrus red mite may be a more important pest than the Texas citrus mite, as this mite infests both the upper and lower leaf surfaces (Jones & Parrella 1984). Rust mite damage to the upper leaf surface may promote less water loss from a leaf than damage to the lower leaf surface because the upper surface lacks stomates, has a highly developed waxy layer, and has a compact palisade parenchyma layer of cells beneath the epidermis that contribute to the prevention of water loss (McCoy 1976). Therefore, the effect on water loss of damage by Texas citrus mites to the upper leaf surface may also be less. Based on research by McCoy (1976), the ultimate cause of premature defoliation of citrus leaves is water loss. Although injury by mites may promote water loss, a good water supply for trees may help prevent premature abscission of leaves damaged by mites. Working in trees with an overhead watering system rarely used during the winter, McCoy (1976) speculated that scant rainfall (0.5 cm per week) promoted premature abscission of leaves

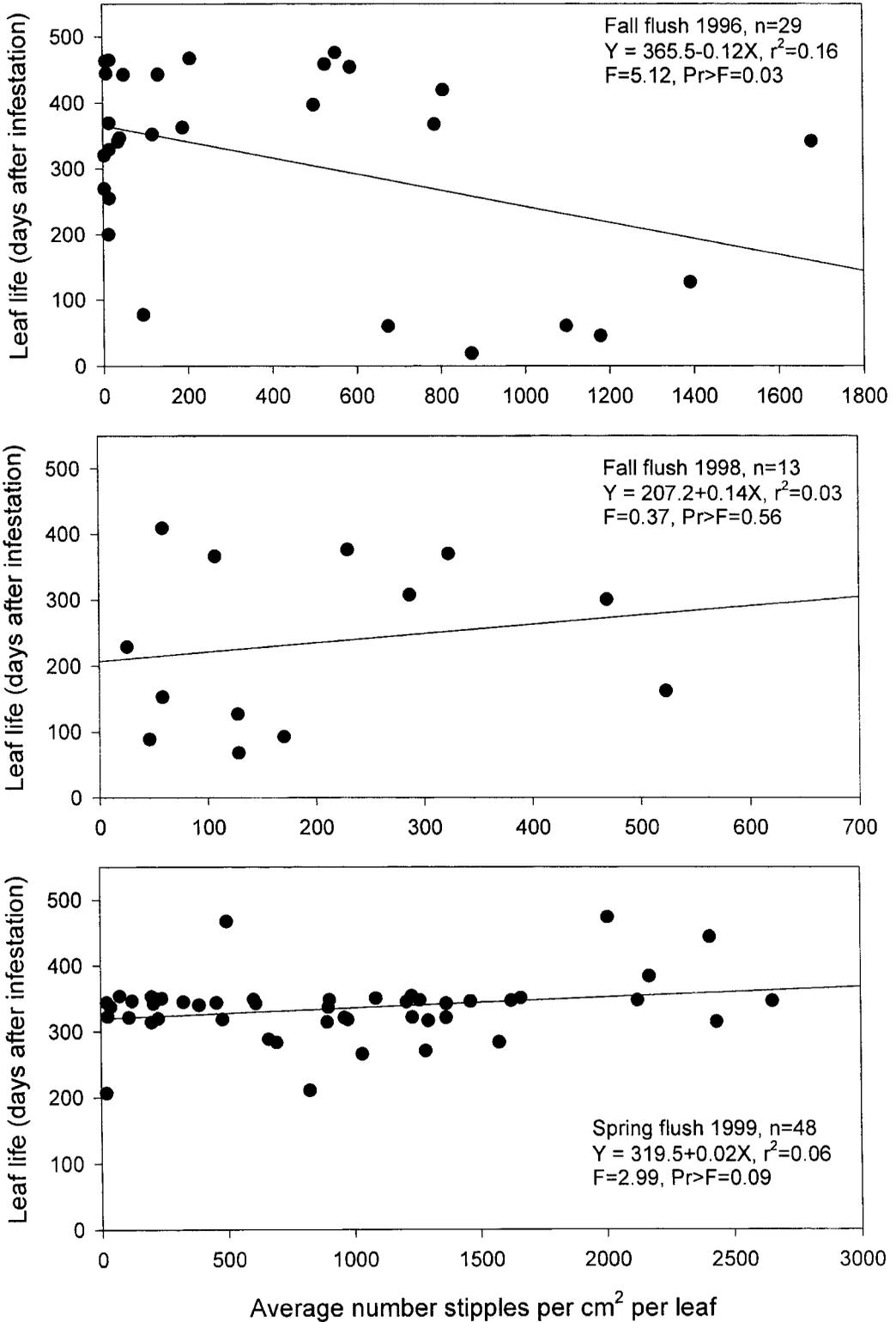


Fig. 5. Relationship between leaf life after mites damaged leaves (days from damage until abscission) and spider mite damage (mean number of stipples per cm²).

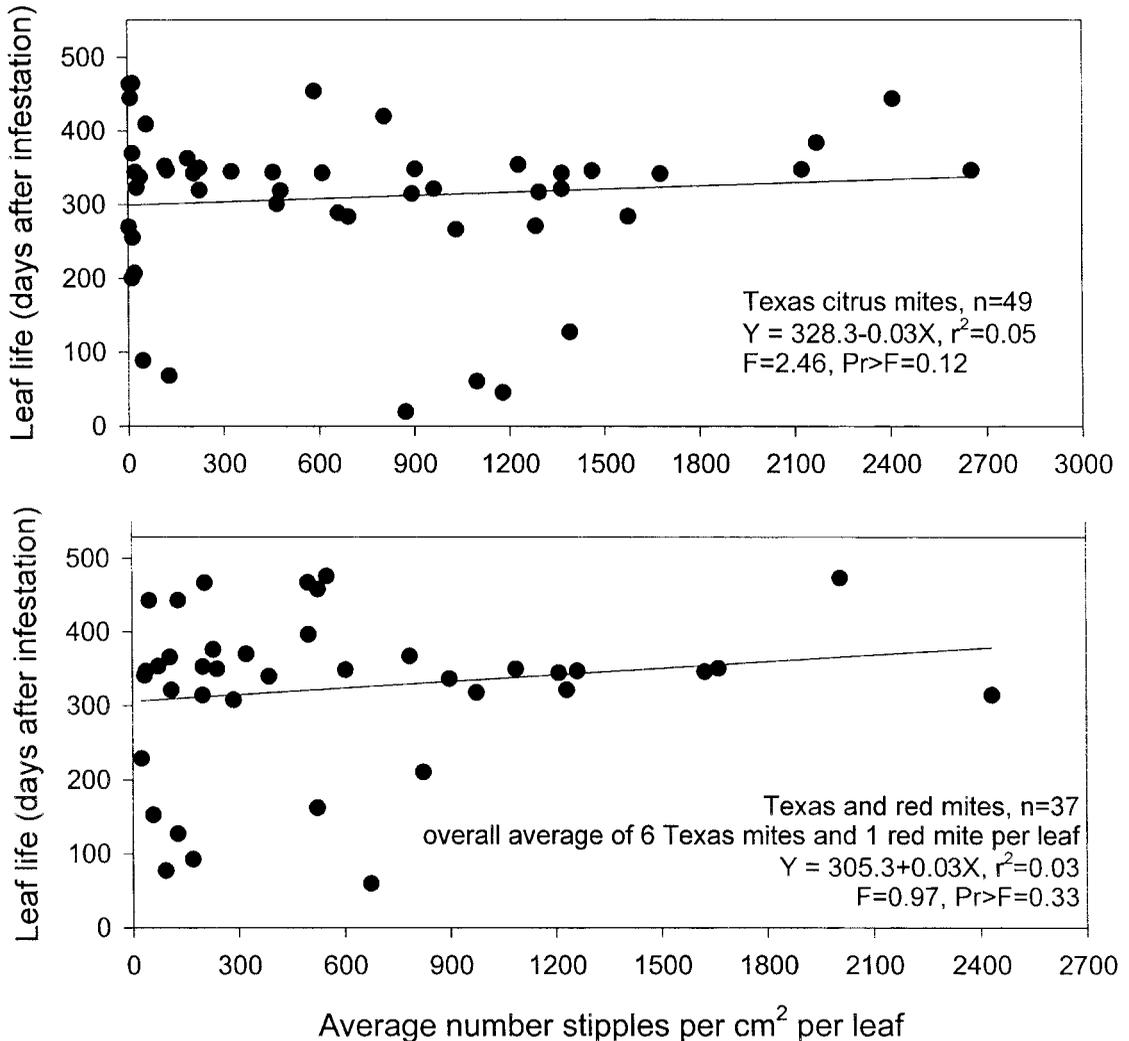


Fig. 6. Leaf life (days) after attack by mites and damage, leaves infested by Texas citrus mites or both Texas citrus and citrus red mites.

damaged by rust mites and that increased water loss from leaves through mite feeding damage to the lower leaf surface may be enough during dry periods to cause leaf abscission. The equivalent of an average of 2.4, 2.5 and 1.2 cm of daily rain in the general vicinity of our study site was associated with periods of time we observed leaf drop among the 1996, 1998 and 1999 cohorts, respectively, considerably more than 0.5 cm per week. But for each cohort, there were one or two 40-day periods during which weekly rainfall at the weather station averaged less than 0.5 cm, and for the 1999 cohort there was one 40-day period during which no rainfall was recorded. Increases in leaf abscission at the study site were not observed during these dry periods. Irrigation during dry periods may have helped prevent premature abscissions of citrus leaves with mite damage

and, therefore, the results of our study may only pertain to irrigated trees.

Healthy citrus leaves can remain on a tree for 2 to 3 years or longer (Kelley & Cummins 1920, Davies & Albrigo 1994). Disease and pest pressures as well as low light levels can significantly reduce leaf longevity (Davies and Albrigo 1994). In a California study, the majority of orange leaves abscised by 17 months and almost all by 24 months (Wallace et al. 1954). Whiteside (1982) speculated that, in the absence of freezes and greasy spot disease, the expected life of citrus leaves in Florida may be 1 to 2 years, similar to what was observed in our study. This supports the conclusion that damage by Texas citrus mites had little influence on leaf longevity under our study conditions. A study of mature 'Valencia' trees indicated that leaf abscission may occur all year long,

with higher abscission rates during late September-November and mid-April to May (Erickson & Brannaman 1960). The greatest abscission rate in citrus normally occurs during the spring flowering period (Erickson 1968). Whiteside (1982) reported that the major seasonal leaf drop from Florida citrus trees begins after the spring growth flush has emerged (usually in March) and may extend until late-May. During our study, abscission of leaves of the spring 1999 cohort generally followed Whiteside's seasonal leaf drop profile while abscission of leaves of the fall 1996 and 1998 cohorts generally did not (Fig. 3).

Based on the study, the amount of physical damage to citrus leaves resulting from an infestation of Texas citrus mites can be projected based on duration of mite densities. If the economic importance of damage by the mite was known, such projections could be useful in making mite control decisions. Whether or not feeding injury by Texas citrus mites to leaves results in economic losses remains to be determined. Although premature leaf abscission could be an important economic problem associated with damage by some pests, our study indicated it was not important with respect to Texas citrus mites in an irrigated citrus orchard.

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POTENTIAL FOR REDUCING OVERFLOODING RATIOS OF STERILE MEDITERRANEAN FRUIT FLIES (DIPTERA: TEPHRITIDAE) WITH THE USE OF GINGER ROOT OIL

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ABSTRACT

The mating behavior of sterile, laboratory-reared, male Mediterranean fruit flies, *Ceratitis capitata* (Wiedemann), was evaluated in field-cage mating competition tests with wild flies after exposing the laboratory males to ginger root oil extract. Without exposure to ginger root oil, sterile males obtained 12.6%, 69.0%, and 72.8% of the total matings with wild females when present in 1:1, 5:1, and 10:1 ratios of sterile males to wild males, respectively. Sterile males, exposed to ginger root oil for 3 h, 1 d before mating trials, in a 1:1 ratio with wild males, achieved 62.3% of the matings with wild females. These data suggest that exposure to ginger oil can elevate sterile male mating competitiveness to a similar degree as elevated ratios of sterile to wild males. Incorporating the use of ginger root oil extract into sterile release programs may thus increase the effectiveness of the sterile insect technique, and/or allow a reduction in the number of sterile flies that are released.

Key Words: sterile insect technique, *Ceratitis capitata*, mating behavior, alpha-copaene

RESUMEN

El comportamiento de copulación en las moscas de la fruta, *Ceratitis capitata* (Wiedemann), mediterráneas estériles, machos criados en el laboratorio, fue evaluado en pruebas de competencia de copula en jaulas de campo fuente con moscas salvajes. Estos pruebas se iniciaron después de que los insectos del laboratorio fueron expuestos al extracto del aceite del raíz de jengibre. Sin la exposición al aceite, los machos estériles obtuvieron 12,6%, 69,0%, y 72,8% de las copulas totales con hembras salvajes cuando fueron presentes en proporciones de 1:1, 5:1, y 10:1 de machos estériles a machos salvajes, respectivamente. Los machos estériles, expuestos 1 d antes de copula al olor del aceite de jengibre para 3 h, en un proporción de 1:1 con machos salvajes, alcanzaron 62,3% de las copulas con hembras salvajes. Estos datos sugieren que la exposición al aceite pueda elevar la competencia sexual del macho estéril a un grado igual a proporciones elevados (ca. 5:1) de machos estériles contra machos salvajes. Incorporando el uso del extracto del aceite en programas de acción de liberar moscas estériles, puede aumentar así la eficiencia de la técnica estéril del insecto, y/o permitira una reducción en el número de moscas estériles liberadas. Translation provided by author.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (medfly), is a multivoltine, polyphagous (250+ plant species) insect pest that could have a devastating economic impact if it were to become established in California or Florida (Metcalf 1994). In these states, the sterile insect technique (SIT) is currently used to inhibit Mediterranean fruit fly colonization. The goal of these programs is for sterile males to mate with any introduced wild females, resulting in the production of infertile eggs (Dowell et al. 2000).

There is an ongoing interest in improving the quality of sterile males used in eradication and control programs for the Mediterranean fruit fly. One way to assess fly quality is through the use of

mating competition tests, where sterile males compete with wild males for wild females. In most cases, mating observations and tests have shown that laboratory-reared male flies are at a disadvantage with wild males when competing for females (Robinson et al. 1986, Shelly et al. 1994, Cayol et al. 1999, Lance et al. 2000, but see Taylor et al. 2001 which found no advantage for wild males). In one case in Hawaii, following apparent intense selection in the field, there was almost complete behavioral resistance by wild females to laboratory reared sterile males (McInnis et al. 1996).

Reduced mating performance of sterile laboratory males is attributed to the mass-rearing pro-

cess, which through artificial selection, may result in the production of flies with qualities different from their wild counterparts (Cayol 2000). There are two options available to increase the mating success of SIT flies. The first is the release of greater numbers of sterile flies so that they vastly outnumber wild males. Recommended sterile/wild fly release ratios vary with each strain, but have been suggested to be 125:1 for the Petapa strain and 100:1 for the Vienna-4/Tol-94 strain based on field cage tests using sterile/wild fly ratios of 1:1, 5:1, 25:1, and 125:1 (Garcia et al. 1999). A second option would be to use "male-only" strains, containing 95%+ males, which have resulted in a significant improvement over releases containing both sterile males and females (McInnis et al. 1986, McInnis et al. 1994, Hendrichs et al. 1995, Rendon et al. 2000). The benefits of "male-only" strains, derived from the virtual elimination of the sterile male and sterile female interaction, are widely accepted and as such, these strains are now used in many rearing and release programs. Both of these options result in improving the success of SIT by altering the probability of interactions between sterile and wild flies. If, in addition, sterile fly mating competitiveness could be improved, then releasing a lower number of sterile flies would result in an even more efficient SIT program.

A survey in the 1950's identified several attractants of male medflies (Beroza & Green 1963) that were later developed for use with medfly trapping and monitoring programs. One of these attractants, trimedlure, was shown to increase the mating success of males after they were exposed to it (Shelly et al. 1996). In addition, exposure of medfly males to ginger root (*Zingiber officinale* Roscoe) oil, which contains alpha-copaene at 0.4% volume, increased their mating competitiveness vs. wild males (Shelly 2001, Shelly & McInnis 2001). Alpha-copaene is a known male attractant that has been identified from angelica oil (Guiotto et al. 1972, Flath et al. 1994a, b, Nishida et al. 2000). Shelly & McInnis (2001) found a several-fold greater mating success of mass-reared, sterile flies exposed to ginger root oil compared with sterile flies not exposed. This study adds to previous research by comparing the mating success of sterile males at different sterile:wild fly ratios, to a 1:1 ratio of sterile (exposed to ginger root oil): wild flies.

MATERIALS AND METHODS

Study Animals

Wild male and female Mediterranean fruit flies were collected as larvae and eggs from coffee, *Coffea arabica* L., from Kauai, Hawaii and from loquats, *Eriobotrya japonica* Thunb., from Kula, Maui, Hawaii in February and March, 2001.

Fruits were placed on screens above vermiculite (22-26°C) that was sifted every 5-7 d for pupae. To obtain virgin flies for mating trials, newly emerged flies were separated by sex <2 d after eclosion. Adult flies were fed honey, sugar, and protein hydrolysate until they were sexually mature (>12 d old). Twenty five adult flies were held collectively in small plastic containers (400 ml) with nylon mesh screening. The source of wild flies for each mating trial was dependent on the availability of host material (coffee and/or loquat) and the number of flies obtained from each host.

Laboratory-reared flies of the Vienna-4 (Toliman) strain (male-only genetic sexing strain, carrying a temperature sensitive lethal (*tsl*) mutation) were obtained from the Tropical Fruit and Vegetable Research Laboratory, USDA-ARS, in Honolulu, HI. The sexing strain was obtained from the mass-rearing facility in Guatemala at El Pino in 1998. The larval rearing protocol followed that of Tanaka et al. (1969) & McInnis et al. (1994). Before irradiation, white pupae (presumed to be females) were removed in order to achieve a higher percentage of males. In preparation for irradiation, which occurred 2 d before eclosion, pupae were placed in hypoxia for 1-2 h. Sterilization was achieved using a dose of 14.5 Kr in a Cobalt⁶⁰ irradiator located at the University of Hawaii, Manoa (McInnis et al. 1996, Rendon et al. 1996). Adult sterile flies were fed honey, sugar, and protein hydrolysate until they were sexually mature (at least 4 d old). Adult flies were held in cages (16 liters, 225-250 flies per container) with nylon mesh screening until testing in the field. (Different size containers were used for wild and sterile, laboratory flies, in part because of the numbers of each type needed for each experiment and also to reduce the mortality of wild flies, which is usually high in the laboratory. Carey et al. (1995) have investigated the affects of different fly densities on mortality.

Mating Tests

On the day before a mating trial, one male type was marked (wild and Vienna-4 were alternated with each replicate) by placing a small drop of enamel paint on the thorax. This allowed later identification of male type in mating pairs. In the ginger root oil treatment, 1 day before the mating trial, 25 laboratory male flies were exposed in each of two small plastic containers (400 ml) for 3 h to 20 µl of ginger root oil (Citrus and Allied Essences Ltd., Lake Success, NY) placed on a 1 cm² piece of blotter paper. These flies were exposed in an isolated room that was distant from all other flies.

Circular, nylon-screened, field cages (2.5 m high, 2.5 m diameter, and containing a 2 m tall guava tree, *Psidium guajava* L.) were used for the mating trials (McInnis et al. 1996). Four treat-

ments were randomly assigned to four field cages for each of five replications over time. The treatments were 1:1, 5:1, and 10:1 ratios of sterile laboratory-reared males to wild males, and a 1:1 ratio of sterile laboratory-reared males exposed to ginger oil to wild males. In each cage, there were always 25 wild males and 25 wild females, so different ratios of laboratory to wild males were obtained by altering the number of laboratory-reared males (i.e., the 1:1 ratio had 25 laboratory-reared males, 5:1 had 125, and 10:1 had 250).

On the day of a mating experiment, males were released first into each of the cages, followed by the females after an interval of 5-10 min. Flies that were dead, incapable of flight, or noticeably damaged in any way at the time of release were replaced. Two field observers, alternating between cages every 15 min, located and removed mating pairs without replacement. Observations were made from approximately 0900 until 1400 h. Temperature ranged from 23-29°C and relative humidity ranged from 47-68% (HOBO® datalogger, Pro Temp/ RH, Onset Computer Corporation, Bourne, MA).

An ANOVA was performed on data, after arcsine transformation, for the three treatments with sterile males not exposed to ginger root oil to determine if there were significant differences in mating based on the sterile:wild fly ratio. A two-way t-test was used to compare transformed data from all four treatments.

RESULTS

Mating Tests

With flies not exposed to ginger root oil, the sterile:wild fly ratio had a significant effect on the percentage of mating pairs involving a sterile male ($F = 14.98$; $df\ 2, 12$; $P = 0.001$; Fig. 1). Results with the 1:1 ratio treatment, with sterile flies not exposed to ginger oil, were significantly different from all other treatments (1:1 to 5:1, $P = 0.0057$, $df = 4$; 1:1 to 10:1, $P = 0.0068$, $df = 4$; and 1:1 to 1:1 ginger exposed, $P = 0.013$, $df = 4$). Pair-wise comparisons among the three remaining treatments (1:1 with ginger exposed sterile males, 5:1, and 10:1) were not significant ($P > 0.05$, $df = 4$).

DISCUSSION

Specially treated mass-reared flies have the potential to outperform wild flies in mating competitiveness trials (Shelly & McInnis 2001). In our study, ginger root oil was responsible for increased mating success with sterile laboratory-reared flies. At a 1:1 ratio of sterile (exposed): wild flies, sterile males had a success (62% of the matings) similar to wild males. In comparison, a 1:1 ratio of sterile (not exposed): wild flies, resulted in sterile males failing to mate in 3 of 5 replicates.

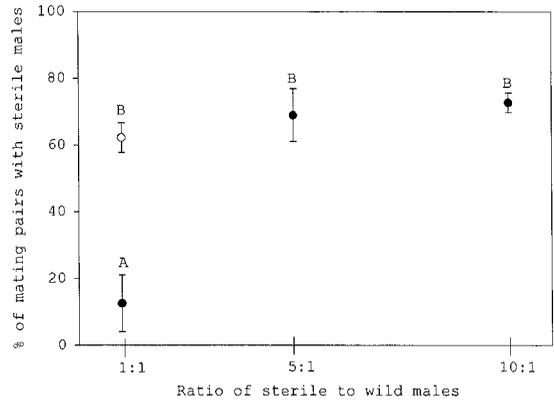


Fig. 1. Mating success (mean \pm SE) of Vienna-4 (Toliman) sterile males not exposed (\bullet), or exposed to ginger root oil (\circ), for 3 h, 1 d before mating competition trials with wild flies. The 1:1 treatment with sterile flies not exposed to ginger oil was significantly different from all other treatments (1:1 to 5:1, $P = 0.0057$, $df = 4$; 1:1 to 10:1, $P = 0.0068$, $df = 4$; and 1:1 to 1:1 ginger exposed, $P = 0.013$, $df = 4$).

Sterile males (not exposed) showed mating success similar to, or above that of, wild males only at elevated ratios of 5:1 and 10:1 (sterile:wild males). These results suggest that exposure to ginger root oil elevates male mating success to a degree comparable with elevated ratios (5:1 or 10:1) of sterile/wild males.

During a medfly infestation, in areas where eradication is the goal (i.e., Florida and California), it is likely that sterile:wild male ratios will be more skewed than the highest ratio that was tested in this study, 10:1, because of daily releases of sterile flies. Assuming that these cage tests are indicative of what may happen during mass releases, there could be as much as a 1/5 reduction in the number of flies released if they were pre-treated with ginger root oil or if numbers of flies remained unchanged, then the flies released would have five times the mating success compared with the present system. The use of ginger root oil should result in a fly that is qualitatively better, regardless of the sterile:wild fly ratio.

In this study, the number of available wild flies was a limiting factor. Using higher numbers of wild flies in each cage would increase the power of the statistical tests, but would also increase the number of sterile flies that would be needed in order to maintain the ratios used. Using more than 300 flies in a field cage of the size we used (see Materials and Methods) could raise concerns about unnaturally high densities of flies, and whether the results could reasonably be extrapolated to open field conditions. Fly density in this study ranged from 15-60 flies/m² (75 to 300 flies in a 4.9 m² cage). The Preventative Release Program

(PRP) in Southern California releases approximately 300 million flies each week over an area of approximately 6,446 km² (CDFA 2001). This corresponds to a density of approximately 0.048 flies/m² (or 1 fly/21 m²), assuming 0% mortality for released flies, 100% mortality for flies released the previous week, and uniform fly distribution. Testing higher ratios of sterile to wild flies, as well as using more total flies could be better accomplished with the use of larger field cages, however, the ability to find almost all mating pairs then becomes more difficult.

Further study is needed to determine how to incorporate ginger root oil into mass-rearing programs. The impact of ginger root oil on mating performance should also be evaluated against other strains of wild flies and using other sterile male strains. In addition, the economics and practical methodology of incorporating ginger root oil exposure into the large Mediterranean fruit fly SIT programs is now being investigated (TES and DOM, unpublished data).

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SEASONAL LIFE STAGE ABUNDANCE OF *DIAPREPES ABBREVIATUS* IN IRRIGATED AND NON-IRRIGATED CITRUS PLANTINGS IN CENTRAL FLORIDA

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ABSTRACT

The seasonal abundance of various life stages of *Diaprepes abbreviatus* (L.), was monitored in separate years in adjacent irrigated and non-irrigated citrus plantings, as well as thickets of Brazilian-pepper located near Poinciana, FL in Osceola County. Adult emergence, estimated by weekly catches in cone-shaped ground traps, occurred throughout the year with a peak in mid-June in both citrus and Brazilian-pepper plantings. Onset of adult emergence coincided with an increase in soil moisture and temperature. Trap counts were highest when soil water potential increased to 3-5 centibars at a depth of 15-30 cm and soil temperature averaged 22-24°C. In the non-irrigated citrus planting, the adult emergence peak was of shorter duration, but of greater magnitude, compared to the irrigated planting. Although the alternate host, Brazilian-pepper, produced fewer weevils than did citrus, the seasonal emergence pattern was virtually the same.

Adult abundance within the citrus plantings was also monitored weekly using modified Tedders traps. The number of adults captured approximated the number caught in ground traps. Adult number caught weekly changed seasonally, particularly in the fall when adult populations were the highest. Ground traps caught a larger number of adults in the spring. The number of egg masses collected weekly in the tree canopy and the number of neonates caught weekly beneath the tree canopy were both correlated with the number of adults captured weekly in modified Tedders traps. These data suggest that adults caught in modified Tedders traps provide a reliable indicator for estimating the seasonal abundance of all life stages within a citrus planting. Larvae of different instars, pupae, and teneral adults were recovered from the soil rhizosphere after periodic tree removal. No diseased or parasitized life stages were observed in the study. Most life stages were present in the soil at each sample date, but the proportion of larvae in various instars changed seasonally. The implications of this study for understanding the population dynamics of *D. abbreviatus* are discussed in relation to current and future IPM strategies.

Key Words: *Diaprepes abbreviatus*, population dynamics, citrus, Brazilian-pepper

RESUMEN

Se realizó un monitoreo sobre la abundancia estacional de varias etapas de vida de *Diaprepes abbreviatus* (L.) en años separados en siembras de cítricos irrigadas y no irrigadas en campos adyacentes, así como en matorrales de la pimienta de Brasil (*Schinus terebinthifolius*) cerca de Poinciana, Florida en el condado de Osceola. La emergencia de los adultos, estimada por el número de adultos atrapados en las trampas de forma de un cono puestas sobre el suelo, aconteció a través del año con el punto más de la población ocurriendo en el medio de junio en siembras de cítricos y en *Schinus terebinthifolius*. El inicio de la emergencia de los adultos coincidió con un aumento en la humedad del suelo y de la temperatura. El número de *Diaprepes* atrapados por trampa fue el más elevado cuando el potencial del agua de suelo aumentó al 3-5 centibarras a una profundidad de 15-30 cm y a un promedio de la temperatura del suelo de 22-24°C. En siembras de cítricos no irrigados, el punto más alto de la emergencia de los adultos duró menos tiempo, pero fue de mayor magnitud, comparada con las siembras no irrigadas. Aunque el hospedero alternativo, *Schinus terebinthifolius* produjo menos picudos de lo que fueron producidos en los cítricos, el patrón de emergencia estacional fue virtualmente el mismo.

También, se realizó un monitoreo semanalmente sobre la abundancia de adultos entre la misma siembra de cítricos usando trampas modificadas del tipo "Tedders". El número de adultos capturados fue aproximadamente el número recolectado en las trampas del suelo. El número de adultos recolectados semanalmente cambió según la estación, particularmente en el otoño cuando la población de adultos fue la más alta. Las trampas de suelo capturaron un mayor número de adultos en la primavera. El número de masas de huevos recolectados semanalmente en la copa del árbol y el número de neonatas (larvas recién nacidas) capturados semanalmente debajo la copa del árbol fueron ambos correlacionados con el número de

adultos capturados semanalmente en las trampas modificadas de tipo "Teddners". Estos datos sugieren que los adultos capturados en las trampas modificadas de tipo "Teddners" proveen un indicador confiable para estimar la abundancia estacional de todas las etapas de vida dentro de una siembra de cítricos.

Larvas de diferentes estadios, pupas y adultos tenebrales fueron recobradas de la rizófera después de la eliminación de árboles hecha periódicamente. No fueron observadas las etapas de vida con enfermedades o parasitadas en el estudio. La mayoría de las etapas estuvieron presentes en el suelo en cada fecha de muestreo, pero la proporción de larvas en las varias etapas cambió según la estación. Se discuten las implicaciones de este estudio para entender la dinámica de la población de *D. abbreviatus* en relación con las estrategias actuales y futuras de Manejo Integrado Plagas (MIP).

Diaprepes abbreviatus (L.) a root weevil native to the Caribbean region (O'Brien & Wibmer 1982) is a major localized pest of commercial citrus, ornamental plants, and some agronomic crops in Florida since its introduction in 1964 (Woodruff 1964, McCoy 1999). It has recently spread to citrus and ornamentals in Texas (French & Skaria 2000) and has been intercepted frequently by regulatory officials in California (Kris Godfrey, personal communication). Currently, it ranks as a high risk pest of California agriculture. The adult, egg, and neonate stages appear on above-ground parts of the host plant and all larval stages, pupae and teneral adults occur below ground (Wolcott 1936). Although this weevil can be univoltine on citrus, the life cycle varies in duration with many overlapping generations and many different life stages may be present simultaneously. Upon hatching, neonates fall from tree and enter the soil. The small neonates feed on fibrous roots, whereas later instars feed on larger structural roots, causing deep grooves as they consume the outer bark and cambium layer. From year to year root injury appears to accumulate, and feeding sites can serve as infection courts for root rot diseases such as *Phytophthora* spp. (Graham et al. 1996, 2002), thereby exacerbating economic losses.

Current integrated pest management (IPM) strategies for weevil suppression include horticultural practices such as irrigation and fertilization, *Phytophthora* control in the soil, and a mix of control tactics aimed at reducing larval and adult populations (McCoy & Duncan 2000). Monitoring of adults using visual counts or modified Tedders traps has been deployed to better time foliar and soil applications of adulticides and larvicides in citrus groves (Stansly et al. 1997, Duncan et al. 2001). Although trapping has been useful in determining seasonal patterns of adult emergence from the soil, a lack of knowledge of the developmental biology and ecology of *D. abbreviatus* has limited our understanding of trap accuracy and population dynamics of various life stages in the field.

The purpose of this 2-yr study was to assess the relative abundance of various life stages of *D. abbreviatus* in adjacent distinct citrus plant-

ings and in Brazilian-pepper thickets surrounding the citrus using different monitoring methods. Brazilian-pepper is a host to both adults and larvae of *Diaprepes* (Simpson et al. 1996) where they feed on leaves and roots, respectively. Relative abundance of different life stages was then related to abiotic factors such as air temperature, soil temperature, rainfall, and soil moisture. No irrigation was deployed in the first year. This information was deemed fundamental to the development and application of various IPM strategies.

