CATERPILLAR (LEPIDOPTERA: NOCTUIDAE) FEEDING ON PASTURE GRASSES IN CENTRAL FLORIDA

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ABSTRACT

Stargrasses (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) and bermudagrasses (*C. dactylon* (L.) Persoon) are important warm-season forage grasses, with several cultivars developed for conditions found in central and southern Florida. Major insect pests of these grasses include grass loopers (*Mocis* spp.) and fall armyworm (*Spodoptera frugiperda* (J. E. Smith)), which annually may impose economic losses for beef cattle and hay producers. Population studies conducted during a 3-year period showed that both species had similar profiles with respect to larval population seasonality but not abundance. Plot studies with 4 stargrass and 4 bermudagrass lines showed that higher grass looper populations were found in stargrasses than bermudagrasses. Laboratory studies found grass loopers and fall armyworm larvae generally developed faster with larger weights on lines of stargrass than lines of bermudagrass. The two fall armyworm host strains also can differ substantially in their larval weight, developmental time, and survivability when grown on different lines of grasses. These results indicate that the selection of pasture grasses made by growers can significantly and differentially affect the population densities of these grass defoliators.

Key Words: *Mocis latipes*, *Mocis disseverans*, *Mocis marcida*, *Spodoptera frugiperda*, larval densities

RESUMEN

Los pastos, *Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) y *C. dactylon* (L.) Persoon, son pastos importantes para las estaciones calidas, con varias variedades desarrolladas para las condiciones encontradas en el centro y sur de la Florida. Las plagas insectiles mayores de estos pastos incluyen: gusanos medidores de pastos (*Mocis* spp.) y el cogollero, *Spodoptera frugiperda* (J. E. Smith), las cuales puedan imponer perdidas económicas para los ganaderos y productores de pastos de corte. Estudios de población realizados durante un periodo de tres años mostraron que ambas especies tuvieron perfiles similares con respecto al periodo de estación en que se encontraban las poblaciones de larvas pero no de su abundancia. Estudios de parcelas con 4 líneas de *C. nlemfuensis* y 4 líneas de *C. dactylon* mostraron que se encuentran poblaciones mas altas del gusano medidor de pasto en *C. nlemfuensis* que en *C. dactylon*. Estudios del laboratorio mostraron que las larvas del medidor y cogollero generalmente se desarrollaron mas rápidamente con peso mayores en las líneas de *C. nlemfuensis* que en las líneas de *C. dactylon*. Las dos variedades de hospederos para el cogollero pueden variar substancialmente en el peso de la larva, el tiempo de desarrollo, y su capacidad para sobrevivir cuando están criados sobre diferentes líneas de pastos. Estos resultados indican que la selección de pastos para pasturas hecha por los agricultores puede afectar significativamente y diferentialmente la densidad de estos defoliadores de pastos.

Several *Cynodon* species are used in the southeastern United States as the base forage by beef and dairy producers. These grasses yield more than bahiagrasses (*Paspalum notatum* Flugge) during short daylength periods (cool season), and depending on temperature and soil fertility, can produce considerable forage during Jan and Feb (Mislevy & Martin 1997). Improved bermudagrass (*C. dactylon* (L.) Persoon) and stargrass (*C. nlemfuensis* var. *nlemfuensis*) (Mislevy 2002) cultivars have been developed and production practices optimized for beef cattle growers in central Florida for many years, and new germplasm lines are continuously screened under grazing conditions (Mislevy et al. 1991; Mislevy et al. 1996).

Mocis spp. larvae or grass loopers are pests of *Cynodon* forage grasses in the southeastern United States (Watson 1933; Ogunwolu & Habeck 1975; Koehler et al. 1977). Meagher & Mislevy (2005) found three *Mocis* species (*disseverans* (Walker), *latipes* (Guenée) (striped grass looper), and *marcida* (Guenée)) in central Florida when developing attractants for adults. *Mocis* spp. also are important pests of both pasture and cultivated grasses in Central America, South America, and the Caribbean (Gibbs 1990; Portillo

et al. 1991; Cave 1992). Determination of life history, biology, and geographic information for *Mocis* spp. has been hampered by misuse of scientific names in the literature and misidentification in the field (Dean 1985; Gregory et al. 1988).

Another lepidopteran pest of pasture grasses is the fall armyworm, *Spodoptera frugiperda* (J. E. Smith). Differential susceptibility to fall armyworm of grasses grown for hay production and grazing has been shown in various trials conducted with bermudagrass lines developed in Georgia, Louisiana, and Oklahoma (Leuck et al. 1968; Lynch et al. 1983; Lynch et al. 1986; Jamjanya et al. 1990). None of the grasses tested in the earlier studies is used in central and southern Florida, but the parents of 'Tifton 85' (an F_1 hybrid pentaploid between the bermudagrass PI 290884 (in the literature as 'Tifton 292') from South Africa and the stargrass (*C. nlemfuensis* Vanderyst) 'Tifton 68', a highly digestible but cold-susceptible hybrid released in 1983 (Burton et al. 1993)) have been compared for resistance. 'Tifton 68' was shown to be susceptible, with high larval weights and high larval survival in feeding trials (Lynch et al. 1983). The other parent, 'Tifton 292', was shown to be highly resistant to larval feeding (Leuck et al. 1968; Lynch et al. 1983) and larvae exhibited nonpreference resistance in comparative tests (Chang et al. 1985).

Research in Louisiana, Georgia, and Florida has shown that there are two host strains (corn strain and rice strain) of fall armyworm (Pashley 1986; Lu et al. 1992; Lu et al. 1994; Levy et al. 2002; Meagher & Gallo-Meagher 2003). In Florida, corn plants are invaded by both host strains, while forage and turf grasses are infested predominately by rice strain larvae (Meagher & Gallo-Meagher 2003; Nagoshi et al. 2006a; Nagoshi et al. 2006b). Pashley et al. (1987) compared feeding of 'Tifton 292' by larvae from a rice strain and a corn strain culture and found that the grass was resistant to corn strain individuals but susceptible to rice strain larvae. Further testing classified 'Tifton 292' as intermediately resistant when fed to rice strain larvae (Jamjanya & Quisenberry 1988), but other factors such as artificial diet (Quisenberry & Whitford 1988) and whether the plants were grown in the field or in the greenhouse affected larval response (Jamjanya et al. 1990; Pitman et al. 2002). Research conducted to improve 'Tifton 292' by producing a bermudagrass with both high quality and fall armyworm resistance, led to the creation of 'Tifton 85' (Burton 2001). Although there have been many published reports on agronomic and grazing attributes of 'Tifton 85', there are no reports comparing fall armyworm feeding on this grass with other forage grasses.

Field sampling and larval feeding studies with *Mocis* spp. or fall armyworm have not been conducted on the grasses grown and developed in Florida. We conducted studies to determine the population densities of *Mocis* spp. and fall armyworm supported by different grass lines in the subtropical environment of central Florida. The results of these field surveys were compared to laboratory studies examining the capacity of the different grasses to support larval development of these species. The grass lines were selected based on their popularity with growers or on field observations that certain lines were highly susceptible to feeding by caterpillars.

Materials and Methods

Field Site and Population Density

Field experiments were conducted at the University of Florida, Range Cattle Research and Education Center (RCREC), Ona (27°26'N, 81°55'W; 26 m elevation). This subtropical center contains over 1150 hectares of natural and improved grasses divided into large pastures and small plots for multi-discipline research in beef cattle and forage grass production.

Sampling of *Mocis* spp. and fall armyworm larval populations was done with sweep nets and was conducted in various bermudagrass and stargrass pastures at the RCREC during 2001, 2002, and 2003.

Grass Lines

This study was designed to compare populations of *Mocis* spp. and fall armyworm larvae on various *Cynodon* spp. *Mocis* spp. larvae can be found in large numbers but separation of larvae by species is difficult. Ogunwolu & Habeck (1979) separated *latipes/disseverans* from *marcida/texana* using the shape and length of the anal setae, but no characters were found to separate individual pairs of species. Therefore, *Mocis* larvae were not identified to species.

The grass lines used in this study are important to growers who raise beef cattle in central Florida. They include cultivars and ecotypes developed at the RCREC, cultivars developed in other locations but are popular with beef cattle growers, or lines that are being considered for use in central Florida. Grass lines (cultivars, ecotypes, and ecotypes released as cultivars (Karaca et al. 2002; Taliaferro et al. 2004)) included the bermudagrasses 'Jiggs', a common bermudagrass selection found growing along the Texas Gulf Coast (Redmon 2002), 'World Feeder', a mutant of 'Alicia' bermudagrass released by Agriculture Enterprises, Inc. in Bethany, OK (Gordon 1989), 'Tifton 85', and a locally-derived ecotype known as Bermudagrass 2000, a daylength-insensitive bermudagrass found growing at the RCREC during the cool season of 1999-2000 (PM, unpublished data). The stargrasses were 'Flo-

rona', found growing in a 'Pensacola' bahiagrass pasture in Ona in 1973 (Mislevy et al. 1989; Mislevy et al. 1993), 'Okeechobee', a local stargrass ecotype that was originally found growing with 'Callie' bermudagrass in Okeechobee Co., FL (PM, unpublished data), and two locally-derived ecotypes known as Stargrass 2000, a highly digestible coarse grass found growing in *Hemarthria altissima* (Poiret) Stapf & C. E. Hubbard, at Ona in 1999 and Ona Pasture #2 (believed to be a natural hybrid developed from a seed from 'Ona' stargrass hay fed to cattle in the middle of a bahiagrass pasture).

Grasses were planted beginning the week of 23 Jul 2001. The experiment was designed as a randomized complete block with 3 blocks and 4 replications of the 8 grass entries arranged in plots (81 m^2). Tilled ground separated plots (1 m) and blocks (10 m) from each other. Lepidopteran larvae can be located either at the ground surface or spatially within the grass canopy (Dean 1985). Therefore, larvae were sampled by either searching a 0.2787 m^2 area of grass (ground samples) or by using a sweep net (38.1 cm diameter) (sweep net samples, 30 sweeps per plot). Ground samples and sweep samples were taken in the experimental plots on 16 Oct, 30 Oct, and 1 Nov, 2001. Analysis of variance of square root (x + 0.5)-transformed data (PROC MIXED, Contrasts, Littell et al. 1996) was used to examine variation among grass plots.

Larval Feeding

This study was designed to compare larval feeding on the different grass lines grown in the field study (except Bermudagrass 2000). Striped grass looper larvae (*M. latipes*) were colonized from individuals collected, reared, and identified from the RCREC in 2002. Larvae were reared on greenhouse- and field-grown grasses in the laboratory. Neonates were placed in plastic tubs, 35 (l) × 24 (w) × 13 (h) cm, containing bermudagrass ('NuMex Sahara', Pennington Seeds, Madison, GA). The tubs were lined with paper towels (Sparkle™, Georgia-Pacific, Atlanta, GA) and the grass was placed on top of a plastic grate (holes at 1.5 cm). After 1 week a metal screen (holes at 0.7 cm) was placed on top of the grate. New grass ('Florona' stargrass, original material from the RCREC) was placed under the screen while the "old" grass was placed on top of the screen. In this way, larvae feeding on the "old" grass could migrate down to the "new" grass. The "old" grass was removed the next day and the larval rearing procedure repeated. This technique slowed the development of mold in the rearing tubs. Pupae were harvested from the grass and paper toweling, sexed, and 8 to 12 pairs of adults were placed in screen cages that were 24 × 24 × 24 cm. Paper towels were attached to 3 sides of the cage for oviposition and adults were supplied distilled water and a 2% sugar-honey solution for nourishment. Larvae and adults were reared in incubators or large rearing units at ≈23°C, 70% RH, and 14:10 photoperiod.

Fall armyworm larvae were from the same cultures described previously (Meagher et al. 2004). Larvae shown to carry the mitochondrial marker of corn strain (Tifton) were from a culture provided by Dr. James Carpenter, USDA-ARS, Tifton, GA. This culture was maintained on a pinto bean artificial diet according to the procedures of Guy et al. (1985). Larvae shown to carry the mitochondrial marker of rice strain (Ona) were from a culture of individuals collected from the RCREC in Jul 2002 (Nagoshi & Meagher 2003), and were maintained on bermudagrass and stargrass grown in Gainesville.

Grass line plants (except Bermudagrass 2000) were grown in 3.8-L pots in a greenhouse at ambient temperature, and were fertilized weekly with Miracle-Gro® 15-30-15 plant food. No pesticides were applied to the plants. New leaf growth was placed on filter paper discs (Whatman®, 90 mm) moistened with ≈1 mL deionized water in a 9-cm diameter polystyrene petri dish (Thomas Scientific, catalog #3488-B32). One neonate larva was placed on plant foliage, and the petri dishes were placed in an incubator at 23.9 ± 2°C with a 14:10 photoperiod. The filter paper in each petri dish was moistened daily with ≈ 1 mL of deionized water for the first 10 d. Larvae were supplied with fresh plant material until time of pupation. Larval weights were measured at 10 d. Development time (in d) from neonate to pupa was calculated and pupal weight was recorded at pupation.

For both *M. latipes* and fall armyworm, 15 larvae were arranged in 3 replications on different dates, and mortality on each host plant was recorded. Analysis of variance of log10-transformed data (PROC MIXED, Contrasts, Littell et al. 1996) was used to examine variation among grass lines.

Results

Population Density

Larval populations were variable both within and across years. In 2001, populations were low until early Sep, when *Mocis* spp. peaked at 90 and fall armyworm peaked at 30 larvae per 30 sweeps on 11 Sep. Larval populations of both species declined to 19.6 and 2.2, respectively, in early Nov (Fig. 1a). In 2002, the increase in larval populations of both species occurred about one month earlier, with the highest number of *Mocis* spp. larvae collected in mid-Aug and comparatively low numbers found through early Nov (Fig. 1b). Populations of fall armyworm were low with fewer than 5 larvae per 30 sweeps collected in mid-Aug.

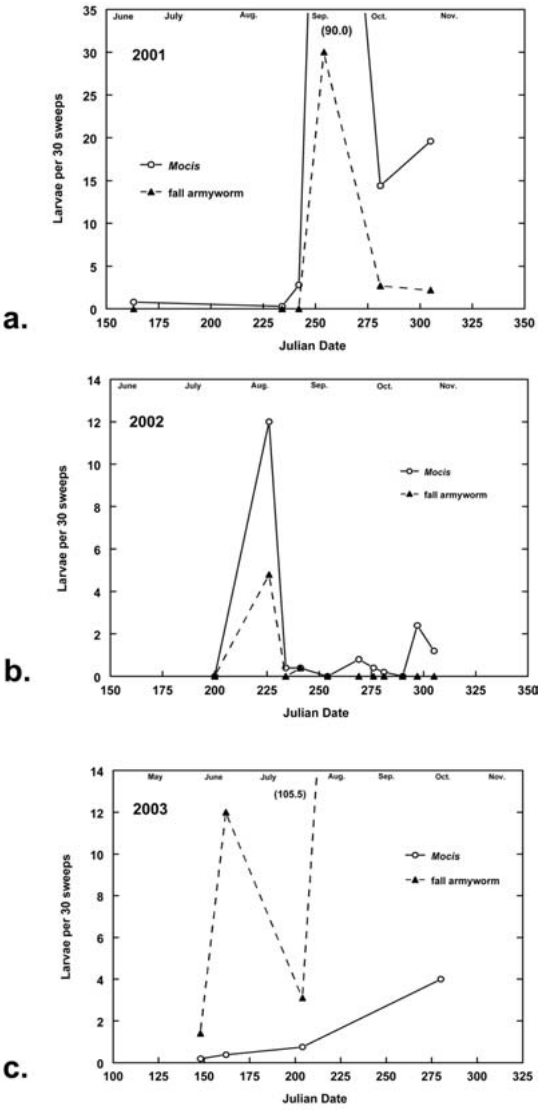

Fig. 1. Populations of *Mocis* spp. and fall armyworm larvae sampled with sweep nets from bermudagrass and stargrass pastures in 2001, 2002, and 2003, Ona, FL.

Substantially different population dynamics was observed in 2003. *Mocis* spp. populations were low throughout the sampling period, not reaching more than 4 larvae per 30 sweeps (Fig. 1c). Fall armyworm larval numbers were high in mid-Jun (12 per 30 sweeps) and very high in early Oct, with over 100 fall armyworm larvae per 30 sweeps collected.

Grass Lines

The effects of different grass germplasm on larval populations were examined by sweep net and ground sampling. Significant differences were found among grasses in number of *Mocis* spp. larvae collected by sweep net and ground samples (Fig. 2). Stargrass plots ('Florona', 'Okeechobee', Ona Pasture #2, and Stargrass 2000) contained more larvae than bermudagrass plots (Bermudagrass 2000, 'Jiggs', 'Tifton 85', and 'World Feeder') (sweep net samples (mean number of larvae per 30 sweeps ± SE), stargrass 25.5 ± 2.3 vs. bermudagrass 13.7 ± 1.7; $F = 14.3$, $df = 1, 14$, $P = 0.0020$; ground samples (mean number of larvae per m^2 ± SE), stargrass 75.6 ± 4.6 vs. bermudagrass 55.6 ± 3.8, $F = 11.3$, $df = 1, 14$, $P = 0.0046$).

Compared to *Mocis* spp., about a 10-fold lower number of fall armyworm larvae was collected and stargrass and bermudagrass plots showed similar numbers of larvae. For sweep net samples, stargrass plots contained 2.1 ± 0.55 larvae per 30 sweeps compared to bermudagrass plots which contained 2.4 ± 0.63 ($F = 0.07$, $df = 1, 14$, $P = 0.8001$). Ground sample stargrass plots had 4.3 ± 0.7 larvae per m^2 vs. bermudagrass plots which had 3.4 ± 0.6 ($F = 0.87$, $df = 1, 14$, $P = 0.3665$). Selected larvae were returned to the laboratory and all were shown to carry the mitochondrial marker for rice strain (Meagher & Gallo-Meagher 2003).

Larval Feeding

There was no difference in striped grass looper larval weights among grass lines ($F = 0.7$, $df = 6, 12$, $P = 0.6326$), however there was a trend for larvae fed stargrasses (31.9 mg ± 4.5) to be heavier than those fed bermudagrasses (21.3 ± 3.7; $F = 3.1$, $df = 1, 12$, $P = 0.1069$). Development time to pupation differed among grass lines, and larvae fed 'Florona' stargrass developed 4 days faster than those fed 'World Feeder' bermudagrass (Table 1). Overall, larvae fed stargrasses developed 2.4 days faster than those fed bermudagrasses. Pupal weights were not different among lines ($F = 1.9$, $df = 6, 12$, $P = 0.1687$), however larvae fed stargrasses (234.8 mg ± 7.6) produced larger pupae than those fed bermudagrasses (206.2 ± 7.0; $F = 6.8$, $df = 1, 12$, $P = 0.0228$). There was no difference in neonate survival among lines ($F = 1.3$, $df = 6, 12$, $P = 0.3373$) or between grass species ($F = 2.1$, $df = 1, 12$, $P = 0.1777$), as survival averaged 0.793 ± 0.03.

Feeding by fall armyworm larvae provided differences between insect cultures (host strains), between grass species, and among grass lines. Rice strain (Ona culture) larvae were heavier and developed faster than corn strain (Tifton culture) larvae (Table 2). Pupal weights and survival were similar between host strains. However, there was a significant insect culture × grass line interaction with larval weight ($F = 4.1$, $df = 6, 26$, $P = 0.0049$), therefore host strains were compared among each grass line, and grass lines were compared within both host strains. The insect culture × grass line interactions for the other variables were not sig-

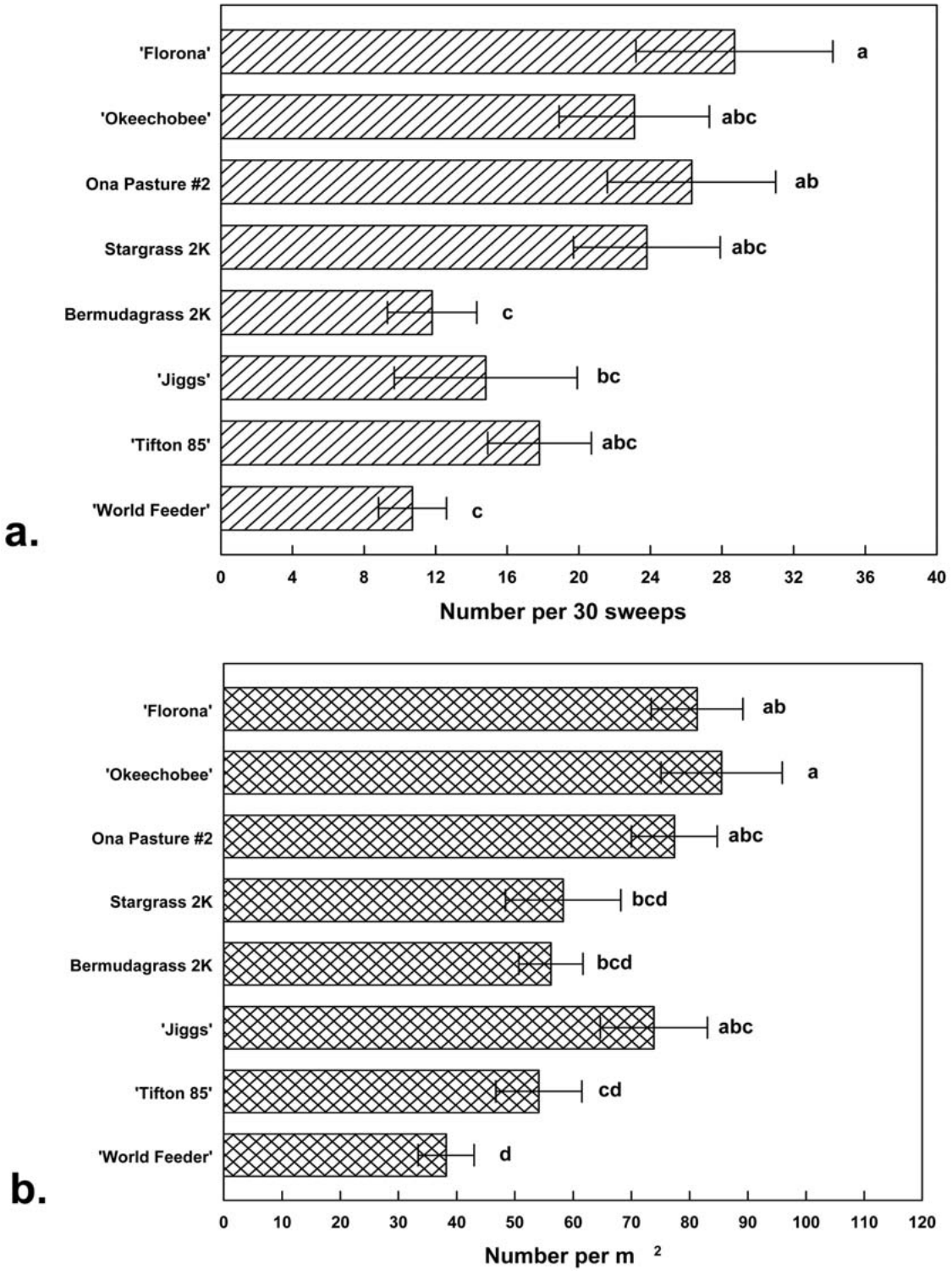

Fig. 2. Number of *Mocis* spp. larvae collected in sweep net (a) and ground samples (b) from different *Cynodon* spp. grasses, Ona, FL, 2001. Means (± SE) with the same letter are not significantly different ($P > 0.05$). The top 4 lines are stargrasses (*C. nlemfuensis* var. *nlemfuensis*); the bottom 4 lines are bermudagrasses (*C. dactylon*).

TABLE 1. DEVELOPMENT TIME TO PUPATION (D) OF STRIPED GRASS LOOPER FED 7 DIFFERENT *CYNODON* SPP. GRASSES. MEANS (± SE) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (CONTRASTS, $P > 0.05$). THE TOP 4 LINES ARE STARGRASSES; THE BOTTOM 3 LINES ARE BERMUDAGRASSES.

Grass line	Development time
'Florona'	21.6 ± 0.3 a
'Okeechobee'	22.6 ± 1.2 ab
Ona Pasture #2	23.8 ± 0.7 abc
Stargrass 2000	22.4 ± 0.1 ab
'Jiggs'	25.0 ± 1.6 bc
'Tifton 85'	24.4 ± 1.0 bc
'World Feeder'	25.6 ± 0.5 c
	$F = 2.9$; $df = 6, 12$; $P = 0.0561$
Stargrasses	22.6 ± 0.38 A
Bermudagrasses	25.0 ± 0.57 B
	$F = 13.0$; $df = 1, 12$; $P = 0.0036$

nificant (development time, $F = 2.5$, $df = 6, 26$, $P = 0.0521$; pupal weight, $F = 0.7$, $df = 6, 26$, $P = 0.6276$; survival, $F = 1.4$, $df = 6, 26$, $P = 0.2363$).