MATERIALS AND METHODS

Experimental Site

Independent studies were conducted near Poinciana, FL in Osceola County in two distinct plantings of Hamlin orange grafted to Swingle citrumelo rootstock set at 6.1 × 8.5 m. These adjacent plantings, designated north (40 acres) and south (50 acres), were planted on two-row beds, in a poorly drained alfisol soil type classified as Floridana fine sand (68.8% sand, 11.8% silt, 19.4% clay). The surface layer was 35.6 cm loam and the subsurface layers 76.2 cm gray fine sand followed by clay. The soil had a low to moderate organic matter content with a pH of 4.8. Both plantings had been infested with *D. abbreviatus* for at least 10 yr according to local reports. In the north planting, an estimate of weevil larvae in the soil was made in early February 2000, shortly before our study was begun. Three trees were removed and soil sieved to recover larvae. These trees produced 83-86 late instar larvae/0.4 m³ of soil. Although tree decline was severe in the north planting at this time, high larval populations in combination with poor horticultural care (no irrigation) resulted in greater tree decline by years end. The rapidity of tree decline and death in the north planting in 2000, necessitated moving our study to the south planting in 2001. In so doing, our original experimental plan to replicate by year was lost.

In the north planting, 150 productive trees with decline symptoms were purposely selected for studies in 2000. These trees were non-irrigated and received no chemical treatments dur-

ing the study period. The grove was mowed regularly to reduce weed growth and experimental trees received a herbicide application (glyphosate at standard rate) as needed to prevent weed growth at the tree canopy margin and beneath the tree. Some trees were pruned lightly to improve accessibility beneath the tree. One fertilizer application was applied to the healthy trees in mid-April using a standard citrus mixture (6-6-6). Brazilian-pepper trees, *Schinus terebinthifolius* Raddi, were growing wild throughout the north planting along with other woody weed hosts of *D. abbreviatus*.

By contrast, the south planting used for study in 2001, was highly productive and had been under regular grove care that included irrigation on a need basis, fertilization twice per year, and regular weed and pest control. Fertilization and weed control only were continued in 2001 following the same schedule.

Monitoring Adult Weevils

In each planting, seasonal adult emergence from the soil and adult abundance in the grove were monitored using cone-shaped screened ground traps (0.9 m base dia.) and modified pyramidal Tedders traps (Tedders & Wood 1994, McCoy et al. 2000), respectively. A trap of each type was placed as pairs opposite each other beneath a tree, midway between the tree trunk and the canopy dripline. Within each planting, 100 randomly selected trees received a pair of traps. Traps were monitored weekly from 17 March through 18 December 2000 and from 6 March through 11 December 2001. In 2001, an additional 100 cone-shaped ground traps covered with mylar plastic to simulate no irrigation, were monitored weekly within the grove from 6 March through 29 August.

The whole citrus planting was bordered on three sides by pasture that harbored various alternate host plants for *D. abbreviatus* such as tallow tree, *Sapium sebiferum* L., coffee weed, *Sesbania exaltata* (Raf.) Rydb. ex A. W. Hill, and the fore-mentioned Brazilian-pepper. In two thickets of Brazilian-pepper located in the adjacent pasture within 90 m of the south citrus planting, 100 ground traps were distributed randomly beneath trees and monitored from 6 March through 11 December 2001 only.

Monitoring Oviposition in the Tree Canopy

In 2001 only, egg mass abundance within the accessible part of the tree canopy was monitored weekly by systematic 15 min visual inspections of each of 20 trees from 6 March through December 2001. Inspection entailed searching for detection of attached or folded leaves glued by female weevils during oviposition.

Monitoring Neonate Fall to the Soil Surface

The seasonal abundance of neonates falling from the trees was assessed using modified pitfall traps (funnel traps). Traps consisted of a 20.3 cm diameter plastic funnel attached to 3.1 × 50.0 cm length of PVC pipe support elevated about 30 cm above the ground. A screw top conical tube (50 ml), containing 5 ml of ethylene glycol, was attached to the funnel tip using duct tape to serve as a collection unit.

Eight traps were placed under each tree in the cardinal directions, four traps at 30 cm from the trunk and 4 traps at 30 cm from the dripline. Larvae were collected from 25 trees in 2000 and 20 trees in 2001; and collection tubes were changed weekly. In the laboratory, neonates were identified and counted. *Diaprepes abbreviatus* neonates were identified by the frontal suture joining the epicranial suture to form an inverted "Y" on the head capsule (Beavers & Woodruff 1971). Trapping was performed weekly from 1 May through 26 December 2000 and from April through 31 December 2001.

Monitoring Weevil Life Stages in the Soil

The seasonal changes in relative abundance of the various life stages of *D. abbreviatus* in the soil was assessed by periodically removing four or more trees and recovering all detectable life stages from the soil beneath the extracted trees. Trees were topped using a chainsaw and the remaining roots and surrounding soil were removed using a back hoe. Most of the soil adhering to the roots was removed by shaking and/or probing with a shovel. Soil from the roots and beneath the tree was then placed into buckets for subsequent sieving. Approximately 0.59 m³ of soil was collected per tree to a depth of 30 cm according to the procedures of Duncan et al. (1996). All life stages of *D. abbreviatus* except larvae younger than fifth instar were visually detectable and recovered from the soil using a motor-driven shaker and 0.64-cm mesh sieve. The numbers of larvae, pupae and adults recovered from each tree were recorded. Larval instar was determined by head capsule width measurement (Quintela et al. 1998). All healthy larvae exhibiting normal behavior were recorded as 'live'. Live larvae exhibiting abnormal behavior and dead larvae were placed in a disposable Petri dish (50 × 9 mm) on moistened filter paper. Cadavers were examined microscopically every other day for 7 d to detect characteristic signs indicative of bacterial, fungal, or nematode infection (Lacey & Kaya 1999).

Weather Monitoring

Soil temperature and moisture were continuously monitored at 15 and 30 cm depths using

paired thermistors and calcium block transducers set to record hourly readings. Rainfall (amount and duration) was measured using a tipping-bucket rain gauge linked to a day recorder. Free water applied via irrigation was also manually recorded. Ambient air temperature and relative humidity were recorded using a weekly chart hygrothermograph. Weekly data were integrated and stored in a T21X micrologger (Campbell Scientific Inc., Logan, Utah).

Statistical Analysis

SAS System for Windows, release 6.11 was used for analysis (SAS Institute Inc. 1990). Proportions of adults captured in the two trap types were compared with contingency table analysis and χ^2 tests using PROC FREQ, SAS System for Windows 6.11 (SAS Institute Inc. 1990). Correlation analysis used PROC CORR.

RESULTS

Adult Weevil Emergence and Abundance

Cone-shaped ground traps, designed specifically to catch emerging adults, captured 385 weevils from mid-July through the end of October in the non-irrigated north planting in 2000. The same type traps caught 428 weevils from mid-May through the end of November in the irrigated south planting in 2001. Peak adult emergence occurred in mid-June in the non-irrigated planting and at approximately the same time the following year in the irrigated planting (Figs. 1A and B). Adult emergence in the non-irrigated planting was closely-related to soil moisture (Fig. 1A). When soil water potential at a depth of 15-30 cm increased to 3-5 centibars emergence began shortly thereafter and continued throughout the summer. In late September, soil temperature began to decline and adult emergence dropped off, even though soil moisture remained at or below 10 centibars (Fig. 1A). Low levels of adult emergence did occur in the early spring when soil temperatures were 2-4EC lower than during the mid-summer period. Adult emergence in the irrigated planting in 2001 was lower in magnitude, but more continuous throughout the year (Fig. 1B). Soil water potential of <-4 centibars combined with soil temperatures ranging from 22-24EC in the summer appeared to favor a longer emergence period in the irrigated grove (Fig. 1B) compared to the non-irrigated grove (Fig. 1A).

Tedders traps, designed to catch both emerging adults and those attracted from the surrounding area, captured 3113 weevils from mid-March to mid-December in the non-irrigated planting in 2000. The same type of trap caught 7337 in the irrigated planting in 2001. Although a greater number of adults were caught in Tedders traps, both

traps detected a strong late spring emergence in both years (Figs. 1 and 2). However, only Tedders traps detected a late season peak in adult abundance in September and October that exceeded the spring peak in magnitude (Figs. 2A and C).

Although there was a significant correlation between the number of adults caught using the 2 trap types when all year 2000 data was pooled ($r = 0.6682$, $df = 39$, $P = 0.0001$), this relationship disappeared during the active emergence period (22 June through 12 October) when >90% of adult captures were recorded ($r = 0.2682$, $df = 15$, $P = 0.2979$). In 2001 in the irrigated planting, there was no significant correlation between catches from the two trap types even when all data were pooled ($r = 0.2305$, $df = 38$, $P = 0.1524$). By comparison, total weevils captured in cone traps were 428, 1297, and 100, respectively, in irrigated, non-irrigated citrus and Brazilian-pepper, respectively, in the south planting in 2001. The seasonality of adult emergence under the three conditions was quite similar when percent cumulative emergence was compared from mid-March to late August (Fig. 3A). However, trap counts for certain sample dates were significantly different for percent cumulative emergence ($P \neq 0.05$) between irrigated citrus and non-irrigated citrus based on contingency table analysis and the χ^2 test. There was no difference between non-irrigated citrus and Brazilian-pepper (Fig. 3A). Exceeding cumulative adult emergence increased from 29 August to mid-November in both irrigated citrus and Brazilian-pepper plantings with no significant difference between sites (Fig. 3B). Sampling was terminated in August in the non-irrigated citrus planting to prevent further water stress to the trees.

Egg Mass Abundance

A total of 811 egg masses of *D. abbreviatus* was detected on leaves in 2001 in the irrigated planting from 13 June through 11 December with a peak in mid-Sept (Fig. 2D). The seasonal pattern for the number of egg masses recovered during weekly monitoring was similar to the number of adults caught in modified Tedders traps (Figs. 2C and D). The correlation between egg mass number and adult number as indicated by modified Tedders trap counts was highly significant ($r = 0.7885$, $df = 38$, $P = 0.0001$).

Neonate Abundance

In 2000 in the non-irrigated planting, 2408 neonates were recovered from modified pitfall traps compared to 4841 in the irrigated planting in 2001. Neonate drop was detected from 12 June through 18 December, 2000 in the non-irrigated planting and 28 May through 31 December 2001 in the irrigated planting (Figs. 2B and E). Neo-

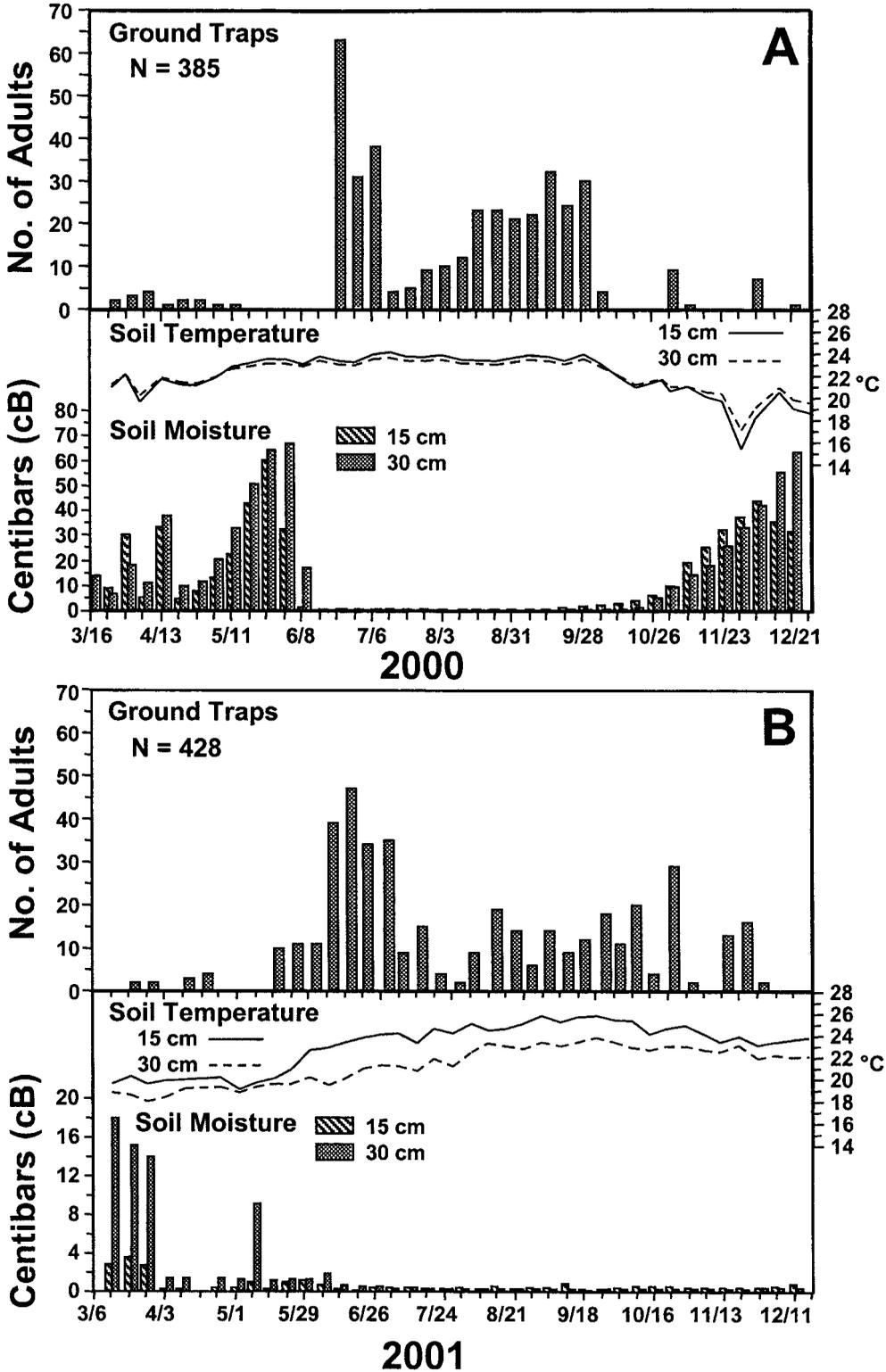


Fig. 1. Weekly records of soil moisture, soil temperatures, and numbers of adult weevils captured in cone traps: (A) non-irrigated citrus planting, 2000, and (B) the irrigated citrus planting, 2001.

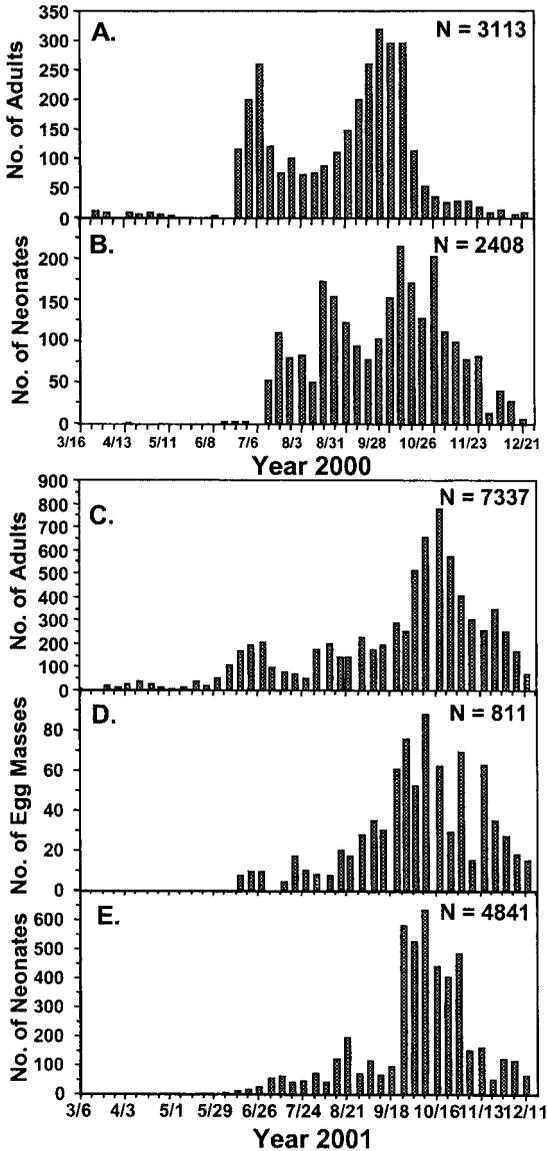


Fig. 2. Weekly numbers of adult weevils captured in Tedders traps, numbers of egg masses counted during systematic visual canopy inspections and numbers of neonates captured in funnel traps during 2000 (A, B) and 2001 (C, D, E).

nate drop was significantly correlated with numbers of adults caught in Tedders traps in both years (year 2000, $r = 0.3971$, $df = 32$, $P = 0.0201$; year 2001, $r = 0.8467$, $df = 32$, $P = 0.0001$) but neither egg mass nor neonate abundance were correlated with numbers of adults caught in cone traps (neonates vs. cone traps, year 2000, $r = 0.0143$, $df = 32$, $P = 0.9360$; year 2001, $r = -0.0884$, $df = 32$, $P = 0.6190$; and egg masses vs. cone traps, $r = 0.0462$, $df = 38$, $P = 0.7769$).

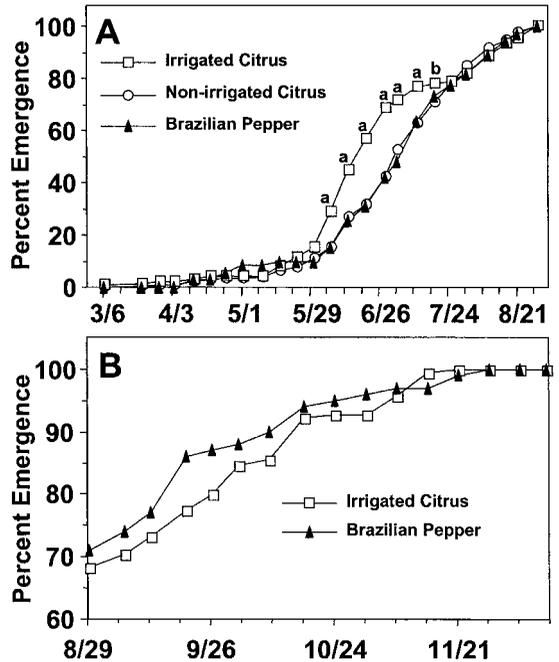


Fig. 3. Comparison of the cumulative percent emergence of adult weevils as estimated by cone traps in irrigated citrus, non-irrigated citrus, and Brazilian-pepper from 6 March through 29 August 2001 (A); and for irrigated citrus and Brazilian-pepper for 29 August through 11 December 2001 (B). An "a" denotes significance (contingency table analysis, χ^2 test, $P = 0.05$ level) for irrigated citrus compared to non-irrigated citrus and Brazilian-pepper with no difference between the latter two; whereas, a "b" denotes significance for irrigated citrus compared to non-irrigated citrus but not Brazilian-pepper, and no difference between the latter two.

Abundance of Subterranean Life Stages

In the non-irrigated citrus planting in 2000, 1253 life stages of *D. abbreviatus* were recovered from 0.59 m³ of sieved soil collected from the tree rhizosphere of each of 32 trees. In the irrigated planting in 2001, 785 life stages were recovered from 28 trees using the same procedures. Many fourth instar larvae and younger stages probably escaped detection. No diseased or parasitized larvae were found among live and dead larvae held in the laboratory for symptom expression. All detectable larval instars were present in the soil rhizosphere throughout most of the year, but, the proportion of the population represented by different life stages often changed seasonally (Figs. 4 and 5). Pupae and teneral adult recovery was more erratic.

In the non-irrigated planting in 2000, the only instar that failed to show significant change in proportional representation during the year was the eighth instar ($n = 319$) whereas, in the irrigated planting in 2001, the only instar failing to

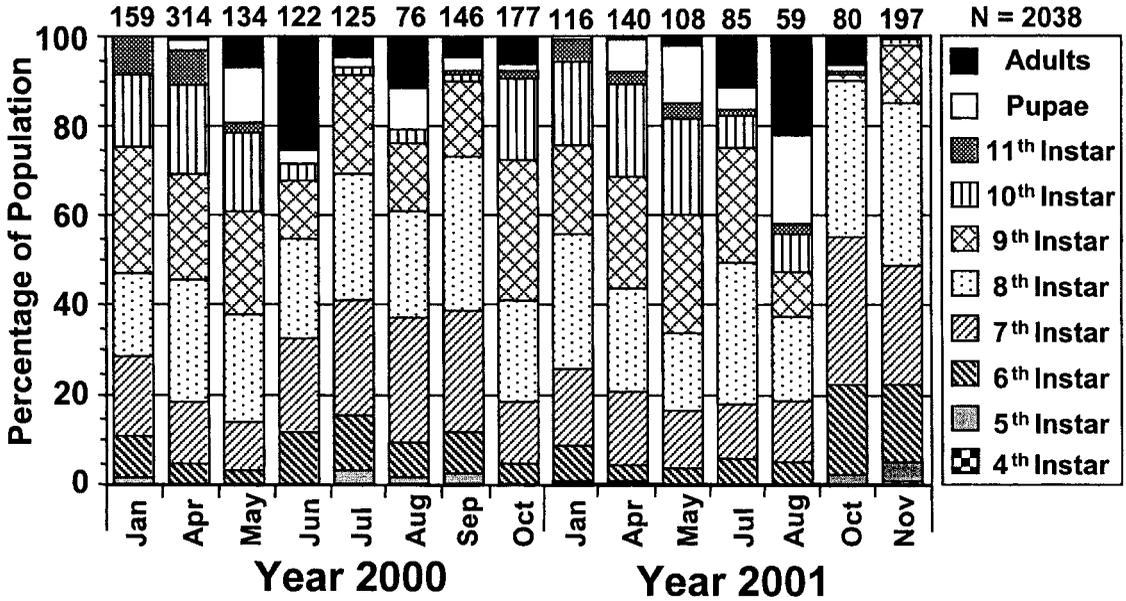


Fig. 4. Percentage of the weevil population in each life stage in the soil rhizosphere in non-irrigated (2000) and irrigated (2001) citrus plantings. The total number of life stages recovered monthly is indicated above the stacked bar for that month, and the overall total is given above the legend.

show this change was the eleventh instar ($n = 17$; contingency table analysis, $P > 0.05$; Fig. 5). The scarcity of tenth and eleventh instars during the emergence period suggest an earlier onset to pupation. Pupae and adults showed strong seasonal peaks in abundance that correlated to peak adult emergence as monitored with ground traps. Moreover, in 2001 in the irrigated planting, ninth instars were extremely rare in October, whereas fourth to eighth instars were unusually abundant at that time.

DISCUSSION

Comparison of data from both irrigated and non-irrigated studies suggest that the onset and magnitude of adult emergence of *D. abbreviatus* can be delayed by soil moisture deficit. This response to moisture can be related to more frequent rainfall (Figs. 1A and B) or free water via irrigation supplemented with rainfall (Fig. 3). A positive effect of soil moisture on weevil emergence has been suggested in the literature from the Caribbean (McCoy 1999) and Florida (Beavers & Selhime 1975). In the laboratory, Lapointe & Shapiro (1999) found that soil moisture ranging from 20-80% affected larval survival and pupation. Our results represent the first field study where soil moisture was linked to seasonal adult emergence. The effect of soil moisture on adult weevil emergence is well-documented for the pecan weevil, *Curculio caryae*

(Horn) (Harris & Ring 1980). Generally, pecan weevils emerge in large numbers after rainfall. Furthermore, Harris & Ring (1980) found that drought hardened clay soil impeded weevil emergence but irrigation allowed normal emergence. In addition, they found that weevil emergence can be postponed by withholding moisture, but cannot be accelerated by the addition of moisture before the normal emergence window.

In many citrus groves located in central and east coastal areas of Florida, one annual peak of adult abundance has been detected using Tedders traps (Stansly et al. 1997, Duncan et al. 2001), single peak population density generally occurred typically in the May/June period. In the non-irrigated and irrigated citrus plantings in 2000 and 2001 respectively, adult abundance estimated using Tedders traps demonstrated as two distinct seasonal peaks, one in late spring and another in late summer, the latter being the greatest. These data suggest that the fall peak cannot be explained by adult emergence. Since adult weevils are relatively long-lived in the field, at least some of the late-summer peak in adult abundance might be due to an accumulation of older weevils. However, this trend has not occurred in other locations where adult abundance was monitored with Tedders traps (Stansly et al. 1997, Duncan et al. 2001). A more likely explanation for the fall peak is adult migration from alternate host plants. As shown in Fig. 3, adults can emerge con-

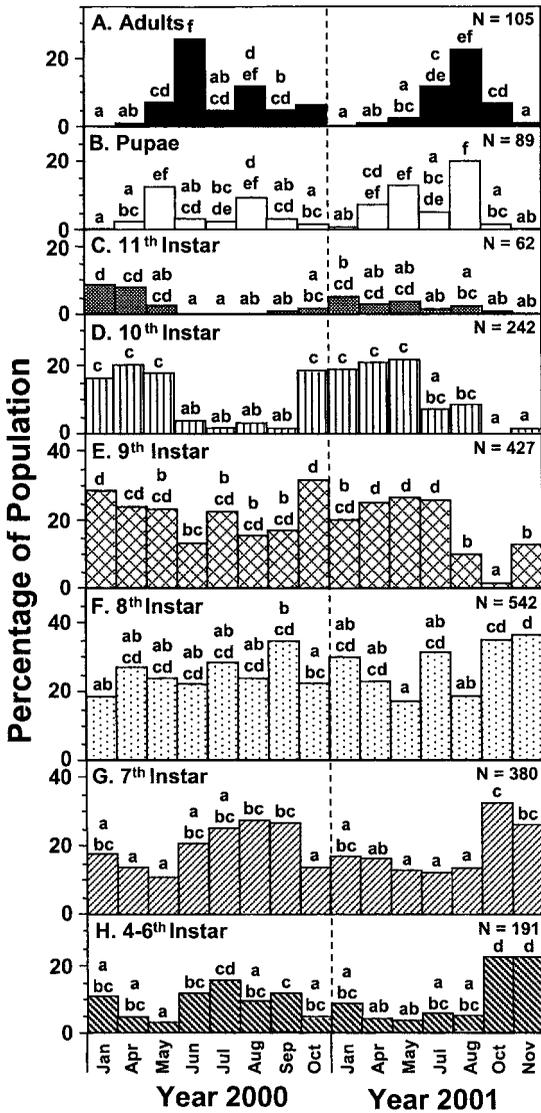


Fig. 5. Statistical comparison of the percentage of the population of each weevil life stage recovered in time from the soil rhizosphere in non-irrigated (2000) and irrigated (2001) citrus plantings. For each life stage, bars with common letters are not significantly different at the $P = 0.05$ level based on contingency table analysis and χ^2 test.

tinuously from alternate hosts such as Brazilian-pepper throughout the summer.

As previously mentioned, the Tedders trap is widely used in scientific research to trap adult weevils (Duncan et al. 2001). When placed beneath the tree midway between the canopy margin and the tree trunk, Tedders traps are effective for measuring changes in adult citrus root weevil abundance within a season, and the onset of adult emergence from the soil (Stansly et al. 1997, Dun-

can et al. 2001, Nigg et al. 2001). These data show conclusively a close association of adult trap counts, egg mass counts, and neonate counts substantiating the conclusions of the above authors. Tedders traps, therefore, provide useful estimates, not only of adult number, but also reproductive activity in the field.

Entomopathogenic fungi and nematodes can reduce weevil populations in the soil under natural conditions; however, their spatial distribution and persistence in the soil is variable (McCoy & Duncan 2000). In independent studies conducted in both the north and south citrus plantings to determine the field efficacy of commercial preparations of entomopathogenic nematodes against *Diaprepes* larvae in the soil, no control was obtained. In addition, only a few larvae recovered from the soil were parasitized by native nematodes and no microbe infections were diagnosed in the laboratory (McCoy et al. 2002). These findings agree with the present data that indicate no soil-inhabiting natural enemies were found in wild larvae collected from the rhizosphere of extracted trees. The alfisol soil type found in these citrus plantings has fine particle size and may be antagonistic to these natural enemies which, in turn, might partially explain why overall weevil densities were exceptionally high and tree injury so severe in both plantings.

Our data on the number of neonates dropping from trees into funnel traps compared to the number of adult weevils being captured under trees in cone-shaped emergence traps suggests that mortality during this part of the life cycle might often exceed 98%. In 2000, in the non-irrigated grove, the number of dropping neonates averaged 376.25 per m^2 ; whereas the number of emerging adults averaged 6.05 per m^2 , for a survival rate of 1.61%. In 2001, in the irrigated grove, the number of dropping neonates averaged 945.51 per m^2 ; whereas the number of emerging adults averaged 6.73 per m^2 , for an average survival rate of 0.71%.

Our data on the various developmental stages of *D. abbreviatus* have important practical implications for timing the application of various control agents used as foliar or soil treatments. Since prolonged dry weather can delay adult emergence and, conversely, extended periods of high soil moisture can extend emergence, seasonal adult emergence can be highly variable from year to year. It would appear that monitoring with ground traps or Tedders traps is worthwhile. Since trapping is not grower friendly, a weather related model might be of benefit in timing foliar treatments to control emerging adults as they enter the tree canopy. If adult emergence is prolonged by favorable soil conditions, the benefits of foliar sprays to control adults will be lost because of their generally short residual activity and the possible negative side effects of multiple applications. Future control strategies might be more effective if they could target the teneral adult stage in the soil.

Peak neonate drop to the soil occurred from mid-July through October in both citrus plantings (Fig. 2), suggesting that soil barrier treatments might be more effective when applied at this time of the year (McCoy & Duncan 2000). As is true for foliar sprays for adult control, the residual effect of a soil barrier treatment with chemicals is too short to assure prolonged efficacy, suggesting the need for multiple applications during the period of highest neonate drop.

A broad range of larval instars occur in the tree rhizosphere throughout the year. The fact that all larval stages are present year round with overlapping generations presents enormous problems when attempting chemical control since susceptibility and appropriate dosage vary with larval age. Although entomopathogenic nematodes also exhibit differential larval susceptibility to *Diaprepes*, they will infect virtually all instars (Shapiro et al. 1999) at levels unachievable by chemicals.

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AN UPDATED LIST OF FLORIDA ANTS (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

A list of ants of Florida published in 1989 is replaced to accommodate 49 additional species now known from Florida, and 34 name changes in species already on the 1989 list. Currently, 218 species of ants are reliably reported from Florida.

Key Words: exotic species, faunistics

RESUMEN

Se presenta una nueva lista de las hormigas de Florida para reemplazar una lista publicada en 1989. La nueva lista incluye 49 más especies y 34 cambios de nomenclatura. Al presente, 218 especies de hormigas son sabidas a hallarse en Florida.

In 1989 Deyrup et al. published a list of the ants of Florida. In the ensuing thirteen years there has been considerable myrmecological activity, both survey work in Florida, and taxonomic descriptions and revisions. Consequently, the 1989 list is drastically out of date: there are 49 additional species to be added to the list, 34 name changes that apply to species already on the 1989 list, and 4 species that have been removed from the list because the records are probably based on misidentifications.

These advances do not mean that there is no need for further work in the inventory and taxonomy of Florida ants. Included in the list are a number of species that are awaiting description by various specialists. The species epithets in the entire genus *Brachymyrmex* are suspect, although it is clear that at least five species of *Brachymyrmex* occur in Florida. It is probable that there are additional species of ants still to be found in Florida. This can be deduced from the fact that there are a number of species on the list that are known from only one or two collections, or from one or two sites; there are probably other equally rare species that nobody has been lucky enough to find. There is reason to suppose that exotic ants will continue to become established in Florida (Deyrup et al. 2000); even now there are likely to be some species of localized exotics that have not yet been reported. On the other hand, there are four species of ants on the list that have not been found for many years and may have been extirpated. These are the native species *Formica subsericea* and *Solenopsis xyloni*, and the exotic species *Myrmelachista ramulorum* and *Tetramorium lanuginosum*.

The 49 species that have been added to the list were overlooked before for various reasons. They are not, fortunately, primarily exotics that have invaded Florida since 1989, although there are

nine exotic species that have been added to the list. Most of the added Florida records are either native species whose Florida populations have been recently discovered, or native species that have recently been described or are awaiting description.

The number of species listed below, 218, is the largest ant fauna known from any state in eastern North America, and is likely to remain so, even after other large states have received as much attention as Florida. The reasons for this lie in the convergence of various faunal elements. There is a set of Antillean species, such as *Leptothorax alldardycei* and *L. torreyi* in the southern Peninsula. There are many tropical exotics, including both Old World species, such as *Technomyrmex albipes* and *Strumigenys emmae*, and New World species, such as *Wasmannia auropunctata* and *Pseudomyrmex gracilis*. There are southeastern coastal plain species such as *Camponotus socius* and *Paratrechina arenivaga*. There are, mostly in north Florida, species probably derived from the southern Appalachians, such as *Pyramica rostrata* and *P. pulchella*. There are species representing western lineages, such as *Pogonomyrmex badius* and *Neivamyrmex texanus*. All this diversity, however, still cannot compare with that of southwestern states such as Arizona, where an almost intact, pre-ice age, dryland fauna (Madrotertiary) is augmented at higher elevations by north temperate species.

The following alphabetical list generally follows the nomenclature in Barry Bolton's catalog (1995), combined with the nomenclature in his revision of the Dacetini (2000). These two works are currently the foundations of North American ant nomenclature. Numbers in parentheses following a name in the list usually refer to variances from the nomenclature or from the lists of species in Bolton 1995 and 2000. These variances are ex-

plained in the section following the list. In cases in which a name appearing in the 1989 list has been changed and the changes are referenced in one of the two works by Bolton mentioned above, the 1989 usage is noted but the original source of the change in nomenclature may be omitted.

An asterisk (*) denotes a species that was absent from the 1989 list.

Florida specimens of all but seven species are deposited in the collection of Archbold Biological Station.

LIST OF FLORIDA ANTS

Acanthomyops claviger (Roger).* Okaloosa Co. Rare.

Acanthomyops interjectus (Mayr).* Liberty Co. Rare.

Amblyopone pallipes (Haldeman). Widespread.
Anochetus mayri Emery. Dade and Palm Beach Cos. Introduced.

Aphaenogaster ashmeadi (Emery). Widespread in north FL, south into Highlands Co.

Aphaenogaster carolinensis Wheeler. North FL; distribution unclear: confounded with *miamiana*. (1)

Aphaenogaster flemingi M. R. Smith. Widespread.

Aphaenogaster floridana M. R. Smith. Widespread in north FL, south into Highlands Co.

Aphaenogaster fulva Roger. Widespread in north FL, south into Orange and Volusia Cos.

Aphaenogaster lamellidens Mayr. Widespread in north FL, south into Highlands and St. Lucie Cos.

Aphaenogaster mariae Forel. Liberty Co. Rare.

Aphaenogaster miamiana Wheeler.* South FL; distribution unclear: confounded with *carolinensis*.

Aphaenogaster tennesseensis (Mayr). Northernmost counties of peninsular Florida.

Aphaenogaster treatae Forel. Widespread.

Aphaenogaster umphreyi Deyrup & Davis.* Scattered sites in north FL, south into Highlands Co. (2).

Brachymyrmex brevicornis Emery?*(Genus in disarray). Columbia Co. Rare. Introduced.

Brachymyrmex depilis Emery? (Genus in disarray). Widespread.

Brachymyrmex minutus Forel?*(Genus in disarray). Dade and Monroe Cos. Introduced.

Brachymyrmex musculus Forel?*(Genus in disarray). Widespread. Introduced.

Brachymyrmex obscurior Forel? (Genus in disarray). Widespread.

Camponotus caryae (Fitch). Liberty Co. Rare.

Camponotus castaneus (Latreille). Widespread.

Camponotus decipiens Emery. Widespread.

Camponotus discolor (Buckley).* Scattered sites in peninsular Florida, Alachua into High-

lands Cos. Records in 1989 list under *sayi* Emery apparently refer to this species.

Camponotus floridanus (Buckley). Widespread.

Camponotus impressus (Roger). Widespread. Listed under *Colobopsis* in 1989 list.

Camponotus nearcticus Emery. Widespread.

Camponotus pennsylvanicus (DeGeer). Northernmost FL, including Panhandle.

Camponotus planatus Roger. South FL, north into Hillsborough and Orange Cos. Introduced.

Camponotus pylartes Wheeler.* North-central Peninsula.

Camponotus sexguttatus (Fabricius).* Dade Co. Rare. Introduced.

Camponotus snellingi Bolton. Widespread. Records under *C. pavidus* Wheeler in 1989 list refer to this species.

Camponotus socius Roger. Widespread, south into Broward Co.

Camponotus tortuganus Emery. South FL, north into Volusia Co.

Cardiocondyla emeryi Forel. Widespread. Introduced.

Cardiocondyla nuda (Mayr). Widespread. Introduced.

Cardiocondyla venustula Wheeler. Widespread. Introduced.

Cardiocondyla wroughtonii (Forel). Widespread in peninsular FL. Introduced.

Cardiocondyla sp.* Widespread in peninsular FL. Introduced. (3)

Cephalotes varians (F. Smith). Dade and Monroe Cos. (4)

Crematogaster agnita Wheeler.* Monroe Co. Rare. Introduced.

Crematogaster ashmeadi Mayr. Widespread.

Crematogaster atkinsoni Wheeler. Widespread.

Crematogaster cerasi (Fitch). Widespread in north FL, south into Highlands Co.

Crematogaster lineolata (Say). Widespread in north FL, south into Hernando and Sumter Cos.

Crematogaster minutissima Mayr. Widespread.

Crematogaster missouriensis Emery.* Panhandle. (5)

Crematogaster pilosa Emery. Widespread.

Crematogaster vermiculata Emery. Scattered sites in north FL, south into Hillsborough Co.

Crematogaster sp. A (pine species).* Widespread in north and central FL, south into Lee and Palm Beach Cos.

Crematogaster sp. B (large species in mangroves).* Monroe Co. Rare.

Cryptopone gilva (Roger). Widespread in north FL, south into Highlands Co.

Cyphomyrmex minutus Mayr. Widespread in south FL, north into Hillsborough and Volusia Cos.

Cyphomyrmex rimosus (Spinola). Widespread. Introduced.

- Discothyrea testacea* Roger. Widespread.
- Dolichoderus mariae* Forel.* Leon Co. Rare.
- Dolichoderus pustulatus* Mayr.* Scattered sites throughout FL. Records in 1989 list under *D. plagiatus* Mayr refer to this species.
- Dorymyrmex bossutus* (Trager)*. Widespread in peninsular FL, west into Leon Co. (6)
- Dorymyrmex bureni* (Trager)*. Widespread. Records in 1989 list under *Conomyrma insana* (Buckley) refer to this species. (6)
- Dorymyrmex elegans* (Trager)*. Central peninsular ridges. (6)
- Dorymyrmex flavopectus* M. R. Smith. Central peninsular FL, south Highlands Co. into Marion Co. Listed under *Conomyrma* in 1989 list. (6)
- Dorymyrmex grandulus* (Forel)*. North FL, south into Citrus Co. (6)
- Dorymyrmex medeis* (Trager)*. Scattered sites in north FL, south into Highlands Co. (6)
- Dorymyrmex reginicula* (Trager)*. Central peninsular FL, southern Highlands Co. north into Volusia Co. (6)
- Eurhopalothrix floridana* Brown & Kempf. Widespread in peninsular FL.
- Forelius pruinosus* (Roger). Widespread.
- Forelius* sp.* Scattered sites in north FL, south into Citrus Co.
- Formica archboldi* M. R. Smith. Widespread in peninsular FL, west into Liberty Co.
- Formica pallidefulva* Latreille. Widespread in north FL, south into Highlands Co.
- Formica schaufussi dolosa* Wheeler. North FL, south into Lake Co.
- Formica subsericea* Say. Liberty Co. Rare, not seen in recent decades.
- Gnamptogenys triangularis* (Mayr). Isolated records from Dade, Broward and Escambia Cos. Rare. Introduced. Record in 1989 list under *G. aculeaticoxae* (Santschi) refers to this species.
- Hypoponera inexorata* (Wheeler). Widespread.
- Hypoponera opaciceps* (Mayr). Widespread.
- Hypoponera opacior* (Forel). Widespread.
- Hypoponera punctatissima* (Roger). South FL, north into Alachua and Bradford Cos. Introduced.
- Lasius alienus* (Foerster). North FL, south into Marion Co.
- Lasius neoniger* Emery. Scattered sites in north FL.
- Lasius umbratus* (Nylander). A few sites in Panhandle.
- Leptogenys manni* Wheeler. Highlands Co., north and west into Leon Co. Records in 1989 list under *L. elongata* (Buckley) refer to this species.
- Leptothorax allardycei* (Mann). Monroe and Lee Cos. Listed under *Macromischa* in 1989 list.
- Leptothorax bradleyi* Wheeler. North FL, south into Highlands Co.
- Leptothorax curvispinosus* Mayr. Northern-most FL.
- Leptothorax pergandei* Emery. Widespread.
- Leptothorax schaumii* Roger. North FL, south into Highlands Co.
- Leptothorax smithi* Baroni Urbani. Scattered sites in north FL, south into Highlands Co. Records under *L. wheeleri* in 1989 list refer to this species.
- Leptothorax texanus* Wheeler. Widespread. (7)
- Leptothorax torrei* (Aguayo). Dade, Monroe, Martin Cos. Listed under *Macromischa* in 1989 list.
- Leptothorax* sp.* Undescribed. Liberty and Leon Cos. (7)
- Linepithema humile* (Mayr). Scattered sites throughout FL. Introduced. Listed under *Iridomyrmex* in 1989 list.
- Monomorium destructor* (Jerdon). Scattered sites in south FL, chiefly in Key West. Introduced.
- Monomorium ebeninum* Forel. Monroe Co. Introduced.
- Monomorium floricola* (Jerdon). South FL, north into Pinellas Co. Introduced.
- Monomorium pharaonis* (Linnaeus). Widespread. Introduced.
- Monomorium trageri* DuBois.* Scattered sites throughout FL. Records listed under *M. minimum* (Buckley) in 1989 list refer to this species.
- Monomorium viride* Brown. Widespread.
- Myrmecina americana* Emery. Widespread.
- Myrmecina* sp.* Undescribed. Bradford Co. Rare.
- Myrmelachista ramulorum* Wheeler. Polk Co. Rare or extirpated. Introduced.
- Myrmica punctiventris* Roger.* Walton, Sta. Rosa, Escambia Cos. Rare.
- Neivamyrmex carolinensis* (Emery). Scattered sites in north FL, south into Highlands Co.
- Neivamyrmex opacithorax* (Emery). Scattered sites throughout FL.
- Neivamyrmex texanus* Watkins. Scattered sites in north FL, south into Highlands Co.
- Ochetellus glaber* (Mayr). Orange and Volusia Cos. Introduced. Listed under *Iridomyrmex* in 1989 list.
- Odontomachus brunneus* (Patton). Widespread in Peninsula, in Panhandle west into Leon Co.
- Odontomachus ruginodis* M. R. Smith. South FL, north into Orange and Co. Introduced.
- Odontomachus* sp. Undescribed. Highlands into Orange, Citrus Cos. Records listed under *O. clarus* in 1989 list refer to this species.
- Pachycondyla stigma* (Fabricius). South FL, north into Orange and Volusia Cos. Introduced.
- Paratrechina arenivaga* (Wheeler). Widespread.
- Paratrechina bourbonica* (Forel). South FL, north into Orange and Hillsborough Cos. Introduced.
- Paratrechina concinna* Trager. Widespread.
- Paratrechina faisonensis* (Forel). Widespread in north FL, south into Highlands Co.

Paratrechina guatemalensis (Forel). South FL, north to Sarasota and Indian River Cos. Introduced.

Paratrechina longicornis (Latreille). South FL, north to St. Johns and Columbia Cos. Introduced.

Paratrechina parvula (Mayr). North FL, south to Volusia Co.

Paratrechina phantasma Trager. Peninsular FL, Alachua Co. south into Palm Beach Co.

Paratrechina pubens (Forel). Dade, Sarasota and Palm Beach Cos. Introduced.

Paratrechina vividula (Nylander). North FL, south into Highlands Co.

Paratrechina wojciki Trager. Widespread.

Paratrechina sp. A.* Undescribed. Workerless parasite of *wojciki*. Highlands Co.

Paratrechina sp. B.* Undescribed. Workerless parasite of *faisonensis*. Hamilton Co.

Pheidole adrianoi Naves. Widespread.

Pheidole bicarinata vinelandica Forel.* North FL, south into Citrus Co.

Pheidole carrolli Naves. Scattered sites in north FL, south into Citrus Co. Rare.

Pheidole crassicornis Emery. Scattered sites in north FL, south into Alachua Co.

Pheidole dentata Mayr. Widespread.

Pheidole dentigula M. R. Smith. Widespread.

Pheidole diversipilosa Wheeler.* A few sites in Panhandle, east into Columbia Co. Rare.

Pheidole flavens Roger.* South FL, north into Martin Co. Introduced.

Pheidole floridana Emery. Widespread.

Pheidole lamia Wheeler. Leon and Jackson Cos. Rare.

Pheidole littoralis Cole. Peninsular FL, west into Franklin Co., south into Highlands Co.

Pheidole megacephala (Fabricius). Scattered sites in south FL, north into Hillsborough Co. Introduced.

Pheidole metallescens Emery. Widespread in north FL, south into Highlands Co.

Pheidole moerens Wheeler. Widespread. Introduced.

Pheidole morrisi Forel. Widespread.

Pheidole obscurithorax Naves*. Panhandle, east into Leon Co. Introduced. (8)

Pheidole tysoni Forel. Alachua and Madison Cos.

Platythyrea punctata (F. Smith). South FL, north into Highlands Co.

Pogonomyrmex badius (Latreille). Widespread.

Polyergus lucidus Mayr. Alachua, Columbia, Leon, Sta. Rosa Cos. Rare.

Ponera exotica M. R. Smith. Scattered sites in north FL, south into Highlands Co.

Ponera pennsylvanica Buckley. North FL, south into Marion Co.

Prenolepis imparis (Say). North FL, south into Orange Co.

Prionopelta antillana Forel. Marion Co. Introduced.

Proceratium crassicorne Emery.* Sta. Rosa and Liberty Cos. Rare. (9)

Proceratium croceum (Roger). Scattered sites in north FL, south into Levy Co.

Proceratium pergandei (Emery). North FL, south into Pinellas and Highlands Cos.

Proceratium silaceum Roger. North FL, south into Highlands Co.

Proceratium sp. A.* Undescribed. Leon and Lafayette Cos. Rare.

Proceratium sp. B.* Undescribed. Liberty Co. Rare.

Pseudomyrmex cubaensis (Forel). South FL, north into Polk Co.

Pseudomyrmex ejectus (F. Smith). Widespread.

Pseudomyrmex elongatus (Mayr). South FL, north to Highlands Co.

Pseudomyrmex gracilis (Fabricius). South FL, north into Alachua Co. Introduced. Records listed under *P. mexicanus* in 1989 list refer to this species.

Pseudomyrmex leptosus Ward. A few sites in Peninsula, north into Alachua Co.

Pseudomyrmex pallidus (F. Smith). Widespread.

Pseudomyrmex seminole Ward. Widespread in south FL, Orange and Bay Cos. in north FL.

Pseudomyrmex simplex (F. Smith). South FL, north into Orange Co.

Pyramica abdita (Wesson & Wesson)* Alachua and Leon Cos. Rare.

Pyramica angulata (M. R. Smith)* A few sites in north FL. Rare.

Pyramica apalachicolensis Deyrup & Lubertazzi.* Leon Co. Rare. (10)

Pyramica archboldi (Deyrup & Cover)* North FL, south into Volusia Co., west into Jefferson Co.

Pyramica bunki (Brown). Scattered sites in north FL, south into Highlands Co. Under *Smithistruma* in 1989 list.

Pyramica carolinensis (Brown). A few sites in north FL. Rare. Under *Smithistruma* in 1989 list.

Pyramica cloydi (Pfitzer)* Lake Co. Rare.

Pyramica clypeata (Roger). North FL, south into Highlands Co. Under *Smithistruma* in 1989 list.

Pyramica creightoni (M. R. Smith). North peninsular FL, south into Highlands Co. Under *Smithistruma* in 1989 list.

Pyramica deyrupi Bolton.* Marion Co.

Pyramica dietrichi (M. R. Smith). Widespread. Under *Smithistruma* in 1989 list.

Pyramica eggarsi (Emery). South FL, north into Alachua Co. Introduced. Under *Strumigenys* in 1989 list.

Pyramica gundlachi Roger. Dade, Monroe, Collier Cos. Introduced. Under *Strumigenys* in 1989 list.

Pyramica hexamera (Brown). Marion and Hernando Cos. Rare. Introduced. Under *Epitritus* in 1989 list.

- Pyramica inopina* (Deyrup & Cover).^{*} Alachua, Marion, Putnam Cos. Rare.
- Pyramica laevinasis* (M. R. Smith). Walton Co. Rare. Under *Smithistruma* in 1989 list.
- Pyramica margaritae* (Forel). Scattered sites in north FL, south into Marion Co. Introduced. Under *Smithistruma* in 1989 list.
- Pyramica membranifera* (Emery). Widespread. Introduced. Under *Trichoscapa* in 1989 list.
- Pyramica missouriensis* (M. R. Smith).^{*} Alachua and Highlands Cos. Rare.
- Pyramica ohioensis* (Kennedy & Schramm). Scattered sites in north FL, south into Alachua Co. Under *Smithistruma* in 1989 list.
- Pyramica ornata* (Mayr). North FL, south into Highlands and Sarasota Cos. Under *Smithistruma* in 1989 list.
- Pyramica pilinasis* (Forel).^{*} Scattered sites in north FL, south into Highlands Co.
- Pyramica pulchella* (Emery). North FL, west into Bay Co., south into Highlands Co. Under *Smithistruma* in 1989 list.
- Pyramica reflexa* (Wesson & Wesson). North FL, south into Polk Co. Under *Smithistruma* in 1989 list.
- Pyramica rostrata* (Emery).^{*} Northern tier of FL counties. Under *Smithistruma* in 1989 list.
- Pyramica talpa* (Weber). North FL, south into Highlands Co. Under *Smithistruma* in 1989 list.
- Pyramica wrayi* (Brown).^{*} Leon and St. Johns Cos. Rare.
- Solenopsis abdita* Thompson.^{*} Widespread.
- Solenopsis carolinensis* Forel. Widespread.
- Solenopsis corticalis* Forel. Monroe Co.
- Solenopsis geminata* (Fabricius). Widespread.
- Solenopsis globularia littoralis* Creighton. South FL, north into Alachua and Franklin Cos.
- Solenopsis invicta* Buren. Widespread. (11)
- Solenopsis nickersoni* Thompson. Collier Co. north into Leon Co.
- Solenopsis pergandei* Forel. Widespread.
- Solenopsis picta* Emery. Widespread.
- Solenopsis tennesseensis* M. R. Smith. Widespread.
- Solenopsis tonsa* Thompson.^{*} North FL, Leon Co. east to Alachua Co., south to Orange Co.
- Solenopsis truncorum* Forel.^{*} Alachua Co. Rare.
- Solenopsis xyloni* McCook. Western Panhandle. Not seen in recent decades, possibly extirpated.
- Solenopsis* sp.^{*} Undescribed parasite of *Pheidole dentata*. Gilchrist Co. Rare.
- Stenammina foveolocephalum* M. R. Smith.^{*} Walton Co. Rare.
- Strumigenys emmae* (Emery). South FL, north into Hillsborough and Volusia Cos. Under *Quadristruma* in 1989 list.
- Strumigenys lanuginosa* Wheeler. Dade, Lee and Monroe Cos. Rare. Introduced.
- Strumigenys louisianae* Roger. Widespread.
- Strumigenys rogeri* Emery. South FL, north into Orange Co. Introduced.
- Strumigenys silvestrii* Emery. A few sites, from Monroe into Gadsden Cos. Rare. Introduced.
- Tapinoma litorale* Wheeler. South FL, north into Pinellas Co.
- Tapinoma melanocephalum* (Fabricius). South FL, north into Brevard Co. Introduced.
- Tapinoma sessile* (Say). Widespread.
- Technomyrmex albipes* (F. Smith).^{*} Widespread. Introduced.
- Tetramorium bicarinatum* (Nylander). Widespread. Introduced.
- Tetramorium caldarium* (Roger). South FL, north into Lake Co. Introduced.
- Tetramorium lanuginosum* Mayr. Hernando, Holmes, Jackson Cos. Rare, extirpated? Introduced. Under *Triglyphothrix* in 1989 list.
- Tetramorium simillimum* (F. Smith). South FL, north into St. Johns Co. Introduced.
- Trachymyrmex jamaicensis* (André). Martin and Monroe Cos. Rare.
- Trachymyrmex septentrionalis* (McCook). Widespread.
- Wasmannia auropunctata* (Roger). South FL, north into Orange Co. Introduced.
- Xenomyrmex floridanus* Emery. South FL, north into Orange Co.

(1). *Aphaenogaster carolinensis* is listed as a subspecies of *A. texana* Wheeler in Bolton 1995. It was raised to species level by Gary Umphrey (1996). *Aphaenogaster carolinensis* is difficult to separate from *A. miamiana*.

(2). *Aphaenogaster umphreyi* is a recently described species (Deyrup and Davis 1998).

(3). There are at least five species of *Cardiocondyla* in Florida, differentiated by structural character states, coloration and habitat preferences. There is also some variation in color and sculpture within apparent species. This could denote additional species, but I have kept in mind that members of this genus are fully capable of founding inbred populations from the introduction of a single female, so variation could result from the introduction of a species from more than one source. If this occurred, there might be variant forms, even sympatric variant forms, that reflected geographic variation within the natural range of the species, or genetic drift. The persistence of these forms would reflect the degree of inbreeding in the population. I have resorted to using Creighton (1950), which leaves one species without a name. There are several names that might be applied to this species, or to other Florida species that I have identified using the Creighton key. These names include *obscurior* Wheeler, *ectopia* Snelling, *mauritanica* Forel, and *minutior* Forel. Inconvenient though it might seem, the most sensible approach to understand-

ing the taxonomy of exotic *Cardiocondyla* would be to begin by looking at specimens from the native ranges of the species.

(4). *Cephalotes varians* was, until recently, placed in the genus *Zacryptocerus*, now synonymized with *Cephalotes* (Andrade and Baroni Urbani 1999).

(5). *Crematogaster missouriensis* (the original description lacks the "o" in Missouri), was considered a subspecies of *C. minutissima* by Creighton (1950). Although the two species are similar, they differ in nest site, in the sculpture of the mesopleuron (Creighton 1950), and are sympatric in a large part of their ranges. A paper on the subgenus *Orthocrema* in eastern North America is in preparation.

(6). Species of *Dorymyrmex* were listed under the genus *Conomyrma* in the 1989 list. In the 1989 list, several species of *Dorymyrmex* reported from Florida by James Trager (1988) were excluded from the list. This was a mistake, and an injustice to Trager's excellent work. Of the seven species known to occur in Florida, four made their way into Bolton's catalog, three did not. Extensive field work in Florida since 1989 has confirmed that there are at least seven species in Florida. Whether any of these are identical with species known from the southwestern U.S. is an open question until the southwestern species have been studied as intensively as those in the Southeast. In 2001, I did a little collecting of *Dorymyrmex* during a short stay in Arizona. The results of even so brief an exposure have convinced me that the southwestern *Dorymyrmex* are as complex and challenging as they are fascinating.

(7). The subspecies *Leptothorax texanus davisi* Wheeler, listed in the 1989 list, was raised to species rank by Mackay (2000). In Florida and elsewhere *davisi* does not seem to be recognizable either as a species or subspecies; a paper dealing with this and the undescribed Florida *Leptothorax* listed above is in preparation.

(8). *Pheidole obscurithorax* is listed in Bolton (1995) as a subspecies of *P. fallax* Mayr. Deyrup et al. (2000) treated *obscurithorax* as a species.

(9). *Proceratium crassicornis* was synonymized with *P. silaceum* by Creighton (1950), but Maria de Andrade will be reviving this species in a forthcoming revision of the genus; she has sent me a

series of identified Florida specimens of both species that seem to justify this treatment.

(10). *Pyramica apalachicolensis* is a recently described species (Deyrup and Lubertazzi 2001).

(11). *Solenopsis invicta* is listed as *S. wagneri* Santschi in the 1995 catalog; the former name has been conserved.

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Many of the species added to the 1989 list were found for the first time in Florida by various entomologists, who recognized them as new records and kindly sent or brought specimens to me. These collectors I gratefully list below; the parenthesis after each name denotes the number of species that each person discovered: Lloyd Davis (7), Stefan Cover (3), Clifford Johnson (3), David Lubertazzi (3), Zachary Prusak (1), Vincent Golia (1), Paul Skelley (1). I thank Lloyd Davis and Hilary Swain for detailed and thoughtful comments on the first draft of this paper.

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THE EFFECT OF HARVESTING AND REPLANTING ON ARTHROPOD GROUND PREDATORS IN FLORIDA SUGARCANE

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ABSTRACT

Arthropod ground predators were sampled with pitfall traps in Florida sugarcane fields. More red imported fire ants, *Solenopsis invicta* Buren, were caught in pitfall traps than all other predators combined. Sugarcane harvesting did not affect pitfall trap catches of arthropod ground predators. However, replanting reduced arthropod catches for five to six months. These data show that for most of its three to five year crop cycle, Florida sugarcane is a stable ecosystem at ground level for arthropod ground predators.

Key Words: Sugarcane, predators, Florida, pitfall traps, ants

RESUMEN

Se muestrearon los depredadores artrópodos del suelo con trampas de suelo ("pitfall", trampas donde la presa cae en un hoyo en el suelo) en campos de caña de azúcar en Florida. Se capturaron más hormigas de fuego importadas, *Solenopsis invicta* Buren, en las trampas que todos los otros depredadores juntos. La cantidad de depredadores artrópodos capturados en las trampas no fué afectada al cosechar la caña de azúcar. No obstante, resembrando redujó la cantidad de artrópodos capturados durante cinco a seis meses. Estos datos muestran que por la mayor parte de su ciclo de cultivo, de tres a cinco años, la caña de azúcar en Florida es un ecosistema estable al nivel de suelo para los depredadores artrópodos del suelo.

Sugarcane (*Saccharum* spp.) is a major field crop in Florida and is primarily grown in the Everglades area of southern Florida. Numerous studies have been published about various biological control agents in Florida sugarcane. A list of many of these studies is provided by Hall (1988). In a later report, Hall & Bennett (1994) discuss in greater detail the overall biological control and IPM of sugarcane pests in Florida sugarcane. However, there are no published reports on the population dynamics of arthropod ground predators in Florida sugarcane. Florida sugarcane is a long-term crop and few tillage practices are required over the entire course of a 3 to 5 year planting (Hall & Bennett 1994). Hence, what the effect of yearly harvesting and eventual replanting of sugarcane is on arthropod ground predators is an interesting question. The objective of this study was to determine the effects of harvesting and replanting on arthropod ground predators in Florida sugarcane.

MATERIALS AND METHODS

Four sugarcane fields in southern Florida were sampled starting in June, 2000. Two of the fields were eighteen months old at the start of sampling. These fields were left in production after harvest (ratooned) and were used to measure the effect of harvest on activity of arthropod ground predators. In this paper, I consider arthropods to be predaceous if they belong to a taxonomic group in which

most members are predaceous. Two of the fields were three and one half years old at the start of sampling. These older fields were replanted to sugarcane (successive planting) after harvest and were used to measure the effect of replanting on activity of arthropod ground predators. The two ratooned fields were harvested during February, 2001. Harvesting consisted of burning the sugarcane to remove litter and removal of sugarcane stalks by mechanical harvesting. The two successively planted fields were harvested and replanted during November, 2000. Harvesting was as described for ratooned fields. Replanting consisted of fields being disced, sugarcane seedpieces placed in furrows, Thimet 20G (AI = phorate) placed in furrows on cane at 4.55 kg AI/hectare, and then seedpieces covered with soil.

Pitfall trap sampling in all four fields started June, 2000 and continued until June, 2001. Each pitfall trap consisted of a nine cm diameter plastic cup containing 100 ml of ethylene glycol. A five cm deep plastic collar was also cut from the 9 cm plastic cups. The top of this collar was taped in the middle of a 26 cm diameter paper plate with it's center removed. This collar was then inserted into the pitfall trap and the plate loosely covered with soil. This arrangement prevented soil subsidence around the trap rim thus allowing arthropods easy access to the trap. A small metal roof was also placed above each trap to prevent rainfall from filling traps. Five traps were used in each field. The first trap was located mid-field in a sug-

arcane row 50 m into the field to avoid possible edge effects. The next four traps were placed 5 m apart in the row into the field. Traps were used for two weeks each month. After each two week period, traps were taken to a laboratory and samples drained into paper towels and frozen. Thereafter, ants (Formicidae), earwigs (Dermaptera), ground beetles (Carabidae), rove beetles (Staphylinidae), spiders (Araneida), and centipedes (Chilopoda) were counted under a microscope. Taxonomic determinations of ants and spiders were made since these were the most abundant predators found in traps. The relative abundance of predators in all traps was determined. For statistical analysis, data from the two ratooned fields were pooled as were data from the two replanted fields. The mean monthly catch of ants, spiders, and total predators in pitfall traps in ratooned fields and replanted fields was compared using Least Significant Difference (LSD) tests (SAS 1996).

RESULTS AND DISCUSSION

A total of 4,255 arthropod ground predators were caught in pitfall traps during the one year study (Table 1). Of these, the vast majority were ants being 67.6% of the total catch. Among ants, the imported fire ant, *Solenopsis invicta* Buren was clearly the dominant ant species being 79.2% of all ants found in traps. These data are consistent with the report of Cherry & Nuessly (1992)

that showed that *S. invicta* had become the dominant ant species in Florida sugarcane since first being found there in 1970. In fact, more *S. invicta* (2,279) were caught in pitfall traps in this study than all other predators combined. There is a wealth of literature on *S. invicta* as a predator in sugarcane and other ecosystems and this is reviewed by Reagan (1986).

Hall & Bennett (1994) have noted that insect pests of sugarcane are good candidates for classical biological control because some pest damage may be generally tolerated, sugarcane is a long term crop, and few tillage practices are required over the entire course of the three to five year planting. They also note that pre-harvest burning is the most disruptive practice that may interfere with biological control. However, the effects of burning on arthropod populations are complex and not always predictable. For example, ants were the most frequently caught predators in this study and MacKay et al. (1991) noted that fire may reduce species richness of ants, increase ant activity, or have no effect on ant populations. Data in Table 2 show that there were significant differences in catches of ants, spiders, and total predator numbers among different months in ratooned fields. However, there were no significant differences in catches of these groups in the month immediately preceding harvest and following harvest in ratooned fields. Also, catches of these groups during the three month post-harvest period were not significantly different than the

TABLE 1. RELATIVE ABUNDANCE OF ARTHROPOD GROUND PREDATORS CAUGHT IN PITFALL TRAPS IN FLORIDA SUGARCANE FIELDS.

Predator	Number	% of total catch
Ants	2877	67.6
<i>Brachymyrmex obscurior</i> Forel	50	1.2
<i>Monomorium pharaonis</i> (Linn.)	52	1.2
<i>Odontomachus ruginodis</i> Wheeler	96	2.3
<i>Pheidole moerens</i> Wheeler	126	3.0
<i>Solenopsis invicta</i> Buren	2279	53.4
<i>Strumigenys louisianae</i> Roger	46	1.1
<i>Tetramorium simillimum</i> Smith	65	1.5
<i>Wasmannia auropunctata</i> (Roger)	60	1.4
Unknown	109	2.6
Earwigs	252	5.9
Ground Beetles	76	1.8
Rove Beetles	89	2.1
Spiders	913	21.5
Corinnidae	116	2.7
Gnaphosidae	49	1.2
Linyphiidae	69	1.6
Lycosidae	633	14.9
Unknown	46	1.1
Centipedes	48	1.1
Total	4255	100.0

TABLE 2. PREDATORS CAUGHT IN PITFALL TRAPS IN RATOONED FIELDS OF FLORIDA SUGARCANE.

Month	Predators ¹		
	Ants	Spiders	Total ²
June - 2000	30.5 ± 34.8 A	3.1 ± 1.5 BC	34.5 ± 35.9 A
July	19.1 ± 19.4 AB	13.2 ± 17.8 A	35.0 ± 29.5 A
August	7.3 ± 8.5 BC	5.4 ± 4.8 BC	13.0 ± 10.1 B
September	7.3 ± 11.2 BC	8.1 ± 4.7 AB	16.8 ± 14.7 B
October	2.3 ± 2.8 C	3.6 ± 2.9 BC	5.9 ± 4.8 B
November	1.1 ± 1.4 C	3.3 ± 6.3 BC	4.4 ± 7.2 B
December	2.7 ± 1.9 C	1.8 ± 1.8 C	5.0 ± 2.9 B
January - 2001	1.7 ± 2.7 C	1.8 ± 1.9 C	3.5 ± 4.3 B
February	Harvest	Harvest	Harvest
March	9.2 ± 10.9 BC	1.9 ± 1.6 C	13.7 ± 11.6 B
April	1.8 ± 1.5 C	4.1 ± 2.9 BC	6.4 ± 4.1 B
May	4.0 ± 4.7 C	2.2 ± 1.9 C	10.7 ± 6.8 B

¹Mean ± SD. Means in a column followed by the same letter are not significantly different (alpha = 0.05) using the LSD test (SAS 1996).