Differences in larval feeding parameters were found between stargrasses and bermudagrasses when host strains were analyzed separately. Both rice strain and corn strain larvae showed significantly enhanced growth and development when fed stargrass leaves (Table 3). Larval survival for rice strain larvae was not different between grass species, but there was a trend for corn strain larvae to have higher survival on stargrasses.

Rice strain larvae fed Stargrass 2000 and 'Florona' were heavier and developed more quickly than the other lines (Table 4). Pupal weights averaged 138.9 mg ± 3.8 and did not differ among lines ($F = 1.8$, $df = 6, 12$, $P = 0.1859$). Larval survival also did not differ among lines, averaging 0.607 ± 0.04 ($F = 2.4$, $df = 6, 12$, $P = 0.0896$). Corn strain larvae were heavier, developed faster, and had larger pupal weights when fed Stargrass 2000 leaves (Table 5). Survival was not different among lines ($F = 2.4$, $df = 6, 12$, $P = 0.0896$), averaging 0.505 ± 0.04. However, there was a trend for corn strain larvae placed on 'Tifton 85' (0.333 ± 0.067) and 'World Feeder' (0.361 ± 0.02) to have very low survival.

DISCUSSION

Surveys of bermudagrass and stargrass pastures showed similar population profiles for *Mocis* spp. and fall armyworm with respect to larval population dynamics. The peaks of larval abundance occurred at different times in each of the 3 years and were typically associated with sharp increases and sudden declines in numbers. In each case the timing of the changes in *Mocis* populations coincided with that of fall armyworm larvae, suggesting that these species were possibly responding to the same environmental factors with respect to oviposition and development on their plant hosts. However, the level of infestation between species was more variable. In the first 2 years, approximately 2-3 fold higher *Mocis* larval numbers were observed than fall armyworm. This changed dramatically in 2003 when high fall armyworm larval density coincided with low *Mocis* infestation. Little is known about what factors influence the severity of infestations in these species and why conditions suitable for high fall armyworm density in 2003 were apparently less for *Mocis*.

Several lines of bermudagrasses and stargrasses were tested for their ability to support *M. latipes* and fall armyworm populations. This was the first examination of *M. latipes* feeding of *Cynodon* spp. germplasm developed or isolated in Florida, and both bermudagrasses and stargrasses are important forage grasses for the beef cattle industry in the central and southern parts of the state. Bermudagrasses as a group were generally associated with lower densities of *Mocis* larvae than stargrasses when tested in field settings. This observation compared well with the results of laboratory feeding studies showing that *M. latipes* larvae were smaller and developed slower on bermudagrasses. *M. latipes* larvae took approximately 2.4 d longer to develop to pupation, which prolongs the period of larval exposure to natural enemies and disease.

Fall armyworm showed a similar preference for a subset of stargrasses over bermudagrasses. Larvae reared on Stargrass 2000 or 'Florona' were heavier and developed 2-4 d quicker than those reared on bermudagrasses. However, this difference was not reflected in changes in population densities in the field. It may be that localized variations between plant hosts in their ability to

TABLE 2. FEEDING PARAMETERS OF FALL ARMYWORM RICE AND CORN STRAIN LARVAE WHEN FED DIFFERENT STARGRASS AND BERMUDAGRASS LINES.

Variable	Rice strain	Corn strain	*F*-value	*df*	*P*-value
Larval weight (mg)	28.5 ± 2.9	23.9 ± 3.7	12.20	1, 26	0.0018
Development (days)	20.6 ± 0.4	23.2 ± 0.6	47.20	1, 26	<0.0001
Pupal weight (mg)	138.9 ± 3.8	140.8 ± 3.2	0.23	1, 26	0.6335
Survival (prop.)	0.607 ± 0.04	0.505 ± 0.04	3.60	1, 26	0.0706

TABLE 3. FEEDING PARAMETERS BETWEEN STARGRASS AND BERMUDAGRASS LINES WHEN FED TO FALL ARMYWORM RICE AND CORN STRAIN LARVAE.

Variable	Stargrass	Bermudagrass	*F*-value	*df*	*P*-value
Rice strain					
Larval weight (mg)	33.4 ± 4.5	22.0 ± 1.9	25.70	1, 12	0.0003
Development (d)	19.9 ± 0.5	21.7 ± 0.4	20.80	1, 12	0.0006
Pupal weight (mg)	143.8 ± 4.7	132.5 ± 5.8	4.30	1, 12	0.0594
Survival (prop.)	0.600 ± 0.064	0.615 ± 0.044	0.05	1, 12	0.8194
Corn strain					
Larval weight (mg)	32.9 ± 5.0	11.9 ± 1.9	69.10	1, 12	<0.0001
Development (d)	21.3 ± 0.5	25.9 ± 0.5	64.30	1, 12	<0.0001
Pupal weight (mg)	149.5 ± 2.4	129.2 ± 4.3	17.00	1, 12	0.0014
Survival (prop.)	0.568 ± 0.053	0.42 ± 0.069	4.60	1, 12	0.0541

support fall armyworm are substantially masked by the mobility of this species, requiring larger plot size and/or sample size to detect statistical differences between grass species.

Fall armyworm strain-specific differences were observed for larvae reared on the various grass lines. In most cases, rice strain larvae were heavier, had better survival, and developed faster than corn strain larvae. Previous studies showed that rice strain also produced larger larvae, faster development times, and higher survival even when grown on plant hosts favored by the corn strain (Meagher et al. 2004). This suggests that the observed differences reflected general characteristics of the strains rather than specific responses to the plant hosts tested.

TABLE 4. LARVAL WEIGHT (MG) AND DEVELOPMENT TIME TO PUPATION (D) OF FALL ARMYWORM RICE STRAIN FED SEVEN DIFFERENT *CYNODON* SPP. GRASSES. THE TOP 4 LINES ARE STARGRASSES AND THE BOTTOM 3 LINES ARE BERMUDAGRASSES. MEANS (± SE) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ($P > 0.05$).

Grass line	Larval weight	Development time
'Florona'	38.5 ± 4.6 b	18.9 ± 0.5 a
'Okeechobee'	22.7 ± 2.9 cd	21.5 ± 0.5 b
Ona Pasture #2	18.7 ± 4.3 d	21.1 ± 0.6 b
Stargrass 2000	53.4 ± 4.6 a	18.0 ± 0.4 a
'Jiggs'	20.8 ± 1.7 d	22.0 ± 1.0 b
'Tifton 85'	27.7 ± 3.6 c	20.7 ± 0.5 b
'World Feeder'	17.4 ± 0.7 d	22.3 ± 0.2 b
	$F = 22.7$, $df = 6, 12$, $P < 0.0001$	$F = 9.8$, $df = 6, 12$, $P = 0.0005$

TABLE 5. LARVAL WEIGHT (MG), DEVELOPMENT TIME TO PUPATION (D), AND PUPAL WEIGHT (MG) OF FALL ARMYWORM CORN STRAIN FED 7 DIFFERENT *CYNODON* SPP. GRASSES. THE TOP 4 LINES ARE STARGRASSES AND THE BOTTOM 3 LINES ARE BERMUDAGRASSES. MEANS (± SE) WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT ($P > 0.05$).

Grass line	Larval weight	Development time	Pupal Weight
'Florona'	24.7 ± 8.1 b	21.3 ± 0.2 ab	146.5 ± 5.3 a
'Okeechobee'	28.8 ± 8.5 b	22.8 ± 1.2 b	151.7 ± 3.8 a
Ona Pasture #2	29.7 ± 8.5 b	21.3 ± 0.4 ab	148.8 ± 4.4 a
Stargrass 2000	48.2 ± 13.5 a	19.7 ± 0.9 a	151.1 ± 7.3 a
'Jiggs'	13.6 ± 5.5 c	26.1 ± 1.1 c	137.5 ± 2.3 ab
'Tifton 85'	12.0 ± 2.6 c	25.7 ± 0.8 c	124.6 ± 4.9 b
'World Feeder'	10.0 ± 2.1 c	25.8 ± 0.7 c	125.4 ± 11.7 b
	$F = 13.6$, $df = 6, 12$, $P < 0.0001$	$F = 12.5$, $df = 6, 12$, $P = 0.0002$	$F = 3.4$, $df = 6, 12$, $P = 0.0346$

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REFERENCES CITED

BURTON, G. W. 2001. Tifton 85 bermudagrass—early history of its creation, selection, and evaluation. Crop. Sci. 41: 5-6.

BURTON, G. W., R. N. GATES, AND G. M. HILL. 1993. Registration of 'Tifton 85' bermudagrass. Crop. Sci. 33: 644-645.

CAVE, R. D. 1992. Inventory of parasitic organisms of the striped grass looper, *Mocis latipes* (Lepidoptera: Noctuidae), in Honduras. Florida Entomol. 75: 592-598.

CHANG, N. T., B. R. WISEMAN, R. E. LYNCH, AND D. H. HABECK. 1985. Fall armyworm (Lepidoptera: Noctuidae) orientation and preferences for selected grasses. Florida Entomol. 68: 296-303.

DEAN, T. W. 1985. Behavioral Biology of the Striped Grass Looper, *Mocis latipes* (Guenée), in North-Central Florida, Ph.D. Dissertation. University of Florida, Gainesville, FL 123 p.

GIBBS, I. H. 1990. The guinea grass moth- an occasional pest of pasture grasses in Barbados. Proc. Barbados Soc. Tech. Agric. 67-69.

GORDON, L. R. 1989. World Feeder bermuda grass. U.S. Plant Patent 7081. Date issued: 19 Dec. 1989.

GREGORY, JR., B. M., C. S. BARFIELD, AND J. B. CHAPIN. 1988. Morphological differences between adult *Anticarsia gemmatalis* and *Mocis latipes* (Lepidoptera: Noctuidae). Florida Entomol. 71: 352-359.

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

JAMJANYA, T., AND S. S. QUISENBERRY. 1988. Fall armyworm (Lepidoptera: Noctuidae) consumption and utilization of nine bermudagrasses. J. Econ. Entomol. 81: 697-704.

JAMJANYA, T., S. S. QUISENBERRY, S. S. CROUGHAN, AND R. N. STORY. 1990. Comparison of bermudagrass lines grown in different cultural conditions and the effect on screening for fall armyworm (Lepidoptera: Noctuidae) resistance. J. Econ. Entomol. 83: 585-590.

KARACA, M., S. SAHA, A. ZIPF, J. N. JENKINS, AND D. J. LANG. 2002. Genetic diversity among forage bermudagrasses (*Cynodon* spp.): evidence from chloroplast and nuclear DNA fingerprinting. Crop Sci. 42: 2118-2127.

KOEHLER, P. G., R. G. GOUGER, AND D. E. SHORT. 1977. Control of striped grass loopers and armyworms in pasture: 1976. Florida Entomol. 60: 103-104.

LEUCK, D. B., C. M. TALIAFERRO, G. W. BURTON, R. L. BURTON, AND M. C. BOWMAN. 1968. Resistance in bermudagrass to the fall armyworm. J. Econ. Entomol. 61: 1321-1322.

LEVY, H. C., A. GARCIA-MARUNIAK, AND J. E. MARUNIAK. 2002. Strain identification of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) insects and cell line: PCR-RFLP of cytochrome oxidase subunit I gene. Florida Entomol. 85: 186-190.

LITTELL, R. C., G. A. MILLIKEN, W. W. STROUP, AND R. D. WOLFINGER. 1996. SAS system for mixed models. SAS Institute, Inc., Cary, NC.

LU, Y., M. J. ADANG, D. J. ISENHOUR, AND G. D. KOCHERT. 1992. Restriction fragment length polymorphism analysis of genetic variation in North American populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Mol. Ecol. 1: 199-208.

LU, Y. J., G. D. KOCHERT, D. J. ISENHOUR, AND M. J. ADANG. 1994. Molecular characterization of a strain-specific repeated DNA sequence in the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Insect Mol. Biol. 3: 123-130.

LYNCH, R. E., W. G. MONSON, B. R. WISEMAN, AND G. W. BURTON. 1983. Bermudagrass resistance to the fall armyworm (Lepidoptera: Noctuidae). Environ. Entomol. 12: 1837-1840.

LYNCH, R. E., W. G. MONSON, B. R. WISEMAN, G. W. BURTON, AND T. P. GAINES. 1986. Relationship of forage quality to developmental parameters of the fall armyworm (Lepidoptera: Noctuidae). Environ. Entomol. 15: 889-893.

MEAGHER, R. L., AND M. GALLO-MEAGHER. 2003. Identifying host strains of fall armyworm (Lepidoptera: Noctuidae) in Florida using mitochondrial markers. Florida Entomol. 86: 450-455.

MEAGHER, R. L., AND P. MISLEVY. 2005. Trapping for *Mocis* spp. adults using different attractants. Florida Entomol. 88: 424-430.

MEAGHER, R. L., R. N. NAGOSHI, C. STUHL, AND E. R. MITCHELL. 2004. Larval development of fall armyworm (Lepidoptera: Noctuidae) on different cover crop plants. Florida Entomol. 87: 454-46.

MISLEVY, P. 2002. Stargrass. Florida Coop. Ext. Serv. SS-AGR-62. 4 p.

MISLEVY, P., AND F. G. MARTIN. 1997. Comparison of Tifton 85 and other *Cynodon* grasses for production and nutritive value under grazing. Soil Crop Sci. Soc. Florida Proc. 57: 77-82.

MISLEVY, P., G. W. BURTON, AND P. BUSEY. 1991. Bahiagrass response to grazing frequency. Soil Crop Sci. Soc. Florida Proc. 50: 58-64.

MISLEVY, P., F. G. MARTIN, G. W. BURTON, AND L. F. SANTOS. 1996. Influence of grazing frequency on production and quality of *Paspalum*, *Brachiaria* and *Setaria* grasses. Soil Crop Sci. Soc. Florida Proc. 55: 97-103.

MISLEVY, P., W. F. BROWN, L. S. DUNAVIN, D. W. HALL, R. S. KALMBACHER, A. J. OVERMAN, O. C. RUELKE, R. M. SONODA, R. L. STANLEY, JR., AND M. J. WILLIAMS. 1989. 'Florona' stargrass. Florida Agric. Exp. Stn. Circ. S-362. 13 p.

MISLEVY, P., W. F. BROWN, L. S. DUNAVIN, D. W. HALL, R. S. KALMBACHER, A. J. OVERMAN, O. C. RUELKE, R. M. SONODA, R. L. STANLEY, JR., AND M. J. WILLIAMS. 1993. Registration of 'Florona' stargrass. Crop Sci. 33: 359-360.

NAGOSHI, R. N., AND R. L. MEAGHER. 2003. *FR* tandem-repeat sequence in fall armyworm (Lepidoptera:

Noctuidae) host strains. Ann. Entomol. Soc. Am. 96: 329-335.

NAGOSHI, R. N., R. L. MEAGHER, G. NUESSLY, AND D. HALL. 2006a. Effects of fall armyworm (Lepidoptera: Noctuidae) interstrain mating in wild populations. Environ. Entomol. 35: 561-568.

NAGOSHI, R. N., R. L. MEAGHER, J. J. ADAMCZYK, JR., S. K. BRAMAN, R. L. BRANDENBURG, AND G. NUESSLY. 2006b. New restriction fragment length polymorphisms in the cytochrome oxidase I gene facilitate host strain identification of fall armyworm (Lepidoptera: Noctuidae) populations in the southeastern United States. J. Econ. Entomol. 99: 671-677.

OGUNWOLU, E. O., AND D. H. HABECK. 1975. Comparative life-histories of three *Mocis* spp. in Florida (Lepidoptera: Noctuidae). Florida Entomol. 58: 97-103.

OGUNWOLU, E. O., AND D. H. HABECK. 1979. Descriptions and keys to larvae and pupae of the grass loopers, *Mocis* spp., in Florida (Lepidoptera: Noctuidae). Florida Entomol. 62: 402-407.

PASHLEY, D. P. 1986. Host-associated genetic differentiation in fall armyworm (Lepidoptera: Noctuidae): a sibling species complex? Ann. Entomol. Soc. Am. 79: 898-904

PASHLEY, D. P., S. S. QUISENBERRY, AND T. JAMJANYA. 1987. Impact of fall armyworm (Lepidoptera: Noctuidae) host strains on the evaluation of bermuda grass resistance. J. Econ. Entomol. 80: 1127-1130.

PITMAN, W. D., S. S. CROUGHAN, AND M. J. STOUT. 2002. Field performance of bermudagrass germplasm expressing somaclonal variation selection for divergent responses to fall armyworm. Euphytica 125: 103-111.

PORTILLO, H. E., H. N. PITRE, D. H. MECKENSTOCK, AND K. L. ANDREWS. 1991. Langosta: a lepidopterous pest complex on sorghum and maize in Honduras. Florida Entomol. 74: 287-296.

QUISENBERRY, S. S., AND F. WHITFORD. 1988. Evaluation of bermudagrass resistance to fall armyworm (Lepidoptera: Noctuidae): influence of host strain and dietary conditioning. J. Econ. Entomol. 81: 1463-1468.

REDMON, L. A. 2002. Forages for Texas. Texas Coop. Ext. SCS-2002-14. 16 p.

TALIAFERRO, C. M., F. M. ROUQUETTE, JR., AND P. MISLEVY. 2004. Bermudagrasses and Stargrasses, pp. 417-475 *In* L. E. Moser, B. L. Burson and L. E. Sollenberger [eds.], Warm-season (C_4) Grasses. Agronomy Mon. #45, ASA, CSSA, SSSA, Madison, WI.

WATSON, J. R. 1933. An outbreak of *Mocis repanda* Fabr. Florida Entomol. 17:15.

MATING BEHAVIOR AND FEMALE-PRODUCED PHEROMONE USE IN TROPICAL SOD WEBWORM (LEPIDOPTERA: CRAMBIDAE)

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ABSTRACT

Research was initiated to develop a pheromone-based monitoring system for the tropical sod webworm, *Herpetogramma phaeopteralis* (Guenée). A laboratory rearing procedure was developed to produce individuals for field tests and behavioral bioassays. Virgin females placed in Unitraps in the field attracted and captured males for 8 d, while no males were captured in unbaited traps. Total male capture ranged from 1 to 24, and there was a slight decrease in capture as females aged. Laboratory mating behavior studies suggested that mating occurs later in the scotophase. Males responded to virgin females in a linear olfactometer throughout the dark period (scotophase), although there was a trend for higher male activity late in scotophase. There was no observed calling behavior, and adults exhibit simple mating behavior. Lack of both calling posture among virgin females and periodicity of male response will make it difficult to determine the optimal time periods for pheromone production, which would facilitate the collection and subsequent identification of pheromone components.

Key Words: *Herpetogramma phaeopteralis*, female calling, male attraction, turf

RESUMEN

Se inicio una investigación para desarrollar un sistema de monitoreo en base de feromonas contra el gusano tropical de césped *Herpetogramma phaeopteralis* (Guenée). Se desarrolló un procedimiento de cría en el laboratorio para producir individuos para pruebas del campo y de bioessayos de comportamiento. Hembras vírgenes puestas en "Unitraps" en el campo atrayeron y capturaron machos por 8 días, mientras que ningún macho fue capturado en trampas sin cebo. El número total de machos capturados fue de 1 a 24, y hubo una menor disminución en el número de machos capturados cuando las hembras envejecieron. Los estudios de comportamiento del apareamiento en el laboratorio sugerieron que el apareamiento ocurre más tarde en la scótofase. Los machos respondieron a las hembras vírgenes en un olfactometro linear a través del periodo oscuro (scótofase), aunque hubo una tendencia para un aumento en la actividad del macho en la parte final de la scótofase. No fue observado el comportamiento de llamar, y los adultos exhibieron un comportamiento del apareamiento sencillo. La falta del la postura de llamar entre las hembras vírgenes y la periodicidad de la respuesta de los machos hace difícil el poder determinar el periodo del tiempo optimo para la producción de feromonas, con la cual facilitaría la identificación y recolección de los componentes de la feromona.

Tropical sod webworm (TSW), *Herpetogramma phaeopteralis* (Guenée) (Lepidoptera: Crambidae), is a pest of Florida turfgrasses. Species within *Herpetogramma* attack grasses in the continental United States, Australia, Hawaii, and Guam, and *H. licarsisalis* (Walker) is the most serious turfgrass pest in Hawaii (Davis 1969; Murdoch & Tashiro 1976; Tashiro 1976). TSW also has a wide tropical distribution and occurs throughout the Caribbean (Wolcott 1936). In the U.S., TSW occurs across the Gulf Coast from Texas to Florida (Kerr 1955).

TSW feeds on a variety of grasses including bermudagrass *Cynodon dactylon* (L.) Persoon, centipedegrass *Eremochloa ophiuroides* (Munro) Hackel, seashore paspalum *Paspalum vaginitium* Swartz, St. Auginegrass *Stenotaphrum secundatum* (Walter) Kuntze, and zoysiagrass *Zoysia japonica* Steudel (Kerr 1955; Reinert 1983; Korndorfer et al. 2004). TSW populations are managed by using primarily chemical and cultural controls (Reinert 1973, 1974, 1976, 1983; Buss & Meagher 2005), although resistant cultivars have been reported (Reinert & Busey 1983).

Few studies have been conducted on the general biology of TSW (Kerr 1955; Reinert & Busey 1983). More recently, Cherry & Wilson (2005) determined that more adults of both sexes rested in

unmowed grass versus mowed grass, and that when disturbed, adults flew only a short distance. Seasonally, more TSW adults were attracted to light traps in the fall (Sep-Nov) than summer, spring, or winter in southern Florida. TSW larvae are sampled by locating damaged turf and conducting soap flushes (Buss & Meagher 2005). Adult monitoring is accomplished by either sweep nets or light traps (Cherry & Wilson 2005), both of which have limitations for turfgrass consultants or homeowners. Studies were initiated in 2004 to develop a pheromone-based monitoring system for this pest. Pheromone monitoring could allow turfgrass managers to predict future damaging populations and prescribe insecticide applications based on action levels. Field studies were conducted to confirm production of female-produced volatile chemicals that could be used for attraction of male moths. Laboratory studies were conducted to evaluate the mating behavior of TSW and periodicity of pheromone release. This study provides information on the biology of this important pest, which will be needed for identification and formulation of a synthetic pheromone lure for field use.

Materials and Methods

TSW Colonies

TSW were colonized at 2 locations from larvae collected from the Everglades Research and Education Center (EREC), Belle Glade, FL. At the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE), Gainesville, FL, adults were placed in screen cages ($24 \times 24 \times 24$ cm) and supplied with distilled water and 2% sugar-honey solution for nourishment. After 2 d, a 237-mL plastic cup containing greenhouse-grown bermudagrass ('NuMex Sahara', Pennington Seeds, Madison, GA) was placed in a cage for oviposition. Cups were left for 2 d, removed, and replaced with new cups of bermudagrass. This oviposition cycle was repeated until adults died. Cups containing grass with TSW eggs were placed on top of a plastic grate within plastic tubs, $35\ (l) \times 24\ (w) \times 13\ (h)$ cm, lined with paper towels (Sparkle™, Georgia-Pacific, Atlanta, GA). When egg hatch was complete, the grass in the cups was cut and the soil and cup were removed from the tub. Bermudagrass was added daily for 7 d. After 7 d field-grown 'Florona' stargrass (*Cynodon nlemfuensis* Vanderyst var. *nlemfuensis*) was added by placing it on a metal screen that was placed on top of the grate. Each day new grass was placed under the screen while the old grass was placed on top of the screen. In this way, larvae feeding on the old grass could move down to the new grass. The old grass was removed the next day. This technique slowed mold development in the rearing tubs. Pupae were harvested from the grass and paper toweling, and the rearing procedure repeated. Larvae and adults were reared in incubators or large rearing units at ≈26°C, 70% RH, and 14:10 (L:D) photoperiod. Rearing procedures were similar at EREC except that larvae were reared on St. Auginegrass and adults were fed 0.25 M sucrose solution.

Field Study

The attraction of male moths to females was tested with Standard Universal Moth Traps, 'Unitraps' (Great Lakes IPM, Vestaburg, MI) baited with virgin females obtained from the EREC colonies. Unitraps are comprised of a green top with a 2.0-cm hole, yellow funnel, and white collecting bucket. The trap is designed to have the attractant placed within an insert (1.8 cm W $\times$ 5.0 cm L) that is put in the hole in the top. Moths that are attracted to the lure become excited and fall through the funnel into the collecting bucket. For our experiments, the traps were modified by placing fine-meshed window screen around the insert and attaching a small vial (2 dram, 1.5 cm W $\times$ 5.5 cm L) with a cotton dental wick to the bottom of the insert. A virgin female (<24 h old) was placed in the insert and had access to the vial, which contained 0.25 M sucrose solution.