²Total predators = all predators noted in Table 1.

three month pre-harvest period. These data show that the sugarcane harvesting, including the burning of the fields, did not reduce overall activity of ants, spiders, or total predator number in ratooned fields.

Predator catches in pitfall traps in successively planted fields of Florida sugarcane are shown in Table 3. Pitfall trap catches of ants, spiders, and total predators all decreased in the month following replanting versus the month immediately before replanting. Also, total predator catches remained low for the first four months after replanting compared to pre-planting catches and then increased dramatically at five to six months after planting. These data make sense since re-

planting is more disruptive to the soil habitat than harvesting because replanting involves not only burning of the field and mechanical harvesting, but also discing, and the use of a soil insecticide.

To summarize, my data show that sugarcane harvesting had no significant effect on total numbers of arthropod ground predators caught in pitfall traps. In contrast, replanting significantly reduced total numbers of ground predators in pitfall traps, but these numbers resurged after 5 to 6 months to preharvest levels. These data show that through most of its 3 to 5 year crop cycle, Florida sugarcane is a stable ecosystem at ground level for most arthropod ground predators.

TABLE 3. PREDATORS CAUGHT IN PITFALL TRAPS IN REPLANTED FIELDS OF FLORIDA SUGARCANE

Month	Predators ¹		
	Ants	Spiders	Total ²
June - 2000	61.6 ± 120.9 A	5.7 ± 3.2 CDE	69.0 ± 120.3 A
July	16.5 ± 19.8 B	11.7 ± 6.9 BC	34.7 ± 28.0 ABC
August	9.5 ± 11.9 B	8.2 ± 6.6 BCD	9.4 ± 17.0 BC
September	10.6 ± 14.2 B	13.2 ± 12.1 B	27.1 ± 18.3 BC
October	21.3 ± 42.0 B	21.6 ± 18.6 A	44.3 ± 48.2 AB
November	Replant	Replant	Replant
December	2.4 ± 2.3 B	2.2 ± 1.7 DE	5.4 ± 3.5 C
January - 2001	1.6 ± 1.8 B	1.2 ± 1.2 E	3.0 ± 2.5 C
February	1.3 ± 1.6 B	0.7 ± 0.8 E	2.7 ± 2.1 C
March	2.1 ± 2.6 B	0.4 ± 0.7 E	4.5 ± 2.6 C
April	14.9 ± 32.3 B	2.0 ± 2.0 DE	18.3 ± 32.5 BC
May	32.3 ± 43.9 AB	0.9 ± 1.0 E	48.2 ± 39.7 AB

¹Mean ± SD. Means in a column followed by the same letter are not significantly different (alpha = 0.05) using the LSD test (SAS 1996).

²Total predators = all predators noted in Table 1.

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COLONIZATION OF *FOPIUS CERATITIVORUS*, A NEWLY DISCOVERED AFRICAN EGG-PUPAL PARASITOID (HYMENOPTERA: BRACONIDAE) OF *CERATITIS CAPITATA* (DIPTERA: TEPHROTIDAE)

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ABSTRACT

Fopius ceratitivorus Wharton is a recently discovered braconid parasitoid of the Mediterranean fruit fly (= medfly), *Ceratitidis capitata* (Wied.). Unlike other parasitoids previously used in medfly biological control, *F. ceratitivorus* was originally collected from medfly in its purported region of origin, east Africa. Shipments of *Ceratitidis* spp. pupae from Kenya to a newly constructed quarantine facility in Guatemala yielded both *F. ceratitivorus* and its congener *F. caudatus* (Szèpliget). Only the former species was successfully colonized through the use of medfly infested coffee berries. In the process of colonization it was determined that *F. ceratitivorus* oviposited into the eggs and recently hatched larvae of medflies and completed development in the hosts' puparia. This is a relatively rare behavior among fruit fly parasitoids and, because tephritid eggs near the surface of fruits are particularly vulnerable to attack, one that might contribute to its success as a biological control agent.

Key Words: biological control, mass-rearing, medfly

RESUMEN

F. ceratitivorus Wharton es un parasitoide Braconido de la mosca del Mediterráneo (= moscamed), *Ceratitidis capitata* (Wied.), recientemente descubierto. A diferencia de otros parasitoides previamente usados en el control biológico de la moscamed, *F. ceratitivorus* fue colectado originalmente de moscamed en su supuesta región de origen, al este de Africa. Envíos de pupa de tephritidos desde Kenia hacia la recientemente construida instalación de Cuarentena en Guatemala, produjeron especímenes de *F. ceratitivorus* y su congener *F. caudatus* (Szepliget). Solo la primera especie fue colonizada exitosamente mediante el uso de frutos de café infestados por moscamed. En el proceso de colonización se determinó que *F. ceratitivorus* oviposita sobre los huevos y larvas recientemente eclosionadas de moscamed, y que completa su desarrollo en la pupa huésped. Este es un comportamiento relativamente raro dentro de los parasitoides de moscas de la fruta, y debido a que los huevos de los tephritidos cercanos a la superficie del fruto son particularmente vulnerables, ello podría contribuir a su éxito como agente de control biológico.

By the end of the 19th century the Mediterranean fruit fly (= medfly), *Ceratitidis capitata* (Wied.), had spread from its African homeland to tropical and subtropical countries around the

world. After finding medfly in the Honolulu area in 1910, the progressive Hawaiian agricultural community of the time financed an African collection of tephritid natural enemies by Silvestri (1914) in order to bring the fly under biological control. By 1918 there were several parasitoids from various parts of the world established in Hawaii, including three species of opiine Braconidae (Pemberton & Willard 1918). Over time, subsequent expeditions resulted in additional Hawaiian establishments (Gilstrap & Hart 1987), the most effective of which for suppression of both medfly and oriental fruit fly (*Bactrocera dorsalis* [Hendel]) proved to be the braconid *Fopius arisanus* (Sonan) (Bess et al. 1961).

While *F. arisanus* is a common parasitoid of medfly in Hawaii, it is an Asian species that was originally obtained from the pupae of oriental fruit fly (Wharton & Gilstrap 1983). In fact, to our knowledge, none of the braconids successfully disseminated for the control of medfly originated from collections of medfly (Wharton & Gilstrap 1983; Ovruski et al. 2000). This shortage of "true-medfly" parasitoids is not due to a lack of candidates since a recent Kenyan survey of *Ceratitis* spp. yielded 10 species of hymenopterous parasitoids (Wharton et al. 2000; see also Steck et al. 1986), but probably reflects the historical difficulty of transporting live insects from Africa to afflicted agricultural areas such as Hawaii or Central America (e.g., van Zwaluwenburg 1937).

We here describe the shipment to Guatemala and subsequent colonization of *Fopius ceratitivorus* Wharton, a recently discovered parasitoid of the medfly that is both a true, African natural enemy of medfly and, like its congener *F. arisanus*, an egg-pupal parasitoid. This combination of characteristics suggests that this species may be a particularly attractive candidate for biological control.

MATERIALS AND METHODS

Origin of Insects

Fopius ceratitivorus has been obtained only from coffee, *Coffea arabica* L., in central Kenya and in particular from plantations at Ruiru (1°5.72'S, 36°54.22'E at 1609 m) and Rurima (0°38.39'S, 37°29.69'E at 1228 m) (Wharton 1999; Wharton et al. 2000). Mean annual rainfalls in these areas are 1.06 m and 0.9 m, respectively, and the mean temperature ranges are 12.8-25 and 15.5-28°C, respectively (Wharton et al. 2000). Collections were made throughout the November-July coffee harvest season. The tephritids in the shipments, in order of abundance, were: *C. capitata*, *C. rosa* Karsch, and *Trirhithrum coffeae* Bezzi (Wharton 1999).

Insect Arrival

Field collection procedures and handling procedures were described by Wharton et al. (2000). Pupae were shipped by air in lots of 4,000-23,000 insects to the Guatemala International Airport, cleared through customs, and then brought by car to the USDA-APHIS/MOSCAMED quarantine facility at San Miguel Petapa, Guatemala (Table 1).

Quarantine Facility

Packages were brought to the USDA-APHIS / MOSCAMED quarantine facility at San Miguel Petapa outside of Guatemala City, Guatemala. Initially, packages holding pupae were removed from containers in a large (0.8 × 0.8 × 0.8 m) sleeved cage separated from the remainder of the quarantine facility by a locked door. Adult parasitoids were captured individually and transferred to smaller (21 × 21 × 21 cm) cages containing honey and a water-wick while adult flies were placed in 70% ethanol and preserved for latter ex-

TABLE 1. THE DATES, SOURCES, AND NUMBERS OF PUPAE IN SHIPMENTS OF *CERATITIS* SPP. PUPAE FROM KENYA TO THE QUARANTINE FACILITY NEAR GUATEMALA CITY, GUATEMALA AS WELL AS THE NUMBERS OF VARIOUS PARASITIDS THE SHIPMENT CONTAINED AND THE % PARASITISM OF THE PUPAE.

Date	Collection	No. Pupae	% Parasitism	Parasitoids		
				<i>F. ceratitivorus</i>	<i>F. caudatus</i>	<i>D. fullaway</i>
9/19/2000	Koru	4,310	4.11	0	177	0
10/31/2000	Koru		2.14	0	215	0
10/31/2000	Ruiru	10,052	0.73	73	0	0
12/22/2000	Koru		0.46	0	95	8
12/22/2000	Ruiru	22,507	1.57	290	0	63
12/22/200	Rurima		0.35	74	0	5
6/26/2001	Koru	15,645	12.77	0	1,998	0
12/21/2001	Koru		0.20	0	16	0
12/21/2001	Ruiru	8,043	3.68	221	0	75

amination. Caged parasitoids were then moved into a larger room within the quarantine facility that had both windows providing natural light and artificial lighting (12L: 12D). Temperature in this room was 26°C and relative humidity 65-75%. All packaging materials and biological wastes were sterilized in an autoclave before removal from the quarantine building. Specimens of all insect species received were preserved in collections at the quarantine facility.

Presentation of Hosts

Several means of host presentation were developed, including artificially placing eggs into slits cut through the skin and pulp of coffee berries. However, the most practical and effective means of presenting hosts to *F. ceratitvorus* consisted of first allowing female medflies to oviposit into firm coffee berries that were mature to the point of color-break. In addition to conserving any host-location cues the ovipositing medfly might leave, this technique minimized fermentation during the presentation. High densities of medflies (~4,000 males and 4,000 females) were kept in 31 × 31 × 31 cm screen cages and allowed to lay eggs in varying numbers of berries for a period of 24 hours. The berries had been previously strung on thread to form "necklaces" of ~180 fruits and then suspended from the ceiling of the cage. Strings of berries were then transferred to screen cages that contained ~600 female parasitoids and a similar number of males. There was an attempt to present infested fruit at a ratio of 3 fruit / female (~120 host egg clutches / female parasitoid). Females at the time of first presentation were ~ 8 days of age and had been kept in the presence of similar aged males since eclosion. During the first 2 days of this mating period cages were placed near windows to approximate a natural light environment. Over the next 6 days the cages were kept under full spectrum lights.

Typically, infested fruits were exposed to parasitoids for 48 hours; however, if berries were small and prone to drying exposures were curtailed after 24 hours. Depending on coffee availability there were 2 to 4 exposures / cage / week. Female survived in the exposure cages for up to 45 days, with 50% percent of females were typically alive after 30 days. Male lifespans were lower in quarantine, with 50% dead after only 15 days.

In addition to coffee, medflies were allowed to oviposit in several other fruits that were subsequently exposed to *F. ceratitvorus* : mangos (*Mangifera indica* L. var. Tommy Atkins), *Spondias* sp., papaya (*Carica papaya* L.), apple (*Malus pumila* Mill.), peach (*Prunus persica* Batsch), and pear (*Pyrus communis* L.). The treatment of these fruits following exposure was as described above for coffee berries.

Holding of Larvae

Following either 24 or 48 hours of exposure to parasitoids, 180-360 berries were placed in 30 × 15 cm trays on dampened paper over a 0.5 kg of moist medfly diet obtained from the USDA-APHIS / MOSCAMED rearing facility at El Pino, Guatemala (Schwarz et al. 1985). After spraying the berries lightly with water, the trays were placed in a 0.6 × 0.4 × 1.0 m rack covered with a black plastic sheet, which allowed temperature and relative humidity to increase to 26-27°C and 98-99%, respectively. After 48 hours, the berries were mixed into an additional 0.5kg of diet. Trays were moved into a cooler room at 23°C and 65-75% RH, and held over sawdust for 13 days (day 19 of the process). At the end of this time, mature larvae had left the diet and completed pupation in the sawdust. Puparia were placed in the 31 cm × 31 cm × 31 cm cages and emerging medflies removed through aspiration. *Fopius ceratitvorus* males began to eclose on day 24 and females on day 26.

Host Stage Parasitized

Standard rearing methods enabled oviposition into both eggs and newly hatched larvae. However, they did not distinguish between these stages. To better determine what stage(s) of medfly was being parasitized we varied parasitoid-exposure schedules to present either eggs alone or larvae alone (Fig. 1). In one case (Fig 1; A), fruit with eggs were presented 21 hours after the initial exposure to medfly females and then removed after 24 hours. This eliminated the possibility that early-instar larvae were present. In the second case (Fig. 1; B), 69 hours elapsed between the initiation of oviposition and exposure of the fruit to parasitoids, and only larvae were available as hosts. In the third case (Fig.1; C), the standard exposure sequence was modified so that parasitism was limited to a 24 hour period rather than the usual 48 hours. This again resulted in only eggs being present during attacks. D represents the standard sequence where both eggs and early instar larvae are potentially present. There were 3 replications of each of the exposure regimes.

RESULTS

Host Stage Parasitized

In the course of the standard rearing procedure, infested berries were removed from exposure to parasitoids prior to or just following egg hatch and larvae were rarely observed when berries were first placed on dampened paper over diet. To better determine the stage of medfly being attacked the exposure procedure was modified to expose only eggs or only recently hatched first in-

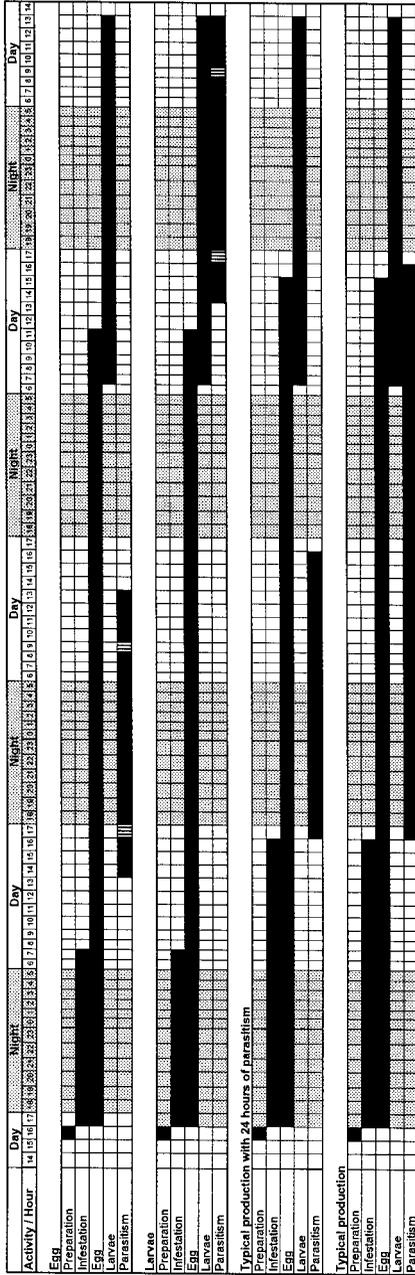


Fig. 1. The timing of exposure of coffee berries containing Mediterranean fruit fly eggs and larvae to the parasitoid *Fopius ceratitivorus*. The various schedules resulted in either eggs (A&C), first instar larvae (B) or both (D) being open to attack. Dark bars refer to time spent in the various activities of preparation of fruit to be exposed to medflies (“preperation”), exposure of fruit to medflies (“infestation”), the period of egg availability (“egg”), the period during which eggs hatch (“larvae”), and the period of exposure to parasitoids (“parasitism”). The time line in hours is at the top of the chart and the light and shaded areas represent alternating periods of light and darkness.

star larvae to parasitoids. Both stages were vulnerable to attack (Table 2).

In addition to *F. ceratitivorus*, the Kenyan shipments contained other Braconidae including *Fopius caudatus* (Szépligeti). Attempts to rear *F. caudatus* were unsuccessful, although colonies were sometimes maintained for up to 6 generations before increasingly male-biased sex ratios resulted in collapse. *Fopius caudatus* was also an egg-pupal parasitoid and when medfly eggs were presented in slits cut in coffee berries it was relatively easy to observe the penetration of the host egg by the parasitoid’s ovipositor. Its capacity to attack early instar larvae is unknown.

Percent Parasitism and Colony Growth

The *F. ceratitivorus* colony increased over time until at present (April 2002) weekly production was 10,000-18,000 adults/week or roughly 2-3 adult parasitoids per berry (Fig. 2). Overall, percent parasitism was 3.5-4%, but was occasionally as high as 21%. Typical sex ratios approximated 1 male:1 female, but were sometimes strongly female or male biased (Fig. 2). For example, in the experiments to identify stage of host attacked only 37% of the adult parasitoids were male. In part, fluctuations in numbers reflected the seasonal changes in the abundance of coffee berries used in the rearing process. *Fopius ceratitivorus* was capable of parasitizing medfly in a variety of fruit species other than coffee (Table 3), and these may be integrated into the rearing program in the future.

DISCUSSION

The establishment of *F. arisanus* in Hawaii is arguably the most successful instance of fruit fly biological control in the world (e.g., Clausen 1978), and it would be useful to employ other parasitoids that possess the characteristics that have made *F. arisanus* so prominent among Hawaiian fruit fly natural enemies. Certainly one the most unusual attributes of *F. arisanus* is that it is an endoparasitic koinobiont that oviposits into the egg, rather than the larva, of its tephritid host (Wharton 1997). The larval parasitoid persists in the first instar until the host’s puparium is formed after which it completes its development (Clausen 1978).The ability to parasitize eggs, as do *F. arisanus*, *ceratitivorus* and *caudatus*, is otherwise rare among fruit fly parasitoids. We are aware of only one other species known to do so, *Utetes canaliculatus* (Gahan), a North American parasitoid of *Rhagoletis* and another opiine braconid (Prokopy & Webster 1978).

Several reasons have been proposed why egg-parasitism might account for the success of *F. arisanus*, including its early presence inside the host compared to other braconids that attack var-

TABLE 2. THE NUMBER OF *F. ARISANUS* DEVELOPING IN HOST COHORTS OF VARIOUS AGES. (A) "STANDARD EGG" REFERS TO THE COLONIZATION PROCEDURE DESCRIBED IN THE TEXT AS IT WAS APPLIED TO SMALL COFFEE BERRIES. BECAUSE SMALL FRUITS ARE SUBJECT TO DRYING THEY WOULD ONLY BE EXPOSED FOR A 24 HOUR PERIOD A DAY AFTER OVIPOSITION AND AS A RESULT WOULD CONTAIN ONLY EGGS. (D) "TYPICAL EGGS & LARVAE" REFERS TO THE 48 HOUR EXPOSURE EMPLOYED WITH LARGER BERRIES. (A) "EGG" REFERS TO A 24 HOUR EXPOSURE BEGUN EARLIER THAN USUAL TO FURTHER GUARANTEE ONLY EGGS ARE PRESENT, AND (B) "LARVAE" TO A DELAYED 24 HOUR EXPOSURE THAT WOULD CONSIST ENTIRELY OF 1ST INSTAR LARVAE

Exposed intervals (h)	Replicates	A	B	C	D
		Egg	Larvae	Standard Egg	Standard Egg & Larvae
		21-45	69-93	24-48	24-72
Adults reared	1	30	7	24	248
	2	143	51	21	92
	3	72	7	123	333
Pupae recovered (cc)	1	120	280	103	153
	2	400	250	68	62
	3	65	80	98	110
% Parasitism	1	0.4	0.0	0.4	2.6
	2	0.8	0.3	0.5	2.5
	3	1.9	0.1	2.1	5.1

ious larval instars (Bess et al. 1961). Because it is the first parasitoid present, a *F. arisanus* larva would be in a position to eliminate or suppress the growth of its competitors. In addition, tephritid eggs located near the surface of a fruit or vegetable are particularly vulnerable to parasitism. Fruit fly larvae that feed in the pulp or seeds of fruit can be difficult for parasitoids to reach with their ovipositors, and there is a well established negative relationship between fruit size and parasitism by larva-attacking braconids (e.g., Sivinski et al. 1997, Lopez et al. 1999).

Thus the capacity to parasitize vulnerable eggs and early instar larvae is potentially a valuable trait for a biological control agent (Bess et al. 1961), and medfly control in Central America may particularly benefit from the availability of an effective natural enemy. At present, there is little parasitism of medfly in the New World by native tephritid parasitoids, and only local and sporadic parasitism by introduced species such as *Diachasmimorpha longicaudata* (Ashmead) (Eskafi 1990; Sivinski et al. unpublished data). Unlike its exemplary performance in Hawaii, *F. arisanus* has either failed to become established in the Americas (Ovruski et al. 2000) or failed to flourish after establishment (Wharton et al. 1981).

In addition to being a potential candidate for establishment, *F. ceratitivorus* might prove to be important in regional eradication programs. Medfly is now widely distributed across the Latin American tropics and subtropics. The northward spread of medfly into Mexico, and ultimately into the United States, has been prevented by a Sterile Insect Technique (= SIT) / insecticide-bait spray barrier maintained along the Mexican / Guatema-

lan border by the international organization MOSCAMED (United States, Mexico, and Guatemala). Recently, this barrier has been expanded and the possibility of regional eradication of the medfly is under consideration (P. Rendon, personal communication.).

In a region-wide eradication program the medfly must be attacked in a variety of environments, some of which may not be amenable to repeated applications of insecticide-bait sprays, such as organic growing areas, urban / suburban locations, water-sheds, and national parks. In these areas it will be important to maximize the impact of the biological components of the various control options. There is accumulating evidence that augmentative parasitoid releases may be an efficacious means of suppressing fruit fly populations, perhaps to a level where SIT can then be used to complete eradication (e.g., Wong et al. 1991, Sivinski et al. 1996, Montoya et al. 2000).

The potential of *F. ceratitivorus* for mass-rearing and augmentative release is unknown. The mean parasitism rate in the present colony of ~4% is an order of magnitude or more lower than the laboratory parasitism rates of better established medfly parasitoids (e.g., Baeza et al. 2002). However, experience suggests that greater familiarity with the species' requirements will improve production. In the meantime a stable colony in Guatemala will allow the experiments to be accomplished that will clarify its usefulness in medfly biological control. These include determination of host range and capacity to persist through seasonal declines in Guatemalan medfly populations when eggs are rare.

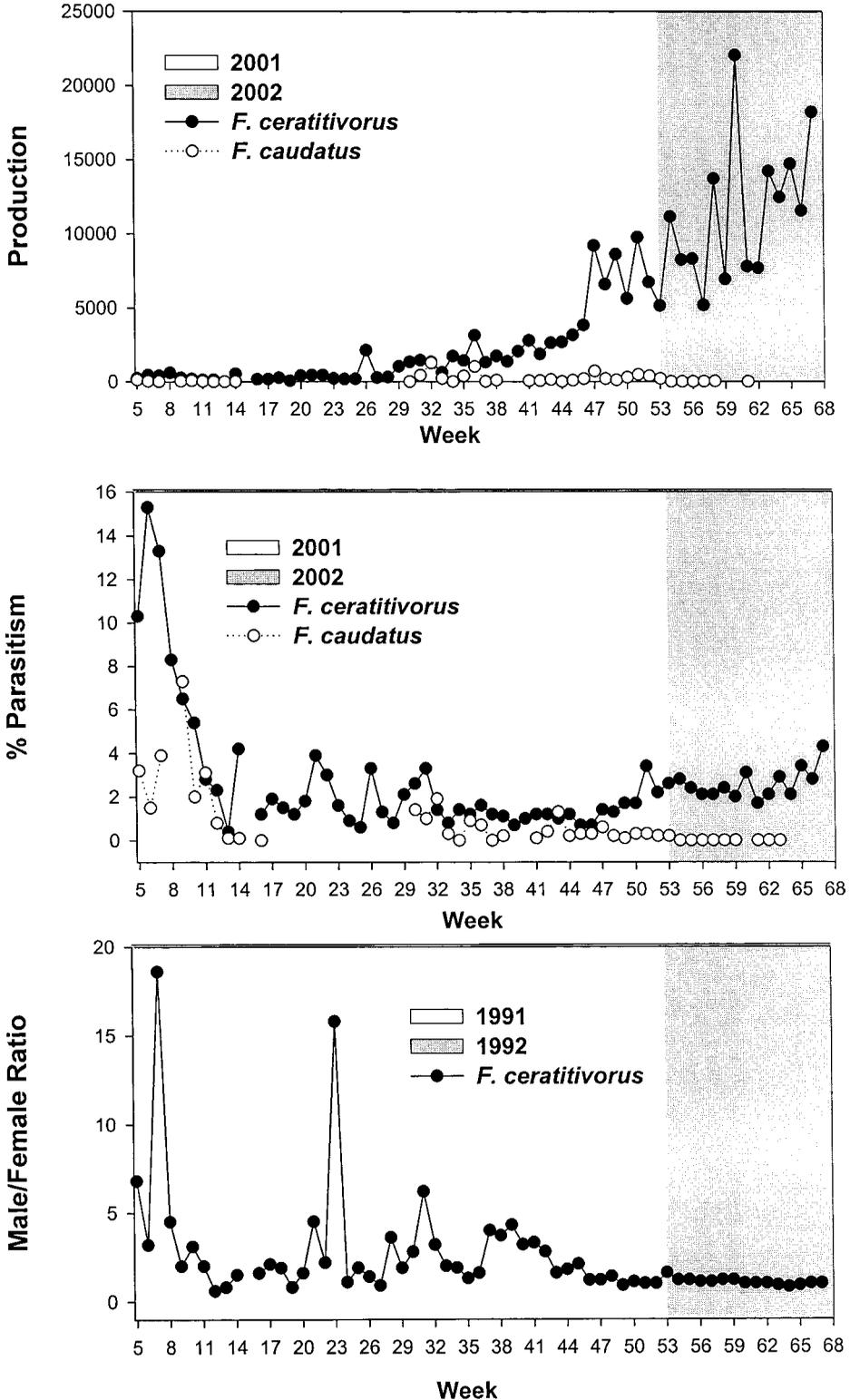


Fig 2. The production, % parasitism, and sex-ratio of *Fopius ceratitivorus* and *F. caudatus* in the Guatemalan quarantine facility over time. Sharp declines are typically due to temporary shortages of coffee berries.

TABLE 3. THE PRODUCTION OF *F. CERATITIVORUS* IN VARIOUS FRUITS INCLUDING THE MEAN NUMBERS OF PUPAE RECOVERED/FRUIT (1 ML = ~50 PUPAE), THE NUMBER OF PARASITOIDS TO EMERGE FROM THE SUMMED FRUITS OF EACH SPECIES AND THE SEX RATIOS OF THE PARASITOIDS.

Host Fruit	Number of Fruit	Pupae per Fruit (ml)	Emerged Parasitoids		% Parasitism	♂:♀ Ratio
			♂	♀		
Mango	60	20	2,398	1,359	5.3	1.8
Pear	46	40	4,254	2,593	6.4	1.6
Coffee	1,260	1	1,511	1,343	3.3	1.1
<i>Spondias</i> sp.	32	2	235	99	7.2	2.4
Papaya	12	9	226	84	4.7	2.7
Peach	1	3	7	0	4.0	
Apple	1	8	0	0		

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PREDATION ON NEONATE LARVAE OF
DIAPREPES ABBREVIATUS (COLEOPTERA: CURCULIONIDAE)
 IN FLORIDA CITRUS: TESTING FOR DAILY PATTERNS OF
 NEONATE DROP, ANT PREDATORS AND CHEMICAL REPELLENCY

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ABSTRACT

The root weevil, *Diaprepes abbreviatus* (L.), is a major pest of Florida citrus. When neonate larvae hatch from egg masses in the citrus canopy and drop to the soil surface before burrowing down to the roots for feeding, they are vulnerable to ant predation. However, neonates are reported to produce a chemical repellent that lasts up to four days and reduces ant predation by about 40%. We assessed the daily pattern of neonate drop from egg masses under laboratory conditions (24°C, 70% RH, L:D = 12:12), examined the role of ants as predators of neonates (<48 h post hatch) on the soil surface in three citrus groves in central Florida, and tested for chemical repellency in the field by comparing predation rates on 5-day versus 1-2 h old neonates. Neonate drop was not well synchronized within or among egg masses, occurred during all hours of the light and dark phases, and extended over 5 to 23 h (mean = 11.97, SE = 0.866) for individual egg masses (n = 29). However, the drop rate was highest during the second half of the light phase (52.4%) and lowest during the second half of the dark phase (8.0%). Predation occurred in 104 of 199 replicates (52.3%) in the three groves with a total of 475 of the 3980 larvae (11.9%) removed by predators within 20 mins. Predation pressure varied within and among groves, and involved eight ant species (Hymenoptera: Formicidae) and a single predation event by a nymph of the big-eyed bug, *Geocoris floridanus* Blatchley (Hemiptera: Lygaeidae). For data pooled among groves, the red imported fire ant, *Solenopsis invicta* Buren, was responsible for 29.5% of the predation, *Pheidole moerens* Wheeler 27.8%, *Dorymyrmex reginacula* (Trager) 9.7%, *Brachymyrmex obscurior* Forel 8.8%, *Dorymyrmex bureni* (Trager) 8.6%, *Cardiocondyla emeryi* Forel 8.0%, *Paratrechina bourbonica* Forel 4.8%, and *Pheidole morrisi* Forel 2.5%. In our test for age-dependent chemical repellency, a total of 2620 of the 3840 neonates (68.2%) were preyed upon within 30 mins but the predation rate on old versus young neonates did not differ at 68.8% and 67.7%, respectively. In this experiment, 368 of the predation events (14.0%) were observed directly with *P. moerens* responsible for 62.5%, *S. invicta* 25.3%, *C. emeryi* 10.1%, *B. obscurior* 1.4%, *Cardiocondyla wroughtonii* (Forel) 0.5%, and *D. bureni* 0.3%. We conclude that ants are important predators of *Diaprepes* neonates in central Florida citrus groves and have potential for a conservation biological control program.

Key Words: predation, ants, biological control, integrated pest management, red imported fire ant, Curculionidae, Formicidae

RESUMEN

El picudo de la raíz, *Diaprepes abbreviatus* (L.), es una plaga importante de cítricos en la Florida. Cuando las larvas neonatas (recién nacidas) esclosionan de las masas de huevos en la copa de los árboles de cítricos y caen a la superficie del suelo escavando hacia abajo en las raíces para alimentarse, ellas están vulnerables a la depredación por las hormigas. No obstante, se reportan que las neonatas producen un químico repelente que dura hasta cuatro días y reduce la depredación por las hormigas por aproximadamente 40%. Nosotros evaluamos el patrón diario de la caída de las neonatas de las masas de huevos bajo condiciones en el laboratorio (24°C, 70% RH, L:D = 12:12) [RH = Humedad Relativa; L:D = Luz:Oscuridad], examinamos el papel de las hormigas como depredadores de las neonatas (<48 h después de esclosionar) sobre la superficie del suelo en tres huertos de cítricos en central Florida, y probamos la habilidad del químico para repelar en el campo comparando las tasas de depredación sobre las neonatas de 5-días versus 1-2 horas de edad. La caída de las neonatas no fué bien sincronizada dentro de la misma o en diferentes masas de huevos, más ocurrió durante todas las horas de las fases de luz y oscuridad, y se extendió de 5 a 23 horas (promedio = 11.97, SE = 0.866) para las masas de huevos individuales (n = 29). No obstante, la tasa de la caída fué la más alta durante la segunda mitad de la fase de luz (52.4%) y la más baja durante la segunda mitad de la fase (8.0%). La depredación ocurrió en 104 de las 199 replicas (52.3%) en tres huertos con un total de 475 de los 3980 larvas (11.9%) eliminadas por los depredadores dentro de 20 minutos. La presión de los depredadores varió dentro del mismo y en dife-

rentes huertos, y abarcó ocho especies de hormigas (Hymenoptera: Formicidae) y un solo evento de depredación por parte de una ninfa del chinche de ojos grandes, *Geocoris floridanus* Blatchley (Hemiptera: Lygaeidae). Por los datos colectados entre todos los huertos, la hormiga de fuego importada, *Solenopsis invicta* Buren, fué responsable por el 29.5% de la depredación, *Pheidole moerens* Wheeler 27.8%, *Dorymyrmex reginica* (Trager) 9.7%, *Brachymyrmex obscurior* Forel 8.8%, *Dorymyrmex bureni* (Trager) 8.6%, *Cardiocondyla emeryi* Forel 8.0%, *Paratrechina bourbonica* Forel 4.8%, y *Pheidole morrisi* Forel 2.5%. En nuestra prueba de la repelencia químico dependiendo de la edad, un total de 2620 de los 3840 neonatas (68.2%) fueron atacadas dentro de 30 minutos pero la tasa de depredación sobre neonatas viejas versus neonatas juvenes no fué diferente siendo 68.8% y 67.7%, respectivamente. En este experimento, 368 de los eventos de depredación (14.0%) fueron observados directamente con *P. moerens* responsable por 62.5%, *S. invicta* 25.3%, *C. emeryi* 10.1%, *B. obscurior* 1.4%, *Cardiocondyla wroughtonii* (Forel) 0.5%, y *D. bureni* 0.3%. Nosotros concluimos que las hormigas son depredadores importantes de neonatas de *Diaprepes* en los huertos de cítricos en central Florida y tienen potencial en un programa de conservación de control biológico.

The root weevil, *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae), is a major pest of citrus, ornamentals, and other crops, and has spread widely in Florida since it was first detected in 1964 (Graham et al. 1996, McCoy 1999, McCoy et al. 2001). Adults are long lived and feed on foliage, especially new growth. Mating occurs in the canopy, and eggs are laid in masses between leaves that are glued together by an adhesive secreted by the female during oviposition. The larvae hatch, escape from the sealed leaf envelope, drop to the soil, and burrow down to the roots where they begin feeding. As they grow, the larvae move to larger roots, and pupate in the soil after 9-11 instars (Woodruff 1985, Quintela et al. 1998, McCoy 1999). In citrus, larval feeding reduces yield, girdles trees, and facilitates infections by plant pathogens such as *Phytophthora* spp. The combination of *Diaprepes* and *Phytophthora* can cause severe tree decline and destroy groves within a few years of an initial infestation (Graham et al. 1996). In developing an effective integrated pest management (IPM) program for *D. abbreviatus*, it is important to maximize the effectiveness of natural enemies. Preliminary research indicates that some of the major mortality agents of *Diaprepes* eggs, larvae, and adults are predators; and that the primary predators are ants (Whitcomb et al. 1982; Richman et al. 1983, Stuart et al. 2002, in press, Stuart & McCoy, in press).

Ants are recognized as important predators of pest insects in various agroecosystems and are subject to conservation in some IPM programs (Way & Khoo 1992, Perfecto & Castiñeiras 1998, Eubanks 2001). Indeed, the use of ants to control citrus pests in Asia dates back to at least 304 AD, is the earliest known example of biological control, and is still practiced today (Way & Khoo 1992). Florida has a rich and diverse ant fauna numbering over 200 species (Deyrup et al. 2000), and ants can be extremely abundant in Florida citrus groves (Whitcomb et al. 1982, Richman et al. 1983; Tryon 1986, Stuart & McCoy, in press, Stuart et al. in press). Under the proper condi-

tions and with appropriate management, ants could constitute a major weapon in our fight against *Diaprepes*, and a conservation biological control program focusing on appropriate ant species might well be the key to controlling this insect (Whitcomb et al. 1982, Jaffe et al. 1990, Stuart & McCoy, in press; Stuart et al. in press). However, at present, it is unclear which ant species are the most effective predators of *Diaprepes* on the soil surface, in the canopy, and below ground, and what strategies might be most effective in promoting and conserving beneficial ant species (Whitcomb et al. 1982, Richman et al. 1983, Stuart & McCoy, in press, Stuart et al. in press). Natural variability in the abundance and distribution of ants, combined with various possible influences of citrus management practices could contribute to considerable variability in predation pressure by ants on *Diaprepes* within and among groves across the state (e.g., see McCoy et al. 2001). Our present research begins to address these issues by assessing the role of ants as predators of *Diaprepes* neonate larvae on the soil surface in citrus groves in central Florida.

The timing of *Diaprepes* egg hatch, neonate escape from sealed leaf envelopes, and neonate drop to the soil surface could influence the relative exposure of neonates to predation (Whitcomb et al. 1982, Richman et al. 1983, Stuart et al. 2002). Jones & Schroeder (1983) found that a considerable period often elapsed between egg hatch and neonate escape from leaf envelopes, estimated average larval age at the time of neonate drop to be about 48 h, and found that neonates dropped between 1100 and 2400 h. Ant foraging on the soil surface during this period is reported to be low compared to early morning hours, and the timing of neonate drop could be an adaptation to avoid peak foraging periods (Whitcomb et al. 1982; Richman et al. 1983). Unfortunately, Jones & Schroeder (1983) examined neonate drop for only five egg masses, and did not report any information on the light cycles used in their laboratory experiments. Additional research on the activity

patterns of predators, the factors that stimulate egg hatch and neonate drop, and the conditions that promote neonate survival in the canopy, on the soil surface, and below ground is necessary for a more thorough understanding of how these factors might shape *D. abbreviatus* life history and survival strategies (Stuart et al. 2002).

Jaffe et al. (1990) observed that first instar *Diaprepes* larvae were preyed upon by various ant species but that the larvae appeared to be somewhat repellent, and Jaffe et al. suggested that the larvae might have chemical defenses. Pavis et al. (1992) investigated this chemical repellency with respect to the fire ant, *Solenopsis geminata* (F.), on the island of Guadeloupe in the Caribbean and identified two bicyclic sesquiterpene aldehydes that appeared to be responsible for the effect. The concentration of the repellent was highest in newly-hatched neonates, decreased with larval age, and was absent after about four days. Pavis et al. (1992) suggested that ant predation on neonate larvae during the first few hours after hatching would be reduced by about 40% because of chemical repellency. Various coccinellid species readily consume *Diaprepes* neonates under laboratory conditions with no indication of repellency (Stuart et al. 2002), and no experimental demonstrations of neonate repellency against other ant species have yet been reported (Stuart & McCoy, in press).

Our objectives in the present study were to (i) assess the daily temporal pattern of neonate drop from *Diaprepes* egg masses under controlled laboratory conditions, (ii) evaluate predation pressure on neonates on the soil surface in a series of citrus groves in central Florida, and (iii) test for neonate age-dependent chemical repellency by comparing predation rates on neonates of different ages under field conditions in central Florida.

MATERIALS AND METHODS

Neonate Drop

Egg masses laid between wax paper strips were obtained from a *Diaprepes* colony that was maintained on citrus foliage in a greenhouse at the Citrus Research and Education Center, Lake Alfred, FL. Egg masses were transferred to environmental chambers (Gaffney Engineering, Gainesville, FL) at least 4-5 days prior to neonate drop and were held in glass funnels (6 cm dia.), each of which was placed over a moving clock face coated with Tanglefoot® (The Tanglefoot Company, Grand Rapids, MI, 40504). The clock face was fashioned from the lid of a plastic Petri dish (150 × 15 mm) on which 24 segments were marked to correspond to a 24-h clock. All one-hour segments will be referred to by the time at the beginning of the hour (e.g., the 0800 h one-hour time segment refers to the period from 0800 h to

0859 h). The clock face was secured to the top of the mechanical clock apparatus of a hygrothermograph (model H-302, Weather Measure Corporation, P.O. Box 41257, Sacramento, CA 95841). The top of the funnel was covered with the base of a small plastic Petri dish (60 × 15 mm) that was secured to the funnel with Parafilm® (American National Can, Menasha, WI 54952). Attached to the inside of the Petri dish was a vial of water sealed with a cotton plug to prevent the egg mass from drying out. The environmental chamber was set to 24°C, 70% RH, L:D = 12:12. Onset of the light phase was at 0800 h and of the dark phase was at 2000 h. We conducted 42 replicates during the period from 14 May through 28 October 2001, and each replicate involved a single egg mass. This included nine replicates that were conducted as controls in which the clock face was immobile to determine whether neonates would move between clock segments under these experimental conditions.

A two-way ANOVA (PROC GLM, SAS Institute Inc. 1990) with seven levels for the factor "date" and 24 levels for the factor "time" was conducted on the percent neonate drop from each egg mass after arcsin transformation. Means comparisons used the LSMEANS procedure (SAS Institute Inc. 1990). A similar ANOVA was conducted for the data pooled into four time intervals representing the first and second halves of the light and dark phases.

Predation

We conducted direct observations of predation on lab-reared *Diaprepes* neonates (<48 h post hatch) placed in citrus groves, and used procedures similar to those of Whitcomb et al. (1982) and Richman et al. (1983). Egg masses were obtained from a *Diaprepes* colony that was maintained on citrus foliage at the Citrus Research and Education Center, Lake Alfred, FL. Egg masses were held at room temperature in plastic containers that were arranged so that neonates hatching in the upper chamber could drop through a screen into the lower chamber. Neonates were removed from the lower chambers on a daily basis at 0800 h for use in experiments conducted that day. Each replicate consisted of 20 neonates placed in an open plastic dish (4 mm high × 48 mm dia, Millipore Petrislide containers, Millipore Corporation, Bedford, MA) on the soil surface under the canopy and observed for 20 min. A thin layer of fine sand that passed through a No. 40 sieve was placed in the bottom of the dish and effectively discouraged neonates from crawling out (see Richman et al. 1983), whereas roughening the outside and inside vertical surfaces of the dish with sand paper facilitated the entrance and exit of predators. A predation event was scored whenever a neonate was removed from the dish.

In most cases, predators were identified visually by field observers based on previously identified samples. In some cases, additional samples were taken to confirm identifications.

We conducted a total of 199 replicates in three groves located near the following municipalities in central Florida: Lake Alfred (63 replicates) and Alturas (58 replicates) in Polk County; and Southport (78 replicates) in Osceola County. In the Lake Alfred grove, which was located at the Citrus Research and Education Center of the University of Florida, we worked in two blocks, one of which was a mature planting of Marsh seedless grapefruit on Swingle citrumelo rootstock whereas the other was a close-set 5-year old planting of Navel orange on Swingle citrumelo rootstock. In the Southport grove, we worked in two mature blocks of Hamlin orange on Swingle citrumelo rootstock. The south block was being managed for commercial production whereas the north block had been abandoned because of severe tree decline, was no longer irrigated, and was subject to relatively little weed control. The Alturas grove was a uniform planting of mature Flame grapefruit on Swingle citrumelo rootstock and was under management for commercial production. Observations were conducted between 1 August and 11 September 2000, between 1200 and 1700 h. Minimum and maximum daily temperatures during this period at Lake Alfred, FL, ranged from 20.1 to 35.8°C.

Statistical comparisons of the proportion of replicates in which predation was observed and the proportion of neonates preyed upon in different groves and blocks were conducted using contingency table analyses and the χ^2 test (PROC FREQ, SAS Institute Inc. 1990).

Chemical Repellency

Twelve assay stations were established under the canopy of mature grapefruit trees (Marsh seedless grapefruit on Swingle citrumelo rootstock) with one station per tree at the Citrus Research and Education Center of the University of Florida, Lake Alfred, FL. *Diaprepes* egg masses laid between sheets of wax paper were obtained from the USDA rearing facility, Fort Pierce, FL. Egg masses were held as in the previous experiment; and neonates were harvested on a daily basis at 0800 h, and hourly on the day of the experiment. At each station, for each replicate, 20 neonates aged five days post hatching and 20 aged 1-2 h post hatching were placed in separate paired open plastic dishes (as described in the previous experiment), ca. 2-4 cm apart, on the soil surface near the trunk, for 30 min. The number of neonates remaining in the dishes was counted under a microscope at the termination of each replicate. Eight replicates were conducted, with the position of the dishes (i.e., left versus right) being randomized for the first replicate at each station,

and then alternated in successive replicates. Data were collected from six stations simultaneously, with stations 1-6 and 7-12 forming two distinct cohorts within the experiment, which were generally run sequentially. Direct observations of predation events, defined as a neonate being removed from an assay dish by a predator, were conducted during the experimental period for all replicates by systematically observing the dishes for 1-2 minute intervals as the test progressed. Observations were conducted during the period 21-26 July 2001, between 0850 and 1550 h. Weather data were recorded during the first 15 mins of each replicate by FAWN (Florida Automated Weather Network, University of Florida, Gainesville, FL) in an open field at the Citrus Research and Education Center, Lake Alfred, FL. These data include air temperature (measured 61.0 cm above the soil surface), soil temperature (measured 7.6 cm below the soil surface), relative humidity, and solar radiation.

A three-way ANOVA (PROC GLM, SAS Institute Inc. 1990) with two levels for the factor "age" (old and young), two levels for the factor "position" (left and right), and twelve levels for the factor "station" (1-12) was conducted on the percent predation in each dish after arcsin transformation. Means comparisons used the LSMEANS procedure (SAS Institute Inc. 1990). Percent predation on old versus young larvae by various ant species was compared using contingency table analysis and the χ^2 test (PROC FREQ, SAS Institute Inc. 1990).

RESULTS

Neonate Drop

The 42 egg masses contained from 21 to 127 eggs (total = 2687, mean = 63.98, SE = 3.983). A total of 491 eggs failed to hatch (18.3%), and an additional 60 eggs hatched but the neonates failed to exit the egg masses (2.2%). In two cases, the entire egg mass failed to hatch (4.8%). A total of 259 neonates dropped in the nine control replicates. Of these, 11 neonates moved across one clock-segment boundary (4.2%), and none moved across more than one boundary. In the 33 experimental replicates, less than 20 neonates dropped in four replicates, and only the remaining 29 replicates were considered in assessing the temporal pattern of neonate drop. In these 29 replicates, 23 to 125 neonates dropped per egg mass (total = 1865, mean = 64.31, SE = 5.410).

Neonates dropped during every hour of the light and dark phases, but there were significant differences in the percentage of neonates dropping among one-hour and six-hour time intervals (Fig. 1A, B). For the analysis involving 24 one-hour time intervals (Fig. 1A), the dates on which replicates were conducted had no influence on the

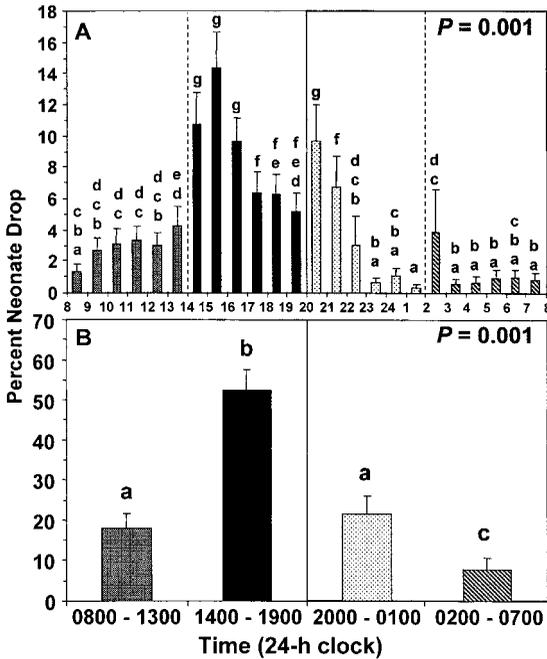


Fig. 1. Comparison of the percentage of neonates (mean + SE) that dropped from egg masses at various times of the day when the onset of the light phase was at 0800 h and the dark phase was at 2000 h with the data partitioned either hourly (A) or into six-hour time intervals (B). Statistical analysis was by way of ANOVA and LSMEANS. Common letters above bars indicate no significant difference at the $P = 0.05$ level.

results (ANOVA: $F = 1.01, df = 6, 528, P = 0.4148$) but time was a significant factor (ANOVA: $F = 12.50, df = 23, 528, P = 0.0001$) and the interaction between date and time was not significant (ANOVA: $F = 1.22, df = 138, 528, P = 0.0676$). Similarly, for the analysis involving four six-hour time intervals (Fig. 1B), the dates on which replicates were conducted had no influence on the results (ANOVA: $F = 0.13, df = 6, 88, P = 0.9928$) but time was a significant factor (ANOVA: $F = 22.32, df = 3, 88, P = 0.0001$) and the interaction between date and time was not significant (ANOVA: $F = 1.47, df = 18, 88, P = 0.1192$). On average, 17.9% of neonates dropped from 0800-1300 h, 52.4% from 1400-1900 h, 21.7% from 2000-0100 h, and 8.0% from 0200-0700 h.

The pattern of neonate drop for individual egg masses over the four six-hour time intervals defined above shows that the timing of neonate drop was not highly synchronized within or among egg masses (Fig. 2). For individual egg masses, neonates dropped over a period of 5 to 23 h (mean = 11.97, SE = 0.866). Of the 29 egg masses referred to above, 28 had neonates drop during both the light and dark phases (96.6%), 12 during all four of the six-hour time intervals (41.4%), 24 during at least three intervals (82.8%), 28 during at least

two intervals (96.6%), and one during only one interval (3.4%). All 29 egg masses had some neonates drop during the peak 6-h time interval from 1400-1900 h, 23 had more than 25% drop during this period (79.3%), 17 had more than 50% drop during this period (58.6%), and 8 had more than 75% drop during this period (27.6%). Two egg masses had more than 50% of their neonates drop from 0800-1300h (6.9%), three had more than 50% drop from 2000-0100 h (10.3%), and one had more than 50% drop from 0200-0700 h (3.4%).

Predation

We observed predation in 104 of 199 replicates (52.3%) with a total of 475 of the 3980 neonates being removed from assay dishes by predators (11.9%). All but one of the observed predation events were by ants (Hymenoptera: Formicidae). The single exception was by a nymph of the big-eyed bug, *Geocoris floridanus* Blatchley (Hemiptera: Lygaeidae), that attacked a neonate in the Lake Alfred grove. There was no significant difference in the proportion of replicates in which predation was observed at the three sites (Fig. 3A), but a greater proportion of neonates was preyed upon at the Alturas grove than at the other sites (Fig. 3B). Significant differences in the level of predation occurred within different areas of the groves. At the Lake Alfred grove, we observed no significant difference in the proportion of replicates that resulted in predation events in the grapefruit block versus the orange block (Fig. 3C), but a greater proportion of neonates was preyed upon in the orange block (Fig. 3D). At the Southport grove, we observed a highly significant difference in the proportion of replicates that resulted in predation events in the abandoned north block versus the cultivated south block (Fig. 3E), and a greater proportion of neonates was also preyed upon in the north block (Fig. 3F).

Eight ant species preyed on neonates in this experiment. For the data pooled from all sites, the red imported fire ant *Solenopsis invicta* Buren and *Pheidole moerens* Wheeler were the most active predators, and accounted for 29.5% and 27.8% of the predation events, respectively. Other predatory ant species in decreasing order of percent predation for the pooled data included *Dorymyrmex reginacula* (Trager) (9.7%), *Brachymyrmex obscurior* Forel (8.8%), *Dorymyrmex bureni* (Trager) (8.6%), *Cardiocondyla emeryi* Forel (8.0%), *Paratrechina bourbonica* Forel (4.8%), and *Pheidole morrisi* Forel (2.5%). Predation pressure by different ant species was variable within and among groves and probably reflects differences in the abundance and distribution of those species (Fig 4A, B). It is noteworthy that the Alturas grove was the site with the highest predation pressure on neonates (Fig. 3B) and was also the site in which *S. invicta* was the most active predator (Fig. 4A, B).

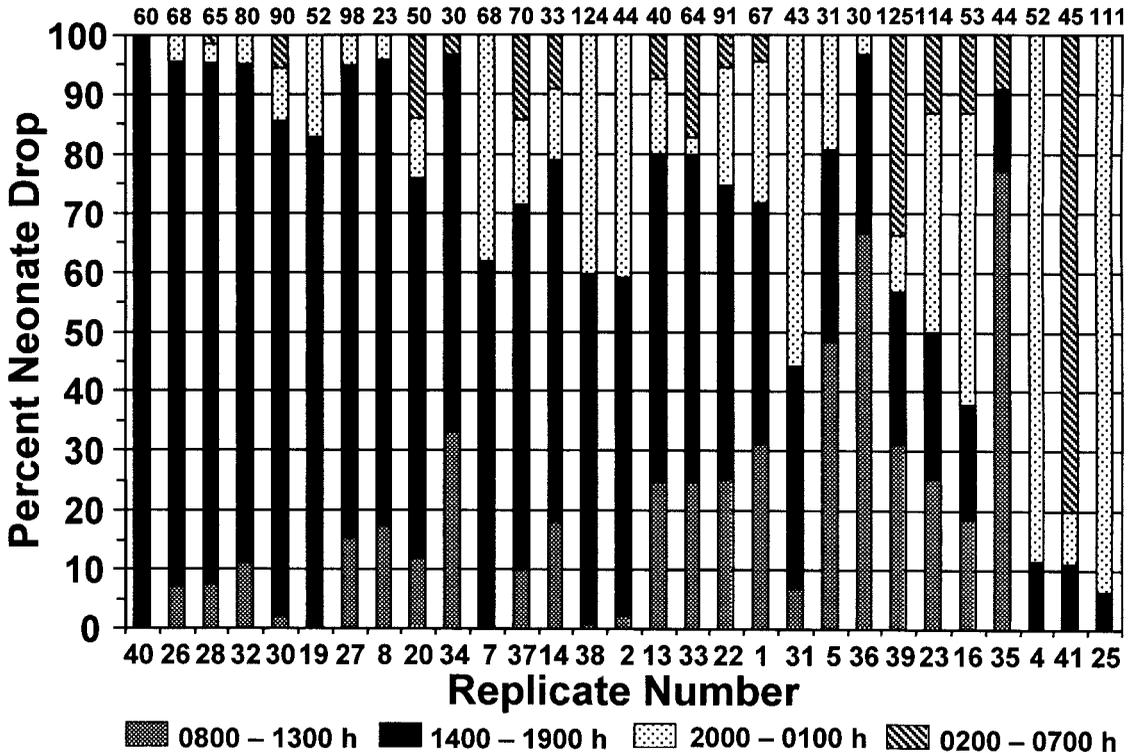


Fig. 2. Comparison of the percentage of neonates that dropped from egg masses at various times of the day for data partitioned into six-hour time intervals and ranked from left to right in descending order of the percentage of neonates that dropped during the peak time interval, 1400-1900 h (see Fig. 1). The replicate number is indicated below each bar, and the number of neonates dropping from the egg mass in that replicate is indicated above the bar.

Casual observations of the behavior of the ants in this study indicate that workers of different species often respond quite differently to *Diaprepes* neonates. Workers of both *Dorymyrmex* species are relatively large and fast moving, and often failed to respond to neonates as they quickly passed through assay dishes. *S. invicta* workers are also relatively large but move more slowly, and responded to neonates more frequently. We also noted that *S. invicta* often appeared to sting neonates before carrying them out of the dish, and that sometimes *S. invicta* workers appeared to have difficulty removing their sting from the body of the neonate and would wander about the dish with the neonate attached to the tip of their abdomen by the sting. Workers of the smaller species (i.e., *P. moerens*, *B. obscurior*, and *C. emeryi*) appeared to detect and respond to neonates more readily than the larger species. However, *P. moerens* workers typically seized and carried off neonates almost immediately upon contact whereas *B. obscurior* was more hesitant, often picked up and dropped neonates repeatedly, and sometimes abandoned the assay dish without a neonate. This handling difficulty is reminiscent of some of the behavior observed by previous researchers and

might be indicative of repellency (Jaffe et al. 1990, Pavis et al. 1992). *C. emeryi* was intermediate to the other two small species in terms of its handling efficiency. We did not observe mass recruitment of nestmates to assay dishes by any ant species in this experiment. Rather, predation appeared to be conducted by individually foraging workers that discovered and carried off a neonate, and often returned repeatedly to an assay dish to prey on additional neonates.

Chemical Repellency

A total of 2620 of the 3840 larvae (68.2%) were preyed upon in this experiment but the predation rate on 5-day old versus 1-2 h old *Diaprepes* neonates showed no significant difference at 68.8% and 67.7%, respectively (ANOVA: $F = 0.44$, $df = 1$, 144 , $P = 0.5067$). Both station and position were significant factors (ANOVA: station, $F = 19.27$, $df = 11$, 144 , $P = 0.0001$; position, $F = 5.56$, $df = 1$, 144 , $P = 0.0197$), and there was a highly significant interaction between station and position (ANOVA: $F = 6.11$, $df = 11$, 144 , $P = 0.0001$). The position of the assay dishes, whether left or right, had a significant impact on the percent predation

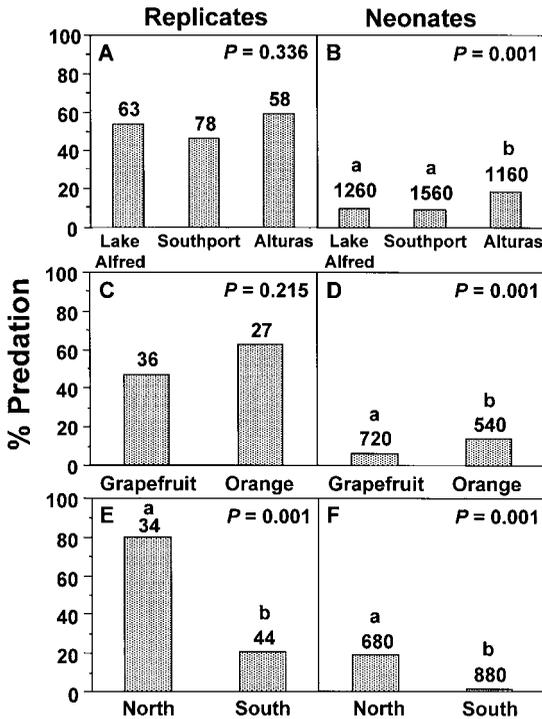


Fig. 3. Comparison of the percentage of replicates in which predation was observed (A, C, E) and the percentage of neonates preyed upon (B, D, F) in the three groves (A, B), and for different areas within two groves, Lake Alfred (C, D) and Southport (E, F). The numbers above the bars indicate the total number of replicates (A, C, E) or neonates (B, D, F). *P*-values indicate the results of χ^2 tests on 2×2 or 2×3 contingency tables. Common letters above bars indicate no significant difference at the *P* = 0.05 level. No letters appear above groups of bars where the overall contingency table analysis was not significant at the *P* = 0.05 level.

at five of the 12 stations, and predation was more intense at some stations than at others (Fig. 5A). At one station (#11), predation was 100% in both dishes in every replicate.

A total of 368 of the 2620 predation events (14.0%) were observed directly. All observed predation was by six ant species, with *P. moerens* and *S. invicta* preying on 62.5% and 25.3% of the neonates, respectively. Other ant species in decreasing order of percent predation included *C. emeryi* (10.1%), *B. obscurior* (1.4%), *Cardiocondyla wroughtonii* (Forel) (0.5%), and *D. bureni* (0.3%). *C. wroughtonii* was not observed as a predator in the previous experiment, and is the ninth predatory ant species reported here. The two predation events by *C. wroughtonii* occurred at the same assay station on successive days between 1520 and 1600 h. A comparison of the number of predation events by *P. moerens*, *S. invicta*, or the other ant species pooled on old versus young larvae revealed no significant differences (2×3 contin-

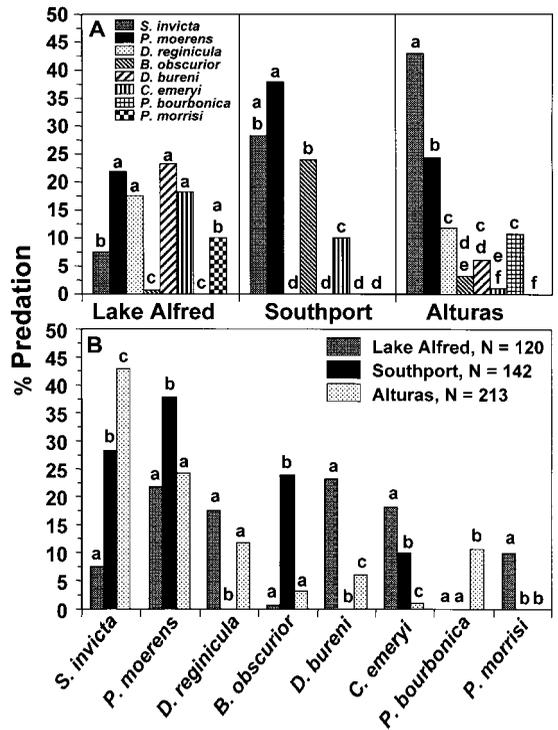


Fig. 4. Comparison of the percent predation by different ant species within and among the three groves. Statistical analysis was by way contingency tables and the χ^2 test. Common letters above bars indicate no significant difference at the *P* = 0.05 level either among species within sites (A) or within species among sites (B).

gency table, $\chi^2 = 4.170$, *df* = 2, *P* = 0.124; Fig. 5B). Thus, we found no evidence for age-dependent repellency of neonates, either overall or for particular ant species.

There was no correlation between time of day and percent predation for data pooled among stations and assay dish positions for stations 1-6 (Fig. 6A) but there was a significant increase in predation rate as the day progressed for stations 7-12 (Fig. 6B). There were no significant correlations between predation rate and air temperature, relative humidity, or solar radiation for either experimental cohort (Table 1). Soil temperature was correlated with predation rate for stations 7-12 but not for stations 1-6 (Table 1).

DISCUSSION

This study indicates that ants are important predators of *Diaprepes* neonates on the soil surface in the citrus groves of central Florida. Our laboratory data indicate that neonate larvae drop from the canopy during all hours of the day and night but that peak drop occurs during the afternoon. Our field tests demonstrate that at least nine ant species prey on neonates on the soil sur-

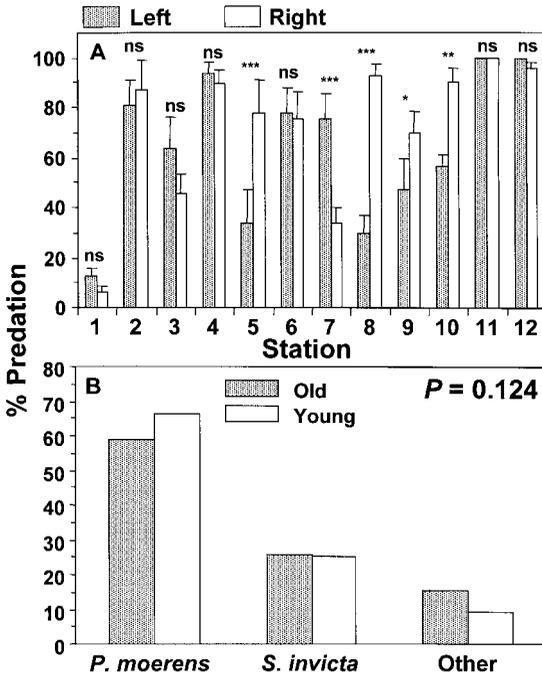


Fig. 5. Comparison of percent predation (mean + SE) on neonate larvae by ants for different assay-dish positions (left or right) at the 12 stations (A), and on old versus young neonate larvae by different ant species (B). Statistical analysis was by ANOVA and LSMEANS (A), or by contingency tables and the χ^2 test (B): ns, $P > 0.05$; *, $P < 0.05$; ** $P < 0.01$; ***, $P < 0.001$.

face, and are active during the peak drop period, but that predation pressure overall and by particular species can be highly variable within and among groves. Ants preying on *Diaprepes* neonates in this study included *Brachymyrmex obscurior*, *Cardiocondyla emeryi*, *C. wroughtonii*, *Dorymyrmex bureni*, *D. reginacula*, *Paratrechina bourbonica*, *Pheidole moerens*, *Ph. morrissi*, and *Solenopsis invicta*. A single predation event on a neonate by a nymph of the big-eyed bug, *Geocoris floridanus*, was also observed. We found no field evidence for differential predation on neonates of different ages by ants in this community and, hence, no evidence for age-dependent chemical repellency of neonates (see Jaffe et al. 1990, Pavis et al. 1992). However, behavioral observations indicate that ant species differ widely in their abilities to detect and handle this prey item and, consequently, the relative importance of particular species as predators of neonates is likely to depart markedly from their relative abundance in this agroecosystem. Our results reinforce the view that ants are among the primary mortality agents of *Diaprepes*, and have potential as the basis for a conservation biological control program (Whitcomb et al. 1982, Jaffe et al. 1990, Stuart & McCoy, in press, Stuart et al. in press).