The experiment was conducted at the EREC during Jul and Aug 2004. Wild TSW adults were observed at the EREC during this time and were present for testing pheromone attraction in the field. Pairs of baited and unbaited traps (10 m apart) were attached to metal poles (1.5 m) placed in mowed grass of different species. Traps were placed in the shade to avoid exposure to the sun. Two to 5 pairs of traps were deployed per week 9 Jul through 25 Aug. Each trap pair was >10 m apart and there were 11 trap pairs. Survival of the females and number of males captured was observed daily until females died. Baited trap capture numbers were compared against number of males captured in unbaited traps (Paired t-test, SigmaStat, Systat Software, Richmond, CA). Additionally, the length of time that females continued to attract males was tested 2 to 3 d, 4 to 6 d, or 7 to 8 d later (One-way analysis of variance, ANOVA, SigmaStat).

Laboratory Mating Behavior

Pupae from CMAVE were shipped to the USDA-ARS Subtropical Horticulture Research Station, Miami, FL (SHRS) for laboratory tests. Newly emerged adults were collected each day and maintained in single-sex cages in separate holding rooms at 25°C and 70% RH until time of testing. The holding rooms had windows to provide natural lighting and were supplemented with room lights set to a photoperiod of 12:12 (L:D) h, with lights off at 2000 h and lights on at

0800 h. Adults were provided with water and a sucrose solution.

Tests were conducted to document aspects of mating behavior under laboratory conditions. All tests were conducted in rooms with windows so that natural light was available and room lights were set to same photoperiod so that bioassay rooms had the same light conditions as the adult holding rooms. Observations on adult mating behavior (e.g., female "calling" or pheromone release posture, periodicity of calling, and time of mating) were made by filming adults under an infra-red light with a low light CCTV camera (BP330, Panasonic Corp., Secaucus, NJ). Moths were placed in clear plastic 140-mL vials (8.6 cm length × 4.8 cm ID) with removable snap-top lids (Thornton Plastics, Salt Lake City, UT). A piece of aluminum window screen (4 cm diam) was attached to the bottom of the clear vial with hot glue, and a piece of filter paper (Whatman #1, 7.62 cm × 7.62 cm) was placed along the back wall of the vial to provide foot-holds for the moths. The vial was inverted with the snap-lid becoming the floor and a moistened piece of cotton wick (~2 cm long) was added to provide water to the adults. Moths were placed in the vials prior to the start of the dark period (scotophase), video output was recorded on a VCR throughout scotophase, and the tapes were reviewed for observations of mating behavior. All moths were dissected at the end of the observation period to confirm sex, mating status, and presence of mature eggs in females. Initial studies evaluated behavior of 10 sets of male and female adults that were 0-5 d old at time of testing.

Linear olfactometers (Analytical Research Systems, Inc., Gainesville, FL) were used to evaluate female behavior during time periods of male response. Individual virgin females were placed in small glass chambers (~12.7 cm × 2.2 cm ID) with a downwind screen that was attached to a glass tube (35.5 cm total length × 2.54 cm ID). A moistened piece of cotton wick (~1.5 cm long) was added to provide water to the females and to add humidity to the air. Purified air was delivered to the chamber containing the female and then into the olfactometer through connectors made from Teflon tubing. A single male was released at the downwind end of the tube prior to the start of scotophase, and there were 4 linear olfactometers used per test. Tests were conducted on 5 different nights, for a total of 20 samples. Activity of females in the small glass chambers and of males in ~5 cm of the upwind ends of the glass tubes (response window) during 0000-0800 h (ET) was recorded by video capture on a VCR. Video tapes were reviewed, entrance and exit times for males into the response windows were recorded, and the difference in entrance and exit time was used to determine number of min that males spent per individual visit. Min per visit were summed for each h to quantify sum total time per h per male. Sum total time per h per male was used as the response variable and effect of time period was analyzed by one-way ANOVA in Proc GLM (SAS Institute 2001). Tapes also were reviewed to evaluate posture and activity of the females during time periods of male response as indications of female calling behavior.

RESULTS

Field Study

Females survived in the traps for up to 8 d. Significantly more males were collected in virgin female-baited traps than in traps with no females (baited traps, mean ± SEM = 9.3 ± 2.5 males per female; unbaited, 0.0 ± 0, t = 3.76 with 10 df, P = 0.004). Total male capture per trap ranged from 1 to 24, with a total of 102 males collected. Males were collected in baited traps up to 8 d after females were placed in traps. Females aged 2-3 d attracted 5.7 ± 1.9 males, those aged 4-6 d attracted 3.6 ± 1.5 males, and those aged 7-8 d attracted 3.3 ± 1.7 males to the traps. Although there was a decline in the number of males captured as females aged, this difference was not significant (F = 0.6; df = 2, 21; P = 0.555).

Laboratory Mating Behavior

Mating occurred in only 3 of the 10 pairs videotaped. Of these, mating occurred at 0218, 0234, and 0310 h, which was 3-4 h before sunrise. Two of these 3 pairs completed mating during the videotaped time period and they remained paired for 78 and 98 min. Eight of the 10 females videotaped were sexually mature, as indicated by presence of mature eggs, and all 3 females that mated were sexually mature. There was no obvious calling posture observed among females that either did or did not eventually mate, and there were no obvious differences in behaviors of successful versus unsuccessful males. TSW used a simple courtship pattern, with behavioral steps most similar to those described for *Amyelois transitella* (Walker) and *Laetilia coccidivora* (Comstock) (Phelan & Baker 1990), although females for both of those species displayed calling behavior. TSW females that eventually mated tended to be positioned close to the bottom of the vial, remained stationary or moved a short distance away when approached by the male but then remained stationary until copulation was successful. As described for *A. transitella* and *L. coccidivora*, the male approached the female from behind, with rapid wing-fanning and walking. The male faced the same direction as the female and attempted copulation with a ventrolateral thrust. If the initial attempt was unsuccessful and the female moved away, the male would follow and make additional attempts. After a successful copulation attempt, the pair moved to a tail-to-tail

position and remained stationary for the duration of the copulation. This behavioral sequence was observed for all 3 successful matings.

Sixteen of the 20 males tested in the linear olfactometer were observed in the upwind end of the glass tube of the linear olfactometer at some time during the sample period. Total time spent in the upwind end of the glass tube over the 8-h test period ranged from 7.4 min to 368 min, with the overall average (± SEM) of 125.2 ± 27.0 min. The highest sum total number of min per h that males were observed in the upwind end of the glass tube occurred from 0600-0700 h (Fig. 1), but males were observed during all time periods and there were no significant differences among time periods ($F = 1.15$; $df = 7, 120$; $P = 0.3352$; square-root $x + 0.5$ transformed data). Females were active periodically throughout scotophase and again there were no obvious calling postures observed throughout scotophase. Females tended to be quiescent during the time periods of greatest male response, but quiescence and male response were not always concurrent.

Discussion

Sex attractants have been identified for other crambid (Crambini) sod webworms including the bluegrass webworm, *Parapediasia teterrella* (Zincken) (Clark & Haynes 1990), the cranberry girdler, *Chrysoteuchia topiaria* (Zeller) (Kamm & McDonough 1979; McDonough & Kamm 1979; Kamm & McDonough 1980), and the western lawn moth, *Tehama bonifatella* Hulst (McDonough et al. 1982). Pheromones also have been identified for several moth species in the same tribe (Spilomelini) as TSW, including *Cnaphalocrocis medinalis* Guenée (Ramachandran et al. 1990; Ganeswara Rao et al. 1995; Kawazu et al. 2000) and 3 species of *Diaphania*. One chemical, (*E*)-11-hexadecenal, was a major component in the pheromone blends of *D. indica* (Saunders) (Wakamura et al. 1998), melonworm *D. hyalinata* (L.) (Raina et al. 1986), and pickleworm *D. nitidalis* (Stoll) (Klun et al. 1986).

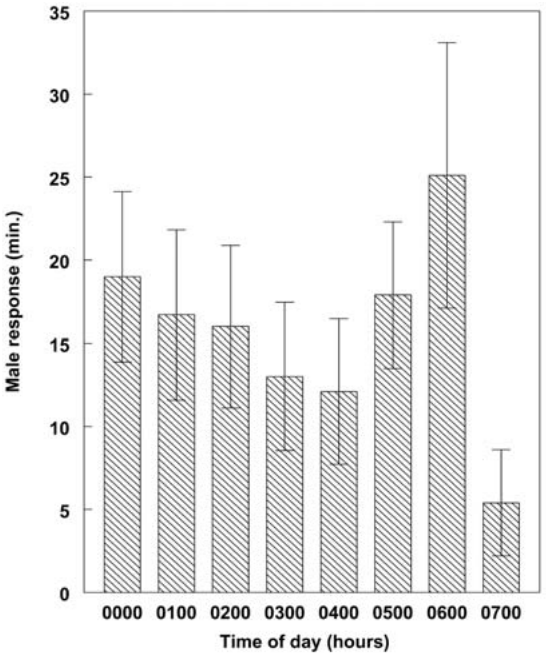

Fig. 1. Number of min (mean ± SE) that virgin males appeared in response to volatile chemicals from virgin female tropical sod webworms ($n = 16$). Number of min per h was determined from videotape recordings of male activity in linear olfactometers 4 h after the start of scotophase.

Our linear olfactometer results suggested that both males and females are active throughout scotophase, with a trend for higher male response 4 to 5 h after the onset of scotophase and at the end of scotophase. Research with other crambids/pyralids has shown variable results relating calling behavior, pheromone production, and mating. In some species, the relationship between female calling and pheromone production was weak, where pheromones appeared to be produced without apparent calling behaviors (Coffelt et al. 1978; Kawazu & Tatsuki 2002). In other species, however, females initiated calling, males responded and mating occurred within a relatively short time period either in early scotophase (Elsey 1982; Valles et al. 1992) or in late scotophase (Hight et al. 2003).

In summary, these results show that female moths release pheromone that is attractive to males and that Unitraps are a suitable trapping system for this species under field conditions. Our field and laboratory studies confirm that female tropical sod webworms use a sex pheromone for chemical communication and if available, a synthetic pheromone lure could be used for trapping males. However, preliminary tests have found that TSW females release very small amounts of pheromone (P.E.A. Teal, personal communication). The lack of calling posture among virgin females and flexibility in the periodicity in the time period of male response to volatile chemicals make it difficult to determine if there is a specific calling period for optimal chemical collection, which would facilitate pheromone chemical identification.

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References Cited

BUSS, E. A., AND R. L. MEAGHER. 2005. Nibbling and notching caterpillars in Florida turfgrass. Florida Pest Pro Jul-Aug: 14-17.

CHERRY, R., AND A. WILSON. 2005. Flight activity of tropical sod webworms (Lepidoptera: Pyralidae). Florida Entomol. 88: 101-103.

CLARK, J. D., AND K. F. HAYNES. 1990. Sex attractant for the bluegrass webworm (Lepidoptera: Pyralidae). J. Econ. Entomol 83: 856-859.

COFFELT, J. A., L. L. SOWER, AND K. W. VICK. 1978. Quantitative analysis of identified compounds in pheromone gland rinses of *Plodia interpunctella* and *Ephestia cautella* at different times of day. Environ. Entomol. 7: 502-505.

DAVIS, C. J. 1969. Notes on the grass webworm, *Herpetogramma licarsisalis* Walker (Lepidoptera: Pyraustidae), a new pest of turfgrass in Hawaii and its enemies. Proc. Hawaiian Entomol. Soc. 20: 311-316.

ELSEY, K. D. 1982. Photoperiod and temperature effects on the occurrence and periodicity of mating in pickleworm moths (Lepidoptera: Pyralidae). Florida Entomol. 65: 466-471.

GANESWARA RAO, A., D. D. R. REDDY, K. KRISHNAIAH, P. S. BEEVOR, A. CORK, AND D. R. HALL. 1995. Identification and field optimisation of the female sex pheromone of the rice leaffolder, *Cnaphalocrocis medinalis* in India. Entomol. Exp. Appl. 74: 195-200.

HEATH, R. R., A. MANUKIAN, N. D. EPSKY, J. SIVINSKI, C. O. CALKINS, AND P. J. LANDOLT. 1993. A bioassay system for collecting volatiles while simultaneously attracting tephritid fruit flies. J. Chem. Ecol. 19: 2395-2410.

HIGHT, S. D., S. BLOEM, K. A. BLOEM, AND J. E. CARPENTER. 2003. *Cactoblastis cactorum* (Lepidoptera: Pyralidae): observations of courtship and mating behaviors at two locations on the Gulf Coast of Florida. Florida Entomol. 86: 400-408.

KAMM, J. A., AND L. M. MCDONOUGH. 1979. Field tests with the sex pheromone of the cranberry girdler. Environ. Entomol. 8: 773-775.

KAMM, J. A., AND L. M. MCDONOUGH. 1980. Synergism of the sex pheromone of the cranberry girdler. Environ. Entomol. 9: 795-797.

KAWAZU, K., AND S. TATSUKI. 2002. Diel rhythms of calling behavior and temporal change in pheromone production of the rice leaffolder moth, *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae). Appl. Entomol. Zool. 37: 219-224.

KAWAZU, K., J. HASEGAWA, H. HONDA, Y. ISHIKAWA, S. WAKAMURA, H. SUGIE, H. KAMIWADA, T. KAMIMURO, Y. YOSHIYASU, AND S. TATSUKI. 2000. Geographical variation in female sex pheromones of the rice leaffolder moth, *Cnaphalocrocis medinalis*: identification of pheromone components in Japan. Entomol. Exp. Appl. 96: 103-109.

KERR, S. H. 1955. Life history of the tropical sod webworm *Pachyzancla phaeopteralis* Guenée. Florida Entomol. 38: 3-11.

KLUN, J. A., B. A. LEONHARDT, M. SCHWARZ, A. DAY, AND A. K. RAINA. 1986. Female sex pheromone of the pickleworm, *Diaphania nitidalis* (Lepidoptera: Pyralidae). J. Chem. Ecol. 12: 239-249.

KORNDORFER, A. P., R. CHERRY, AND R. NAGATA. 2004. Effect of calcium silicate on feeding and development of tropical sod webworms (Lepidoptera: Pyralidae). Florida Entomol. 87: 393-395.

MCDONOUGH, L. M., AND J. A. KAMM. 1979. Sex pheromone of the cranberry girdler, *Chrysoteuchia topiaria* (Zeller) (Lepidoptera: Pyralidae). J. Chem. Ecol. 5: 211-219.

MCDONOUGH, L. M., J. A. KAMM, D. A. GEORGE, C. L. SMITHHISLER, AND S. VOERMAN. 1982. Sex attractant for the western lawn moth, *Tehama bonifatella* Hulst. Environ. Entomol. 11: 711-714.

MURDOCH, C. L., AND H. TASHIRO. 1976. Host preference of the grass webworm, *Herpetogramma licarsisalis* to warm season turfgrasses. Environ. Entomol. 5: 1068-1070.

PHELAN, P. L., AND T. C. BAKER. 1990. Comparative study of courtship in twelve Phycitine moths (Lepidoptera: Pyralidae). J. Insect Behavior 3: 303-326.

RAINA, A. K., J. A. KLUN, M. SCHWARZ, A. DAY, B. A. LEONHARDT, AND L. W. DOUGLASS. 1986. Female sex pheromone of the melonworm, *Diaphania hyalinata* (Lepidoptera: Pyralidae), and analysis of male responses to pheromone in a flight tunnel. J. Chem. Ecol. 12: 229-237.

RAMACHANDRAN, R., P. CABALLERO, AND Z. R. KHAN. 1990. Pheromone components of rice leaffolders (LF) *Cnaphalocrocis medinalis* and *Marasmia patnalis*. Int. Rice Res. Newsl. 15: 25-26.

REINERT, J. A. 1973. Sod webworm control in Florida turfgrass. Florida Entomol. 56: 333-337.

REINERT, J. A. 1974. Tropical sod webworm and southern chinch bug control in Florida. Florida Entomol. 57: 275-279.

REINERT, J. A. 1976. Control of sod webworms (*Herpetogramma* spp. and *Crambus* spp.) on bermudagrass. J. Econ. Entomol. 69: 669-672.

REINERT, J. A. 1983. Field experiments for insecticidal control of sod webworms (Lepidoptera: Pyralidae) in Florida turfgrass. J. Econ. Entomol. 76: 150-153.

REINERT, J. A., AND P. BUSEY. 1983. Resistance of bermudagrass selections to the tropical sod webworm (Lepidoptera: Pyralidae). Environ. Entomol. 12: 1844-1845.

SAS INSTITUTE. 2001. SAS/STAT guide for personal computers, version 8.2 ed. SAS Institute, Cary, NC.

TASHIRO, H. 1976. Biology of the grass webworm, *Herpetogramma licarsisalis* (Lepidoptera: Pyraustidae) in Hawaii. Ann. Entomol. Soc. Am. 69: 797-803.

VALLES, S. M., R. R. HEATH, AND J. L. CAPINERA. 1992. Production and release of sex pheromone by *Diaphania nitidalis* (Lepidoptera: Pyralidae): periodicity, age, and density effects. Ann. Entomol. Soc. Am. 85: 731-735.

WAKAMURA, S., N. ARAKAKI, K. KINJO, AND T. YASUDA. 1998. Sex pheromone of the cotton caterpillar, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae): identification and field attraction. Appl. Entomol. Zool. 33: 429-434.

WOLCOTT, G. N. 1936. Insectae borinquenses. J. Agr. Univ. Puerto Rico. 20: 1-600.

THE BIOLOGY OF *DIATRAEA FLAVIPENNELLA* (LEPIDOPTERA: CRAMBIDAE) REARED UNDER LABORATORY CONDITIONS

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ABSTRACT

Aspects of the biology of the sugarcane pest *Diatraea flavipennella* (Box 1931) (Lepidoptera: Crambidae), locally named *broca-pequena da cana-de-açúcar*, reared and maintained under laboratory conditions and fed on an artificial diet have been investigated. The larval stage, which involved 7 instars, continued for a mean period of 34.87 d. Each instar could be characterized by the size of the cephalic capsule, which increased 1.28-fold on average between instars. The mean duration of the pupal stage was 12.75 d. The pupae exhibited sexual dimorphism in that the females were larger than the males, while the latter exhibited a genital pore that was absent in the females. In adult insects, the female/male ratio was 1:1.3. Adult females were on average 28.73 mm in size while the mean value for adult males was only 20.80 mm. Females commenced oviposition on the first d of their adult life and were able to oviposit until d 6. On average each female produced 431.05 eggs during her lifetime, although the majority of eggs were deposited during the first 2 d after emergence.

Key Words: artificial diet, sugarcane borer, biological aspects, lepidoptera, *Diatraea flavipennella*, Crambidae

RESUMEN

Aspectos da biologia da broca-pequena da cana-de-açúcar, *Diatraea flavipennella* (Lepidoptera: Crambidae), foram investigados em condições de laboratório (26 ± 1°C; 80 ± 10% U.R.; fotoperíodo de 12h) e alimentada em dieta artificial. O estágio larval apresentou um período médio de 34,87 dias e 7 instares, sendo cada estádio separado pela largura da cápsula cefálica, a qual aumentou a cada ínstar numa razão de 1,28. O estágio pupal durou um período médio de 12,75 dias. As pupas apresentaram dimorfismo sexual, onde as fêmeas foram maiores do que os machos, os quais exibiram um poro genital, ausente em fêmeas. A razão sexual entre adultos foi de 1:1,3. As fêmeas adultas apresentaram-se maiores que os machos, com envergadura, em média, de 28,73 cm e 20,80 cm para machos. A oviposição iniciou-se no primeiro dia de vida da fêmea e estendeu-se até o sexto dia, com uma média geral de 431,05 ovos/fêmea, apresentando uma maior produção de ovos nos dois primeiros dias de vida.

Translation provided by the authors.

Within the world economy, sugarcane (*Saccharum officinarum*) constitutes one of the most important crops in terms of annual production and as a major source of employment. Sugarcane biomass is the raw material for the production of alcohol (for beverages and fuel) and animal feed, as well as for sugar and various derived products. However, the production of sugarcane is not straightforward by virtue of the considerable problems caused by numerous pests that can devastate the crop and diminish the yield. Insects of the genus *Diatraea* (Lepidoptera: Crambidae) cause the most damage to sugarcane crops resulting in significant losses of revenue.

Commercially available pesticides are, unfortunately, not efficient for the control of *Diatraea* spp. on sugarcane for a variety of reasons mainly associated with the continuous presence of the host plant in the field throughout the whole year, the simultaneous occurrence of mature and immature forms of the insect, and the feeding habits of the insect. An alternative strategy is that of integrated pest management (IPM), which involves biological control of the insect together with a range of tactics including manual collection of the larvae, introduction of resistant varieties of sugarcane, and the use of pheromone baits. So far, IPM has been the most efficient method of controlling *Diatraea* spp. infestation.

In a number of regions of Brazil, *Diatraea flavipennella* (Box, 1931), popularly known as *broca pequena da cana-de-açúcar*, is considered to be

the main sugarcane pest. This insect can not only kill a plant directly by damaging the apical buds, but it can also cause indirect damage through infiltration of the larvae into the culms, leading to the ingress of phytopathogenic organisms into the plant (Mendonça 1996). The duration of complete metamorphosis of *D. flavipennella* is very irregular and depends on numerous factors such as the climate and the host plant (Guagliumi 1972/73).

Studies concerning the morphology, physiology, and biology of insect pests are very important since they provide valuable insights into aspects of pest management including damage potential, population dynamics and fluctuation, growth rate, and spatial distribution. Such knowledge permits the establishment of appropriate control measures. However, most studies have focused on overall understanding of the genus *Diatraea*. The objective of the present investigation was, therefore, to examine the specific biology of *D. flavipennella* through determination of defined parameters including the number of ovipositions per female, number of eggs per oviposition, viability and incubation time of eggs, development of larvae and pupae, male/female ratio, and longevity of adults.

Materials and Methods

Initiation and Maintenance of the Insect Population

Larvae of *D. flavipennella* were obtained from infested sugarcane plants located in commercial plantations in the State of Alagoas, Brazil, and transported to the Laboratório de Química Entomológica at the Universidade Federal de Alagoas. Eggs, pupae, and adults were maintained in the laboratory at 22 ± 1°C, 70 ± 10% relative humidity and 12 h photoperiod, while larvae were maintained at 26 ± 1°C, 80 ± 10% relative humidity and 12 h photoperiod. Larvae received an artificial diet developed by Hensley & Hammond (1968) and modified in collaboration with the Laboratório de Assistência Fitossanitária e Controle Biológico (FITOSSAN Maceió—AL, Brazil), according to the following description: ascorbic acid (7.0 g); agar-carrajeenate (26.0 g: 12.0 g), vitamin solution (60 mL), sucrose (162 g), and sugarcane culms in powder (40 g). Adult insects were fed with 10% sucrose solution. All of the described experiments were conducted with insects that had been reared and maintained under laboratory conditions.

Incubation of Eggs, Emergence of Larvae, Pupae and Adults, and Viability of the Immature Forms

The number of eggs in each of 10 newly deposited egg masses was determined with use of a Wild Leica model M3B stereomicroscope. Each egg mass was placed in a separate glass tube (8.5 length × 2.5 cm diam) containing artificial diet and observed daily until larvae emerged. Newly emerged larvae (n = 110; 0 to 24 h old) were placed individually into acrylic dishes (1.5 cm depth × 6.0 cm diameter) containing artificial diet and observed daily. The following aspects relating to the development of larvae were recorded: occurrence of pupation, presence of a cephalic capsule, the size of the cephalic capsule (measured with a stereomicroscope containing an ocular micrometer), and the number of dead larvae. Pupae (n = 140) originating from the egg masses mentioned above, were grouped by sex, measured with a calliper and maintained in acrylic dishes (1.5 cm depth × 6.0 cm diameter) until emergence of adult insects. The following aspects relating to the development of pupae were recorded daily: occurrence of metamorphosis, the number of males and females that emerged, and the number of dead pupae.

Longevity and Reproduction of Adult Insects

Twenty four newly emerged adults originating from the egg masses mentioned above were placed in pairs (1 male with 1 female) in glass cages (15 cm × 30 cm × 20 cm). Each group was observed daily and the numbers of dead males and females were recorded. Measurements of adult size were performed at this stage.

Measurements of Adult Size

Twenty two pairs (1 male with 1 female) of newly emerged adults were placed in PVC tubes (10 cm length × 10 cm diameter) that had been lined with greaseproof paper. The paper lining was removed each day and the number of eggs present was determined.

Statistical Analysis

All experiments were conducted in a randomized design. The results concerning the sizes of male and female pupae and the longevity of male and female adults were submitted to analysis of variance, and differences between mean values of each sex were determined by Tukey's test at the 5% probability level.

Results

Number of Eggs per Oviposition, Incubation Period, and Viability of Eggs

Females of *D. flavipennella* deposited egg masses containing between 3 and 58 (mean 33.2 ± 2.53 SEM) elliptical-shaped, milky-white eggs per oviposition. The eggs gradually became dark yellow in color as the embryos matured, and eventually turned black at the stage when the larvae

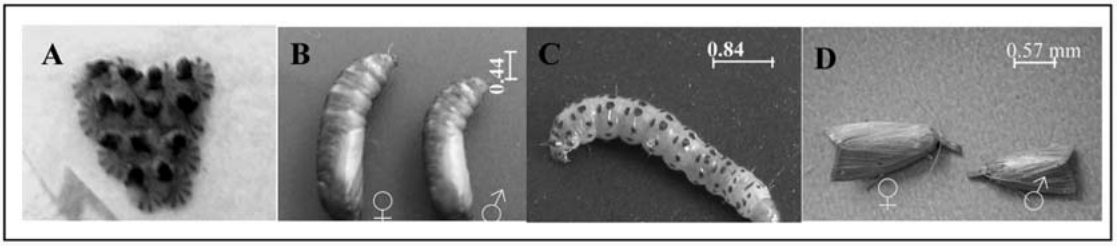

Fig. 1. Stages of the life cycle of *Diatraea flavipennella* reared and maintained under laboratory conditions: A—an egg mass (160-fold increased); B—pupae; C—a larva; and D—male and female adults.

emerged (Fig. 1A). The viability of the eggs per mass varied between 54 and 100% (mean 87.8%), and the average incubation period was 8.35 d (± 0.17) (Table 1).

Development and Viability of Larvae and Pupae

The larvae of *D. flavipennella* were yellowish in color with brown spots that did not appear to form any uniform pattern along the dorsal surface of the insect (Fig. 1B). The cephalic capsule was yellow or brownish color. The mean duration of the larval stage was 34.87 d (± 0.41) and the average viability of larvae was 75.46% (Table 1). Assuming that metamorphosis from one instar to another is indicated by the release of the cephalic capsule, the total number of instars was determined to be 7 (Fig. 2). The mean durations of the instars (Table 2) varied between 6.20 ± 0.37 d (1st instar) and 2.89 ± 0.65 d (7th instar), while the average sizes of the cephalic capsules ranged between 0.32 ± 0.01 mm (1st instar) and 1.50 ± 0.04 mm (7th instar). The mean size of the last instars was 26.0 ± 0.4 mm although some were as large as 32 mm.

The average duration of the pupal stage (Fig. 1C) was 12.75 d ± 0.42 and the mean viability was 77.63% (Table 1). Male and female pupae had 8 tergites and could be identified from the difference in the external genitalia because males had a distinct pore that was absent in females. The size of the pupae varied between 12 and 21 mm, with a mean value of 16.13 ± 0.17 mm; however, female pupae were larger (mean 17.90 ± 0.22 mm) than males (mean 14.77 ± 0.11 mm), and the difference was significant ($P < 0.05$).

Longevity of Adult Insects and Number of Eggs Deposited per Female

The female/male ratio in adult insects was 1:1.3. Adults were milky white in color and varied in size from 18-33 mm, with average dimensions of 28.73 (± 0.25) mm for females and 20.80 (± 0.86) mm for males. The wings were striated and the central part of the frontal wings bore a black spot (Fig. 1D). The average life span of adult insects was 9.17 ± 0.69 d. The lifespan of male insects was not different statistically ($P > 0.05$) from female insects. Females began to oviposit on d 1 after emergence and continued until the d 6. The maximum production of eggs occurred during the first 2 d (Fig. 3). The lifetime-number of eggs laid by the females varied between 96 and 585, with an average of 431.05 ± 30.28.

Discussion

Diatraea flavipennella was able to complete its life cycle successfully within a population reared and maintained under experimental conditions. The number of eggs laid by adult females of this

Table 1. The duration and viabilities of eggs, larvae, and pupae, and life span of adults of *Diatraea flavipennella* reared on an artificial diet regime.

Phase	Number of specimens examined (*n*)	Duration of stage (d)	Viability (%)
Eggs	110 (masses)	8.35 ± 0.17	87.80
Larvae	110	34.87 ± 0.41	75.46
Pupae	140	12.75 ± 0.42	77.63
Adults	24	9.17 ± 0.69	—

Mean values ± SEM are shown.

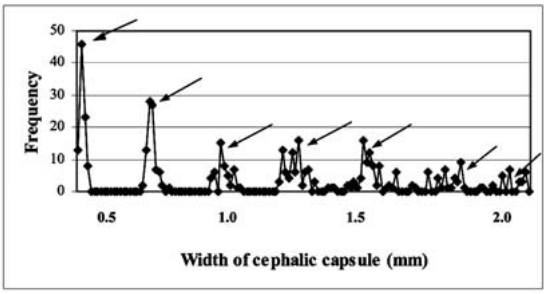

Fig. 2. Frequency distribution of the sizes of cephalic capsules during the larval stage of *Diatraea flavipennella*. The 7 instars were characterized by the most frequently occurring sizes of cephalic capsules (arrowed).

TABLE 2. DURATION OF EACH INSTAR AND SIZE OF THE CEPHALIC CAPSULE DURING THE DEVELOPMENT OF LARVAE OF *DIATRAEA FLAVIPENNELLA*.

Instar	Duration of instar (d)	Size of cephalic capsule (mm)
1st instar	6.20 ± 0.37	0.32 ± 0.01
2nd instar	5.80 ± 0.94	0.46 ± 0.01
3rd instar	4.93 ± 0.40	0.61 ± 0 02
4th instar	5.56 ± 1.04	0.91 ± 0.03
5th instar	4.95 ± 0.45	1.22 ± 0.03
6th instar	4.54 ± 0.72	1.44 ± 0.04
7th instar	2.89 ± 0.65	1.50 ± 0.04

Mean values ± SEM are shown.

species was similar to that found for *D. saccharalis* (Holloway et al. 1928). The 7 instars detected during the larval stage of *D. flavipennella* were within the range expected for the order Lepidoptera, which is normally 5 to 6 but which can vary between 3 and 11 owing to intrinsic and extrinsic factors for each species (Parra & Haddad 1989). Variations in the number of instars have been previously reported for a number of species including *D. saccharalis*, *Lacanobia oleracea*, *Delterollyta majuscule*, and *Copitarsia incommoda* (Guagliumi 1972/73; Corbitt et al. 1996; Acatitla-Trejo et al. 2004; Nava et al. 2004). The size of the cephalic capsule varied with each instar and increased between instars by a mean ratio of 1.28 in agreement with Dyar (1980), who reported that the size ratio between instars can vary between 1.1 and 1.9. The size of the cephalic capsule can thus be used as a precise indication of each instar.

The duration of the pupal stage in *D. flavipennella* was longer than that previously reported for *D. saccharalis* (Holloway et al. 1928; Guagliumi 1972/73). The pupae of *D. flavipennella* exhibited sexual dimorphism that was characterized mainly by differential size in which the females were significantly larger than the males. This feature is typical of insects of the order Lepidoptera (Slansky & Scriber 1985), and has been observed in other species of the same genus, i.e., *D. saccharalis* (Holloway et al. 1928) and *D. grandiosella* (Chippendale & Sorenson 1997).

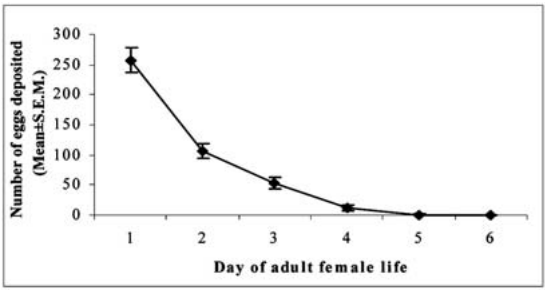

Fig. 3. Mean (± SEM) numbers of eggs deposited per d by individual females of *D. flavipennella* during their adult life span.

Adult females of *D. flavipennella* were able to oviposit for 6 d, although most eggs were deposited during the first 2 d of adult life. In contrast, oviposition in *D. saccharalis* is reported to last for only 4 d (Holloway et al. 1928).

The viabilities of larvae, pupae, and adults of *D. flavipennella* were found to be satisfactory, providing this species with a high reproductive potential and permitting the facile maintenance of an insect population both under laboratory and natural conditions. These findings are very similar to those previously reported for *D. saccharallis* (Filho & Lima 2001). However, for *D. flavipennella*, the periods necessary for the development of eggs, larvae, and pupae subjected to an artificial diet were different from those reported for insects fed on natural diet (Guagliumi 1972/73).

The present results contribute to the understanding of the biology of *D. flavipennella* and will be of value in the context of further studies concerning the reproductive behavior, survival rate, feeding habits, and pheromone production in this species, as well as in the establishment of biological control programs for this detrimental pest.

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REFERENCES

ACATITLA-TREJO, C., N. BAUTISTA-MARTINEZ, J. VERA-GRAZIANO, J. ROMERO-NÁPOLES, AND H. G. CALYECAC-CORTERO. 2004. Ciclo biológico y tasas de supervivencia y reproducción de *Copitarsia incommoda* Walker (Lepidoptera: Noctuidae) en cinco dietas artificiales. Agrociencia 38: 355-363.

CHIPPENDALE, G. M., AND C. E. SORENSON. 1997. Biology and management of the Southwestern corn borer *In* E. B. Radcliffe and W. D. Hutchison [eds.], Radcliffe's IPM World Textbook. University of Minnesota, St. Paul. http://ipmworld.umn.edu/chapters/chippen.htm.

CORBITT, T. S., G. BRYNING, S. OLIEFF, AND J. P. EDWARDS. 1996. Reproductive, developmental and nutritional biology of the tomato moth, *Lacanobia oleracea* (Lepidoptera: Noctuidae) reared on artificial diet. Bull. Entomol. Res. 86: 647-657.

DYAR, H. G. 1890 The number of molts of lepidopterous larvae. Psyche 5: 420-433.

FILHO, M. L., AND J. O. G. LIMA. 2001. Massas de ovos de *Diatraea saccharalis* (Fabr.) (Lepidoptera: Pyralidae) em cana-de-açúcar: Número de ovos e porcent-

agem de parasitismo por *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) em condições naturais. Neotropical Entomol. 30: 483-488.

GUAGLIUMI, P. 1972/73. Pragas da cana-de-açúcar (Nordeste do Brasil). Rio de Janeiro, Instituto do açúcar e do álcool. Coleção canavieira Rio de Janeiro Brazil 10. 622 pp.

HENSLEY, S. D., AND A. M. HAMMOND. 1968. Laboratory techniques for rearing the sugarcane borer on an artificial diet. J. Econ. Entomol. 6: 1742-1743.

HOLLOWAY, T. E., W. E. HALEY, U. C. LOFTIN, AND C. HEINRICH. 1928. The Sugar-cane Borer in the United States, USDA Technical Bulletin No. 41.

MENDONÇA, A. F. 1996. Pragas da cana-de-açúcar. Maceió, Insetos & Cia.

NAVA, D. E., A. D. NEVES, G. I. DIEZ-RODRIGUEZ, J. C. GONÇALVES, AND J. R. P. PARRA. 2004. Biologia e tabela de vida de fertilidade de *Deuterollyta majuscula* (Lep.: Pyralidae) em abacateiro (*Persea Americana* Mill.). Revista Brasileira de Fruticultura 26: 234-236.

PARRA, J. R. P., AND M. L. HADDAD. 1989. Determinação do número de ínstares de insetos. FEALQ Piracicaba, Brazil.

SLANSKY, F., AND J. M. SCRIBER. 1982. Food consumption and utilization—Part 4, pp. 87-163 *In* G. A. Kerkut and L. I. Gilbert [eds.], Comprehensive Insect Physiology, Biochemistry and Pharmacology. Pergamon Press, Oxford, London.

RESIDENTIAL COMPOSTING OF INFESTED FRUIT: A POTENTIAL PATHWAY FOR SPREAD OF *ANASTREPHA* FRUIT FLIES (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Composting plant waste is a beneficial practice commonly used by American gardeners, but disposal of infested fruit directly into the environment creates a potential pathway for introduction of insect pests. This study estimates the likelihood of adult emergence for exotic fruit flies (Tephritidae) from residential composting in south Florida. Ripe grapefruits, *Citrus* × *paradisi* Macfad., were infested with the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Half of the infested fruit was placed onto outdoor compost piles and half was maintained under controlled laboratory conditions. Adult fly emergence was recorded daily for 30 d from both the compost piles and control bins. Compost temperature, air temperature, relative humidity, and precipitation were monitored, and the study was repeated 4 times under different seasonal conditions. Despite high mortality of flies from the composted fruit relative to control fruit, the overall risk of a potentially mated female emerging from composted fruit was calculated to be ~10%. Of the environmental factors evaluated, compost temperature was found to have a significant effect on adult emergence. Mortality approached 100% in piles with maximum compost temperatures ≥48°C. This report provides experimental data in support of quantitative risk analysis for a tephritid-compost pathway.

Key Words: Caribbean fruit fly, risk assessment, pathway analysis, quarantine pest

RESUMEN

Amontonar la desperdición de las plantas para convertirla en abono es una práctica beneficiosa y común usada por los jardineros americanos, pero disponer de fruta infestada directamente en el ambiente crea un pasaje potencial para la introducción de insectos pestilentes. Éste estudio estima la probabilidad de la aparición de las moscas de fruta exóticas (Tephritidae) del abonamiento residencial en el sur de Florida. Toronjas maduras, *Citrus* × *paradisi* Macfad., fueron infestadas con la mosca de fruta del Caribe, *Anastrepha suspensa* (Loew). Mitad de la fruta fué desechada en los montones de abono afuera y la otra mitad fué mantenida en condiciones controladas en el laboratorio. La aparición de las moscas adultas fué registrada diariamente por 30 días en los montones y en los recipientes de control. La temperatura dentro del abono, la temperatura del aire, la humidad relativa, y la precipitación fueron vigiladas, y el estudio fué repetido cuatro veces en diferentes condiciones en várias épocas del año. Aunque la mortalidad de las moscas en las frutas abonadas fué muy alta en comparación a la mortalidad de las moscas en las frutas de los controles, el riesgo de entrada de una hembra pareada de la fruta abonada fué calculado a ~10%. La temperatura del abono fué el factor con un efecto significante asociado con la aparición de las moscas adultas. La mortalidad aproximó 100% en montones con temperatura máxima ≥48°C. Éste reporte provee data experimental para soportar un análisis cuantitativo del riesgo de un pasaje de introducción de moscas tefrítidas por medio de abonamiento.

Translation provided by the authors.

In pest risk analysis, a pathway is any means that allows the entry or spread of a pest species. Pathway risk analysis entails identification of viable pathways, assignment of probabilities to the sequence of events involved (e.g., entry, reproduction, establishment, etc.), and assessment of the consequences of pest introduction (Hennessey 2004). Invasive fruit flies, family Tephritidae, constitute a serious threat to U.S. agriculture, as evidenced by a congressional appropriation of $57 million to fruit fly risk management programs in 2005 (USDA-APHIS 2006a). This threat is pronounced in Florida due to a favorable climate, availability of hosts, and the large volume of foreign produce entering the state's ports. From Dec 2003 to Sep 2006, there were over 1200 interceptions of live tephritids at the Miami International Airport, primarily from larval-infested fruit concealed within baggage

(2048 larvae, 40 puparia, 15 adults; USDA-APHIS 2006b). The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most destructive pests worldwide. Several outbreaks of *C. capitata* have occurred in Florida, with the most recent invasion in 1997-1998 (Silva et al. 2003). Of the 198 recognized species of *Anastrepha* fruit flies (USDA-ARS 2007), only 1 species of economic importance is established in Florida, the Caribbean fruit fly, *A. suspensa* (Loew). Restricted to the Bahamas and Greater Antilles until the 1960s (reviewed in Weems et al. 2001), *A. suspensa* is now common in the southern half of the peninsula where it impacts production of citrus, guava (*Psidium guajava* L.), and other subtropical fruits (Greany & Riherd 1993). Several other *Anastrepha* species pose a threat to Florida, including *A. ludens* (Loew), *A. obliqua* (Macquart), *A. fraterculus* (Wiedemann), *A. striata* (Schiner), and *A. grandis* (Macquart). *Anastrepha ludens* represents a special concern because it has an affinity for grapefruit (*Citrus* × *paradisi* Macfad.) and Florida is one of the world's leading producers (Weems et al. 2004).

Anastrepha females have well-developed ovipositors, inserting their eggs beneath the skin of host fruits, where larvae feed and develop within the flesh (White & Elson-Harris 1992). Consequently, larval infestations are difficult to detect through visual inspections of intact fruit. At ports of entry, quarantine inspectors check incoming shipments by cutting open a small sample of fruit (typically no more than 2 percent) and searching for eggs or larvae (USDA-APHIS 2006c). The efficacy of this procedure has been evaluated in laboratory tests, and Gould (1995), using trained agricultural inspectors, concluded that only about 35% of grapefruits infested with *A. suspensa* could be detected through manual dissection. A comparison of 6 different inspectors revealed considerable variability, with the percentage of larvae detected ranging from 8% to 49% (Gould 1995). Thus, it is highly likely that some infested fruit may evade detection and, if not subjected to appropriate quarantine treatments, contain viable eggs or larvae when distributed to consumers. Subsequent to entry as immature stages within host fruit, pest *Anastrepha* may spread if that fruit is discarded directly into the environment.

Two risk assessment studies have estimated the amount of infested fruit discarded by consumers into the environment. Wearing et al. (2001), reviewing potential spread of codling moth (*Cydia pomonella* L.) via imported cherries, determined that up to 5% was discarded in suburban New Zealand. For urban Japan, Roberts et al. (1998) calculated 0.5% disposal of apples infected with *Erwinia amylovora* (Burr.), the pathogen of fire blight. Although no data are available for the amount of fruit infested with fruit flies that is discarded by Americans, the USDA-APHIS (2004) has estimated it to be 5% for avocados imported from Mexico, choosing the higher proportion from the 2 former studies so as not to underestimate the risk.

With today's emphasis on environmentally-friendly practices there is increased interest in organic gardening, including composting of plant waste. A quick internet search will yield much information on residential composting, disseminated to the public by federal, state, and local agencies (e.g., EPA 2006, USDA-NRCS 2004, Sarasota County 2006, UF-IFAS 2004). But unlike commercial organic recycling facilities which are regulated in Florida, composting in backyard, micro-scale, and farm sites is exempt from state regulation, Florida Administrative Code 62-709.300(10). As a result, compost practices vary widely among consumers, creating a potential means for spread of pests into susceptible areas. This study simulated disposal of *Anastrepha*-infested grapefruit on backyard compost piles in south Florida to estimate the likelihood of emergence of mated females. The results provide experimental data to facilitate quantitative risk analysis for a tephritid-compost pathway, information currently lacking in the scientific literature.

Materials and Methods

Infestation

Anastrepha suspensa were obtained from a laboratory colony maintained at the USDA-ARS, Subtropical Horticulture Research Station, Miami, FL. Insects were reared at 25°C (±2), 70% RH, and a photoperiod of 12:12 h (L:D), by methods previously described (Kendra et al. 2006). Approximately 3500 sexually mature (10-12 d old), mated females were placed in each of 2 infestation cages (94 × 51 × 51 cm) constructed from PVC frames covered with mesh pollination bags (Delstar Technologies, Middletown, DE). Each cage contained 60 ripe, Florida-grown grapefruit (*Citrus* × *paradisi* Macfad., cv White Marsh), arranged in a single layer, and oviposition was allowed for 7 d under the same environmental conditions used for rearing. Infested fruit (120 total) was then removed from the cages and randomly divided into 3 groups: Compost fruit (50), Control fruit (50), and Inspection fruit (20). All grapefruits were held in the laboratory while the Inspection fruits were cut open at 2-3 d intervals (5 fruits per sample) to monitor progress of larval development and to approximate the level of infestation. When the majority of the larvae had reached the third (final) instar, typically 9 d after oviposition, field tests were initiated.

Field Tests

Ten replicate compost piles (~1.5 m^3 each) were constructed for each field test. Piles were contained within square wooden frames (1.2 × 1.2 m), spaced 5 m apart, located under partial shade.

Compost material consisted of a 1:1 mixture of wood chips and fresh grass clippings, applied in alternating layers, according to the guidelines provided by the USDA Natural Resources Conservation Service (USDA-NRCS 2004). Piles were turned weekly to promote active decomposition, then left undisturbed once grapefruit had been added. Compost piles received no manual watering during the study, only natural rainfall.

For each of the 10 compost replicates, 5 infested fruit were placed on top of the pile, nestled 4-5 cm deep, and left in place for 30 d. For the first 5 d, the piles were left exposed to allow access of potential predators and competitors. Then each pile was covered with a pyramidal screen cage (modified from Raney & Eikenbary 1969) fitted on top with the upper assembly from a boll weevil trap (Great Lakes IPM, Vestaburg, MI). The cages were sealed with hook-and-loop tape (Velcro Industries, Manchester, NH), and the screen at the base of each cage was pulled tightly over the wooden frame and secured with bricks and soil, creating a closed system for the remainder of the field test. Adult fly emergence (number and sex) was recorded daily for the next 25 d. Temperature of compost beneath the fruit (15 cm depth) was monitored throughout the 30-d period, using compost thermometers (Reotemp, San Diego, CA). Air temperature, relative humidity, and rainfall data were obtained from the SHRS weather station. At the completion of the tests, the upper layer of compost and any remaining fruit was collected for sampling with Berlese funnels.

To accompany each field test, 10 replicate controls were set up in polyethylene bins ($51 \times 31 \times 15$ cm; U.S. Plastic Corp., Lima, OH) maintained in the laboratory under the same conditions used for rearing the *Anastrepha* colony. Each bin contained 5 infested fruit placed above vermiculite (8 cm deep), and bins were enclosed in clear plastic cages (Bug-Dorm-2; BioQuip Products, Rancho Dominquez, CA). Vermiculite was sifted weekly to collect wandering larvae and puparia, which were held for adult emergence for the same 30-d period monitored in the field. The controls showed that 30 d was sufficient to allow all larvae to complete development.

The study consisted of 4 field tests (and controls), conducted from the late summer through early spring of 2004-2005. The 30-d monitoring periods were as follows: Summer test from 23 Aug to 22 Sep 2004; Early Fall test from 26 Sep to 26 Oct 2004; Late Fall test from 29 Oct to 28 Nov 2004; and Spring test from 28 Feb to 30 Mar 2005. This time period allowed us to evaluate composting of infested fruit under a variety of environmental conditions, with the first 2 tests conducted during the wet season, and the second 2 tests conducted during the dry season in south Florida.

Statistical Analysis

The levels of infestation (number of larvae per fruit) varied among the 4 field tests, therefore adult emergence counts were converted to percent emergence from compost fruit relative to control fruit prior to statistical analysis. Differences in percent emergence among the tests were analyzed by one-way analysis of variance (ANOVA) followed by Tukey-Kramer HSD test for mean separation ($P = 0.05$), with JMP (SAS Institute 2006). To evaluate environmental factors during the 4 tests, ANOVA followed by mean separation was performed initially. If significant differences were found among tests, then regression analysis was used to explore the relationship with percent fly emergence, which was then graphed with SigmaPlot 10 (Systat Software, Inc. 2006). Additionally, two-way ANOVA was used to assess potential interaction effects of environmental factors on percent emergence. Actual fly counts were analyzed in 1 instance, to determine if there was a difference in adult emergence based on sex. Two-way ANOVA with interaction, followed by Student's *t*-test, was performed separately on compost and control treatments to evaluate the number of males and females emerging in each test.

Results and Discussion

Manual grapefruit dissections indicated the following levels of infestation at the start of the 4 field tests: 19.0, 22.4, 34.8, and 8.2 larvae per fruit, respectively. In light of Gould's (1995) conclusions regarding the reliability of this detection procedure, these values were used only as relative indicators of the degree of infestation among the tests. Infestation was considered to be moderate for the Summer and Early Fall tests, high for the Late Fall test, and low for the Spring test. Differences in levels of infestation were not unexpected since Marsh grapefruit susceptibility to *A. suspensa* infestation has been shown to vary seasonally in relation to fruit senescence (Greany et al. 1985). All 3 instars were represented in the infested fruit of each test, but on average, 62.4% of the larvae were in the third instar when field tests were initiated. The percentages for the 4 tests were 75.8, 50.9, 66.8, and 56.1, respectively. This developmental stage was chosen to provide conditions comparable to those used in previous investigations of *Anastrepha*-infested grapefruits (e.g., Hallman et al. 1990; Hallman 1994; Gould 1995), but more significantly, use of fruit infested with third instars represented a scenario that would maximize the likelihood of adult emergence and thereby estimate the greatest potential risk of pest escape and establishment via a compost pathway. Shortly after infested fruit was placed on the piles, third instars would exit the fruit, burrow into the compost, and pupate. By restricting the field tests to this time window, any reduction in adult emergence would result primarily from conditions in the compost environment. Also, a third instar/wandering larva scenario would

represent the point at which consumers would most likely notice an insect and discard the fruit.