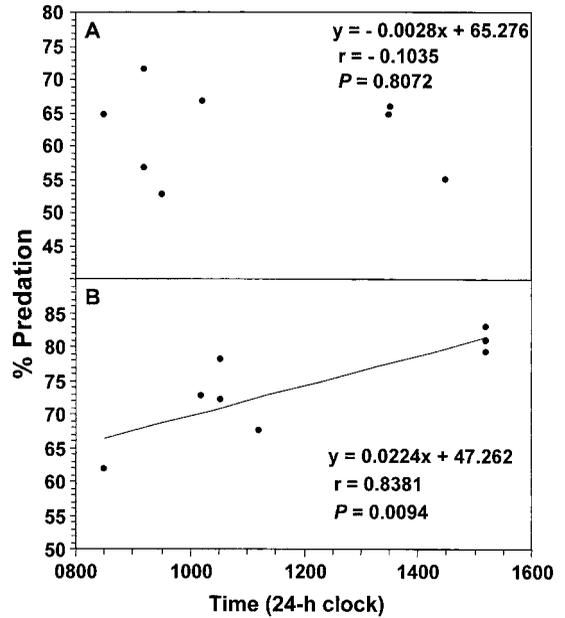


Fig. 6. Relationship between percent predation on neonate larvae and time of day for stations 1-6 (A) and 7-12 (B) of the chemical repellency experiment. The equation for the regression line, the Pearson correlation coefficient (r), and the P -value are given.

Previous research on ant predation on *Diaprepes* neonates in a central Florida citrus grove in Forest City, Seminole County, identified *Pheidole dentata* Mayr, *P. floridana* Emery, and *Tetramorium simillimum* Roger as the primary species involved (Whitcomb et al. 1982, Richman et al. 1983, Tryon 1986) but none of these species were detected in the present study. Moreover, whereas *S. invicta* and *P. moerens* were relatively minor predators in the previous studies, they were major predators in the present study. It is unclear whether this difference represents mere variability among sites or whether the ant fauna in central Florida citrus groves has undergone a dramatic change during this period. Florida has over 200 ant species, more than 50 of which are thought to be introduced, exotic species (Deyrup et al. 2000). Both *S. invicta* and *P. moerens* are exotic, and were classified by Deyrup et al. (2000) as "possible ecological villains" since they occur in both disturbed and undisturbed habitats, appear to dominate their trophic roles, and might displace native competitors. Indeed, both *S. invicta* and *P. moerens* appear to have spread "explosively" in Florida since they were first reported in 1950 and 1975, respectively (Deyrup et al. 2000). However, whereas *S. invicta* is considered a major invasive pest in a broad range of habitats and is often subject to intensive control efforts (see Vinson 1997), *P. moerens* has remained obscure (Deyrup et al. 2000). Notably, of the predatory ants

TABLE 1. SUMMARY AND CORRELATION ANALYSIS FOR THE PERCENT PREDATION AND WEATHER CONDITIONS IN EXPERIMENTS CONDUCTED AT STATIONS 1-6 AND 7-12 OF THE CHEMICAL REPELLENCY FIELD STUDY.

Station No.	Variable	N	Mean	SE	Range	Correlation with % Predation	
						R	P
1-6	Predation (%)	8	62.15	2.326	52.5-71.3	—	—
	Air Temperature (°C)	8	28.31	0.873	26.0-33.2	0.2660	0.5244
	Soil Temperature (°C)	8	28.61	0.462	26.9-31.1	-0.4654	0.2452
	Relative Humidity (%)	8	72.78	3.785	51.5-84.9	-0.3654	0.3734
	Solar Radiation (W/m ²)	8	416.14	87.233	61.0-722.0	0.2637	0.5281
7-12	Predation (%)	8	74.33	2.557	61.7-82.9	—	—
	Air Temperature (°C)	8	28.67	1.138	24.3-33.7	0.2842	0.4951
	Soil Temperature (°C)	8	28.76	0.370	27.2-30.6	0.7543	0.0306
	Relative Humidity (%)	8	72.04	4.905	52.0-90.7	-0.4979	0.2093
	Solar Radiation (W/m ²)	8	411.63	89.858	46.0-853.0	0.0287	0.9463

recorded in the present study, only the two *Dorymyrmex* species, *P. morrisi*, and possibly *B. obscurior*, are considered native (Deyrup et al. 2000); and very little is known concerning the ecological dynamics of the highly synthetic ant community we now find in Florida citrus.

The results of the present study show how variable ant predation pressure on *Diaprepes* neonates can be in Florida citrus groves. Our experiments detected variation in predation rates among groves, among blocks within groves, among assay stations under a series of grapefruit trees, and between paired assay dishes only a few centimeters apart. This spatial variability probably reflects heterogeneity in the distribution and abundance of ant nests of various species. Unfortunately, there is little information on factors that might influence the distribution, growth, and activity levels of ant colonies of most species in this particular agroecosystem, but such information is necessary for the selective manipulation of species within a conservation biological control program. One exception is the red imported fire ant, *Solenopsis invicta*, since many aspects of its biology are well known (Vinson 1997). However, *S. invicta* is sometimes considered a citrus pest since it feeds on foliage and bark, can girdle young trees, tends aphids and scales, and disrupts harvesting by stinging grove workers (Banks et al. 1991, McCoy 1999, Michaud et al. 2002). Chemical suppression of *S. invicta* tends to increase ant diversity (McCoy et al. 2001) but, since *S. invicta* is such a voracious predator, it is unclear whether manipulating the ant community in this manner has a positive or a negative impact on the biological control of insect pests in general or of *Diaprepes* in particular. Whatever the case, *S. invicta* has become the dominant ant in many Florida citrus groves (Banks et al. 1991) and further studies of the positive and negative impacts of this and other ant species in this agroecosys-

tem seem warranted. In particular, we know virtually nothing about the biology of *P. moerens* (Deyrup et al. 2000) and its appearance as a major predator of *Diaprepes* neonates in the present study justifies further research.

Our results on the temporal pattern of neonate drop complement and extend those obtained previously. On the basis of five egg masses, Jones & Schroeder (1983) concluded that neonate drop occurred from 1100 to 2400 h but provided no further information on the temporal pattern observed, variability within or among egg masses, or on the light cycle under which these data were obtained. With a larger data set of 29 egg masses and controlled laboratory conditions (24°C, 70% RH, L:D = 12:12), we found that a peak in neonate drop occurred during the second half of the light phase but that neonate drop could occur during all hours of the light and dark phases, and that there was considerable variability in the timing of neonate drop within and among egg masses. In central Florida, neonate drop is closely associated with adult abundance and has been recorded from mid June to mid December (McCoy et al. in press, Nigg et al. in press). Further research is necessary to explore patterns of neonate drop under the range of light cycles and environmental conditions that occur during this period. At present, it is unclear what proximate or ultimate factors influence neonate drop, and to what extent temporal patterns are under genetic, developmental, or environmental control. Such patterns might constitute adaptive responses to the activity patterns of predators (Whitcomb et al. 1982, Richman et al. 1983, Stuart et al. 2002), or be influenced by environmental conditions such as rainfall, which could have an important impact on the ability of neonates to penetrate soil (Jones & Schroeder 1983).

Daily cycles in the foraging activity of ants in Florida citrus groves could be an important factor

regulating predation pressure on *Diaprepes* neonates. When Whitcomb et al. (1982) examined ant predation on neonates in a central Florida citrus grove using similar procedures to ours, they found no predation at 1200 h and 1500 h but 62%, 44% and 49% predation at 0700 h, 1800 h, and 2400 h, respectively. Richman et al. (1983) found predation levels of only 9.6% from 1200 to 1530 h in the same grove as the previous researchers but, since they used 50 neonates per dish rather than 20, their result might better be adjusted to 24.0% for a reasonable comparison. In the present experiments, we found a predation rate of 11.9% from 1200 to 1700 h when using a 20 min exposure period similar to those cited above, but a rate of 68.2% from 0850 to 1550 h when using a 30 min exposure period. Moreover, in the latter case, there was no evidence of a decline in predation rate during the day as suggested by the previous researchers. Indeed, in one cohort, there was a significant increase over the course of the day.

Current data suggest that ant foraging activity could be highly variable temporally and spatially in Florida citrus groves, and might depend more on the species involved, their relative abundance, and ambient environmental conditions than on time of day *per se*. According to Hölldobler & Wilson (1990), every ant species can be expected to have a distinctive foraging schedule. In some species, circadian rhythms have been demonstrated but can apparently be over-ridden or phase shifted by colony hunger, patterns of food availability, or other factors. To some extent, different foraging schedules among similar sympatric species might ultimately reflect coevolution and the temporal partitioning of resources, but could be based proximately on different humidity and temperature preferences or tolerances. However, in a detailed study of the foraging patterns of *Solenopsis invicta*, Porter & Tschinkel (1987) found that soil temperature, season, and rainfall explained most of the variation in foraging activity as indicated by food discoveries and recruitment to baits. Factors like time of day, even the difference between day and night, were not related to foraging activity. *S. invicta* foraged when soil temperatures at a depth of 2 cm ranged from 15 to 43°C, and exhibited maximum foraging rates between 22 and 36°C. Foraging was unusually low in late fall and was reduced during periods of rainfall. In the present research, we found that predation rates were related to time of day and soil temperature at some assay stations but not at others. Unfortunately, our weather records were based on measurements made in a nearby open field rather than under the citrus canopy where the experiments took place, and more detailed information regarding environmental conditions in this particular microhabitat might have been revealing. Further research is necessary to elucidate the foraging patterns of other ant spe-

cies in this community, the factors that regulate them, and how this might affect *Diaprepes* survival strategies.

This study indicates that there is no differential predation on 5-day versus 1-2 h old *Diaprepes* neonates by ants in a central Florida citrus grove. Thus, there is no evidence that a quantitative decrease in the chemical ant repellents produced by neonates over this time period as reported by Pavis et al. (1992) has any influence on the intensity of predation by this ant community. Our results are not necessarily in conflict with those of Pavis et al. (1992) since the repellents might be equally effective when present in small or large amounts, or might be totally ineffective against the ant species in this study, which did not include the species in their study, *Solenopsis geminata*. Furthermore, since Pavis et al. (1992) conducted their research on the island of Guadeloupe in the Caribbean, and since the *Diaprepes* population in Florida was introduced, perhaps as a few small founder populations (Bas et al. 2000), it is possible that the weevils in the two studies differ genetically and that the dynamics of the purported chemical ant-repellent system is different as well. Coccinellids are reported to be deterred from attacking alfalfa weevil larvae by their defensive wriggling (Kalaskar & Evans 2001), and it is unclear whether wriggling contributes to defense for *Diaprepes* larvae. Further research is necessary to explore these possibilities.

The predation assay used in the present study might underestimate predation pressure on *Diaprepes* neonates. This assay would miss any "sit and wait" predators (e.g., ant lions, various spiders) present under trees, and presents a novel substrate to mobile predators that might deter prey searching. It often appeared in our experiments that ants more readily walked around assay dishes than through them. However, these assay dishes might also facilitate predation by depriving neonates of cracks, crevices, and other complex elements of the leaf litter environment that might shelter them from predators or facilitate soil penetration. Jones & Schroeder (1983) found that the presence of grass stems and leaves placed vertically in soil did not promote soil penetration, and that neonates failed to penetrate dry soil. They also found that half of the groups of 20 neonates in their experiments that were aged 9 and 72 h required 80 and 105 mins, respectively, to penetrate below the surface of moist soil, and that all of the larvae in these groups disappeared below the surface within 180 mins. These results indicate that neonates might often remain on the soil surface for relatively long periods and that exposure times of 20 or 30 mins as used in our experiments are not excessive. Our experiments presented neonates to predators at an initial density of 20 neonates per assay dish, a density that would appear to constitute a relatively diffuse food resource and that

seems justified by the temporal pattern of neonate drop from individual egg masses observed in the present study. At this density, we found that ants discovering a neonate and carrying it off to their nest did not engage in the mass recruitment of nestmates but would often return to the assay dish repeatedly to prey on additional neonates, a foraging response common to many ant species and known as *Ortstreue* (Hölldobler & Wilson 1990). Neonates experimentally presented to predators at higher densities are reported to induce mass recruitment by some ant species (Whitcomb et al. 1982), and predation rates observed in such experiments might be considered artifacts of an unrealistically high neonate density. Consequently, we suggest low densities of the kind used here as a more realistic mode of neonate presentation for future experiments.

In general, the extent to which ants prey on various life stages of *Diaprepes* and are capable of controlling this insect will likely depend on the abundance and diversity of the ant species present. In turn, the structure of the ant community in particular citrus groves probably depends on an array of factors including local environmental conditions, grove management practices, and the dynamics associated with interactions among the various native and exotic species that become established within groves. Given the possible complexity of these factors and their interactions, it is premature to speculate upon what factors might have contributed to the variability in predation rates among groves and blocks within groves that we observed in the present study. Nonetheless, since ants are often extremely abundant in Florida citrus groves, and can be such important predators of a broad range of insect pests, considerable benefits could be derived from further studies of this ant community, the positive and negative impacts of different species, and how they might best be managed for pest control within the framework of a comprehensive IPM program. It might seem unusual to consider incorporating invasive exotic ant species into such a program but many of these species are now well established components of the ecological landscape in Florida and exploiting their beneficial aspects, especially in agricultural habitats, might be an extremely practical and cost-effective way of using these resources.

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**NEOTERMES PHRAGMOSUS, A NEW DAMPWOOD TERMITE
(ISOPTERA: KALOTERMITIDAE) FROM SOUTHEASTERN CUBA**

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ABSTRACT

Neotermes phragmosus n. sp. is described from the imago and soldier castes. The imago head capsule of *N. phragmosus* has a distinctly phragmotic and concave frons. Plesiomorphic characters of *N. phragmosus* unique among the Kalotermitidae include partial separation of the otherwise fused first and second marginal teeth of the left imago/worker mandible, long subcosta and radius, and increased number of antennal articles in both imagos and soldiers. This species is confined to the xeric coastal habitats of southeastern Cuba.

Key Words: new species, taxonomy, West Indies, Greater Antilles, Caribbean

RESUMEN

El *Neotermes phragmosus* n. sp. es descrito de la casta imago y la casta soldado. La cápsula de la cabeza del imago *N. phragmosus* tiene el frente distintivamente fragmótico y cóncavo. Las características plesiomórficas del *N. phragmosus* son únicas entre los Kalotermitidae incluyen la separación parcial de los primeros y segundos dientes marginales de la mandíbula izquierda del imago/trabajador, que en otros casos se encuentra fundidos; un subcosta y un radio largados; y un mayor número de artículos en las antenas en los imagos y los soldados. Esta especie está restringida a la zona árida costera del sureste de Cuba.

A species of *Neotermes*, collected in extreme southeastern Cuba, was originally listed as *Neotermes* sp. nr. *mona* (Banks) (Scheffrahn et al. 1994). A subsequent redescription of *N. mona* (Krecek et al. 2000) revealed that the Cuban *Neotermes* was a new species that is described herein from the winged imago and small and large soldier castes. *Neotermes phragmosus* n. sp. is the fourth *Neotermes* species recorded from Cuba and the sixth from the Greater Antilles. *Neotermes phragmosus* and *N. cubanus* (Snyder) are endemic solely to Cuba (Snyder 1956, data herein). Of the two additional Cuban species, *N. castaneus* (Burmeister) is also recorded from the Bahamas, Cayman Islands, Florida, Hispaniola, Jamaica, and Turks and Caicos Islands (Scheffrahn et al. unpublished), while *N. jouteli* (Banks) ranges into the Bahamas, Cuba, Florida, and Mexico (Scheffrahn et al. 2000). The remaining Greater Antillean species include *N. platyfrons* Krecek and Scheffrahn (2001) from Hispaniola, and *N. mona* from the Bahamas, Hispaniola, Puerto Rico, Turks and Caicos, and Virgin Islands (Krecek et al. 2000).

MATERIALS AND METHODS

The description of *N. phragmosus* is based on 87 colony samples from the authors' collection taken from 23 localities in Guantánamo Province, Cuba, as part of a survey of termites of Cuba and the West Indies (Fig. 4). Collection localities were mapped using ArcView GIS version 3.0a software and relevant map data from Digital Map of the World version 1.0 (Environmental Systems Re-

search Institute, Inc. Redlands, CA). Morphometric data from specimens preserved in 85% ethanol were obtained using a stereomicroscope fitted with an ocular micrometer. Scanning electron micrographs were scanned at 300 dpi, the specimen outline captured with photograph-enhancing software (Adobe Photoshop Elements, Adobe Systems Inc., San Jose, CA), the background converted to black, and the scale bar digitally redrawn. The imago head capsule photomicrograph was obtained using a digitized three-dimensional imaging system (Auto-Montage, Syncroscopy Inc. Frederick, MD) and further enhanced as mentioned above.

The holotype alate and paratype large and small soldier will be deposited at the American Museum of Natural History, New York. The additional alate and soldier paratypes will be submitted to the National Museum of Natural History (Smithsonian Institution), Washington, D.C., and to the Florida State Collection of Arthropods, Florida Department of Agriculture and Consumer Services, Division of Plant Industry, Gainesville, Florida. The remaining paratypes will be held in the authors' collection at the University of Florida Research and Education Center, Fort Lauderdale, Florida.

NEOTERMES PHRAGMOSUS, NEW SPECIES

Neotermes sp. nr. *mona* (Banks); Scheffrahn et al. 1994: 217 (Cuba).

Imago (Figs. 2 and 3, Table 1).

In dorsal view, head capsule ferruginous orange, except for slightly darker ferruginous ante-

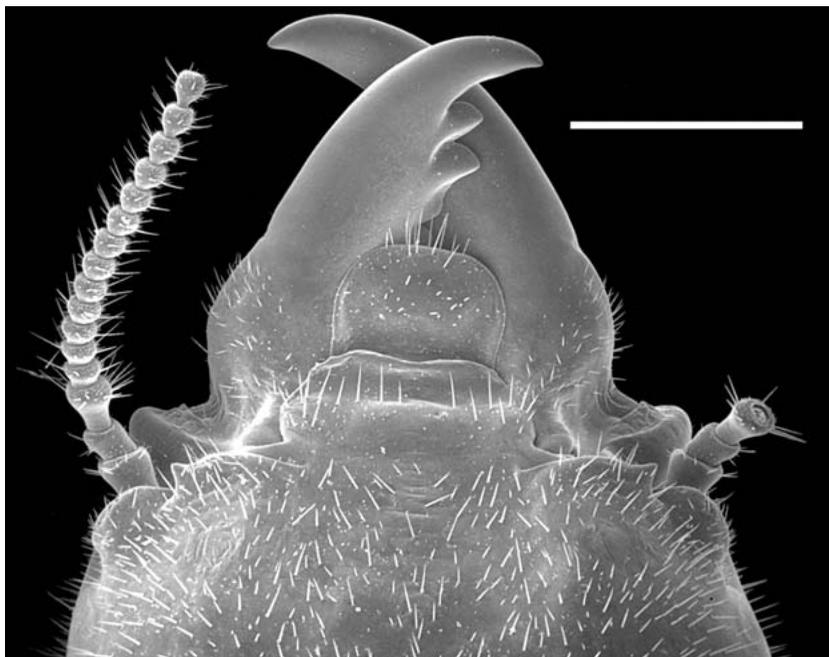


Fig. 1. Scanning electron micrograph of anterior of the large soldier head (dorsal view) of *Neotermes phragmosus* n. sp. from Tortuguilla, Guantánamo Province, Cuba. Scale bar equals 1 mm.

rior frons and postclypeus. Compound eyes almost black. Mandibles chestnut brown. Antennal articles 1-3 ferruginous; remaining articles ferruginous orange. Anteclypeus yellowish. Ferruginous orange chevron patterns formed by wing scales on pterothorax faint and wide; remaining dorsum of body pale orange-yellow. Sclerotized wing venation ferruginous orange, remainder of wings and abdominal sternites yellowish.

In dorsal view, head capsule suboval with sides along and anterior to eyes slightly concave; posterior of head capsule broadly rounded. Head converging to anterior in ventral aspect. In oblique view, frons phragmotic, broadly excavated; depression sharply delimited by moderately raised ridge; surface of frons covered by dense wrinkling of variable orientation (Fig. 2). A pair of tiny tubercles behind ocelli; lateral branches of epicranial suture near tubercles. In lateral view, plane of frons margin slopes weakly toward a slightly convex vertex. Compound eyes large and protruding, subcircular; eye margins narrowly subrectate along ocelli and along posteroventral area, and broadly subrectate or slightly concave along antennal sockets. Ocelli slightly protruding, large, elliptical; contacting or very narrowly separated from eyes; distinctly converging anteriorly. Mandibular bases and anterolateral corners of head capsule with distinct striations. Left mandible with slight hump at basal two-fifths; basal hump with several ~0.03 mm long setae; first and

second marginal teeth partially separated; each with separate pointed apex (Fig. 3); third marginal tooth with sinuous anterior and posterior margins. Right mandible with molar plate longer than posterior margin of second marginal tooth and composed of ca. 20 ridges (Fig. 3).

Several dozen setae of medium length (~0.05mm) dispersed on head, pronotum, wing scales, abdominal tergites, and sternites. Antennae with 18-24 articles, 75% (n = 64) with 22-24 articles, 10% with 24; relative length formula $2 > 3 > 4 = 5$ or $2 = 3 > 4 = 5$. Pronotum robust, about twice as wide as its median length. Pronotum with anterior margin evenly concave, lateral margins faintly convex, posterolateral margins subtruncate or faintly concave, and posterior margin slightly concave medially; anterior and lateral margins with raised and rounded rim. Fore wing with very long subcosta and radius; subcosta terminating at costal margin usually beyond 1/2 of wing length from suture and near intersection of radius and costal margin at 2/3 of wing length. Radial sector with 4-6 branches that fork in apical third of wing just beyond intersection of radius into costal margin. Median vein sclerotized and with about four sclerotized and short posterior branches; branches dissolve gradually into membrane except for usually the two most distal branches, that terminate at wing margin. Wing membrane faintly and irregularly nodulate with some nodules fused. Arolia distinct.



Fig. 2. Photomicrograph of the oblique view of imago head of *Neotermes phragmosus* n. sp. from the U.S. Naval Base, Guantánamo, Cuba, showing deeply excavated and phragmotic frons. Scale bar equals 1 mm.

Comparisons.

The *N. phragmosus* imago is unique among congeners in that its frons is characteristically truncated, depressed, encircled by a ridge, and rugose. Imagos of *N. phragmosus* and the allopatric *N. mona* are the largest among the West Indian Kalotermitidae. The *N. phragmosus* imago has less dense pilosity than *N. mona* on the head, pronotum, and wing scales. Few short setae on basal hump of mandibles present in *N. phragmosus* imago are absent both in *N. mona* and *N. jouteli*.

Compared to the sympatric *N. jouteli*, *N. phragmosus* alates differ primarily in size, the first species being distinctly smaller than the second one, usually without any overlapping. Those most distinctive characters are: 1.77-2.16 mm for head length with labrum of *N. jouteli*, versus 2.24-2.74 mm for *N. phragmosus*; labrum width maximum 0.60-0.70 mm versus 0.74-0.83 mm; pronotum maximum length is 1.06-1.32 mm of *N. jouteli*, but 1.44-1.81 in *N. phragmosus*; and

pronotum width with 1.75-2.05 mm, while 2.10-2.59, respectively. Total body length is also useful; 13.92-16.05 mm in *N. jouteli*, versus 15.80-19.04 mm in *N. phragmosus*.

Soldier. (Fig. 1, Tables 2 and 3).

The soldier caste consists of two distinct morphs, large and small, both usually present in mature colonies. Other than size, there are few distinguishing characters that separate small and large soldiers of *N. phragmosus* compared with some congeners and species in several other kalotermitid genera.

Head capsule and labrum ferruginous orange in dorsal view. Antennae ferruginous orange; three proximal articles ferruginous. Anteclypeus pale yellowish. Mandibles glossy, almost black; basal areas dark chestnut. Epicranial sutures faint or absent. Eyes dark gray. Thorax, including femora and abdominal dorsum and sternum pale yellowish. Tibiae and genae pale orange-yellow. Postmentum pale ferruginous.

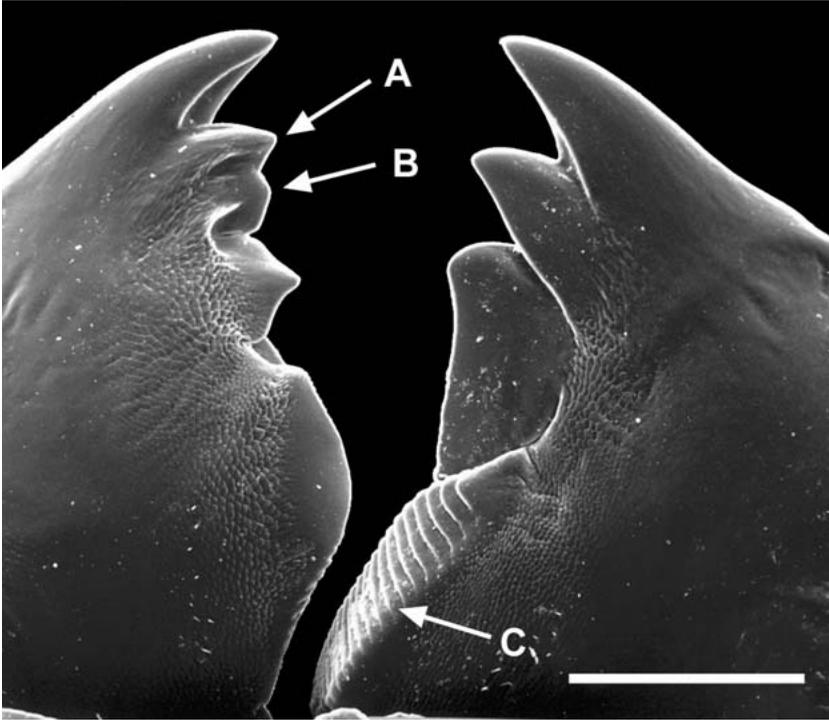


Fig. 3. Scanning electron micrograph of imago mandibles of *Neoterme phragmosus* n. sp., dorsal view, from Tortuguilla, Guantánamo Province, Cuba. Labels: first marginal tooth (A), second marginal tooth (B) of left mandible, and molar plate (C) of right mandible. Scale bar equals 0.5 mm. Labrum removed for clarity.

In dorsal view, head capsule subsquare, with sides subparallel, faintly convex in large soldiers, slightly convex in small morph; posterior corners rounded and posterior margin widely rectate in both morphs. Head capsule, thorax, and abdomen covered with dense mat of long setae (~0.1 mm); occiput glabrous. Frons depressed, faintly submerged, and broadly continuous with postclypeus; depressed area faintly striate. Frontal carinae tapered into distinctly protruding tubercle near antennal carinae. Labrum broadly linguiform; apex slightly convex. Mandibles elongate and relatively robust, with remarkably pilose basal humps; dentition distinct. Small soldier antennae with 17-21 articles, usually 18; large morph with 16-20 articles, usually 18 or 20; third antennal article subclavate, terminal articles usually slightly elongate; antennal formula $2 < 3 > 4 = 5$. Antennal carinae protruding and faintly rugose. Pronotum papilionaceous, noticeably wider than head, and more than twice as wide as long in middle. Anterior margin of pronotum deeply and evenly concave; anterolateral corners abruptly rounded, sides of pronotum subparallel, faintly convex; posterior margin weakly emarginate. Pterothorax with posterolateral sides subtruncate, more so in small soldiers than in large soldiers. All soldiers with short wing buds.

In lateral view, head capsule dorsoventrally flattened; principal plane of frons occupying about half of head capsule length in small soldiers; about one third in large morph. Frons slopes $\approx 15^\circ$ from plane of vertex; mandibles noticeably curved upward; eyes large and vertically oriented; without peripheral satellite facets. Pilosity of frons and anterior vertex dense. Hind femora moderately broadened in small soldiers and noticeably inflated in large morphs.

Comparisons.

No single measurement in either soldier morph is diagnostic for separating *N. phragmosus* from its nearest congener, *N. mona*. Nevertheless, the small morph of *N. phragmosus* is larger in the majority of measurements than that of *N. mona*. The mandibular hump pilosity of *N. phragmosus* is considerably more conspicuous than that of both *N. mona* and *N. jouteli*. The *N. phragmosus* soldiers possess a distinctly protruding tubercle on each frontal carina, which, both in *N. mona* and *N. jouteli*, are rudimentary. Striations of frons in *N. phragmosus* are considerable, while absent or very faint in *N. mona*. The rugosity of antennal carinae is faint in *N. phragmosus*, while being well developed in *N. mona*. The eyes of *N.*

TABLE 1. MEASUREMENTS OF *NEOTERMES PHRAGMOSUS* IMAGO.

Measurement in mm (n = 9 males, 10 females from 6 colonies)	Range	Mean \pm S.D.	Holotype
Head length with labrum	2.24-2.74	2.58 \pm 0.12	2.64
Head length to postclypeus	1.56-2.00	1.86 \pm 0.12	1.93
Head width, maximum at eyes	1.83-2.27	2.11 \pm 0.093	2.12
Head height without postmentum	1.00-1.19	1.14 \pm 0.050	1.18
Labrum width, maximum	0.74-0.83	0.80 \pm 0.026	0.80
Eye diameter with sclerite, maximum	0.56-0.68	0.64 \pm 0.034	0.67
Eye to head base, minimum from sclerite	0.27-0.38	0.33 \pm 0.029	0.34
Ocellus diameter, maximum	0.18-0.26	0.23 \pm 0.020	0.22
Ocellus diameter, minimum	0.16-0.20	0.19 \pm 0.0098	0.19
Eye sclerite to ocellus, minimum	0-0.016	0.0090 \pm 0.0066	0.0082
Pronotum, maximum length	1.44-1.81	1.67 \pm 0.10	1.77
Pronotum, maximum width	2.10-2.59	2.44 \pm 0.13	2.59
Total length with wings	15.80-19.04	17.88 \pm 0.75	17.69
Total length without wings	8.91-13.10	11.35 \pm 1.09	12.29
Fore wing length from suture	11.07-13.77	12.93 \pm 0.61	13.23
Fore wing, maximum width	3.08-4.07	3.79 \pm 0.28	4.07
Hind tibia length	1.60-1.90	1.78 \pm 0.089	1.83

phragmosus do not display peripheral facets, which are typical of *N. mona*. Finally, the antennae of *N. phragmosus* soldiers have more articles compared to those of *N. mona*, in which the range is 13-19, 12-18 in *N. jouteli*, while in *N. phragmosus* it is 16-21.

Compared with the sympatric *N. jouteli*, *N. phragmosus* soldiers of both forms differ in having a much wider and much more deeply concave anterior margin of the pronotum. The character is particularly distinctive in large soldiers (pronotum width in *N. jouteli* ranges between 2.61-3.03 mm, while the same measurement in *N. phragmosus* is

3.32-3.96 mm). Pronotal length of *N. phragmosus* large soldiers ranges between 2.15-2.52 mm while in *N. jouteli* the length is 1.71-1.85 mm. Both soldier morphs of *N. phragmosus* are more pilose than *N. jouteli* around the anterior portion of the head including mandible bases. The maximum head width (2.93-3.46 mm) and left mandible length (2.64-2.90 mm) of *N. phragmosus* large soldiers do not overlap with those respective measurements (2.34-2.70 and 2.17-2.42 mm) in *N. jouteli*. Although some small soldier measurements overlap for both species, the *N. phragmosus* small soldier is larger overall than that of *N. jouteli*.