Overall, the mean emergence of adult flies (males and females) from composted fruit was 11% of that observed emerging from the control treatments (Table 1). In terms of risk assessment, the critical component is the percentage of females that survive the composting process, successfully mate, and escape into the environment. For the purposes of this study, 2 criteria were adopted to address that component. First, risk was defined as the presence of a single mated female emerging from compost. Second, a female was considered potentially mated if at least 1 male emerged from the same compost pile. These are valid assumptions since a single *A. suspensa* female (wild strain) has been shown to lay an average of 1.9 (±0.3) eggs/day over a lifespan of 73.6 (±4.9) days (Sivinski 1993), and males of *A. suspensa* have been documented to engage in multiple matings (Teal et al. 2000). Based on these criteria, percent emergence of potentially mated females was calculated for each field test, and presented in Table 2. The probability of a mated female emerging from compost was estimated to be10% for the study, with the greatest risk (22% emergence) observed during the Spring 2005 test. The lowest risk was seen with the Early Fall 2004 test, when no mated females emerged. In addition, the compost treatments had a significant difference in mean number of adults emerging based on sex ($F = 6.27$; $df = 1, 79$; $P = 0.014$). The average number of females emerging from compost piles was 1.15 times greater than the number of males ($t = 2.50$; $df = 75$; $P = 0.014$), suggesting differential survival of females within the composting environment. This bias was not observed in fly emergence from the control fruits ($F = 3.43$; $df = 1, 79$; $P = 0.068$).

Adult emergence data indicated approximately 90% mortality of *Anastrepha* in the late larval and pupal stages when exposed to composting conditions. Factors in the physical environment during the 4 seasonal tests (Table 3) were assessed for potential effects on fly emergence. The Spring 2005 test, which had significantly higher percent emergence than the other replicates ($F = 6.40$; $df = 3, 39$; $P = 0.002$, Table 1), was characterized by having the lowest compost temperatures and the lowest precipitation. Conversely, the test conducted in Early Fall 2004 had the lowest fly emergence, and the highest values for both compost temperature and precipitation. Therefore, these 2 environmental factors were analyzed further. For compost temperature, the mean maximum values were used for analysis, since maximum temperatures were more likely to contribute to fruit fly mortality. The interaction between maximum compost temperature and precipitation had no effect on the percentage of flies that emerged ($F = 1.66$; $df = 1, 39$; $P = 0.205$), but there was a significant effect due to maximum compost temperature ($F = 6.52$;

TABLE 1. AVERAGE EMERGENCE (MEAN ± SE) OF ADULT *A. SUSPENSA* FROM INFESTED GRAPEFRUITS PLACED ON OUTDOOR COMPOST PILES OR HELD IN THE LABORATORY UNDER CONTROLLED CONDITIONS FOR 30 D.

	Number of flies emerged						Percent emergence from compost relative to controls		
	Compost piles			Control bins					
Season	Male	Female	Total	Male	Female	Total	Male	Female	Total
Summer	2.2 ± 0.5	3.3 ± 0.7	5.5 ± 1.1	39.3 ± 4.9	57.9 ± 8.0	97.2 ± 12.4	5.6 ± 1.3 ab	5.7 ± 1.3 a	5.7 ± 1.1 a
Early Fall	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	19.6 ± 6.8	23.5 ± 7.5	43.1 ± 14.3	0.5 ± 0.5 a	0.4 ± 0.4 a	0.5 ± 0.3 a
Late Fall	0.7 ± 0.4	2.4 ± 1.2	3.1 ± 1.5	16.3 ± 5.5	17.6 ± 6.1	33.9 ± 11.2	4.3 ± 2.2 a	13.6 ± 6.7 ab	9.1 ± 4.5 a
Spring	1.5 ± 0.6	3.3 ± 0.8	4.8 ± 1.4	5.5 ± 1.0	11.7 ± 1.8	17.2 ± 2.7	27.3 ± 10.9 b	28.2 ± 6.9 b	27.9 ± 7.9 b
Overall	1.1 ± 0.2	2.3 ± 0.4	3.4 ± 0.6	20.2 ± 3.1	27.7 ± 4.2	47.9 ± 7.2	9.4 ± 3.2	12.0 ± 2.9	10.8 ± 2.8

Means within a column followed by the same letter are not significantly different (Tukey-Kramer HSD test, $P = 0.05$).

TABLE 2. EMERGENCE OF POTENTIALLY MATED FEMALE *A. SUSPENSA* FROM INFESTED GRAPEFRUITS PLACED ON OUTDOOR COMPOST PILES IN MIAMI, FLORIDA.

Season	Number emerged	Compost pile replicate 1	2	3	4	5	6	7	8	9	10	Percent emergence[1] of mated females[2] (Mean ± SE)
Summer	Male	0	0	2	2	5	3	2	1	3	4	
	Female	0	0	3	3	4	6	5	5	6	1	
	Mated female[2]	0	0	3	3	4	6	5	5	6	1	5.7 ± 1.3 ab
Early Fall	Male	0	1	0	0	0	0	0	0	0	0	
	Female	0	0	1	0	0	0	0	0	0	0	
	Mated female[2]	0	0	0	0	0	0	0	0	0	0	0.0 ± 0.0 a
Late Fall	Male	0	0	0	0	0	2	2	0	0	3	
	Female	0	0	0	0	0	10	4	2	0	8	
	Mated female[2]	0	0	0	0	0	10	4	0	0	8	12.5 ± 6.9 ab
Spring	Male	0	2	4	0	0	0	5	3	1	0	
	Female	3	5	6	0	3	1	8	3	4	0	
	Mated female[2]	0	5	6	0	0	0	8	3	4	0	22.2 ± 8.2 b
											Overall	10.1 ± 2.9

[1]Percent emergence from compost relative to controls. Means followed by the same letter are not significantly different (Tukey-Kramer HSD test, $P = 0.05$).

[2]Females were considered potentially mated if at least 1 male emerged from the same compost pile.

$df = 1, 39$; $P = 0.015$). The mean maximum temperature of compost piles was significantly different for each of the 4 seasons ($F = 137.6$; $df = 3, 39$; $P < 0.0001$; Table 3). The relationship between maximum compost temperature and percent emergence (Fig. 1) showed a marked clustering of the compost treatments: the Spring 2005 test with the highest emergence was at the lower end of the temperature scale, and the Early Fall test with the lowest emergence was at the upper end. Adult emergence decreased with increasing compost temperature, predicting mortality to approach 100% as the temperature rises above 48°C. Since the host fruits were placed 5 cm into the compost, and *A. suspensa* larvae typically pupate at a depth of 1-3 cm into the substrate (Hennessey 1994), the actual temperatures experienced by the insects were probably slightly less than that recorded by the compost thermometers inserted to 15 cm. It should also be noted that the heat of the compost, since generated by biologically active decomposing organic matter, is relatively stable and not subject to rapid changes due to fluctuations in weather conditions. Thus, the insects within an active compost environment are exposed to long periods of sustained high temperatures. Under "hot composting" conditions, as defined by the USDA-NRCS (2004), internal compost can reach 43-71°C, temperatures sufficient to kill most insects, weed seeds, and plant pathogens. The compost temperature of 48°C estimated for fly mortality in this study is consistent with the range of temperatures used in quarantine heat treatments of commercial fruit, 43-48°C (Hallman 1994), and documented in *A. suspensa* laboratory studies for mortality of isolated third instars and pupae, 43°C (Hallman 1996), or for mortality of third instars in host grapefruits, 43-46°C (Hallman et al. 1990).

In addition to the abiotic conditions of the compost system, several factors contributing to mortality were identified from Berlese sampling of the arthropod community. Competitors on the host fruit included adult and larval sap beetles, *Lobiopa insularis* (Cast.) and *Carpophilus* spp., which were often quite abundant. Predators and parasitoids detected in the compost included species known to feed on dipteran larvae, such as a macrochelid mite, *Glyptholaspis fimicola* (Sellnick) (Krantz 1998) and a rove beetle, *Belonuchus pallidus* Casey (Frank 2004); plus several species which have been documented to attack *A. suspensa* larvae and pupae, including a parasitoid wasp, *Diachasmimorpha longicaudata* (Ashmead) (Lawrence et al. 1976), the ringlegged earwig, *Euborellia annulipes* (Lucas) (Hennessey 1997), and the red imported fire ant, *Solenopsis invicta* Buren (Hennessey 1997). Predation by ants has been shown to be an important biotic mortality factor for *Anastrepha* larvae during the wandering prepupal stage (Aluja et al. 2005). Fungal growth, common on the surface of host fruits, was another potential mortality factor since it contributed to rapid breakdown of fruit on compost piles, especially in tests conducted during the wet season.

TABLE 3. AVERAGE VALUES (MEAN ± SE) FOR THE ENVIRONMENTAL CONDITIONS AND COMPOST TEMPERATURE (15 CM DEPTH) MEASURED DURING THE 4 SEASONAL FIELD TESTS.

	Weather data			Compost temperature (°C)		
Season	Air temp. (°C)	Rel. humidity (%)	Precip. (cm)	Mean	Minimum	Maximum
Summer	28.6 ± 0.2 a	83.6 ± 0.7 a	0.53 ± 0.18 a	38.3 ± 0.3 a	34.2 ± 0.2 a	42.9 ± 0.6 a
Early Fall	26.8 ± 0.3 b	81.8 ± 1.1ab	0.61 ± 0.36 a	41.5 ± 0.5 b	30.7 ± 0.4 b	50.0 ± 0.8 b
Late Fall	24.3 ± 0.3 c	78.4 ± 1.3 b	0.12 ± 0.08 a	34.8 ± 0.8 c	30.6 ± 0.5 b	39.3 ± 1.1 c
Spring	21.5 ± 0.7 d	79.3 ± 1.5 ab	0.08 ± 0.03 a	25.9 ± 0.2 d	21.2 ± 0.2 c	28.3 ± 0.2 d

Means within a column followed by the same letter are not significantly different (Tukey-Kramer HSD test, $P = 0.05$).

In summary, this study estimates a 10% likelihood for emergence of a mated female *Anastrepha* through a residential composting pathway. This risk applies to the spread of the established exotic, *A. suspensa*, into areas of Florida or other states that are currently fly-free. It applies equally to spread of exotic *Anastrepha* species should infested fruit evade detection and quarantine measures, as was the case in 2003 with manzano peppers imported from Mexico (Thomas 2004). Peppers infested with *A. ludens* cleared customs in Texas in Apr, were distributed to several U.S. locations, including 2 in Florida, and an adult *A. ludens* was captured in Orlando in May (Thomas 2004). Should pest entry occur despite current safeguards, the risk of spread via composting can be reduced by burying produce deep and promoting high internal compost temperatures (e.g., keeping piles moist and turning them often). Gould & Maldonado (2006) recently reported data on the likelihood of escape of *Copitarsia decolora* (Lepideptera: Noctuidae) larvae from disposal of infested asparagus, estimating that ~1.2% of first instars were able to escape from a commercial garbage dumpster during a 1-week period. These data were critical components of a pathway risk assessment estimating the likelihood of establishment of *C. decolora*. To our knowledge, this is the only other experimental study which quantifies risk of insect pest escape as a result of discarded produce. Comparable studies assessing tephritid emergence from fruit discarded in dumpsters or landfills are needed to support comprehensive pathway risk analysis for invasive fruit flies in Florida.

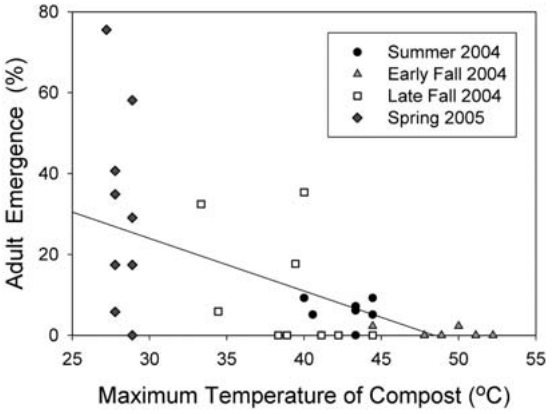

Fig. 1. Relationship between maximum compost temperature and percent emergence of adult *A. suspensa* from infested grapefruits placed on outdoor compost piles in Miami, FL. Each point represents 1 compost pile (5 fruit/pile, $n = 40$ piles); percent emergence and compost temperature (recorded at 15 cm depth) were monitored for 30 days. (Regression with arcsin (√y) transformation of percent emergence: $y = -0.02x + 1.09$; $r^2 = 0.41$. Nontransformed data depicted in graph.)

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REFERENCES CITED

ALUJA, M., J. SIVINSKI, J. RULL, AND P. J. HODGSON. 2005. Behavior and predation of fruit fly larvae (*Anastrepha* spp.) (Diptera: Tephritidae) after exiting fruit in four types of habitats in tropical Veracruz, Mexico. Environ. Entomol. 34: 1507-1516.

EPA. 2006. Composting. U.S. Environmental Protection Agency. http://www.epa.gov/composting/.

FRANK, J. H. 2004. *Belonuchus agilis*, a fourth species of this genus (Coleoptera: Staphylinidae) reported from Florida. Florida Entomol. 87: 92-93.

GOULD, J. R., AND M. H. MALDONADO. 2006. *Copitarsia decolora* (Lepidoptera: Noctuidae) larvae escaping from discarded asparagus: Data in support of a pathway risk analysis. J. Econ. Entomol. 99: 1605-1609.

GOULD, W. P. 1995. Probability of detecting Caribbean fruit fly (Diptera: Tephritidae) infestation by fruit dissection. Florida Entomol. 78: 502-507.

GREANY, P. D., AND C. RIHERD. 1993. Preface: Caribbean fruit fly status, economic importance, and control (Diptera: Tephritidae). Florida Entomol. 76: 209-211.

GREANY, P. D., P. E. SHAW, P. L. DAVIS, AND T. T. HATTON. 1985. Senescence-related susceptibility of Marsh grapefruit to laboratory infestation by *Anastrepha suspensa* (Diptera: Tephritidae). Florida Entomol. 68: 144-150.

HALLMAN, G. J. 1994. Mortality of third-instar Caribbean fruit fly (Diptera: Tephritidae) reared at three temperatures and exposed to hot water immersion or cold storage. J. Econ. Entomol. 87: 405-408.

HALLMAN, G. J. 1996. Mortality of third instar Caribbean fruit fly (Diptera: Tephritidae) reared in diet or grapefruits and immersed in heated water or grapefruit juice. Florida Entomol. 79: 168-172.

HALLMAN, G. J., J. J. GAFFNEY, AND J. L. SHARP. 1990. Vapor heat treatment for grapefruit infested with Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 83: 1475-1478.

HENNESSEY. M. K. 1994. Depth of pupation of Caribbean fruit fly (Diptera: Tephritidae) in soils in the laboratory. Environ. Entomol. 23: 1119-1123.

HENNESSEY, M. K. 1997. Predation on wandering larvae and pupae of Caribbean fruit fly (Diptera: Tephritidae) in guava and carambola grove soils. J. Agric. Entomol. 14: 129-138.

HENNESSEY, M. K. 2004. Quarantine pathway pest risk analysis at the APHIS Plant Epidemiology and Risk Analysis Laboratory. Weed Technol. 18: 1484-1485.

KENDRA, P. E., W. S. MONTGOMERY, N. D. EPSKY, AND R. R. HEATH. 2006. Assessment of female reproductive status in *Anastrepha suspensa* (Diptera: Tephritidae). Florida Entomol. 89: 144-151.

KRANTZ, G. W. 1998. Review reflections on the biology, morphology, and ecology of the Macrochelidae. Exp. Appl. Acarol. 22: 125-137.

LAWRENCE, P. O., R. M. BARANOWSKI, AND P. D. GREANY. 1976. Effect of host age on development of *Biosteres* (= *Opius*) *longicaudatus*, a parasitoid of the Caribbean fruit fly, *Anastrepha suspensa*. Florida Entomol. 59: 33-39.

RANEY, H. G., AND R. D. EIKENBARY. 1969. A simplified trap for collecting adult pecan weevils. J. Econ. Entomol. 62: 722-723.

ROBERTS, R. C., T. VAN DER ZWET, C. MILLER, AND S. REDLIN. 1998. The potential for spread of *Erwinia amylovora* and fire blight via commercial apple fruit: A critical review and risk assessment. Crop Protection 17: 19-28.

SARASOTA COUNTY. 2006. Florida's online composting center. Sarasota County Government. Environmental Services, Solid Waste Division, Resource Conservation Section. http://www.compostinfo.com/.

SAS INSTITUTE. 2006. JMP 6. SAS Institute, Cary, NC.

SILVA, J. G., M. D. MEIXNER, B. A. MCPHERON, G. J. STECK, AND W. S. SHEPPARD. 2003. Recent Mediterranean fruit fly (Diptera: Tephritidae) infestations in Florida—a genetic perspective. J. Econ. Entomol. 96: 1711-1718.

SIVINSKI, J. M. 1993. Longevity and fecundity in the Caribbean fruit fly (Diptera: Tephritidae): Effects of mating, strain and body size. Florida Entomol. 76: 635-644.

SYSTAT SOFTWARE, INC. 2006. SigmaPlot 10 user's manual. Systat Software, Inc., Port Richmond, CA.

TEAL, P. E. A., Y. GOMEZ-SIMUTA, AND A. T. PROVEAUX. 2000. Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. PNAS 97: 3708-3712.

THOMAS, D. B. 2004. Hot peppers as a host for the Mexican fruit fly *Anastrepha ludens* (Diptera: Tephritidae). Florida Entomol. 87: 603-608.

UF-IFAS. 2004. Gardening with Florida yards and neighborhoods: Composting. University of Florida. Institute of Food and Agricultural Sciences. Extension Service. http://cfyn.ifas.ufl.edu/compost.html.

USDA-APHIS. 2004. Importation of avocado fruit (*Persea americana* Mill. var. 'Hass') from Mexico: A risk assessment. U.S. Department of Agriculture. Animal and Plant Health Inspection Service. Plant Protection and Quarantine.

USDA-APHIS. 2006a. Exotic fruit fly strategic plan FY 2006-2010. U.S. Department of Agriculture. Animal and Plant Health Inspection Service. Plant Protection and Quarantine. http://www.aphis.usda.gov/ppq/ep/ff/ffstrategicplan0206.pdf.

USDA-APHIS. 2006b. National pest interception record database. U.S. Department of Agriculture. Animal and Plant Health Inspection Service. Plant Protection and Quarantine (Online database maintained by the Systematic Entomology Laboratory: www.sel.barc.usda.gov).

USDA-APHIS. 2006c. Plant import: Nonpropagative manuals: Fruits and vegetables. U.S. Department of Agriculture. Animal and Plant Health Inspection Service. Plant Protection and Quarantine. http://www.aphis.usda.gov/ppq/manuals/online_manuals.html.

USDA-ARS. 2007. The Diptera site, Tephritidae main page. U.S. Department of Agriculture. Agricultural Research Service. Systematic Entomology Laboratory. http://www.sel.barc.usda.gov/Diptera/tephriti/Anastrep/Anastrep.htm.

USDA-NRCS. 2004. Backyard conservation tip sheet: Composting. U.S. Department of Agriculture. Natural Resources Conservation Service. http://www.nrcs.usda.gov/feature/backyard/compost.html.

WEARING, C. H., J. HANSEN, C. WHYTE, C. E. MILLER, AND J. BROWN. 2001. The potential for spread of codling moth (Lepidoptera: Tortricidae) via commercial sweet cherry fruit: A critical review and risk assessment. Crop Protection 20: 465-488.

WEEMS, H. V. JR., J. B. HEPPNER, T. R. FASULO, AND J. L. NATION. 2001. Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Insecta: Diptera: Tephritidae). University of Florida Featured Creatures. http://creatures.ifas.ufl.edu/fruit/tropical/caribbean_fruit_fly.htm.

WEEMS, H. V. JR., J. B. HEPPNER, G. J. STECK, T. R. FASULO, AND J. L. NATION. 2004. Mexican fruit fly, *Anastrepha ludens* (Loew) (Insecta: Diptera: Tephritidae). University of Florida Featured Creatures. http://creatures.ifas.ufl.edu/fruit/tropical/mexican_fruit_fly.htm.

WHITE, I. M., AND M. M. ELSON-HARRIS. 1992. Fruit flies of economic significance: Their identification and bionomics. CAB International, Wallingford, UK in association with ACIAR (The Australian Centre for International Agriculture Research), Canberra, Australia.

IMPROVED FECUNDITY IN THE PREDATOR *ORIUS INSIDIOSUS* (HEMIPTERA: ANTHOCORIDAE) WITH A PARTIALLY PURIFIED NUTRITIONAL FACTOR FROM AN INSECT CELL LINE

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ABSTRACT

A specific factor that stimulates egg production in the predator *Orius insidiosis* (Say) was earlier shown to be present in eggs of the Indian meal moth, *Plodia interpunctella* (Hübner). We investigated whether the embryonic cell line IPLB-PiE, derived from eggs of the Indian meal moth *P. interpunctella* also produces a specific factor that improves fecundity of the predator. We fractionated cells by preparative isoelectric focusing in a pH gradient of 3-10 and bioassayed the resultant fractions in test diets to determine their effects on egg production. Rates of oviposition were determined by placing adult predators on the test diets the third d after eclosion, allowing them to feed for 3 d, and then providing them with ovipositional substrates for 24 h on d 7. Six out of 20 fractions with isoelectric points between pH 5.2 and 7.3 had significant activity relative to the control diet. The nature of the factor(s) is unknown but corresponds to a partially purified fecundity factor from eggs of *Ephestia kuehniella* Zeller with an isoelectric point of pH 5 in an earlier study. The results indicate that the cell line, which was originally derived from embryos of *P. interpunctella*, has retained a differentiated function in culture by producing products similar to those produced in the *P. interpunctella* egg.

Key Words: artificial diet, factor, insidious flower bug, *Plodia interpunctella*, cell line, *Orius insidiosus*, *Ephestia kuehniella*, eggs

RESUMEN

Un factor específico que estimula la producción de huevos del depredador *Orius insidiosis* (Say) anteriormente se mostro el estar presente en los huevos de la palomilla, *Plodia interpunctella* (Hübner). Nosotros investigamos si la línea celular IPLB-PiE de los embriones derivada de huevos de la palomilla, *P. interpunctella* también produce un factor específico que mejore la fecundidad del depredador. Desde entonces hemos distribuido las células por medio de un preparativo isoeléctrico enfocandose en un gradiente de pH de 3-10 y realizamos un bioensayo sobre las fracciones en pruebas de dieta para determinar sus efectos sobre la producción de huevos. Las tasas de oviposición fueron determinadas por medio de la alimentación de los depredadores adultos con las dietas de prueba al tercer día después de la eclosión, permitiendo que ellos se alimentaran por 3 dias, y luego proveyendolos con sustratos oviposicionales por 24 h en el dia 7. Seis de los 20 fracciones con puntos isoeléctricos entre pH 5.2 y 7.3 tenian una actividad significativa mayor relacionada con la dieta de control. La naturaleza del factor(es) es desconocida pero se corresponde a un factor de fecundidad purificado parcialmente de los huevos de *Ephestia kuehniella* Zeller con un punto isoeléctrico de pH 5 en un estudio anterior. Estos resultados indican que la línea celular que fuera derivada originalmente de los embriones de *P. interpunctella* ha retenido una función diferenciada en el cultivo por la producción de productos similares a los producidos en el huevo de *P. interpunctella*.

Reduced fecundity is a general problem associated with the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) (Ferkovich & Shapiro 2004a), and a number of other species of predators reared on artificial diets without insect components (Cohen 1985a, 1985b, 1992; 2000; De Clercq & Degheele 1992, 1993a, 1993b; Cohen & Staten 1994; De Clercq et al. 1998; Adams 2000a, 2000b; Rojas et al. 2000; Wittmeyer & Coudron 2001; Coudron et al. 2002). Insect hemolymph and tissue extracts have been used to improve artificial diets (Grenier et al. 1994). The use of established insect cell lines as replacements is a relatively recent approach (Rotundo et al. 1988; Ferkovich & Oberlander 1991; Ferkovich et al. 1994; Hu et al. 1999; Ferkovich & Shapiro 2004b; Ferkovich & Lynn 2005; Heslin et al. 2005a, 2005b). The advantages of using cell lines in developing and/or improving artificial diets will be realized when the technology for large-scale cell production with cost-effective serum-free media is available. Cell lines could substitute for hemolymph or other insect materials (e.g., an embryonic cell line could substitute for insect egg homogenates), where those materials are critical for optimal development of insects. Also, the use of cell lines in

chemically defined media could simplify downstream purification and identification of growth-inducing factors, fecundity-inducing factors, and others found naturally in insect hosts and prey.

In an earlier work, we addressed the fecundity problem by supplementing artificial diet for *O. insidiosus* with 2 embryonic cell lines. One line, Ek-x4 was derived from eggs of *Ephestia kuehniella* Zeller (Lynn & Ferkovich 2004), which are generally used to rear *Orius* species by commercial insectaries, and 1 line, IPLB-PiE was derived from eggs of *Plodia interpunctella* (Hübner) (Tsang et al. 1985). Although the resultant fecundity was comparable with both cell lines, the growth characteristics of the IPLB-PiE cell line were conducive to larger scale production of those cells. In a recent study, we fractionated *P. interpunctella* egg proteins and bioassayed the fractions in diet, demonstrating the existence of a specific factor that stimulates egg production. Correspondingly, in this study we have examined the IPLB-PiE line derived from whole egg embryos to determine if a similar fecundity factor is also produced by the IPLB-PiE cells.

Materials and Methods

Orius Rearing

A colony of *O. insidiosus*, originating from a Florida strain collected in Bronson, FL in 2002, was maintained on eggs of *E. kuehniella* Zeller (received frozen from Beneficial Insectary, Redding, CA). Briefly, freshly laid eggs of *O. insidiosus* (about 500 eggs in 1-3 green beans) were placed in 400-mL canning jars, each covered with a 15 × 15-cm square of nylon ripstop cloth. Each jar received 0.3 mL of *E. kuehniella* eggs, 1.25 mL of Hydrocapsules® (1-2 mm dia.; Analytical Research Systems, Gainesville, FL), and 2 granules of local pollen, (Buzzn Bee, Inc., West Palm Beach, FL). After the *O. insidiosus* eggs hatched (approximately 5 d), the green beans were replaced with fresh beans, *E. kuehniella* eggs, and additional pollen every other day. Adults started to emerge in approximately 3 weeks and, when adults began to oviposit in the green beans, new jars were set up with green beans containing eggs. The insects were held at 25.5 ± 1°C, with 70 ± 5% RH, and light: dark cycle of 16:8 h.

Artificial Diet

Artificial diet was prepared under aseptic conditions in a clean room and encapsulated in stretched Parafilm© domes (25 µL) with a diet encapsulation apparatus (Analytical Research Systems, Gainesville, FL) described by Ferkovich et al. (1999). Diet ingredients were 330 mg brewers yeast, 30 mg sucrose, 180 mg soy protein acid hydrolysate, 3.8 mg of 99% palmitic acid (all from Sigma, St. Louis, MO), 40 mg chicken egg yolk, and 80 mg honey in 1.2 mL of distilled water. Palmitic acid was mixed with the egg yolk component before adding it to the diet.

Cells

The embryonic cell line (IPLB-PiE), originally derived from embryonated eggs of *P. interpunctella* by Tsang et al. (1985), was cultured in TNM-FH insect medium (Sigma, St. Louis, MO) in 25-cm^2 culture flasks for 7 d as described by Lynn (1996). For large-scale production of the cells, they were cultured in 250-mL magnetic spinner flasks (Bellco Glass, Vineland, NJ) at 24.9°C for 14 d. The cell suspension was centrifuged (1370 × *g* for 3 min) in a graduated conical centrifuge tube to obtain a soft pellet of cells. The pellet was resuspended in 1.0 mL purified water, washed twice and homogenized with a hand-held homogenizer in 1.0 mL purified water. The homogenate was then sonicated for 60 s with a Polytron© unit (model W-375, Heat Systems-Ultrasonic, Inc., Plain View, NY). Twenty µL were removed and assayed for protein by the Lowry procedure (Protein Assay Kit, Sigma, St. Louis) and the remainder of the cell suspension was saved for the isoelectric procedure.

Electrophoresis and Protein Assay

The Lowry procedure (Protein Assay Kit, Sigma, St. Louis, MO) was used to assay the soluble proteins in the egg protein solution and in the fractions after isoelectric focusing. Gradient SDS-PAGE (4-20%) was carried out in vertical minigels (Bio-Rad) as described by Shapiro et al. (2000).

Preparative Isoelectric Focusing

The cell homogenate (approx 0.98 mL) and 3 mL of ampholyte solution (pH range 3-10, Bio-Rad, Hercules, CA) were mixed in 58 mL of distilled water. The protein solution was run in a Rotofor Cell© isoelectric focusing unit (Bio-Rad, Hercules, CA) for 2.5 h at 12 W constant power at 4°C. Twenty fractions were collected and their volumes (approx. 2 mL each) and pH values measured. Ampholytes were removed by bringing each fraction to 1 M NaCl for 15 min and then aliquots of 10-20 µL of each fraction were used for protein analysis. After the fractions were analyzed for protein, they were combined based on the protein profile. Fractions with low protein levels were combined, and ones with higher protein concentrations were kept as individual fractions. The fractions were combined as follows: 1-5, 12-16 and 17-20. Fractions 6-11, which were cloudy and noted to contain minor precipitates, were kept as individual fractions. The combined and individual fractions

were then concentrated to 0.5 mL in Centriprep© concentrators (10k molecular weight (MW) cutoff; Millipore, Bedford, MA) and 10-20 µL of each fraction were used to analyze for soluble protein.

Proteinase K Digestion of Cells

One mL of pelleted PiE cells was homogenized in 1 mL of PBS (0.15 M sodium chloride/0.1 M sodium phosphate, pH 7.0), centrifuged for 2 min, and the supernatant was applied to 5 mL- Zebra© desalt spin columns (Pierce, Rockford, IL). The resultant desalted protein solution was incubated with 20 mg proteinase K (immobilized on agarose beads) per 30 mg cell protein and held on a shaker-bath at 37°C for 18 h. A second 1-ml sample without proteinase K was incubated at the same temperature. The proteinase K sample was centrifuged to remove the beads and 40 µL were removed for the protein assay and PAGE analysis. Dry diet ingredients were then added to both of the samples for the diet bioassay.

Diet Bioassay of IEF Fractions

Newly emerged (24 h after eclosion) adults of mixed sexes were collected with a camel hair brush and maintained on *E. kuehniella* eggs for 3 d before they were placed on the diet capsules containing the IEF fractions. Each replicate consisted of 6 females and 4 males in a 100-mL plant tissue culture jar (Sigma, St Louis, MO) with 4 jars per treatment. Each jar contained 0.6 mL of Hydrocapsules®, 2 capsules of treatment diet (each 25 µL), and 3 crumpled strips of wax paper (5 × 80 mm) as substrates. *Ephestia kuehniella* eggs and beads of water and diet were replaced daily and mortality was recorded. At the end of d 6, one 7-cm section of green bean pod, used as a substrate for oviposition, was placed in each jar for 24 h. Eggs deposited in the green beans were then counted under a microscope. The insects were held in a growth chamber at 25.5 ± 1°C, with 75 ± 5% RH, and a photoperiod of 15:9 (L:D) h. Diet treatments consisted of the following: (1) Eggs (standard)—jars each contained whole *E. kuehniella* eggs (3 mg, approx. 150 eggs), which were used as a standard in the bioassay because they are widely used by commercial insectaries for rearing predators; (2) Diet (control)—jars contained artificial diet with no additional substances; and (3) Diet (amended)—jars contained artificial diet supplemented with combined fractions 1-5, 12-16, or 17-20, or individual fractions 6 through 11 as separate treatments.

Data Analysis

Each treatment was replicated 4 times. Egg counts (eggs/female) were adjusted for female mortality within each treatment. Data were analyzed by ANOVA with StatMost software (Dataxiom Software, Inc.). Dunnett's test was used to determine if the number of eggs laid per female on each of the diet treatments supplemented with the isoelectric focusing fractions was significantly greater than the number of eggs laid per female on the Diet (control).

Results

Figure 1 shows the protein profile versus pH of the IPLB-PiE proteins separated in a pH gradient of 3-10. The average rate of eggs oviposited per female was highly significant relative to the Diet (control) ($P < 0.01$) in fractions 7 through 9 with isoelectric points ranging from pH 5.2 to 6.1 and significant ($P < 0.05$) in fractions 10 and 11, pH 6.8 and 7.3 (Fig. 2). The active fractions contained 12.6 mg or 40.3% of the total protein (31.4 mg) recovered in all the fractions; the remaining fractions contained 18.8 mg of the total protein and no associated activity. Recovery of the total protein applied to the gradient was 82.6% (38 mg applied; 31.4 mg recovered); light precipitates in fractions 7-11 after isoelectric focusing resulted in a loss of protein in these fractions when the samples were dialyzed and concentrated. SDS-PAGE analysis of the fractions is shown in Fig. 3. Of the active fractions (fractions 7-11), fractions 10 and 11 had fewest bands and displayed 4 easily discernible bands in a relative molecular weight range of 34-133k. Treatment of the cell homogenate with proteinase K revealed a loss of the cellular proteins on an SDS-PAGE gel (Fig. 4); however, females fed proteinase K-treated cells showed oviposition rates similar to those fed untreated cells (Fig. 5).

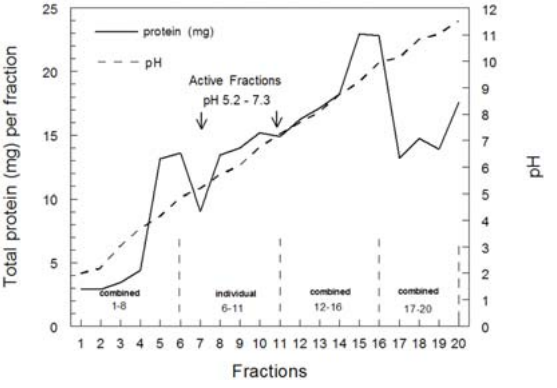

Fig. 1. Protein profile of IPLB-PiE cellular homogenate separated by isoelectric focusing on a pH gradient of 3-10. Fractions that were combined for bioassay in artificial diet are shown by vertical dotted lines. Arrows indicate range of fractions with ovipositional stimulating activity and pH values indicate isoelectric points.

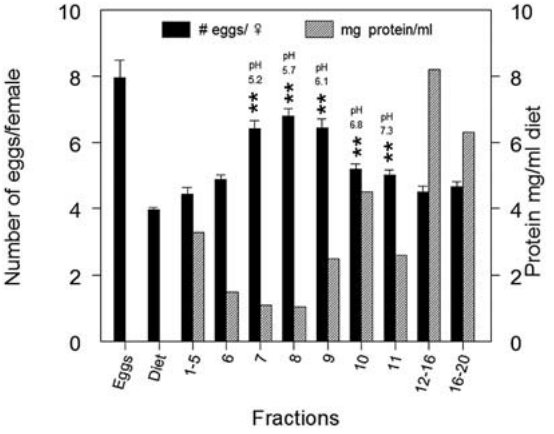

Fig. 2. Average number of eggs oviposited by females of *O. insidiosus* after being fed artificial diet supplemented with fractions from isoelectric focusing separation of IPLB-PiE cellular homogenate shown in Fig. 1. Eggs (standard), whole eggs of *E. kuehniella*; Diet (control); Diet (amended), combined and individual fractions were each biossayed in separate diet treatments; error bars refer to standard error; ** indicates $P < 0.01$.

Discussion

The fecundity of *O. insidiosis* females was improved when fed diet supplemented with 6 of the 20 fractions from isoelectric focusing separation of the homogenate of IPLB-PiE cells. This indicated that the effect was not due to a protein deficiency, since all of the fractions contained protein. Moreover, the activity was limited to fractions in a pH range of 5.2-7.3 and was associated with 40% of the total protein recovered. Since multiple bands were present in all the active fractions, the fecundity-promoting activity could not be attributed to a specific polypeptide. However, active fractions 10 and 11 contained fewer bands, with 2 densely staining bands at MW 156k, 69k, 42, and 34k. A recent study that used whole eggs of *E. kuehniella* and the same procedure employed in this study found only 1 active fraction associated with 16% of the total recovered protein at a pH of 5 (Ferkovich & Shapiro 2005). In addition, SDS-PAGE analysis of the whole egg fractions revealed the presence of 1 major protein band with MW 47k and other faint bands at 163k, 51k, 39k, 31k, and 27k. Other egg components such as extracts of egg lipids and nucleic acids (DNA and RNA) had no effect on the ovipositional rate nor did other non-insect proteins such bovine serum albumin and hen egg albumin tested at similar concentrations.

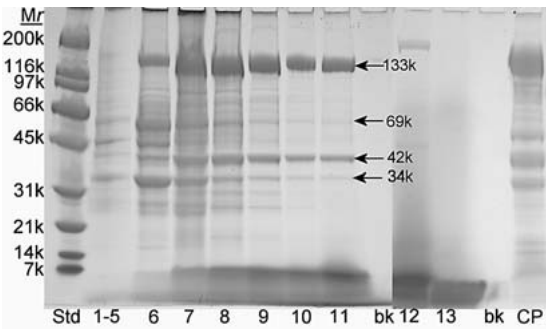

Fig. 3. SDS-PAGE analysis of fractions separated as shown in Fig. 1. MW standards (Std); combined fractions 1-5; individual fractions 6-11; blank (bk); combined fractions 12-16; blank (bk); combined fractions 17-20; blank; crude protein (CP). Twenty micrograms of protein applied per lane.

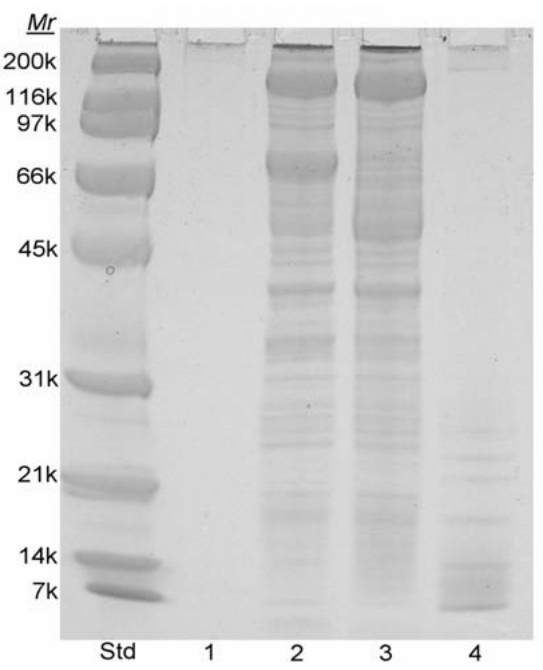

Fig. 4. SDS-PAGE analysis of fractions separated as shown in Fig. 1. MW standards (Std); lanes: 1) blank, 2) untreated, 3) without proteinase K but incubated at 37° for 18 h, and 4) with proteinase K at 37°C for 18 h. Ten micrograms of protein applied per lane.

The results of this study suggest that the IPLB-PiE cell line, originally derived from embryos of *P. interpunctella*, has retained a differentiated function in culture and produces products similar to those synthesized in the *P. interpunctella* egg. We now know that the active material is at least associated with cellular proteins. Digestion of the proteins did not result in a loss of activity, indicating that none of the basic nutritional components of the cell homogenate were destroyed by the enzyme. The activity could be associated with a peptide fragment resulting from the

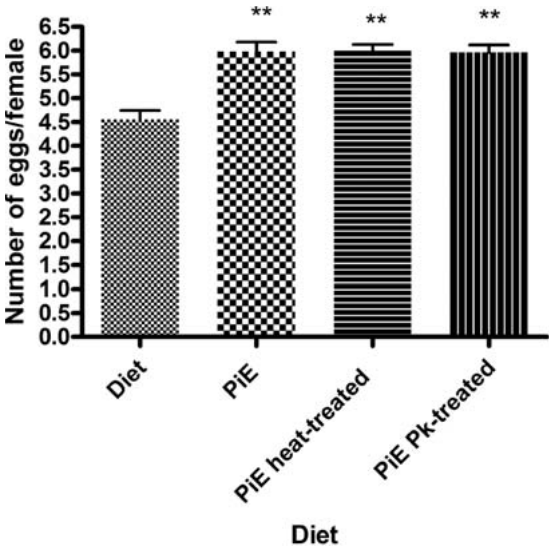

Fig. 5. Average number of eggs oviposited by females of *O. insidiosus* after being fed artificial diet alone (Diet), and diet supplemented with fresh PiE cells (PiE), PiE cells incubated at 37°C for 18 h (PiE heat-treated), and PiE cells incubated with proteinase K at 37°C for 18 h (PiE Pk-treated). Error bars refer to standard error; ** indicates $P < 0.01$.

proteolytic digestion or a ligand carried by one the proteins. Proteins in diets not only provide amino acids as nutrients, but also serve to functionally bind lipids, ions, enzyme co-factors, and flavors, act as emulsifiers and film-formers between diet components, and have buffering and stabilizing effects on diet components (Cohen 2004), none of which was addressed in this study. An alternative possibility for the enhanced fecundity that was observed is that proteins in insect diets may provide other needed nutrients or factors such as the "token stimuli" described by Cohen (2004), which stimulate predators to feed on diets. Although the nature of active material is unknown, this information provides a new avenue for isolation and identification of the fecundity-enhancing substance.

Acknowledgments

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Literature Cited

Adams, T. S. 2000a. Effect of diet and mating on oviposition in the twospotted stink bug *Perillus bioculatus* (F.) (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Amer. 93: 1288-1293.

Adams, T. S. 2000b. Effect of diet and mating status on ovarian development in a predaceous stink bug, *Perillus bioculatus* (F.) (Hemiptera: Pentatomidae). Ann. Entomol. Soc. Amer. 93: 529-535.

Cohen, A. C. 1985a. Metabolic rates of two hemipteran members of a predator prey complex. Comp. Biochem.Physiol. 81A: 833-836.

Cohen, A. C. 1985b. Simple method for rearing the insect predator *Geocoris punctipes* (Heteroptera: Lygaeidae) on a meat diet. J. Econ. Entomol. 78: 1173-1175.

Cohen, A. C. 1992. Using a systematic approach to develop artificial diets for predators, pp. 77-91 *In* T. E. Anderson and N. C. Leppla [eds.], Advances in Insect Rearing for Research and Pest Management. Westview Press, Boulder.

Cohen, A. C. 2000. Feeding fitness and quality of domesticated and feral predators: effects of long-term rearing on artificial diet. Biol. Control 17: 50-54.

Cohen, A. C. 2004. Insect Diets: Science and Technology. CRC Press, Boca Raton.

Cohen, A. C., and R. T. Staten. 1994. Long-term culturing and quality assessment of predatory big-eyed bugs, *Geocoris punctipes*, pp. 122-132 *In* S. K. Narang, A. C. Bartlett, and R. M. Faust [eds.], Applications of Genetics to Arthropods of Biological Control Significance. CRC Press, Boca Raton.

Coudron, T. A., J. Wittmeyer, and Y. Kim. 2002. Life history and cost analysis for continuous rearing of *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae) on a zoophytophagous artificial diet. J. Econ. Entomol. 95: 1159-1168.

De Clercq, P., and D. Degheele. 1992. A meat-based diet for rearing the predatory stinkbugs *Podisus maculiventris* and *Podisus sagitta* (Heteroptera, Pentatomidae). Entomophaga 37: 149-157.

De Clercq, P., and D. Degheele. 1993a. Quality assessment of the predatory bugs *Podisus maculiventris* (Say) and *Podisus sagitta* (Fab) (Heteroptera, Pentatomidae) after prolonged rearing on a meat-based artificial diet. Biocont. Sci. Technol. 3: 133-139.

De Clercq, P., and D. Degheele. 1993b. Quality of predatory bugs of the genus *Podisus* reared on natural and artificial diets. pp. 129-142 *In* G. Nicoli, M. Benuzzi, and N. C. Leppla [eds.], IOBC Working Group on Arthropod Mass Rearing.

De Clercq, P., F. Merlevede, and L. Tirry. 1998. Unnatural prey and artificial diets for rearing *Podisus maculiventris* (Heteroptera: Pentatomidae). Biol. Control 12: 137-142.

Ferkovich, S. M., and D. E. Lynn. 2005. Enhanced egg laying in adult predators fed artificial diet supplemented with an embyonic cell line derived from eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Florida Entomol. 88: 329-331.

Ferkovich, S. M., J. A. Morales-Ramos, M. G. Rojas, H. Oberlander, J. E. Carpenter, and P. Greany. 1999. Rearing of ectoparasitoid *Diapetimorpha introita* on an artificial diet: supplementation with insect cell line-derived factors. Biocontrol 44: 29-45.

Ferkovich, S. M., and H. Oberlander. 1991. Stimulation of endoparasitoid egg development by a fat body cell line: activity and characterization of factors that induce germ band formation and hatching. pp. 181-187 Tissue Culture Assoc., Columbia, MD.

Ferkovich, S. M., H. Oberlander, C. Dillard, and E. Leach. 1994. Embryonic development of an endoparasitoid, *Microplitis croceipes* (Hymenoptera, Braconidae) in cell line conditioned media. In Vitro Cellular & Developmental Biology-Animal 30A: 279-282.

FERKOVICH, S. M., AND J. P. SHAPIRO. 2004a. Comparison of prey-derived and non-insect supplements on egg-laying of *Orius insidiosus* maintained on artificial diet as adults. Biol. Control 31: 57-64.

FERKOVICH, S. M., AND J. P. SHAPIRO. 2004b. Increased egg-laying in *Orius insidiosus* (Hemiptera: Anthocoridae) fed artificial diet supplemented with an embryonic cell line. Biol. Control 31: 11-15.

FERKOVICH, S. M. AND J. P. SHAPIRO. 2005. Enhanced oviposition in the insidious flower bug, *Orius insidiosus* (Hemiptera: Anthocoridae) with a partially purified nutritional factor from prey eggs. Florida Entomol. 88: 253-257.

GRENIER, S., P. D. GREANY, AND A. C. COHEN. 1994. Potential for mass release of insect parasitoids and predators through development of artificial culture techniques. pp. 181-205 *In* D. Rosen, F. C. Bennett, and J. L. Capinera [eds.], Pest Management in the Subtropics: Biological Control- A Florida Perspective. Intercept, Andover.

HESLIN, L. M., R. A. KOPITTKE, AND D. J. MERRITT. 2005a. Refinement of a cell line based artificial diet for rearing the parasitoid wasp, *Trichogramma pretiosum*. Biol. Control 33: 278-285.

HESLIN, L. M., R. A. KOPITTKE, AND D. J. MERRITT. 2005b. The role of insect cell lines in an artificial diet for the parasitoid wasp, *Trichogramma pretiosum*. Biol. Control 33: 186-193.

HU, J. S., D. B. GELMAN, R. A. BELL, AND D. E. LYNN. 1999. In vitro rearing of *Edovum puttleri*, an egg parasitoid of the Colorado potato beetle, on artificial diets: Effects of insect cell line-conditioned medium. Arch. Insect Biochem. Physiol. 40: 173-182.

LYNN, D. E. 1996. Development and characterization of insect cell lines. Cytotechnology 20: 3-11.

LYNN, D. E. AND S. M. FERKOVICH. 2004. New cell lines from *Ephestia kuehniella*: Characterization and susceptibility to baculoviruses. 5 pp. J. Insect Sci. 4: 9. Available on line at insectscience.org/4.9.

ROJAS, M. G., J. A. MORALES-RAMOS, AND E. G. KING. 2000. Two Meridic Diets for *Perillus bioculatus* (Heteroptera: Pentatomidae), a Predator of *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Biol. Control 17: 92-99.

ROTUNDO, G., R. CAVALLORO, AND E. TREMBLAY. 1988. In vitro rearing of *Lysiphlebus fabarum* (Hym.: Braconidae). Entomophaga 33: 264-267.

SHAPIRO, J. P., H. A. WASSERMAN, P. D. GREANY, AND J. L. NATION. 2000. Vitellin and vitellogenin in the soldier bug, *Podisus maculiventris*: Identification with monoclonal antibodies and reproductive response to diet. Arch. Insect Biochem. Physiol. 44: 130-135.

TSANG, K. R., G. B. WARD, A. H. MARDAN, P. K. HAREIN, M. A. BROOKS, AND L. JACOBSON. 1985. Establishment and characterization of a cell line from embryos of the Indianmeal moth, *Plodia interpunctella*. J. Invertebr. Pathol. 46: 180-188.