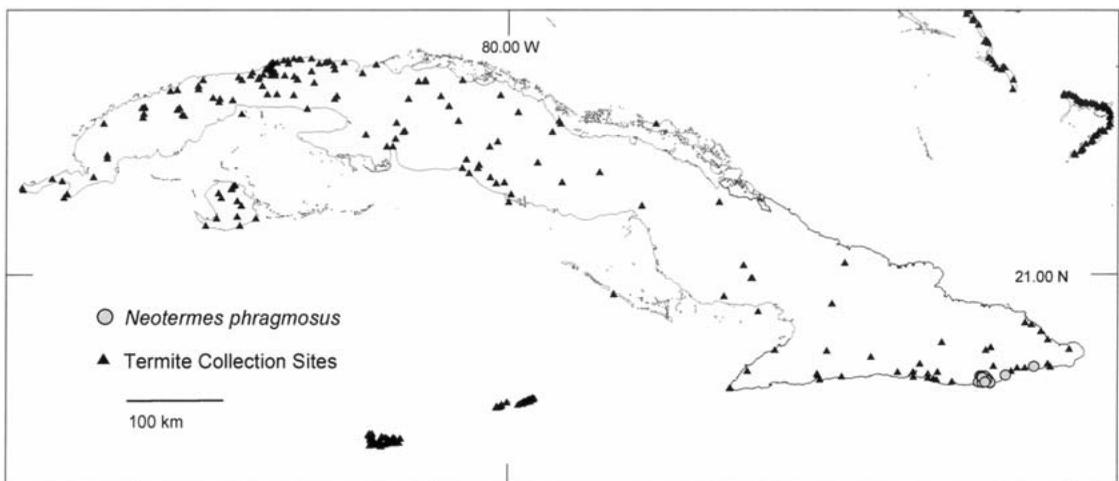


Fig. 4. *Neotermes phragmosus* n. sp. localities and termite collection sites on Cuba and neighboring islands.

TABLE 2. MEASUREMENTS OF *NEOTERMES PHRAGMOSUS* SMALL SOLDIER.

Measurement in mm (n = 12 from 7 colonies)	Range	Mean \pm S.D.
Head length to tip of mandibles	3.91-5.30	4.69 \pm 0.40
Head length to postclypeus	2.43-3.47	3.04 \pm 0.32
Head width, maximum	2.28-3.10	2.77 \pm 0.24
Antennal carinae, outside span	2.04-2.60	2.35 \pm 0.16
Head height, excluding postmentum	1.34-1.83	1.53 \pm 0.15
Labrum, maximum width	0.64-0.82	0.73 \pm 0.053
Postclypeus width, maximum	0.87-1.10	0.98 \pm 0.066
Left mandible length, tip to most distant visible point of ventral condyle	2.17-2.69	2.42 \pm 0.15
Postmentum, length in middle	1.88-2.47	2.17 \pm 0.20
Postmentum, maximum width	0.80-1.11	0.93 \pm 0.087
Postmentum, minimum width	0.49-0.60	0.54 \pm 0.045
Pronotum, maximum width	2.69-3.36	3.07 \pm 0.19
Pronotum, maximum length	1.63-2.20	1.93 \pm 0.16
Hind tibia length	1.38-1.95	1.71 \pm 0.15
Total length	9.72-14.85	12.48 \pm 1.67

Etymology.

The species name reflects the unique and striking phragmosis of the imago frons; possibly the most developed for this character among the Isoptera.

Remarks.

The holotype colony was collected in a very xeric coastal habitat from the dead wood of living *Calotropis procera* Aiton (Asclepiadaceae), an exotic shrub. The colony penetrated into xylem elements within the living cambium. Other colonies were collected from dead branches and trunks of mangroves, buttonwood, and other littoral woods. The dispersal flight season of *N. phragmosus* is unknown, but we suspect nocturnal autumn flights similar to those of others congeners as

alates were collected in late August and early November.

Type material.

Holotype colony series. **Cuba.** Guantánamo Province; Tortuguilla; 19.98°N, 74.93°W; 20-VIII-1974; coll. J. Krecek; 1 female alate holotype, 13 alate paratypes, 6 paratype small soldiers and 6 paratype large soldiers (CU-968).

Paratype colonies series. All material originates from Guantánamo Prov.: Imias; 20.07°N, 74.64°W; VIII-1975; coll. L. de Armas; 1 paratype small and large soldier (CU-1038). The following samples were collected at the U.S. Naval Base Guantánamo Bay by J. Chase, J. Mangold, and R.H. Scheffrahn 2-XI-2001 to 6-XI-2001: Kittery Beach; 19.906°N, 75.089°W; 1 paratype imago (CU-1076); N. Kittery Beach; 19.905°N, 75.088°W;

Table 3. Measurements of *Neotermes phragmosus* large soldier.

Measurement in mm (n = 11 from 6 colonies)	Range	Mean \pm S.D.
Head length to tip of mandibles	5.30-6.09	5.69 \pm 0.22
Head length to postclypeus	3.61-4.16	3.87 \pm 0.17
Head width, maximum	2.93-3.46	3.25 \pm 0.17
Antennal carinae, outside span	2.54-2.97	2.78 \pm 0.13
Head height, excluding postmentum	1.83-2.30	2.15 \pm 0.14
Labrum, maximum width	0.70-0.83	0.78 \pm 0.049
Postclypeus width, maximum	1.06-1.21	1.13 \pm 0.046
Left mandible length, tip to most distant visible point of ventral condyle	2.64-2.90	2.77 \pm 0.080
Postmentum, length in middle	2.57-3.03	2.79 \pm 0.14
Postmentum, maximum width	0.93-1.14	1.06 \pm 0.078
Postmentum, minimum width	0.47-0.65	0.58 \pm 0.063
Pronotum, maximum width	3.32-3.96	3.66 \pm 0.16
Pronotum, maximum length	2.15-2.52	2.33 \pm 0.11
Hind tibia length	1.75-2.10	2.01 \pm 0.10
Total length	12.83-16.07	13.96 \pm 1.13

1 paratype small and large soldier (CU-1343); Old Chief's Club; 19.925°N, 75.131°W; 1 paratype imago (CU-1374); Boat landing, leeward mangroves; 19.941°N, 75.152°W; 1 paratype large soldier (CU-1401); 1 paratype small soldier (CU-1408); Naval Station Brig; 19.936°N, 75.124°W; 1 paratype small soldier (CU-1430); 1 paratype imago (CU-1433); Evan's Point; 19.921°N, 75.141°W; 1 paratype imago (CU-1447); 1 paratype small and large soldier (CU-1448); Leeward mangroves, *Coccothrinax* habitat; 19.958°N, 75.165°W; 1 paratype imago and small soldier (CU-1521), 1 paratype large soldier (CU-1523).

DISCUSSION

The characters of *Neotermes phragmosus* require that morphological definitions for the Kalotermitidae be broadened for both the imago and soldier. Plesiomorphic traits of *N. phragmosis* outside of Krishna's (1961) imago diagnosis include: 1) a maximum of 24 antennal articles (increase of 3), 2) separation of the second and third marginal teeth of the left mandible, 3) the molar plate of the right mandible longer than the posterior margin of the second marginal tooth, and 4) fore wing subcosta extending to at least mid wing with radius intersecting costal margin well beyond mid wing. In the soldier, the number of antennal articles is increased from 19 to 21. It is noteworthy that for the soldier, Kambhampati & Eggleton (2000) use the threshold gap of 20-22 antennal articles to separate the Termopsidae from the Kalotermitidae.

Although a weak frontal concavity and rudimentary phragmosis occur in several Neotropical *Neotermes* imagos, i.e. *N. jouteli* (Scheffrahn et al. 2000), *N. mona* (Krecek et al. 2000), and *N. platyfrons* (Krecek & Scheffrahn 2001), the degree of its development in *N. phragmosus* is remarkable and suggests apomorphism for defense of incipient colonies against predatory ants or competition by termites vying for nuptial microhabitats. The evolutionary significance of pilosity of the mandibular humps in the soldier is unclear. Mandibular basal pilosity is not uncommon in *Neotermes*; it appears also in *Glyptotermes*, *Paraneotermes*, and *Incisitermes*, but this trait reaches its maximum expression in *N. phragmosus*.

Together with the *Antillitermes subtilis* (Scheffrahn & Krecek 1993), *Constrictotermes guantanamensis* Krecek et al. (1996), *Cryptotermes spathifrons*, and *C. cymatofrons* (Scheffrahn & Krecek 1999), *N. phragmosus* is the fifth species recently described from southeastern Cuba.

All species but *C. cymatofrons* are confined to xeric habitats.

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LEGUMINOUS COVER CROPS AND THEIR INTERACTIONS WITH CITRUS AND *DIAPREPES ABBREVIATUS* (COLEOPTERA: CURCULIONIDAE)

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ABSTRACT

Three legume species with potential as cover crops in citrus groves were studied for their effect on the developmental biology of the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) in greenhouse studies. All 3 cover crops were hosts for the Diaprepes root weevil. *Cajanus cajan* (pigeon pea) was a superior host for development of *D. abbreviatus* compared with citrus rootstocks. *C. cajan* appeared to be allelopathic; the root mass of uninfested citrus was greatly reduced when grown in association with *C. cajan* compared with citrus grown alone. Association of citrus with *C. cajan* or *Arachis pintoi* (perennial peanut) reduced chlorophyll fluorescence, a measure of photosynthesis, compared with citrus associated with *Crotalaria pallida* (rattlebox) or with another citrus seedling. When grown in close association with *A. pintoi*, citrus produced the same amount of root mass as citrus seedlings grown alone. Infestation with larval *D. abbreviatus* reduced chlorophyll fluorescence of citrus by 26%. None of the 3 legume species tested reduced the feeding damage caused by *D. abbreviatus* to citrus. Larvae reared in pots with *A. pintoi*, associated with citrus or alone, gained weight at the same rate as larvae reared on the citrus rootstocks alone. Larvae recovered from pots containing *C. pallida* associated with citrus weighed significantly more than larvae reared on citrus alone. *C. cajan* appears to be particularly inappropriate as a cover crop because of its positive effect on larval growth and reduction of citrus root mass. None of the 3 legume species tested had a negative effect on *D. abbreviatus* or on feeding damage.

Key Words: Citrus, *Diaprepes abbreviatus*, cover crops, perennial peanut, *Arachis pintoi*, pigeon pea, *Cajanus cajan*, *Crotalaria pallida*

RESUMEN

Tres especies de leguminosas forrajeras con potencial como cobertura en cítricos fueron evaluadas por su efecto sobre el desarrollo del cucarrón *Diaprepes abbreviatus* (L.) en un invernadero. Las tres especies sirvieron como hospedantes para el insecto. *Cajanus cajan* (gandul) fue un hospedante superior para el desarrollo de *D. abbreviatus* comparado con patrones de cítricos. *C. cajan* fue alelopático; la masa de raíces de cítricos no-infestadas fue menor en asociación con *C. cajan* comparado con cítricos solos. La asociación de cítricos con *C. cajan* o *Arachis pintoi* (mani forrajero) resultó en una reducción de fluorescencia de clorófilo, una medida de fotosíntesis, comparado con cítricos asociados con *Crotalaria pallida* o con otra plántula de cítricos. Cítricos asociados con *A. pintoi* produjeron la misma cantidad de raíces que los cítricos solos. La infestación con larvas de *D. abbreviatus* resultó en una reducción de 26% en fluorescencia de clorófilo de plántulas de cítricos. Ninguna de las tres especies de leguminosas redujo el daño causado por alimentación de *D. abbreviatus* en cítricos. Las larvas en potes con *A. pintoi* (asociado con cítricos o solo) aumentaron de peso de la misma manera que larvas sobre raíces de cítricos solo. Larvas recuperadas de potes que contenían *C. pallida* asociado con cítricos pesaron más que larvas sobre cítricos solo. Parece que *C. cajan* en particular no es recomendable como cobertura debida a su efecto positivo sobre el crecimiento de larvas y su efecto negativo sobre la masa radicular de cítricos. Ninguna de las tres leguminosas tuvo un efecto negativo sobre *D. abbreviatus* o redujo su daño. Translation provided by author.

Leguminous cover crops can contribute to increased and sustainable crop productivity through erosion and weed control, biological nitrogen fixation, and by providing refuge for natural enemies of arthropod pests (Hokkanen 1991). Cover crops such as perennial peanut (*Arachis* spp.) have been suggested for use in citrus groves (Prine et al. 1981), but adoption of this practice will depend on an unequivocal demonstration of benefits to grove managers. Cover crops should be

selected, at a minimum, that do not harbor key pests and may be selected to divert or deter pests and contribute to the diversity and abundance of natural enemies (Altieri 1995, Risch 1981).

A major concern to Florida citrus producers is the highly polyphagous Diaprepes root weevil, *Diaprepes abbreviatus* (L.) (Simpson et al. 1996). Cover crops or trap crops could contribute to citrus productivity and control of damage from *D. abbreviatus*. It is equally possible, however, that

introduction into the citrus cropping system of an additional plant resource for a highly polyphagous pest could result in higher pest population density (Aindow 1991), particularly in the case of *D. abbreviatus* in Florida where natural enemies are insignificant (Hall et al. 2001)

As a first attempt to study the potential influence of cover legumes on the biology of *D. abbreviatus*, I examined the response of larvae to 3 legume species in a greenhouse. *Arachis pintoii* Krapovickas & Gregory is increasingly used as a tropical forage and as a cover in diverse tropical tree crops (de la Cruz et al. 1993) and has been considered for use in Florida citrus groves. *Cajanus cajan* Millspaugh (pigeon pea) is widely grown in Puerto Rico where anecdotal observations suggest a strong preference for this species by *D. abbreviatus*. *Crotalaria pallida* Ait. (rattlebox) has been used extensively in Florida as a green manure and has become naturalized. I report here the effect of these species alone and associated with citrus on development of *D. abbreviatus*.

MATERIALS AND METHODS

Trial I. Effect of Plant Associations in 3.8-L-pots.

Seed of *C. cajan*, *A. pintoii* and *C. pallida* were planted in germination trays in a soilless potting mix (Metromix 500, Scotts, Marysville, OH) and transplanted at approximately 1 mo after germination. Seedlings of 'Carrizo' citrange (*C. sinensis* (L.) Osbeck × *P. trifoliata*) were germinated in fine, sterile sand (Bonsal Play Sand, W. R. Bonsal Co., Charlotte, NC) and transplanted to 3.8-L-pots at 4 mo after germination. All plants were transplanted to 3.8-L-pots containing sterile sand. The pots were lined with a nylon mesh cloth to prevent escape of larvae through the drainage holes. Pots were planted with 2 seedlings per pot in the following combinations: two seedlings of either 'Carrizo', *A. pintoii*, *C. cajan*, or *C. pallida*; or one seedling of 'Carrizo' and a companion plant of *A. pintoii*, *C. cajan*, or *C. pallida*. An additional combination consisted of one plant of *C. cajan* and one plant of *A. pintoii*. Treatments consisted of 3 weevil-infested and 3 noninfested pots of each of 8 plant combinations in a randomized block design. A total of 48 pots were arranged randomly on greenhouse benches within infested and noninfested blocks. Ten early instar larvae of *D. abbreviatus* weighing 20 ± 5 mg each were added to infested pots on 30 June 1998. Larvae were obtained from a laboratory colony maintained by the U.S. Horticultural Research Laboratory, Orlando, FL and reared according to Lapointe & Shapiro (1999). Early instars were used instead of neonates to avoid escape or movement of larvae between pots. Citrus seedlings were one year old and legume seedlings were 3 mo. old at the begin-

ning of the infestation period. Plants were maintained throughout the experiment on elevated benches in a greenhouse with an average diurnal temperature cycle of 35°C maximum and 23°C minimum. No supplemental light was supplied. Maximum photosynthetic photon flux in the greenhouse was 800 mol·s⁻¹·m⁻². Plants were watered with a dilute fertilizer mix weekly using water-soluble 20N-10P-20K at a rate of 150 mg·liter⁻¹N.

At the end of the infestation period, an OS5-FL modulated chlorophyll fluorometer (Opti-Sciences, Tyngsboro, MA, USA), was used to measure yield of chlorophyll fluorescence of light-adapted leaves with saturation intensity of ~2.7 kuE for 0.8 sec. Yield (relative units) is an indicator of quantum yield of photosynthesis and is often used in measuring plant stress (Schreiber & Bilger 1987, van Kooten & Snel 1990). Chlorophyll fluorescence was measured during the late morning hours (9:00-11:00). All readings (Y values) were taken from the center of a leaf, and always from the center leaflet in the case of trifoliolate leaves. Readings were taken from 3 positions on each plant: 'top' was taken from the first fully expanded leaf, 'bottom' was taken from the lowest available intact leaf, and 'middle' was taken from the leaf at the mid-point between 'top' and 'bottom'.

The plants and sand were removed from the pots and sieved to recover larvae 37 d after infestation on 6 August 1998. Recovered larvae were counted and weighed before and after drying in an analytical oven at 60°C for ≥48 h to obtain fresh and dry weights. Roots were washed and separated from the above-ground portion by cutting at the point where the first (uppermost) lateral root emerged from the central root. Roots were allowed to air-dry and weighed.

The effect of plant combination on total larval weight per pot, and the effect of infestation and plant combination on citrus root weight and chlorophyll fluorescence were compared by ANOVA. Where appropriate, means were compared by Tukey's Honestly Significant Differences (HSD) test (Abacus Concepts 1996).

Trial II. Effect of Plant Associations in 76-L-pots.

Eighteen 1-year-old seedlings of the rootstock 'Sun Chu Sha' mandarin (*C. reticulata* Blanco) were transplanted to 76-L-pots containing sterile sand in May, 1998. Availability of plants determined choice of rootstock for this trial. However, 'Carrizo' and 'Sun Chu Sha' have been shown to be equivalent in terms of larval weight gain of *D. abbreviatus* reared on potted seedlings, and in terms of damage to roots of seedlings infested with *D. abbreviatus* (Lapointe et al. 1999).

Plant combinations in the pots consisted of either a single tree of 'Sun Chu Sha' or a seedling of 'Sun Chu Sha' surrounded by 10 seedlings of

A. pinto or 6 seedlings of *C. cajan*. Insect treatments consisted of 3 infested and 3 noninfested pots of each plant combination. Pots were infested 1 July 1998 with 50 larvae each with a mean (\pm SEM) individual weight of 71.7 ± 1.9 mg. Roots and larvae were recovered 40 d later on 10 August 1998. To estimate larval number per pot, soil was sifted and scanned for 15 min. Of the larvae recovered, 15 were randomly selected from each pot, weighed, dried in an analytical oven and weighed again. Roots were allowed to air dry and then weighed.

Larval weights were summed for each pot. The effect of plant combination on total larval weight per pot, and the effect of infestation and plant combination on citrus root weight were compared by ANOVA. Experimental design was a 2×3 factorial with 2 levels of infestation and 3 plant associations. Where appropriate, means were compared by Tukey's Honestly Significant Differences (HSD) test and groups of means (e.g., all pots containing ≥ 1 plant of *C. cajan*) by post-hoc orthogonal contrasts (Abacus Concepts 1996).

RESULTS

Trial I. Effect of Plant Associations in 3.8-L-pots.

Both companion plant and state of infestation had a significant effect on chlorophyll fluorescence of 'Carrizo' leaves. There was no significant effect from leaf position ($F = 2.4$; $df = 2, 72$; $P = 0.10$), interaction between infestation and companion plant ($F = 1.8$; $df = 3, 72$; $P = 0.16$) or between infestation and position ($F = 0.5$; $df = 2, 72$; $P = 0.59$). For analysis of effects of infestation and companion plant, the measures of chlorophyll fluorescence for top, middle, and bottom 'Carrizo' leaves were pooled.

Infestation with larval *D. abbreviatus* reduced chlorophyll fluorescence of the 'Carrizo' plants by $26 \pm 8\%$ compared with noninfested controls ($F = 23.7$; $df = 1, 72$; $P < 0.01$). The species of companion plant also significantly affected chlorophyll fluorescence of 'Carrizo' ($F = 3.3$; $df = 3, 72$; $P = 0.03$). 'Carrizo' planted with another 'Carrizo' plant or planted with *C. pallida* had higher levels of chlorophyll fluorescence compared with 'Carrizo' plants planted with either *A. pinto* or *C. cajan*. Association with *A. pinto* or *C. cajan* reduced photosynthesis fluorescence in 'Carrizo' by 20% (Table 1).

There was neither an effect of companion ($F = 0.9$; $df = 2, 66$; $P = 0.40$) nor of infestation ($F = 0.1$; $df = 1, 66$; $P = 0.73$) on the chlorophyll fluorescence of *C. cajan*. There was an effect of leaf position on chlorophyll fluorescence ($F = 9.1$; $df = 2, 66$; $P < 0.01$). Top and middle *C. cajan* leaves had higher levels of fluorescence (0.514 ± 0.015 and 0.482 ± 0.024 , respectively) than lower leaves (0.389 ± 0.024) (Tukey's HSD, $\alpha = 0.05$).

TABLE 1. YIELD OF CHLOROPHYLL FLUORESCENCE (MEAN RELATIVE VALUE \pm SE) OF A CITRUS ROOT-STOCK ('CARRIZO') PLANTED IN 3.8-L-POTS WITH 3 SPECIES OF LEGUME COMPANION PLANTS.

Companion	Yield	n
<i>A. pinto</i>	0.302 ± 0.028 a	18
<i>C. cajan</i>	0.308 ± 0.032 a	18
Citrus	0.386 ± 0.020 b	36
<i>C. pallida</i>	0.392 ± 0.045 b	18

Means followed by the same letter are not significantly different at $P = 0.05$ by Tukey's HSD after a significant ANOVA ($F = 3.3$; $df = 3, 72$; $P = 0.03$).

The chlorophyll fluorescence of *A. pinto* was unaffected by companion ($F = 2.3$; $df = 2, 66$; $P = 0.11$) and infestation ($F = 0.9$; $df = 1, 66$; $P = 0.35$). As in the case of *C. cajan*, there was a significant effect of leaf position ($F = 4.5$; $df = 2, 66$; $P = 0.01$); top leaves had a higher rate (0.433 ± 0.019) compared with middle and lower leaves (0.344 ± 0.024 and 0.350 ± 0.028 , respectively) (Tukey's HSD, $\alpha = 0.05$).

There was no effect of companion (citrus or another *C. pallida*) ($F = 0.54$; $df = 1, 49$; $P = 0.47$) nor of infestation ($F = 0.004$; $df = 1, 49$; $P = 0.95$) on the chlorophyll fluorescence of *C. pallida*. There was a significant effect of leaf position ($F = 4.2$; $df = 2, 49$; $P = 0.02$); top leaves had a higher rate (0.465 ± 0.027) than lower leaves (0.332 ± 0.038) and middle leaves were intermediate (0.372 ± 0.032), not significantly different from either top or lower leaves (Tukey's HSD, $\alpha = 0.05$).

Both companion plant ($F = 7.1$; $df = 3, 22$; $P < 0.01$) and infestation ($F = 66.9$; $df = 1, 22$; $P < 0.01$) significantly affected final root weight of 'Carrizo' plants in 3.8-L-pots, and there was a significant interaction between companion plant and infestation ($F = 3.9$; $df = 3, 22$; $P = 0.02$). For this reason, root weights were analyzed separately for infested and noninfested groups (Table 2). Noninfested 'Carrizo' plants in 3.8-L-pots had a smaller root mass when associated with *C. cajan*, but not when associated with *A. pinto* or *C. pallida*, compared with 'Carrizo' plants associated with another 'Carrizo'. 'Carrizo' root weight was greater when grown with *A. pinto* than with *C. pallida* or *C. cajan* (Table 2).

The root mass of all 'Carrizo' plants infested with *D. abbreviatus* was significantly reduced. In the case of 'Carrizo' associated with 'Carrizo', pots contained 2 plants and therefore twice as much citrus root mass was available compared with 'Carrizo' associated with a legume species. Apparently, weevils fed equally on 'Carrizo' regardless of companion plant, i.e., none of the associations resulted in reduced feeding damage to 'Carrizo' (Table 2). If the interaction term is ignored by setting α at 1%, the main effect of infestation by *D. abbreviatus* reduced 'Carrizo' root weight by $68 \pm 7\%$

TABLE 2. MEAN FRESH WEIGHT (\pm SE) OF ROOTS OF CITRUS SEEDLINGS INFESTED WITH LARVAL *D. ABBREVIATUS* AND GROWN IN 3.8-L-POTS WITH 3 SPECIES OF COMPANION LEGUMES OR WITH A SECOND CITRUS SEEDLING.

Companion	Citrus root weight (g)		n
	Infested ^a	Noninfested ^b	
<i>C. pallida</i>	0.23 \pm 0.03 a	1.75 \pm 0.38 ab	3
<i>C. cajan</i>	0.39 \pm 0.17 ab	1.19 \pm 0.10 a	3
<i>A. pintoii</i>	0.49 \pm 0.19 ab	2.99 \pm 0.47 c	3
Citrus	1.12 \pm 0.21 b	2.21 \pm 0.18 bc	6

Means in a column followed by the same letter are not significantly different at $P = 0.05$ by Tukey's HSD after a significant ANOVA.

^a $F = 4.6$; $df = 3, 11$; $P = 0.03$.

^b $F = 6.0$; $df = 3, 11$; $P = 0.01$.

compared with noninfested 'Carrizo'. Similarly, the main effect of companion plant was highly significant ($F = 7.1$; $df = 3, 22$; $P < 0.01$). When *C. cajan* or *C. pallida* were the companion plants, root weight of 'Carrizo' was reduced by 53 and 41%, respectively. However, when associated with *A. pintoii*, root weight of 'Carrizo' was equal to the root weight of individual 'Carrizo' trees planted with a second 'Carrizo' ($\alpha = 0.05$, Tukey's HSD).

There was a significant effect of plant association on total wet weight of larvae recovered from each pot ($F = 35.4$; $df = 7, 15$; $P < 0.01$), total dry weight of recovered larvae ($F = 59.7$; $df = 7, 15$; $P < 0.01$) and on the number of larvae recovered per pot ($F = 8.3$; $df = 7, 16$; $P < 0.01$). In the 3 associations that included *C. cajan*, more ($F = 46.9$; $df = 1$; $P < 0.01$) and larger (wet weight: $F = 242.0$; $df = 1$; $P < 0.01$) larvae were recovered compared with pots containing 2 'Carrizo' plants (Table 3). The other 2 legume species did not affect the number or size of larvae recovered compared with pots containing 2 'Carrizo' plants.

Trial II. Effect of Plant Associations in 76-L-pots.

There was a significant effect of association ($F = 9.1$; $df = 2, 12$; $P < 0.01$) and of infestation ($F =$

11.1; $df = 1, 12$; $P < 0.01$) on weight of citrus roots. There was also a significant interaction between these effects ($F = 4.3$; $df = 2, 12$; $P = 0.04$). Citrus root weight differences between noninfested and weevil-infested trees differed between citrus alone and citrus with *A. pintoii* compared with citrus associated with *C. cajan*. There was no significant difference in citrus root weights between infested or noninfested citrus in association with *C. cajan*, whereas citrus root weights of noninfested citrus alone or in association with *A. pintoii* were increased by 47-55% over infested citrus (Fig. 1).

The effect of infestation was therefore tested for each treatment (association) separately, and treatment means were compared within infested and noninfested groups. Uninfested 'Sun Chu Sha' plants associated with *C. cajan* had much smaller root mass compared with 'Sun Chu Sha' alone or 'Sun Chu Sha' associated with *A. pintoii* (Fig. 1). Root mass of 'Sun Chu Sha' was equivalent for all 3 infested treatments ('Sun Chu Sha' alone, 'Sun Chu Sha'/*A. pintoii*, and 'Sun Chu Sha'/*C. cajan*).

The effect of plant association on final larval fresh weight of 15 larvae recovered from each pot was significant at $P = 0.06$ (ANOVA). However,

TABLE 3. MEAN NUMBER AND TOTAL WEIGHT (\pm SE, $N = 3$) OF LARVAL *D. ABBREVIATUS* RECOVERED FROM 3.8-L-POTS CONTAINING 8 DIFFERENT PLANT ASSOCIATIONS.

Association	No. larvae recovered ^a	Larval weight (mg)	
		Wet ^b	Dry ^c
Citrus/Citrus	1.7 \pm 0.3 a	70.4 \pm 23.4 a	16.2 \pm 7.7 a
<i>C. pallida</i> / <i>C. pallida</i>	1.7 \pm 0.9 a	87.8 \pm 23.4 a	13.6 \pm 4.2 a
Citrus/ <i>C. pallida</i>	1.7 \pm 0.3 a	161.1 \pm 24.0 a	38.8 \pm 8.2 b
<i>A. pintoii</i> / <i>A. pintoii</i>	3.3 \pm 0.9 ab	166.5 \pm 47.0 a	30.0 \pm 9.4 a
Citrus/ <i>A. pintoii</i>	3.3 \pm 0.3 ab	210.7 \pm 7.1 a	40.1 \pm 0.6 a
Citrus/ <i>C. cajan</i>	5.0 \pm 0.6 b	852.8 \pm 53.7 b	280.8 \pm 32.4 b
<i>C. cajan</i> / <i>C. cajan</i>	5.0 \pm 0.6 b	966.9 \pm 145.8 b	311.2 \pm 42.5 bc
<i>C. cajan</i> / <i>A. pintoii</i>	5.7 \pm 0.3 b	1027.0 \pm 95.0 b	399.4 \pm 7.3 c

Means in a column followed by the same letter are not significantly different at $P = 0.05$ by Tukey's HSD after a significant ANOVA.

^a $F = 8.3$; $df = 7, 16$; $P < 0.01$

^b $F = 35.4$; $df = 7, 15$; $P < 0.01$

^c $F = 59.7$; $df = 7, 15$; $P < 0.01$

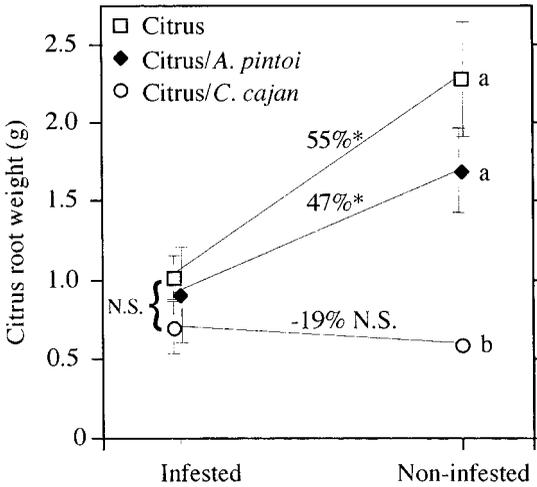


Fig. 1. Interaction plot of effect of infestation with larval *D. abbreviatus* on fresh root weight (\pm SE, $n = 3$) of citrus trees grown in 76-L-pots alone or in association with 2 legumes. Means followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey's HSD); percent reduction of root weight for infested vs. noninfested associations was significant (*) or not (N.S.) by unpaired t test ($\alpha = 0.05$).

the effect of plant association on final larval dry weight of 15 larvae was highly significant ($F = 23.5$; $df = 2, 6$; $P < 0.01$). The dry weight of larvae recovered from pots containing 'Carrizo'/*C. cajan* was 38% greater than that of larvae from the other 2 treatments (Table 4).

DISCUSSION

Damage by subterranean larvae is difficult to detect and plant damage often is not evident until days or weeks after feeding occurs. In this trial, we were unable to visually detect differences between infested and noninfested plants over the period of infestation (1 mo). Indeed, it is not un-

common in these tests to find seedlings with extensive root damage without any visual foliar symptoms. However, we were able to detect a significant reduction in chlorophyll fluorescence due to larval feeding using a fluorometer. This method may be useful in citrus groves as an indicator of tree health in general and presence of root weevils in particular.

Ground covers offer advantages and disadvantages when incorporated into agricultural production systems. In recent years, the tropical forage *A. pinto* has shown potential for use as a cover crop in tropical tree crops such as coffee, banana, oil palm, macadamia, and heart-of-palm (de la Cruz et al. 1993) and has been proposed for use in Florida's subtropical citrus groves (Prine et al. 1981). While slow to establish, *A. pinto* is effective at weed suppression, has a non-twining growth habit, and is efficient at fixation of atmospheric nitrogen (Thomas 1993). For citrus, these attributes must be considered in relation to the potential for nutrient competition, ease of management, and effect on pests and diseases. This study indicates that *A. pinto* is the most appropriate of the 3 species studied here as a cover crop in citrus in terms of its effect on a major pest, *D. abbreviatus*.