WITTMEYER, J. L., AND T. A. COUDRON. 2001. Life table parameters, reproductive rate, intrinsic rate of increase, and estimated cost of rearing *Podisus maculiventris* (Heteroptera: Pentatomidae) on an artificial diet. J. Econ. Entomol. 94: 1344-1352.

A COMPARISON OF TRAPS AND STEM TAP SAMPLING FOR MONITORING ADULT ASIAN CITRUS PSYLLID (HEMIPTERA: PSYLLIDAE) IN CITRUS

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ABSTRACT

Studies were conducted at 2 different field sites to compare yellow sticky card traps, blue sticky card traps, Multi-Lure traps, and CC traps (red, blue, black, white, yellow, and dark green bases) for monitoring adult Asian citrus psyllid, *Diaphorina citri* Kuwayama, in citrus. The Multi-Lure and CC traps were charged with either ethylene glycol or a dichlorvos kill strip to kill psyllids entering the trap. We also investigated a stem tapping method for monitoring adult *D. citri*. Yellow sticky card traps captured significantly more adults than blue sticky card traps over a 4-week period in one study but not the other. Over all sample weeks, each of these traps captured significantly greater numbers of adults than any of the other traps. Yellow and blue sticky traps were equally effective in detecting the presence of adults in trees given the infestation levels present at the 2 study sites. The CC and Multi-Lure traps captured so few adult psyllids and provided such poor detection of trees infested by adults that they appeared to have no value for monitoring *D. citri*. Tap sampling was easy to conduct and provided relatively good detection of trees infested by adults given the infestation levels present at the 2 groves. An advantage to stem tap sampling over sticky trap sampling is that tap sampling provides information on the presence and relative abundance of adult *D. citri* during a single visit to a block of trees while sticky trap sampling requires 2 visits. Research to develop standard protocols for sticky trap and stem tap sampling for adult *D. citri* in citrus would be advantageous.

Key Words: trapping, sampling, sticky traps, Multi-Lure, CC trap

RESUMEN

Se realizaron estudios en dos campos diferentes para comparar las trampas de tarjetas pegajosas del color amarillo, trampas de tarjetas pegajosas de color azul, trampas "Multi-lure" y trampas "CC" (con bases de color rojo, azul, negro, blanco, amarillo y verde oscuro) para el monitoreo de adultos del psílido Asiático de los cítricos, *Diaphorina citri* Kuwayama en cítricos. Las trampas Multi-Lure y CC fueron cargadas con un "kill strip" (una plancha para matar) que contenia ya sea etilenglicol o diclorovos para matar los psílidos que entraban a las trampas. También, investigamos un método de trampa de pegado al tallo para el monitoreo de los adultos de *D. citri*. Las trampas de tarjetas pegajosas de color amarillo capturaron significativamente más adultos que las trampas de tarjetas pegajosas de color azul durante el periodo de 4 semanas en uno de los estudios pero no en el otro. En todas las semanas de muestreo, cada una de estas trampas amarillas capturaron significativamente un número mayor de adultos que cualquiera de las otras trampas. Las trampas pegajosas de los colores amarillo y azul fueron igualmente efectivas en detectar la presencia de adultos en árboles teniendo en cuenta el nivel de infestación presente en los dos sitios del estudio. Las trampas de "CC" y "Multi-lure" capturaron muy pocos adultos de psílidos y proveyeron una detección tan pobre de árboles infestados que pareciera indicar que no tienen ningún valor para realizar un monitoreo de *D. citri*. El muestreo de pega a los tallos fue fácil de realizar y proveyó una detección relativamente buena de árboles infestados por adultos teniendo en cuenta el nivel de infestación presente en los 2 huertos. Una ventaja del muestreo de pega de tallos sobre el muestreo usando trampas pegajosas es que el muestreo de pega de tallos provee información sobre la presencia y la abundancia relativa de los adultos de *D. citri* durante una sola visita al bloque de árboles mientas que el muestreo usando trampas pegajosas requiere 2 visitas más. Investigaciones para desarrollar protocolos estandardizados para el muestreo de adultos de *D. citri* usando trampas pegajosas y de pega de tallos de los cítricos serian de gran provecho.

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, was first discovered in Florida during Jun 1998 (Tsai et al. 2000), and it subsequently dispersed throughout the state (Michaud 2004). *D. citri* has a wide host range within the plant family Rutaceae, including citrus and citrus rela-

tives such as orange jasmine, *Murraya paniculata* (L.) Jack (Halbert & Manjunath 2004). Mature citrus plants fed upon by *D. citri* can sustain damage to growing shoots, while young plants can suffer death during high psyllid populations (Aubert 1987; Michaud 2004). Additionally, *D. citri* vectors the causative bacterial agents (*Candidatus* Liberibacter asiaticus, *C.* L. africanus, and *C.* L. americanus) of citrus greening disease (huanglongbing), one of the world's most serious diseases of citrus (McClean & Schwartz 1970; Bové 2006). Trees infected by this devastating disease may only live 5 to 8 years, during which time they produce misshapen, inedible, and unmarketable fruit (Bové 2006). Halbert & Manjunath (2004) provide a comprehensive overview of citrus greening disease and *D. citri* biology. Citrus greening was discovered in southern Florida during late Aug 2005 and has since been detected at a number of locations across the state's citrus growing region (FDACS 2006). This sets the stage for the spread of the disease into other citrus-producing areas in North America.

A simple and efficient sampling procedure for *D. citri* is vital to the development of a successful IPM program aimed at controlling citrus greening disease. The presence and relative abundance of adult *D. citri* in a planting of citrus or orange jasmine can be determined by counting adults on plant samples (Tsai et al. 2000; Tsai et al. 2002). Adults can be observed by tapping an infested branch with a stick, which promotes adults to drop onto a surface (e.g., a board or pan) held beneath the branch. A similar stem-tapping method has been shown useful for monitoring pear psylla, *Cacopsylla pyricola* (Foerster) in pear (Horton & Lewis 1997). Sticky traps can be used to detect and gauge the relative abundance of *D. citri* (Aubert & Quilici 1988; Aubert & Hua 1990). Preliminary research by Quilici & Trahais (1990) and Aubert & Hua (1990) indicated *D. citri* was more attracted to yellows than other colors, but specific information on the attractiveness of other colors was not presented. Working with sticky traps hung 50 cm above the canopy of an orange jasmine planting, Aubert & Hua (1990) tested sticky Rebell traps (similar to those marketed by Great Lakes IPM, Vestaburg, MI) that were uniformly Saturn yellow, bright yellow, orange yellow, brown yellow, black or white in color and traps that were checkered brown-yellow and bright yellow. These authors did not clarify the difference between Saturn and bright yellow. Brown-yellow colored traps performed best during cloudy weather conditions, while bright yellow functioned best during sunny conditions (Aubert & Hua 1990). A plastic cup trap referred to as the CC trap (named after C. Chu who developed the trap) has been shown to be useful for monitoring thrips, whiteflies and leafhoppers (Chu et al. 2000; Chu et al. 2006), and unidentified adult psyllids have occasionally been caught in these traps in St. Vincent (M. Ciomperlik, unpublished). The Multi-Lure trap (Better World Manufacturing, Inc., Fresno, CA) has been useful in citrus for monitoring fruit flies (Diptera: Tephritidae) (Hall et al. 2005), but its efficacy for monitoring adult *D. citri* is not known.

Although published information indicated yellow to be the most attractive color to adult *D. citri* (Aubert & Hua 1990), quantitative data on the relative attractiveness of blue sticky cards were lacking. Therefore, the purpose of the research presented here was to compare the efficacy of yellow and blue sticky traps, the Multi-Lure trap, and the CC trap along with a stem tapping technique for monitoring adult *D. citri* in citrus.

Materials and Methods

The following traps were compared with respect to numbers of adult *D. citri* trapped weekly: yellow sticky cards, blue sticky cards, Multi-Lure trap (clear top with standard yellow and white base), and 6 CC traps (clear top with a blue, yellow, white, dark green, black, or red base). The yellow sticky cards (7.62 × 12.7 cm) (a bright yellow hue similar to S-G-390 by Behr Process Corp., Santa Ana, CA), blue sticky cards (trimmed to 7.62 × 12.7 cm) (hue similar to 550B-6 by Behr Process Corp.), and Multi-Lure traps were obtained from Great Lakes IPM (Vestaburg, MI). The CC traps were supplied by the Pest Detection Diagnostics and Management Laboratory, Edinburg, TX (USDA, APHIS, Plant Protection and Quarantine, Center for Plant Health Science and Technology). Information on the spectral reflectance of the CC trap colors is provided by Chu et al. (2000). The colors of the CC trap bases were similar to the following Behr Process Corp. hues: blue 3C-20; yellow 310B-6; and red S-G-170. The dark green CC trap base was a hue similar to green 07GG 08/244 by Glidden (Cleveland, OH).

Experiment 1

The study was conducted in a USDA-ARS grove near Ft. Pierce in St. Lucie County, Florida. The block of trees chosen for the study contained 'Hamlin' orange trees (*Citrus sinensis* L.) (4 yr old, ~2 m tall, row spacing 8 m, tree spacing 3 m). No systemic or foliar hard insecticides were applied prior to the study during 2006 or during the course of the study. Each trap was hung near the exterior of a tree canopy about 1.5 m above ground, 1 type of trap per tree. Sixteen trees along each of 5 rows (replications) were randomly assigned one of the traps. Each row consisted of 21 to 40 trees. The test followed a randomized complete block design with 5 replications. The traps were deployed on May 11, 2006, and checked weekly for 4 weeks. At the beginning of

each week, the traps along each row were re-randomized. One set of CC and Multi-Lure traps was charged with 15 mL of a 50% pre-mixed solution of ethylene glycol and water (Super Tech antifreeze, Bentonville, AR) as an entrapment and preservative fluid for adult psyllids, and one set was charged with Hercon Vaportape (10% dichlorvos, 0.229 g ai/cm^2, 2.54 × 4.5-cm strip) (obtained from Great Lakes IPM, Inc., Vestaburg, MI) as a toxicant to kill adults entering a trap. A hole at the center of the top of each CC trap allowed a string to be attached to hang the trap from a branch. No kick plates (Chu et al. 2006) were used with the CC traps in this experiment. Sticky cards were suspended from branches near the outer edge of the canopy with a twist tie. When the CC and Multi-Lure traps were checked for psyllids, all the contents from the traps were emptied into vials and transported to a laboratory. The number of psyllid adults per trap was tabulated weekly. New sticky card traps were deployed each week. The CC and Multi-Lure traps were washed with soap and water each week before they were redeployed in the field.

Data on number of adults per trap per week were analyzed by a multi-observation (measurements over time) analysis of variance, and Tukey's studentized range (honestly significant difference, HSD) was used to determine significant differences ($\alpha = 0.05$) among traps. Prior to these analyses, Levene's test was used to verify homogeneity of variances ($\alpha = 0.05$), and the data were log-transformed where appropriate. The percentage of trees in which adult *D. citri* was detected with each type of trap was computed each week. An analysis of variance over all weeks was conducted on percentage detection (on arcsine square-root-transformed data where appropriate based on Levene's test), and Tukey's studentized range (HSD) was used to determine significant differences ($\alpha = 0.05$) among traps. All analyses of variance were conducted in PROC GLM (SAS Institute, 2002) with the Levene and Tukey options.

In addition to trapping psyllids, adult *D. citri* were monitored with stem tap samples in the same trees in which the above traps were deployed. This allowed a measure of adult abundance in each tree based on both trap and tap samples. A white metal pan (20.32 × 20.32 × 10.16 cm; length, width, and depth, respectively) was held several cm under a haphazardly-chosen branch (1.0-1.5 m above ground), and a polyvinyl chloride (PVC) pipe (0.6 m length, 1.27 cm i.d., 2.13 cm o.d.) was used to tap the branch 3 times. All adult psyllids falling in the pan were counted. Tap sampling was conducted on May 11 when the above traps were deployed and at the end of each week when traps were checked for adult psyllids. The mean number of adult *D. citri* per tap sample was computed for each tree from samples taken at the beginning and end of each sample week. Data were subjected to analyses of variance, and Tukey's studentized range (HSD) was used to investigate for significant differences ($\alpha = 0.05$) in numbers of adults per tap sample among trees assigned the different trap types and to evaluate dispersion of adults among trees. Prior to these analyses, Levene's test was used to verify homogeneity of variances ($\alpha = 0.05$), and the data were log-transformed where appropriate. For trees assigned to each trap type, the percentage of trees in which adult *D. citri* was detected each week with tap sampling was computed. An analysis of variance over all weeks was conducted among trees assigned each type of trap on the percentage of trees in which adult *D. citri* were detected by tap sampling (on arcsine square-root-transformed data where appropriate based on Levene's test). Tukey's studentized range (HSD) was used to determine significant differences ($\alpha = 0.05$) among trees assigned each type of trap with respect to the percentage infested based on tap sampling. All analyses of variance were conducted in PROC GLM (SAS Institute, 2002) with the Levene and Tukey options.

Experiment 2

A second study was conducted near Vero Beach in Indian River County, Florida in a block of 'Temple' orange trees [*C. reticulate* Blanco × *C. sinensis* (L.) Osbeck] (36 yr old, ~3.4 m tall, row spacing 9 m, tree spacing 5 m). No systemic or foliar hard pesticides were applied prior to the study during 2006 or during the course of the study. An application of a nutritional spray including 470 oil (71 L per ha) was applied ~1 h before the traps were placed in the field during the first week of the study. However, the intent of the experiment was to judge relative numbers of adult *D. citri* collected at traps and during tap sampling, not to assess the effects of the treatment against the psyllid. This study was similar to Experiment 1 in all respects except each of the 5 replicates consisted of 3 rows of trees (21 to 26 trees per replicate), and a yellow CC trap with a kick plate (Chu et al. 2006) and charged with ethylene glycol was added to the study. This study was initiated Jun 29, 2006, and ran for 4 weeks.

Results

Experiment 1

Heterogeneity in variances was detected in numbers of adult *D. citri* per trap per week for data over all sample weeks ($F = 3.39$, $Pr > F = <0.0001$, 15 *df*) and for data from week 3 ($F = 2.70$, $Pr > F = 0.0030$, 15 *df*) (analyses on other weeks not presented). Heterogeneity was detected in mean numbers of adults captured from week-to-week for data from blue sticky card traps ($F =$

3.30, Pr > F = 0.05, 3 *df*) but not for data from yellow sticky card traps (F = 1.76, Pr > F = 0.20, 3 *df*) (analyses on other traps not presented). Variances were homogeneous with respect to numbers of adults per stem tap sample for data over all sample weeks (F = 0.92, Pr > F = 0.54, 15 *df*) and for data from week-to-week (F = 0.78, Pr > F = 0.52, 3 *df*). Data analyses on percentages of trees in which adult *D. citri* were detected with traps and stem tap sampling indicated heterogeneity in variances associated with traps (F = 3.32, Pr > F = 0.001, 15 *df*) but not tap sampling (F = 1.47, Pr > F = 0.16, 15 *df*).

There was no significant difference over the 4-week study with respect to numbers of adults captured at yellow and blue sticky card traps (Table 1, F = 4.73, Pr > F < 0.0001, 318 *df*). Each of these sticky card traps captured significantly more adult *D. citri* than any of the other traps. There were no significant differences from week-to-week in captures of adults at yellow sticky traps (F = 1.67, Pr > F = 0.21, 19 *df*) or blue sticky traps (F = 1.60, Pr > F = 0.23, 19 *df*) (data for other traps not presented). Low numbers of adults were captured in the CC and Multi-Lure traps, and there were no significant differences among any of these traps with respect to numbers of adults captured. There was no evidence of any difference with respect to charging CC and Multi-Lure traps with ethylene glycol or dichlorvos strips. Means ± SEM of 1.6 ± 0.1, 1.2 ± 0.1, 1.1 ± 0.1, and 1.3 ± 0.1 adults per stem tap sample were observed across all trees during sample weeks 1, 2, 3 and 4, respectively. There were no significant differences among these weekly means (F = 1.45, Pr > F = 0.22, 39 *df*). Means of 1.6 ± 0.4, 1.0 ± 0.3, 0.7 ± 0.4 and 0.9 ± 0.2 adults per tap sample were observed in trees with yellow sticky traps during weeks 1, 2, 3 and 4, respectively, and means of 2.4 ± 0.7, 1.0 ± 0.3, 2.1 ± 0.5 and 0.6 ± 0.2 adults per tap sample were observed in trees with blue sticky traps during the same respective weeks. Mean numbers of adults per tap sample did not differ significantly from week-to-week in trees with yellow sticky traps (F = 1.67, Pr > F = 0.21, 19 *df*) nor in trees with blue sticky traps (F = 2.55, Pr > F =0.09, 19 *df*) (analyses for tap samples taken in trees with other trap types not presented). No significant differences (F = 1.07, Pr > F = 0.34, 319 *df*) were observed in mean numbers of adult psyllids per tap sample among trees assigned the different types of traps (Table 1). Adult *D. citri* were collected on yellow and blue sticky card traps in every tree sampled (Table 2). Percentage detection of trees infested by adults with the other trap types ranged from 10 to 40% (no significant differences).

TABLE 1. NUMBER OF ADULT *DIAPHORINA CITRI* COLLECTED WEEKLY AT DIFFERENT TRAPS AND DURING WEEKLY TAP SAMPLING IN 'HAMLIN' ORANGE TREES.[a]

	Mean number (SEM) adults per trap per tree[b]					Mean number (SEM) per tap sample per tree[c]
Type of trap in tree[d]	Week 1	Week 2	Week 3[e]	Week 4	Overall[e]	Overall
Yellow sticky card	9.2 (2.4) a	10.8 (4.3) a	3.6 (1.4) a	5.2 (0.9) a	7.2 (1.4) a	1.1 (0.2) a
Blue sticky card	6.2 (0.6) a	6.0 (1.3) ab	4.2 (1.4) a	2.8 (0.7) a	4.8 (0.6) a	1.5 (0.3) a
Yellow CC EG	0.4 (0.4) b	0.8 (0.6) bc	0.6 (0.2) b	0.4 (0.4) b	0.6 (0.2) b	1.0 (0.2) a
Multi-Lure DC	0.8 (0.4) b	1.0 (0.5) bc	0.2 (0.2) b	0.2 (0.2) b	0.6 (0.2) b	1.0 (0.2) a
Blue CC DC	0.6 (0.4) b	1.0 (0.3) bc	0.2 (0.2) b	0.0 (0.0) b	0.5 (0.2) b	1.5 (0.2) a
Red CC EG	0.8 (0.4) b	0.6 (0.2) bc	0.2 (0.2) b	0.0 (0.0) b	0.4 (0.1) b	1.3 (0.3) a
Green CC DC	0.6 (0.2) b	1.0 (0.5) bc	0.0 (0.0) b	0.0 (0.0) b	0.4 (0.2) b	1.1 (0.1) a
Green CC EG	0.6 (0.6) b	0.2 (0.2) c	0.2 (0.2) b	0.0 (0.0) b	0.3 (0.2) b	1.4 (0.2) a
Black CC DC	0.0 (0.0) b	0.2 (0.2) c	0.6 (0.2) b	0.2 (0.2) b	0.3 (0.1) b	1.2 (0.2) a
Black CC EG	0.2 (0.2) b	0.0 (0.0) c	0.0 (0.0) b	0.4 (0.2) b	0.2 (0.1) b	1.2 (0.2) a
White CC EG	0.4 (0.4) b	0.4 (0.2) bc	0.0 (0.0) b	0.0 (0.0) b	0.2 (0.1) b	1.5 (0.2) a
White CC DC	0.0 (0.0) b	0.4 (0.2) bc	0.0 (0.0) b	0.2 (0.2) b	0.2 (0.1) b	1.6 (0.2) a
Yellow CC DC	0.2 (0.2) b	0.2 (0.2) c	0.0 (0.0) b	0.2 (0.2) b	0.2 (0.1) b	1.3 (0.2) a
Red CC DC	0.2 (0.2) b	0.4 (0.4) bc	0.0 (0.0) b	0.0 (0.0) b	0.2 (0.1) b	1.3 (0.2) a
Blue CC EG	0.2 (0.2) b	0.2 (0.2) c	0.0 (0.0) b	0.0 (0.0) b	0.1 (0.1) b	1.3 (0.2) a
Multi-Lure EG	0.2 (0.2) b	0.0 (0.0) c	0.2 (0.2) b	0.0 (0.0) b	0.1 (0.1) b	1.2 (0.2) a

[a]Means in the same column followed by the same letter are not significantly different (α = 0.05), Tukey's test.

[b]For traps—1 trap per tree, 16 trees with traps per replication, 5 replications. Tap sampling was conducted weekly in each tree with a trap.

[c]Weekly mean number of adult *D. citri* observed in tap samples taken in the trees assigned to each specific type of trap.

[d]CC = CC trap; CC and Multi-Lure traps were charged with either EG (ethylene glycol) (15 ml of a 50% solution) or DC (dichlorvos kill strip).

[e]Analyses on log-transformed data, raw means presented.

TABLE 2. PERCENTAGE OF 'HAMLIN' ORANGE TREES IN WHICH ADULT *DIAPHORINA CITRI* WERE DETECTED WITH TRAPS AND STEM TAP SAMPLES.[a]

Type of trap in tree[c]	Mean (SEM) percentage trees in which adults were detected[b]	
	Traps[d]	Tap samples[e]
Yellow sticky card	100.0 (0.0) a	80.0 (8.2) a
Blue sticky card	100.0 (0.0) a	90.0 (5.8) a
Multi-Lure DC	40.0 (4.2) b	80.0 (8.2) a
Yellow CC EG	35.0 (9.6) b	80.0 (8.2) a
Blue CC DC	35.0 (17.1) b	100.0 (0.0) a
Red CC EG	35.0 (15.0) b	85.0 (5.0) a
Green CC DC	30.0 (17.3) b	100.0 (0.0) a
Black CC EG	25.0 (12.6) b	100.0 (0.0) a
Green CC EG	15.0 (5.0) b	85.0 (9.6) a
Black CC DC	15.0 (9.6) b	90.0 (10.0) a
White CC EG	15.0 (9.6) b	95.0 (5.0) a
White CC DC	15.0 (9.6) b	95.0 (5.0) a
Yellow CC DC	15.0 (5.0) b	85.0 (9.6) a
Red CC DC	10.0 (5.8) b	90.0 (10.0) a
Blue CC EG	10.0 (5.8) b	85.0 (9.6) a
Multi-Lure EG	10.0 (5.8) b	80.0 (11.5) a

[a]For traps—1 trap per tree, 16 trees with traps per replication, 5 replications. Tap sampling was conducted weekly in each tree with a trap.

[b]Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$), Tukey's test.

[c]CC = CC trap; CC and Multi-Lure traps were charged with either EG (ethylene glycol) (15 ml of a 50% solution) or DC (dichlorvos kill strip).

[d]Analyses on arcsine square-root transformed percentages (raw percentages presented).

[e]Percentage of trees in which adult *D. citri* were detected in tap samples taken in the trees assigned to each specific type of trap.

Overall, tap sampling indicated 88.8% of the trees studied were infested by adults. There were no significant differences among trees with each type of trap with respect to the percentage identified as being infested by tap sampling (Table 2). Stem tap samples failed to detect a small percentage of infested trees that were identified as being infested by yellow and blue sticky traps.

Experiment 2

Heterogeneity in variances was detected in numbers of adult *D. citri* per trap per week for data over all sample weeks ($F = 4.12$, Pr > F = <0.0001, 16 *df*) and for data from each of the 4 weeks separately (analyses on individual weeks not presented). Heterogeneity was detected in mean numbers of adults captured from week-to-week for data from both blue ($F = 44.19$, Pr > F = <0.0001, 3 *df*) and yellow sticky traps ($F = 8.73$, Pr > $F = 0.001$, 3 *df*) (analyses on other traps not presented). Variances were homogeneous with respect to numbers of adults per stem tap sample for data over all sample weeks ($F = 0.72$, Pr > $F = 0.77$, 16 *df*) and for data from week-to-week ($F = 0.3.1$, Pr > $F = 0.06$, 3 *df*). Data analyses on percentages of trees in which adult *D. citri* were detected indicated no heterogeneity in variances associated with either traps ($F = 0.86$, Pr > $F = 0.61$, 16 *df*) or tap sampling ($F = 1.56$, Pr > $F = 0.12$, 16 *df*).