C. pallida did not decrease chlorophyll fluorescence or root growth by the citrus rootstock. The larvae recovered from pots containing *C. pallida* associated with rootstock, however, weighed significantly more than larvae reared on rootstock alone. This, combined with the upright, annual growth habit of *C. pallida* make this legume a less desirable option as a cover crop compared with *A. pinto*.

C. cajan (pigeon pea) has been reported to be attractive to *D. abbreviatus* (Barrow 1924). In the tests reported here, *C. cajan* was a superior host for development of *D. abbreviatus* compared with 'Carrizo'. More and larger larvae survived in pots when *C. cajan* was present, regardless of association with another plant species (Tables 3 and 4). In addition, the root mass of noninfested rootstock seedlings was greatly reduced when grown in as-

TABLE 4. MEAN WEIGHT (\pm SE, $N = 3$) OF 15 LARVAL *D. ABBREVIATUS* RECOVERED FROM 76-L-POTS CONTAINING 3 DIFFERENT PLANT ASSOCIATIONS.

Association	Larval weight (mg)		
	Initial ^a	Final wet ^b	Final dry ^c
Citrus/ <i>A. pinto</i>	73.1 \pm 2.6 a	2480.3 \pm 185.3 a	714.3 \pm 60.4 a
Citrus/Sun Chu Sha	71.1 \pm 2.8 a	2604.4 \pm 218.1 a	767.3 \pm 66.2 a
Citrus/ <i>C. cajan</i>	70.9 \pm 5.1 a	3207.3 \pm 128.8 a	1191.3 \pm 27.3 b

^aMeans are not significantly different by one-way ANOVA ($F = 0.1$; $df = 2, 6$; $P = 0.90$).
^bMeans are not significantly different by one-way ANOVA ($F = 4.5$; $df = 2, 6$; $P = 0.06$).
^cMeans in a column followed by the same letter are not significantly different at $\alpha = 0.05$ by Tukey's HSD following a significant ANOVA ($F = 23.5$; $df = 2, 6$; $P < 0.01$).

sociation with *C. cajan* compared with rootstock grown alone or in association with *A. pintoii* (Table 2, Fig. 1). The combination of increased growth of *Diaprepes* root weevil and apparent allelopathic effects on citrus makes *C. cajan* a particularly inappropriate choice for a cover crop.

When *A. pintoii* was grown in close association with a citrus rootstock in 3.8-L-pots, the rootstock produced the same amount of root mass as citrus plants grown alone (Table 2, Fig. 1). Although none of the 3 legume species tested reduced the feeding damage caused by *D. abbreviatus* to the rootstock, larvae reared in associations that included *A. pintoii* gained weight at the same rate as larvae reared on citrus alone (Table 3). Similarly, larvae reared in pots with *A. pintoii* alone gained the same amount of weight as larvae reared on citrus alone (Table 3). Simpson et al. (1996) reported larvae feeding on the roots of peanut (*Arachis hypogaea*) and the results presented here indicate that *A. pintoii* is also a host of *D. abbreviatus*. This presents the danger of increased pest populations in citrus/*A. pintoii* polycultures, but also the possibility of diversion of larval infestation from the principal crop (citrus) to the cover crop. Andow (1991) surveyed published reports of the effect of crop diversity on pest density and found that a minority (15%) of species were more abundant in polycultures while 52% were less abundant compared with monocultures. Attempts to establish cover crops in citrus should monitor key pests such as *D. abbreviatus*. Although none of the species tested here had negative effects on *D. abbreviatus* larvae, legumes are known to be a rich source of phytochemicals with diverse insect antifeedant and toxic properties (Simmonds et al. 1990) and should be surveyed for their activity against *D. abbreviatus* for possible inclusion in citrus production systems.

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PREDATION BY NATIVE ARTHROPODS
ON THE AFRICAN PARASITOID *PROROPS NASUTA*
(HYMENOPTERA: BETHYLIDAE) IN COFFEE PLANTATIONS OF MEXICO

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The establishment or colonization of natural enemies is a critical period of adjustment for the introduced individuals in the target area. Successful colonization and its effectiveness in control, depend on the intrinsic capabilities of the species and the interaction of physical and biotic factors (Callan 1969). Although the concept of establishment is simple, in practice it is a difficult task. From 4,769 introductions of predators and parasitoids made up to 1990, only 1,445 (30.3%) were established (Greathead & Greathead 1992). Diverse reasons have been mentioned of the traits likely to reduce the establishment of biological control agents. Among the most important are: adverse climatic condition, insufficient genetic variation, poor searching capacity, interference from chemicals or cultural practices, inadequate numbers of individuals introduced, lack of synchrony with the host and interference by native organisms (Hopper et al. 1993, Hopper 1996).

Prorops nasuta Waterston is an African parasitoid of the coffee berry borer (CBB) *Hypothenemus hampei* (Ferrari) (Coleoptera: Scolytidae), which is considered as the main pest of coffee worldwide (Le Pelley 1968, Baker 1999). *P. nasuta* females usually spend most of its life inside of a coffee fruit infested by the CBB. The wasp feeds on all juvenile stages, but only paralyzes and oviposits on the full-grown larvae and pupae. The larva starts to feed externally after hatching and one host is sufficient for the development of each wasp. When the parasite larva is fully developed, it spins a cocoon and enters to the pupal stage (Hargreaves 1935; Infante 1998). The life cycle of *P. nasuta* from egg to adult lasts 28 days at 22°C (Infante 2000), but adults remain in the coffee fruit for a few days more in order to copulate. As with other bethylids, this species has males emerging before their sisters with which they mate. The new generation of wasps leave the berry during the day, searching for infested coffee fruits (Hargreaves 1935, Murphy & Moore 1990).

In the last few years this parasitoid has been introduced to Mexico, Guatemala, El Salvador, Honduras, Ecuador, Colombia, Jamaica, Indonesia and India. In all these countries *P. nasuta* has been released and is presently under evaluation as a biological control agent (Klein-Koch et al. 1988, Barrera et al. 1990, Baker 1999). In the case

of Mexico, repeatedly releases of parasitoids have been carried out in Chiapas since 1992. Recovery surveys indicated that up to now, there is no evidence of the establishment of *P. nasuta* in the country (Infante et al. 2001). Identifying the factors that interfere in the establishment of *P. nasuta* is essential to the success of biological control programmes for the CBB. For that reason, the present paper reports on some native arthropods that were found preying on this species in coffee plantations of Chiapas.

The wasps used in releases were reared in the laboratory on borer infested coffee fruits. Parasitoids were taken to the field as adults and released from one liter plastic jars directly onto the branches of coffee trees. From 1992 to 1996, ca. 156,000 individuals were released in the field usually in the morning (before 12:00 hr). Colonization sites for releases consisted of 22 locations, each approximately one-fourth of a hectare. Because few people observe insects under field conditions (P. S. Baker, personal communication), we stayed in the field for about 1-2 hours following parasitoid liberations, to observe the wasps and any possible interaction with other organisms. The organisms detected as interacting with *P. nasuta* were collected and taken to the laboratory for identification.

A list of arthropods preying on adults of *P. nasuta* is presented in Table 1. Meantime females were searching for infested fruits, they were easy prey for ants, which normally were present on coffee trees. It was possible to detect at least five species of ants preying on adults of *P. nasuta*. Also six species of spiders caught parasitoids on their webs. As spiders were collected in alcohol, it was not possible to verify their predation on *P. nasuta*. However, we would assume they do, considering they are generalist predators. Recent studies have reported that spiders, such as, *Cyclosa caroli*, *Leucauge mariana* and *L. vetusta* capture and consume individuals of the CBB and its bethylid parasitoid *Cephalonomia stephanoderis* (Henaut et al. 2001).

Because of the small size of *P. nasuta* (2mm), observations were especially difficult to carry out. We could not measure the intensity in which predators were acting, but presumably they are only important during releases, or maybe when the

TABLE 1. SOME SPECIES OF ANTS AND SPIDERS IDENTIFIED AS PREDATORS OF *P. NASUTA* UNDER FIELD CONDITIONS IN CHIAPAS, MEXICO.

Species	Family	Location	Observations
<i>Pseudomyrmex</i> sp.	Formicidae	El Encanto	There were three species. Individuals of one species foraged inside coffee fruits. Possible predator of the CBB, as well.
<i>Azteca</i> sp.	Formicidae	S. Enrique	Predator of adults only.
<i>Tapinoma</i> sp.	Formicidae	Laboratory	Ants foraged inside fruits and predated on juveniles of wasps and borers. Possibly the wasp was dead before ants entered.
<i>Uluborus</i> nr <i>campestratus</i>	Uloboridae	La Gloria	Adult parasitoids were found in the web.
<i>Cyclosa caroli</i>	Araneidae	S. Anita	Adult parasitoids were found in the web.
<i>Dolichognatha</i> sp.	Tetragnathidae	R. Izapa	Adult parasitoids were found in the web.
<i>Leucauge</i> sp.	Tetragnathidae	R. Izapa	Adult parasitoids were found in the web.
<i>Theridion</i> nr <i>nudum</i>	Theridiidae	R. Izapa	Some spiders make their web in the base of the bunch of fruits.
<i>Chrysso</i> <i>cambridgei</i>	Theridiidae	Maravillas	Adult parasitoids were found in the web.

new progeny of wasps leave the coffee fruits in searching for new hosts. Arthropods preying on *P. nasuta* were associated with old coffee trees and abundant shade. It is possible there are other species of ants and spiders preying on *P. nasuta*, as there is a great diversity and abundance of these organisms associated with coffee trees in Chiapas (Ibarra-Núñez 1990, Ibarra-Núñez & García-Ballinas 1998). Callan (1969) emphasized the jeopardy due to ants when colonizing natural enemies for classical biological control. Notwithstanding, Le Pelley (1968) stated that this sort of predation is incidental and only has a transitory effect, since there is no continuing association between predator and prey. According to the poor results in the establishment of *P. nasuta* in Mexico (Infante et al. 2001), is possible that predation is only a part of several factors that impede the establishment of *P. nasuta*. On the other hand, releasing parasitoids in the adult stage may not be favorable for this species. Because of that, it would be worth trying to release other biological stage. For instance, parasitoids could be taken from the laboratory to the field, in the pupal stage while they are still inside the coffee fruits. Coffee fruits could be placed inside a small cage hanging on a branch of coffee with a thread covered with grease to avoid ants and non-flying organisms. In this way, the wasps would emerge when favorable conditions exist. This sort of release might overcome some problems with predators and it would have the additional advantage of giving refuge and shelter to the wasps.

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SUMMARY

Prorops nasuta is an African parasitoid that has been imported into Mexico for the biological

control of the coffee berry borer, *Hypothenemus hampei*. After being released for several years, the establishment of this parasitoid was never achieved. In the present paper we report some native arthropods that were found interfering with *P. nasuta* in coffee plantations of Chiapas during the release of parasitoids. Presumably predation by ants and spiders on adults of *P. nasuta* is only a component of several factors that impede the establishment of *P. nasuta* in this country. Releasing *P. nasuta* in the pupal stage instead of adults is discussed in order to enhance the potential of this species as a biocontrol agent.

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DISTRIBUTION AND PLANT ASSOCIATION RECORDS FOR *HOMALODISCA COAGULATA* (HEMIPTERA: CICADELLIDAE) IN FLORIDA

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The glassy-winged sharpshooter, *Homalodisca coagulata* (Say) is the focus of a major classical biological control program in California. This insect presents a serious threat to several agricultural commodities and potentially native plants as well because of its ability to vector the xylem-inhabiting bacterium *Xylella fastidiosa*, the causative organism of "scorch like" diseases such as Pierce's Disease of grapes and oleander leaf scorch, a serious malady of oleanders (Purcell & Saunders 1999). *Homalodisca coagulata* is an invasive pest in California and its native range is the southeastern and northeastern regions of the USA and Mexico, respectively (Triapitsyn & Phillips 2000). *Homalodisca coagulata* probably was translocated to southern California as egg masses via the movement of ornamental plants in the late 1980's (Sorensen & Gill 1996) and without an accompanying natural enemy fauna; inordinate populations of glassy-winged sharpshooters have resulted.

During foreign exploration by MSH and SVT for *H. coagulata* and associated egg parasitoids in Florida in August 2001, the authors visited the Florida State Collection of Arthropods, Bureau of Entomology, Florida Department of Agriculture and Consumer Services in Gainesville. Following discussion with colleagues there, specimen receipt vouchers for *H. coagulata* were provided that had been sent in for identification by lay people, ornamental, horticultural, and agricultural growers from around Florida. A total of 229 receipts were catalogued for adult *H. coagulata* over the period 1958-2001 inclusive, and chits contained information on date of collection, locality, host plant, and sex of specimens. These data were used to determine possible host plant records, distribution densities, and submission frequencies for *H. coagulata* for different areas of Florida.

Homalodisca coagulata was collected from at least 72 plant species in 71 genera contained in 37 families and *Citrus* spp. were the most common plants from which adult *H. coagulata* were captured (Table 1). Of these plant association records in Table 1 it is uncertain which can support development of *H. coagulata* from egg to adulthood. Adult *H. coagulata* are vagile and known to be highly polyphagous while the relatively immobile immature stages have a narrower host range (Turner & Pollard 1959). Citrus may be over-represented in this dataset because of regular pest surveys in this economically im-

portant crop. To determine if regional differences in numbers of *H. coagulata* specimens sent in for identification existed, Florida was divided into thirds: (1) top third was north of 29° Latitude; (2) middle third was 27°-29°; and (3) the bottom third was south of 29°. Specimen receipts for each county in each section of the state were assumed to have been submitted for identification according to a poisson distribution and proportions were compared using a Log-likelihood Ratio Test (i.e., G-test). Pair-wise comparisons between regions from which specimens were received were made using χ^2 as sample sizes were large (Sokal & Rohlf 1995). The G-test was also used to determine if the frequency with which samples were submitted from each region significantly differed. Significant differences in the number of specimens received by region existed ($\chi^2 = 11.03$; $df = 2$; $P = 0.004$). Significantly more specimens were received for identification from north Florida, intermediate numbers from central Florida, and fewest specimens came from south Florida (Fig. 1). No signifi-

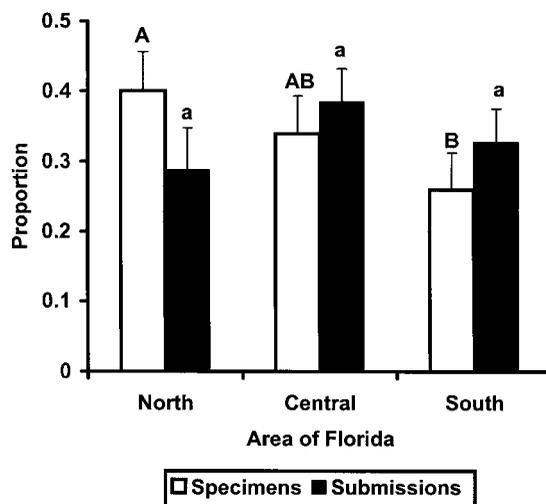


Fig. 1. Proportion ($\pm 95\%$ CI) of specimens and submissions of adult *Homalodisca coagulata* from north, central, and south Florida to the Florida State Collection of Arthropods in Gainesville for identification over the period 1958-2001. Bars with the same letters (upper case [specimen comparisons] or lower case [submission comparisons]) are not significantly different from each other at the 0.05 level of significance.

TABLE 1. HOST PLANT RECORDS AND FREQUENCY OF COLLECTION FOR ADULT *HOMALODISCA COAGULATA* IN FLORIDA.

Plant family	Plant species	No. of collected <i>H. coagulata</i> specimens
Aceraceae	<i>Acer rubrum</i>	1
Agavaceae	<i>Sansevieria</i> sp.	1
	<i>Yucca aloifolia</i>	1
Anacardiaceae	<i>Mangifera indica</i>	1
	<i>Schinus terebinthifolius</i>	1
Apocynaceae	<i>Nerium oleander</i>	2
Aquifoliaceae	<i>Ilex</i> spp.	2
Araliaceae	<i>Brassaia actinophylla</i>	1
Asteraceae	<i>Eupatorium capillifolium</i>	2
	<i>Helianthus annuus</i>	3
	<i>Solidago altissima</i>	3
Begoniaceae	<i>Begonia</i> sp.	1
Bignoniaceae	<i>Catalpa</i> sp.	1
	<i>Spathodea campanulata</i>	1
Caesalpiniaceae	<i>Ditremexa occidentalis</i>	1
Casuarinaceae	<i>Casuarina</i> spp.	5
Clusiaceae	<i>Clusia</i> sp.	1
Combretaceae	<i>Bucida buceras</i>	1
	<i>Concarpus erectus</i>	1
Convolvulaceae	<i>Ipomaea</i> spp.	3
Cycadaceae	<i>Cycas</i> sp.	1
Euphorbiaceae	<i>Aleurites fordii</i>	1
	<i>Codiaeum variegatum</i>	3
	<i>Ricinus communis</i>	1
Fabaceae	<i>Albizia julibrissin</i>	1
	<i>Bauhinia punctata</i>	1
	<i>Caesalpinia pulcherrima</i>	1
	<i>Cercis</i> sp.	1
	<i>Glycine max</i>	2
	<i>Medicago sativa</i>	1
	<i>Mimosa</i> sp.	2
	<i>Parkinsonia aculeata</i>	1
	<i>Pisum</i> sp.	1
	<i>Psopocarpus tetragonolobous</i>	1
	<i>Tetragonolobous</i> sp.	1
	<i>Wisteria</i> sp.	1
Fagaceae	<i>Quercus laevis</i>	1
	<i>Q. virginiana</i>	1
Gramineae	<i>Pennisetum purpureum</i>	1
Juglandaceae	<i>Carya illinoensis</i>	1
	<i>Juglans regia</i>	1
Lauraceae	<i>Persea americana</i>	1
Lythraceae	<i>Lagerstroemia indica</i>	4
Magnoliaceae	<i>Magnolia grandiflora</i>	1
Malvaceae	<i>Abelmoschus esculentus</i>	1
	<i>Hibiscus rosa-sinensis</i>	9
Meliaceae	<i>Swietenia mahagoni</i>	1
Moraceae	<i>Ficus benjamina</i>	3
Myrtaceae	<i>Callistemon viminalis</i>	1
	<i>Eucalyptus</i> spp.	3
	<i>Melaleuca quinquenervia</i>	2
	<i>Myrtus communis</i>	1
	<i>Psidium</i> spp.	5
Nyctaginaceae	<i>Mirabilis jalapa</i>	1
Oleaceae	<i>Olea</i> sp.	1
Polypodiaceae	<i>Hemionitis arifolia</i>	1
Proteaceae	<i>Leucadendron</i> sp.	1

TABLE 1. HOST PLANT RECORDS AND FREQUENCY OF COLLECTION FOR ADULT *HOMALODISCA COAGULATA* IN FLORIDA.

Plant family	Plant species	No. of collected <i>H. coagulata</i> specimens
Rosaceae	<i>Malus spp.</i>	2
	<i>Photinia sp.</i>	1
	<i>Prunus persica</i>	1
	<i>Pyracantha sp.</i>	1
	<i>Pyrus sp.</i>	1
	<i>Rosa sp.</i>	1
Rubiaceae	<i>Gardenia sp.</i>	1
Rutaceae	<i>Citrofortunella microcapra</i>	1
	<i>Citrofortunella mitis</i>	1
	<i>Citrus spp.</i>	68
	<i>Fortunella sp.</i>	3
Salicaceae	<i>Populus sp.</i>	1
	<i>Salix spp.</i>	2
Sapotaceae	<i>Manilkara roxburghiana</i>	1
Vitaceae	<i>Ampelopsis arborea</i>	1
	<i>Vitis spp.</i>	4

cant differences ($\chi^2 = 3.23$; $df = 2$; $P = 0.20$) in frequency of submissions from each region were observed (Fig. 1).

When taken together, these data suggest that more *H. coagulata* were caught and submitted for each identification event from North and Central Florida but the rate of submission was similar across the entire state. These data support MSH and SVT's observations that *H. coagulata* is more abundant and easier to collect in northern Florida in comparison to central and southern Florida. Possible constraints on the southern distribution of *H. coagulata* could be related to temperature, humidity, and rainfall clines or interspecific competition with other proconiine sharpshooters (e.g., *Oncometopia nigricans* [Walker] [Hemiptera: Cicadellidae: Cicadellinae: Proconiini]) that have similar habitat requirements.

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SUMMARY

Information from identification receipt vouchers prepared by the Florida State Collection of Arthropods, Bureau of Entomology, Florida

Department of Agriculture and Consumer Services in Gainesville over the period 1958-2001 for *Homalodisca coagulata* were analyzed for information on host plants and distribution in Florida. *Homalodisca coagulata* was recorded from at least 72 plant species in 37 families and greater numbers of *H. coagulata* were sent in for identification from northern Florida even though there were no significant difference in specimen submission frequencies from north, central, and south Florida.

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CYRTOBAGOUS SALVINIAE (COLEOPTERA: CURCULIONIDAE) SUCCESSFULLY OVERWINTERS IN TEXAS AND LOUISIANA

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Salvinia molesta D. S. Mitchell, an invasive floating fern, has invaded 12 states in the U.S. and is now well established in Texas and Louisiana (Jacono 1999). This plant quickly colonizes the surface of slow moving, fresh water bodies causing severe ecological and economic problems (Harley & Mitchell 1981). Successful biological control programs targeting this weed have been conducted in at least 13 countries worldwide using a small weevil, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (Julien & Griffiths 1998). This species was first collected in southeastern Brazil in 1979 (Forno & Bourne 1984) and, after extensive host range testing, was released in Australia in 1980 where it reduced a large infestation of *S. molesta* by more than 95% after 13 months (Room et al. 1981). Since then *C. salviniae* has been introduced into different countries with infestations of *S. molesta* where it successfully reduced the weed to acceptable levels in most cases (Julien & Griffiths 1998).

Although *C. salviniae* has been present in Florida since at least 1960 (Kissinger 1966) where it reproduces on common salvinia, *S. minima* Baker, attempts to transfer it to Texas and Louisiana sites infested with *S. molesta* in 1999 met with mixed results (Tipping, unpublished data). A comparison of gene sequence data between the Florida and Brazilian populations (ex Australia) revealed some differences, the biological significance of which remain under study (Goolsby et al. 2000). Consequently, further releases of the Florida population were discontinued in favor of the Brazilian population that had been imported from Australia. After additional host range testing confirmed its specificity to *S. molesta*, a general release permit was obtained for a designated area in eastern Texas and western Louisiana.

The first releases were conducted during October 10-11, 2001 when a total of 880 *C. salviniae* adults was released at four sites (220 per site). No more releases were done until March 2002 in order to determine if the insects could survive the winter. Subsequent visits to these sites plus four control sites were conducted in December 2001 and March 2002. During December 4-6, 2001, one hundred plants from the mat of *S. molesta* confined within a floating 1 m² square of PVC pipe frame, were selected without bias and hand-searched and the number of adults found was recorded and left in place. Adults were recovered from three of the four sites with up to nine adults detected at one site from the 100 plant sample. Hand-searching was used because it is non-destructive. However, de-

structive methodologies like Berlese funnels usually extract 5-10 times more adults than hand searching (Tipping, unpublished data) so the weevil density may have been up to 90 adults in the sampled area at one site.

Further sampling was halted during the winter and resumed on March 25-27, 2002. Two of the four release sites yielded adult *C. salviniae* during hand searches with three and four adults found at those sites. It is unknown if these weevils were the original adults or progeny from the October, 2001 release. Sands et al. (1986) found that adults held under constant temperatures of 23, 27, and 31°C lived an average of 163.1, 116.9, and 101.5 days, respectively. These adults were found inside the original release square but sampling within paired 0.1 m² quadrats, one along either side of a transect line, 1, 5, and 10 meters away from the release square, yielded no *C. salviniae*. It is noteworthy, therefore, that adults of *C. salviniae* were able to persist or propagate over a period of 166 days despite air temperatures that reached as low as -9.1°C and -7.1°C at Toledo Bend reservoir and Lake Texana, respectively. The former site is the most northern and the latter the most southern.

Temperature data were not available in the immediate vicinity of these research sites so the nearest recording stations were used (Table 1). In addition, unlike air temperatures, water temperatures were only available on selected dates which varied between the Toledo Bend and Lake Texana sites. In the area around Toledo Bend reservoir, there were 26 days during October 2001 through March 2002 when the minimum air temperatures were below 0°C. In contrast, only 7 days had minimal air temperatures below 0°C in the Lake Texana area. These temperatures probably were not maintained for long; in every case the daily maximum temperature was above 0°C. Whiteman & Room (1991) found that *Salvinia molesta* was killed when its buds were exposed to temperatures less than -3°C for 2-3 h. In many locations at these two sites, the plants were in coves and backwater areas sheltered from direct winds and protected by overhanging vegetation from the shoreline or adjacent floating plants like water hyacinth, *Eichhornia crassipes* (Mart.) Solms. These conditions likely buffered any negative temperature effects on the plants and insects. As expected, water temperatures lagged behind the colder air temperatures (Table 1).

This finding indicates that the Brazilian population of *C. salviniae* can survive the winter climate where *S. molesta* is extant in Texas and Louisiana.

TABLE 1. AIR AND WATER TEMPERATURES (°C) ON SELECTED DATES FOR SAMPLE SITES NEAR AND IN TOLEDO BEND RESERVOIR AND LAKE TEXANA.

Toledo Bend Reservoir				Lake Texana			
Date	Air Temperature ¹		Water Temperature ²	Date	Air Temperature ³		Water Temperature ⁴
	Max	Min	Surface		Max	Min	Surface
10/09/01	25.9	17.2	22.7	10/13/01	26.7	15.6	25.3
11/13/01	24.3	12.3	20.7	11/08/01	28.8	15.5	23.2
12/11/01	10.9	0.4	15.2	12/06/01	26.5	19.5	20.5
01/08/02	17.8	-2.3	9.5	01/15/02	18.8	3.4	12.9
02/12/02	15.2	-1.3	9.5	02/14/02	20.0	3.7	13.6
03/12/02	14.4	6.6	12.5	03/28/02	28.7	11.7	17.1

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⁴Lavaca-Navidad River Authority, site #13985.

SUMMARY

Cyrtobagous salviniae survived the winter of 2001-2002 in eastern Texas and western Louisiana after its release in October 2001 on giant salvinia, *Salvinia molesta*. Adults were recovered from Toledo Bend reservoir and Lake Texana in March 2002, up to 166 days after they were released. Although minimum air temperatures were recorded at or below 0°C on at least 26 days at Toledo Bend reservoir, water temperatures likely buffered these extreme conditions. Weather conditions at Lake Texana, a more southern site, were more benign.

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A SIMPLE DEVICE TO ASSIST WITH PITFALL TRAP SAMPLING

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Pitfall traps are commonly used to sample ants and other ground-crawling arthropods. In some cropping systems, pitfalls are the best means of collecting arthropods such as red imported fire ants (*Solenopsis invicta* Buren) that are active on the soil surface (Kharboutli & Mack 1993). One interesting application for pitfall traps may be to collect ant species as biological indicators (Peck et al. 1998).

Pitfall traps usually consist of a vial or similar container buried up to the rim in the soil. A killing agent (e.g., ethanol or propylene glycol) is placed in the container to capture crawling insects for study. Pitfall traps can yield species richness, species composition, and relative abundance of foraging ants (Bestelmeyer et al. 2000).

Some pitfall traps consist of a container installed permanently (or semi-permanently) in the soil, into which the actual trapping container is placed for easy removal. In larger studies involving hundreds of pitfall traps, however, this may not be possible. For large-scale, area-wide studies of black imported fire ant (*Solenopsis richteri* Forel) and native ants in Mississippi, we use small (2.54 cm I.D.) plastic vials. A cordless drill with a 2.86 cm diameter auger bit is used to drill a hole into the soil, and the vial is placed snugly into the hole with the top flush to the soil surface. This method presents 3 problems. First, vials can be very difficult to remove when soil dries around them. Secondly, soil is frequently brushed into the vials during removal. Finally, stooping/kneeling is necessary to remove the vials.

A device was constructed to address the problems listed above (Fig. 1). A shaft (61 cm long), handle (14 cm long), and trigger (14 cm long) were constructed of 1.27 cm diameter stainless steel tubing. The lower handle was welded to the main shaft, and the trigger was articulated on a short, upright piece of tubing welded to the lower handle. The upright tubing was split and flattened at the top to accept the trigger. A hole was drilled through the upright tubing and the trigger at the point of articulation to accommodate a hex head cap screw. A threaded rod (0.48 cm diameter) was inserted into the main shaft and through a rubber head constructed of four, 0.64 cm thick rings cut from sheets of pure gum rubber. The upper end of the threaded rod was secured to a nut welded to the end of the trigger, and a second nut was used to lock the threaded rod into position once it was

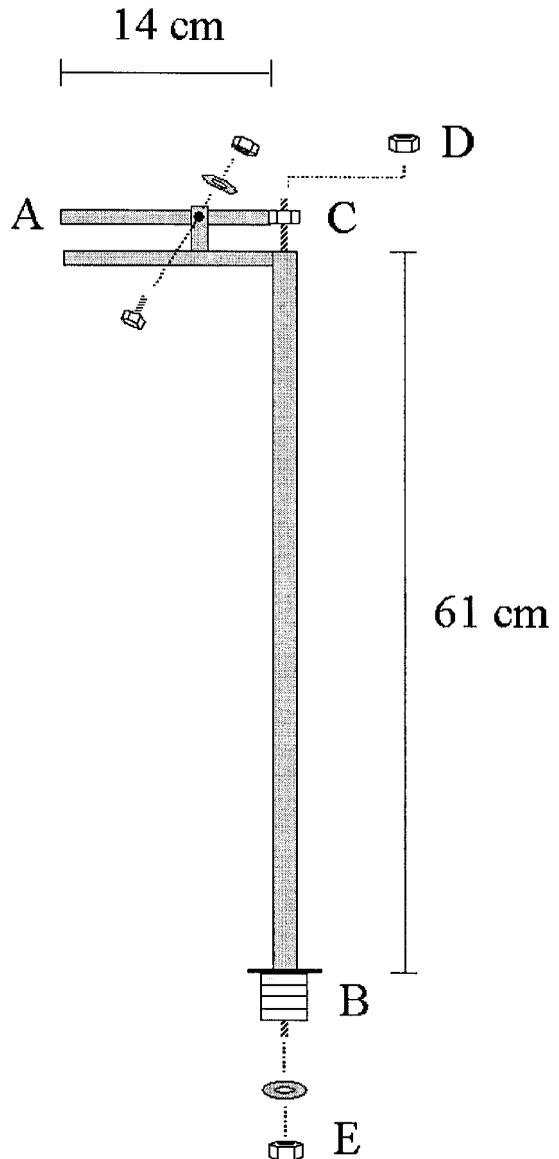


Fig. 1. Schematic of pitfall retriever. When the trigger (A) is depressed, the rubber head (B) expands to grip the inside of the pitfall trap. The trigger has a nut welded to one end (C), through which a threaded rod is placed, and locked in position with a second nut (D). An additional nut (E) secures the lower end of the threaded rod to the rubber head. Figure is not drawn to scale.

properly adjusted. A washer (1.8 cm diameter) secured with a nut to the bottom of the threaded rod and another washer (3.4 cm diameter) welded to the bottom of the shaft held the rubber head in place. The upper washer should be larger than the inner diameter of the pitfall trap to prevent the device from being inserted too far, and the lower washer must be smaller in diameter than the rubber head.

When the rubber head of the device is inserted into a pitfall trap, the trigger is squeezed, the head is compressed vertically and expands laterally, and the trap is pulled free. The rubber head expands and grips traps tightly without harming them, preventing debris from entering the trap during removal, and the 61 cm shaft reduces stooping and effort. The trigger is squeezed until the collector brings the trap up to grasp it, then the trigger is released, and the trap can be capped. Researchers interested in using this device for vials of different size could easily alter the diameter of the rubber head to fit their needs. Some adjustment may be necessary to get sufficient expansion and grip without cracking plastic vials. As configured, our device works well with 9-dram, crystal plastic vials (Bioquip, Gardena, CA, USA).

Other uses for this device might include placement and retrieval of traps in hard to reach places (e.g., below plant canopies, in crevices, etc.), provided a long drilling instrument could be used to drill an appropriate hole for placing the traps.

The length of the device could be altered to suit the needs of the researcher.

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SUMMARY

A device is described for rapid, easy removal of pitfall traps embedded in the soil. The device prevents debris from entering traps during removal and reduces stooping and effort involved in pitfall trap retrieval.

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