Yellow sticky card traps captured significantly more adult *D. citri* over the 4-week study than blue sticky card traps, and each of these traps captured significantly more adult *D. citri* over the 4-week study than any of the other traps (Table 3, $F = 2.78$, Pr > F <0.0001, 338 *df*). There were no significant differences in numbers of adults collected each week at yellow and blue sticky traps. One blue sticky card trap was found to be missing when the traps were checked on Jul 20. There were no significant differences from week-to-week in captures of adults at blue sticky traps ($F = 2.14$, Pr > $F = 0.13$, 18 *df*), but significantly greater numbers of adults were collected at yellow sticky traps during weeks 1 and 4 than during weeks 2 and 3 ($F = 3.97$, Pr > $F = 0.02$, 19 *df*) (data for other traps not presented). Numbers of adults captured at the CC and Multi-Lure traps were consistently low, and there were no significant differences among any of these traps with respect to numbers of adults captured. There was no evidence of any difference with respect to charging CC and Multi-Lure traps with ethylene glycol or dichlorvos strips. Means of 2.36 ± 0.2, 1.2 ± 0.1, 0.8 ± 0.1, and 2.2 ± 0.3 adults per stem tap sample were observed across all trees during sample weeks 1, 2, 3 and 4, respectively, and significant differences were found between these weekly means ($F = 2.55$, Pr > $F = 0.03$, 39 *df*). Means of 2.2 ± 0.3, 0.5 ± 0.2, 0.9 ± 0.3 and 2.2 ± 1.0 adults per tap sample were observed in trees with yellow sticky traps during weeks 1, 2, 3 and 4, respectively, and means of 2.9 ± 0.7, 1.1 ± 0.4, 1.6 ± 0.9 and 2.1 ± 0.8 adults per tap sample were observed in trees with blue sticky traps during the same respective weeks. Week-to-week means varied significantly for data from trees with yellow sticky traps ($F = 4.38$, Pr > $F = 0.02$, 19 *df*) but not for data from trees with blue sticky traps ($F = 2.11$, Pr > $F = 0.14$, 18 *df*) (analyses for tap samples taken in trees with other trap types not presented). No significant differences (overall F = 1.35, Pr > $F = 0.04$, 339 *df*; main effect trap F = 0.40, Pr > $F = 0.98$, 16 *df*) were observed in mean numbers of adult psyllids per tap sample among trees assigned the different types of traps (Table 3). Adult *D. citri* were collected on yellow and blue sticky card traps in 95 and 85%, respectively, of the trees sampled (Table 4). Percentage detection of trees infested by adults with the other trap types ranged from 15 to 50% (no significant differences). Overall, tap sampling indicated 81.5% of the trees studied were infested during the study. There were no significant differences among trees

TABLE 3. NUMBER OF ADULT *DIAPHORINA CITRI* COLLECTED WEEKLY AT DIFFERENT TRAPS AND DURING WEEKLY TAP SAMPLING IN 'TEMPLE' ORANGE TREES.[a]

	Mean number (SEM) per trap per tree[b]					Mean number (SEM) per tap sample per tree[c]
Type of trap in tree[d]	Week 1	Week 2	Week 3	Week 4	Overall	Overall
Yellow sticky card	20.6 (4.7) a	5.8 (2.9) a	4.0 (1.6) a	29.0 (13.7) a	14.8 (4.2) a	1.5 (0.3) a
Blue sticky card	10.3 (4.2) ab	4.0 (1.9) ab	2.8 (1.2) ab	5.0 (0.8) ab	5.3 (1.6) b	1.9 (0.4) a
Yellow CC KP EG	3.2 (1.2) bc	0.2 (0.2) c	0.4 (0.2) bc	2.4 (2.2) bc	1.6 (0.6) c	1.6 (0.4) a
Yellow CC EG	1.8 (0.6) cd	0.6 (0.4) bc	0.2 (0.2) bc	1.6 (1.6) c	1.1 (0.4) c	1.7 (0.3) a
Multi-Lure DC	0.8 (0.2) cd	0.4 (0.2) c	0.2 (0.2) bc	1.6 (0.8) bc	0.8 (0.2) c	1.4 (0.2) a
White CC EG	1.0 (0.6) cd	0.2 (0.2) c	0.8 (0.6) bc	0.2 (0.2) c	0.6 (0.2) c	1.8 (0.3) a
Blue CC EG	1.6 (0.6) cd	0.2 (0.2) c	0.2 (0.2) bc	0.2 (0.2) c	0.6 (0.2) c	1.5 (0.4) a
Green CC DC	0.6 (0.4) cd	0.4 (0.2) c	0.2 (0.2) bc	0.8 (0.5) c	0.5 (0.2) c	1.8 (0.5) a
Multi-Lure EG	0.8 (0.2) cd	0.4 (0.2) c	0.2 (0.2) bc	0.4 (0.2) c	0.5 (0.1) c	1.3 (0.3) a
Black CC EG	1.4 (0.9) cd	0.0 (0.0) c	0.2 (0.2) bc	0.2 (0.2) c	0.5 (0.2) c	1.4 (0.3) a
Red CC EG	0.6 (0.4) cd	0.2 (0.2) c	0.2 (0.2) bc	0.4 (0.2) c	0.4 (0.1) c	1.7 (0.4) a
Green CC EG	0.2 (0.2) d	0.8 (0.4) bc	0.0 (0.0) c	0.2 (0.2) c	0.3 (0.1) c	1.4 (0.3) a
Yellow CC DC	0.6 (0.2) cd	0.2 (0.2) c	0.2 (0.2) bc	0.0 (0.0) c	0.3 (0.1) c	1.4 (0.3) a
White CC DC	0.0(0.0) d	0.4 (0.2) c	0.0 (0.0) c	0.6 (0.4) c	0.3 (0.1) c	1.7 (0.7) a
Blue CC DC	0.4 (0.4) cd	0.0 (0.0) c	0.0 (0.0) c	0.6 (0.4) c	0.3 (.01) c	1.7 (0.4) a
Black CC DC	0.6 (0.2) cd	0.0 (0.0) c	0.0 (0.0) c	0.4 (0.4) c	0.3 (0.1) c	2.0 (0.4) a
Red CC DC	0.2 (0.2) d	0.2 (0.2) c	0.0 (0.0) c	0.2 (0.2) c	0.2 (0.1) c	2.0 (0.4) a

[a]Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$), Tukey's test.
[b]For traps—1 trap per tree, 17 trees with traps per replication, 5 replications. Tap sampling was conducted weekly in each tree with a trap. Analyses on log-transformed data, raw means presented.
[c]Weekly mean number of adult *D. citri* observed in stem tap samples taken in the trees assigned to each specific type of trap.
[d]CC = CC trap; KP = kickplate atached; CC and Multi-Lure traps were charged with either EG (ethylene glycol) (15 ml of a 50% solution) or DC (dichlorvos kill strip).

with traps with respect to the percentage identified as being infested by tap sampling (Table 4). Stem tap samples failed to detect a small percentage of infested trees that were identified as being infested by yellow sticky traps. Blue sticky traps failed to identify a small percentage of trees that were identified as being infested by tap sampling.

DISCUSSION

Numerically greater numbers of adult *D. citri* were usually captured each week with yellow sticky card traps than blue sticky card traps, but statistical differences in numbers captured were only found during the second study across all 4 weeks of the study. Significant differences over all study weeks during the first study and during the individual weeks of each study might have been found had we used more than 5 replications of each type of trap. Previous studies indicated yellow sticky traps capture more adult *D. citri* than sticky traps of other colors, and traps of a bright yellow hue captured more adults than traps of a brown yellow hue under sunny conditions (Aubert & Hua 1990). We did not investigate the occurrence of clouds during our studies, but sunlight may have contributed to increased captures of adults at yellow sticky traps during some weeks. Yellow and blue sticky traps were equally effective in detecting the presence of adult *D. citri* in trees given the infestation levels present. The CC and Multi-Lure traps studied captured so few adult psyllids and provided numerically such low levels of percentage detection of trees infested by adults that they appeared to have no value for monitoring *D. citri*. Additional advantages for sticky cards to detect psyllids were that they were inexpensive, readily available, and relatively easy to work with.

Significant fluctuations from week-to-week were observed in numbers of adult *D. citri* collected at yellow sticky traps during the second study, and these fluctuations were reflected in stem tap samples across all trees with traps. We attributed these fluctuations to suppression of psyllids by the spray oil treatment. By the fourth week, developing nymphs had matured to adults, thus contributing to the increased adult population. No significant fluctuations from week-to-week were observed in numbers of adults captured at blue sticky traps during the second study. Reasons were unknown why increased numbers of adult *D. citri* were observed at the end of the second study both at yellow sticky traps and during tap sampling but not at blue sticky traps. These differences may have been related to

TABLE 4. PERCENTAGE OF 'TEMPLE' ORANGE TREES IN WHICH ADULT *DIAPHORINA CITRI* WERE DETECTED WITH TRAPS AND STEM TAP SAMPLES.[a]

Type of trap in tree[c]	Mean (SEM) percentage trees in which adults were detected[b]	
	Traps	Tap samples[d]
Yellow sticky card	95.0 (5.0) a	85.0 (5.0) a
Blue sticky card	85.0 (9.6) ab	95.0 (5.0) a
Yellow CC KP EG	50.0 (17.3) abc	75.0 (15.0) a
Multi-Lure DC	50.0 (12.9) abc	80.0 (11.5) a
Yellow CC EG	40.0 (14.1) bc	80.0 (8.2) a
Multi-Lure EG	45.0 (12.6) bc	75.0 (9.6) a
Blue CC EG	35.0 (15.0) c	70.0 (19.1) a
Green CC DC	35.0 (5.0) c	85.0 (15.0) a
White CC EG	30.0 (5.8) c	85.0 (9.6) a
Red CC EG	30.0 (5.8) c	75.0 (15.0) a
Black CC EG	25.0 (12.6) c	80.0 (8.2) a
Green CC EG	25.0 (12.6) c	75.0 (12.6) a
Yellow CC DC	25.0 (12.6) c	85.0 (9.6) a
White CC DC	20.0 (11.5) c	90.0 (5.8) a
Blue CC DC	20.0 (9.6) c	75.0 (15.0) a
Black CC DC	20.0 (14.1) c	85.0 (15.0) a
Red CC DC	15.0 (5.0) c	90.0 (5.8) a

[a]For traps—1 trap per tree, 17 trees with traps per replication, 5 replications. Tap sampling was conducted weekly in each tree with a trap.

[b]Means in the same column followed by the same letter are not significantly different ($\alpha = 0.05$), Tukey's test.

[c]CC = CC trap; KP = kickplate atached; CC and Multi-Lure traps were charged with either EG (ethylene glycol) (15 ml of a 50% solution) or DC (dichlorvos kill strip).

[d]Percentage of trees in which adult *D. citri* were detected in tap samples taken in the trees assigned to each specific type of trap.

sunlight or other environmental factors that affect the attractancy of the yellow traps more than blue traps. Additionally, adults may actually be less attracted to blue traps but subject to being accidentally captured at these traps during their movement within trees, as supported by the fact that the blue sticky traps caught similar numbers of adults at each study site. However, it remained possible that significant differences might have been found from week-to-week in numbers of adult *D. citri* on blue sticky traps had we studied more than 5 blue traps each week.

We observed some non-target insect species on both yellow and blue sticky traps but did not identify or quantify these. Various species of Diptera including the love bug, *Plecia nearctica* Hardy, have sometimes been captured in large numbers on yellow sticky card traps during other trapping studies in citrus (D. G. Hall, unpublished). The presence of other insects on sticky traps can interfere with finding and counting adult *D. citri* on the traps and may also interfere with captures of *D. citri*. Whether blue traps might have less impact on non-target insects in citrus than yellow traps remains to be investigated. Other researchers have reported that color influences captures of non-target insects. For example, Knight & Miliczky (2003) reported that the choice of trap color affected numbers of honeybee (*Aphis mellifera* L.) and non-target muscoid flies captured at sticky delta traps used to monitor codling moth (*Cydia pomonella* L.).

Our traps were hung directly in citrus trees. Other researchers working with *D. citri* have placed sticky traps on poles near plants or suspended them above plants (Aubert & Hua 1990). Where traps are placed in a citrus tree or grove may affect their relative efficiency for monitoring adult *D. citri* as well as other insects. This was demonstrated by Dowell & Cherry (1981), who reported that the location of sticky traps in citrus trees affected captures of parasitoids and predators of citrus blackfly, *Aleurocanthus woglumi* (Ashmead). Research to establish guidelines for using sticky traps to detect and monitor adult *D. citri*, including numbers of traps to operate and how these traps should be allocated within trees and across an area of trees, would be beneficial to both growers and researchers.

Stem tap sampling was easy to conduct and provided relatively good detection of trees infested by adults, at least at the infestation levels present at the 2 groves. Data from tap sampling indicated adult psyllids were uniformly dispersed among the trees studied, supporting the conclusion that differences in numbers of adults collected at the various types of traps were due to differences in trap efficiency. An obstacle to stem tap sampling was defining the force at which a branch should be hit. Also, some adults flew before falling to the pan, and it was sometimes difficult to count all adults in the pan before they took flight. Although week-to-week fluctuations in mean numbers of adult *D. citri* per tap sample followed the same trend in trees with yellow and blue sticky traps during the second study, differences were only significant for data from trees with the yellow traps. Larger numbers of trees and tap samples per tree may be required for mean estimates with less variability than were obtained with a sample size of 1 tap sample per week in 5 trees. Overall, however, the stem tap sampling method appeared to provide a good relative measure of the presence and abundance of adult *D. citri* and might be improved by placing a cloth (e.g., see Horton & Lewis 1997) or sticky card in the pan. An advantage to stem tap sampling over sticky trap sampling is that tap sampling provides information on the presence and relative abundance of adult *D. citri* during a single visit to a block of trees. Sticky trap sampling requires 2 visits to a block of trees with a period of time between visits (7 d in our study). Captures of non-target insects was less an issue with stem tap sampling to monitor adults than sticky trap sam-

pling. Research to develop formal protocols for tap sampling would be advantageous. Of interest would be optimum numbers of tap samples to take across an area of trees and how these samples should be allocated within and among trees. The ultimate decision of whether to use sticky traps or stem tap sampling for adult *D. citri* in citrus may depend on the intent of sampling and cost. If one is simply interested in whether or not adults are present in trees, stem tap sampling may be preferable, at least at the infestation densities of adults observed during these studies.

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REFERENCES CITED

AUBERT, B. 1987. *Trioza erytreae* Del Guercio and *Diaphorina citri* Kuwayama Homoptera: Psylloidea), the two vectors of citrus greening disease: biological aspects and possible control strategies. Fruits 42: 149-162.

AUBERT, B., AND X. Y. HUA. 1990. Monitoring flight activity of *Diaphorina citri* on citrus and *Murraya* canopies, pp. 181-187 *In* B. Aubert, S. Tontyaporn, and D. Buangsuwon [eds.], Rehabilitation of citrus industry in the Asia Pacific Region. Proc. 4th International Asia Pacific Conference on Citrus Rehabilitation, Chiang Mai, Thailand, 4-10 February 1990. FAO-UNDP, Rome.

AUBERT B., AND S. QUILICI. 1988. Monitoring adult psyllas on yellow traps in Reunion Island, pp. 249-254 *In* L. W. Timmer, S. M. Garnsey, and L. Navarro [eds.], Proc. 10th Conference of the International Organization of Citrus Virologists, Valencia, Spain, 17-21 November 1986. University of California, Riverside.

BOVÉ, J. M. 2006. Huanglongbing: a destructive, newly-emerging, century-old disease of citrus. J. Plant Pathol. 88: 7-37.

CHU, C. C., P. J. PINTER, JR., T. J. HENNEBERRY, K. UMEDA, E. T. NATWICK, Y. WEI, V. R. REDDY, AND M. SHREPATIS. 2000. Use of CC traps with different trap base colors for silverleaf whiteflies (Homoptera: Aleyrodidae), thrips (Thysanoptera: Thripidae), and leafhoppers (Homoptera: Cicadellidae). J. Econ. Entomol. 93: 1329-1337.

CHU, C. C., M. A. CIOMPERLIK, N. T. CHANG, M. RICHARDS, AND T. J. HENNEBERRY. 2006. Developing and evaluating traps for monitoring *Scirtothrips dorsalis* (Thysanoptera: Thripidae). Florida Entomol. 89: 47-55.

DOWELL, R. V., AND R. H. CHERRY. 1981. Survey traps for parasitoids and coccinellid predators of the citrus blackfly, *Aleurocanthus woglumi*. Ent. Exp. & Appl. 29: 356-361.

FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES (FDACS). 2006. http://www.doacs.state.fl.us/pi/index.html.

HALBERT, S. E., AND K. L. MANJUNATH. 2004. Asian citrus psyllids (Sternorrhyncha: Psyllidae) and greening disease of citrus: a literature review and assessment of risk in Florida. Florida Entomol. 87: 330-353.

HALL, D. G., R. E. BURNS, C. C. JENKINS, K. L. HIBBARD, D. L. HARRIS, J. M. SIVINSKI, AND H. N. NIGG. 2005. A field comparison of chemical attractants and traps for Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae), in Florida citrus. J. Econ. Entomol. 98: 1641-1647.

HORTON, D. R., AND T. M. LEWIS. 1997. Quantitative relationship between sticky trap catch and beat tray counts of pear psylla (Homoptera: Psyllidae): seasonal, sex, and morphotypic effects. J. Econ. Entomol. 90: 170-177.

KNIGHT, A. L., AND E. MILICZKY. 2003. Influence of trap colour on the capture of codling moth (Lepidoptera: Tortricidae), honeybees, and non-target flies. J. Entomol. Soc. Brit. Columbia. 100: 65-70.

MCCLEAN, A. P. D., AND R. E. SCHWARTZ. 1970. Greening of blotchy-mottle disease in citrus. Phytophylactica 2: 177-194.

MICHAUD, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biological Control 29: 260-269.

QUILICI, S., AND B. TRAHAIS. 1990. Experiments on color attractivity for the adults of *Diaphorina citri* Kuwayama, pp. 198-202 *In* Proc. 4th International Asia Pacific Conference on Citrus Rehabilitation, Chiang Mai, Thailand 4-10 February 1990. FAO-UNDP, Rome.

SAS INSTITUTE. 2002. SAS Procedures Guide, Version 9. SAS Institute, Cary, NC, USA.

TSAI, J. H., J. J. WANG, AND Y. H. LIU. 2000. Sampling of *Diaphorina citri* (Homoptera: Psyllidae) on orange jessamine in southern Florida. Florida Entomol. 83: 446-459.

TSAI, J. H., J. J. WANG, AND Y. H. LIU. 2002. Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. Florida Entomol. 85: 446-451.

MORTALITY OF *RHAGOLETIS POMONELLA* (DIPTERA: TEPHRITIDAE) EXPOSED TO FIELD-AGED SPINETORAM, GF-120, AND AZINPHOS-METHYL IN WASHINGTON STATE

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ABSTRACT

The effects of field-aged residues of the new semi-synthetic spinosyn insecticide spinetoram (Dow AgroSciences, Indianapolis, IN) and the spinosad bait GF-120 (Dow AgroSciences, Indianapolis, IN) on mortality of apple maggot fly, *Rhagoletis pomonella* (Walsh), were determined in Washington State. Mortality caused by spinetoram (100 g a.i./ha) sprayed on apples and aged 7 d was significantly lower than that caused by fresh spinetoram. Spinetoram (100 and 75 g a.i./ha) aged for 7 d caused less than or as much mortality as spinosad (100 g a.i./ha) aged for 7 d. Fresh spinetoram and azinphos-methyl aged 7 or 14 d caused similar mortality, but aged spinetoram and spinosad caused lower mortality than azinphos-methyl. Apples treated with spinetoram (75 g a.i./ha) and with spinosad aged 7 d and exposed to flies produced a few larvae. However, even though spinetoram (100 g a.i./ha) aged 14 d did not kill all female flies, no larvae emerged from apples sprayed with this treatment. In separate tests, 0-d, 3-d, and 7-d old GF-120 on apple leaves caused greater mortality than 14-d old GF-120. Results show that spinetoram and GF-120 when fresh are highly toxic to *R. pomonella*, but that both have relatively short residual activities under the hot, dry conditions typical of central Washington in summer. Ingredients that prolong their toxicities or make their toxins available to flies longer may be needed to optimize their performance. Also, results suggest that adult fly mortality caused by spinetoram is not an accurate predictor of larval emergence from apples, and that possible non-lethal effects caused by spinetoram need to be examined.

Key Words: apple maggot, bait sprays, spinosyn formulations, organophosphate, residual activity

RESUMEN

Se determinaron los efectos de residuos envejecidos en el campo del insecticida spinosyn semi-sintético spinetoram (Dow AgroSciences, Indianapolis, IN) y el cebo de spinosad GF-120 (Dow AgroSciences, Indianapolis, IN), sobre la mortalidad de la mosca de la manzana, *Rhagoletis pomonella* (Walsh) en el estado de Washington (EEUU). La mortalidad causada por spinotoram (100 g i.a./ha) aplicada en manzanas y madurado por 7 dias fue significativamente más baja que la mortalidad causada por spinetoram fresco. Spinetoram (100 y 75 g i.a./ha) madurado por 7 d causó una mortalidad menor o igual que spinosad (100 g i.a../ha) madurado por 7 d. Spinetoram fresco y azinofosmetíl madurado por 7 o 14 d causó una mortalidad similar, pero el spinetoram madurado y spinosad causaron una mortalidad mas baja que azinofosmetíl. Las manzanas tratadas con spinetoram (75 g i.a./ha) y con spinosad madurado por 7 d y expuestas a moscas producieron muy pocas larvas. Sin embargo, aunque el spinetoram (100 g i.a./ha) madurado por 14 d no mató todas las hembras de moscas, ninguna larva emergió de manzanas rociadas con este tratamiento. En pruebas separadas de GF-120 madurado por 0-d, 3-d y 7-d, en hojas de manzana causó una mayor mortalidad que en GF-120 madurado por 14-d. Los resultados muestran que el spinetoram y GF-120 cuando estén frescos son altamente tóxicos al *R. pomonella*, pero ambos tienen una actividad residual relativamente corta bajo condiciones de alta temperatura y secas típicas del centro del estado de Washington en el verano. Ingredientes que prolongan su toxicidad o hagan que sus toxinas sean disponibles a las moscas por mas tiempo puede ser requeridos para optimizar su desempeño. También, los resultados sugerieron que la mortalidad de las moscas adultas causada por spinetoram no es un pronosticador preciso para la emergencia de larvas de las manzanas, y por otro lado se necesita examinar los posibles efectos no letales causados por spinetoram.

The apple maggot fly, *Rhagoletis pomonella* (Walsh), is a major pest of apple, *Malus domestica* (Borkh.) Borkh., in eastern North America and is an emerging pest in residential apple and hawthorn trees in central Washington State. The fly has established in low numbers in this region,

based on larvae found in hawthorn and apple on residential trees in 2003 and 2004. These findings resulted in export quarantines in areas within Kittitas and Yakima counties (Washington State Department of Agriculture 2005), which are part of the major apple-growing region in central Washington. The establishment of the fly in this region has major economic implications and threatens the export of commercial apples from Washington. The apple industry in Washington was estimated at US$1.11 billion in 2004 (Garibay 2005). There is a zero tolerance for *R. pomonella* larvae in apples transported within the state and to many overseas markets (Washington State Department of Agriculture 2001). Trap captures of *R. pomonella* adults in central Washington result in the spraying of host trees to suppress fly populations and reduce chances that flies will move into commercial apple orchards. Commercial apple orchards in central Washington to date have been free of *R. pomonella*.

Conventional insecticides are considered the leading candidates for controlling *R. pomonella* in central Washington. Currently, the organophosphate imidan (phosmet) is being used in residential trees in this region. Similarly, the organophosphates malathion and azinphos-methyl have been used for many years to control *R. pomonella* in the eastern (Neilson & Maxwell 1964; Neilson & Sanford 1974) and western U.S. (Mohammad & AliNiazee 1989). Despite the effectiveness of organophosphates, alternative materials are increasingly important because of the impending phaseout of organophosphate use due to the federal Food Quality & Protection Act (FQPA) (1996). Because of their relatively high mammalian toxicity, organophosphate insecticides may be hazardous to use, especially around homes or near water where fly-infested apple or hawthorn trees can occur. Effective organophosphates for controlling *R. pomonella* are not available for residential use.

Newer and safer insecticides need to be tested against *R. pomonella*. Laboratory bioassays with the newer insecticides imidacloprid, indoxacarb, pyriproxyfen, spinosad (85% spinosyn A and 15% spinosyn D), thiacloprid, and thiamethoxam showed that imidacloprid reduced oviposition the most and that imidacloprid and spinosad were the most toxic (Reissig 2003). However, these materials were not aged, and none of them appears to equal the organophosphates in toxicity. The residual toxicity after field aging for any material needs to be studied for several reasons. Some materials may be highly toxic initially when fresh but lose that toxicity quickly. This affects the frequency of insecticide applications and spray costs. No study has determined the effects of aging newer materials under the hot, dry central Washington conditions during summer on the toxicity of these materials to *R. pomonella*. In addition, even though resistance has never been documented in *R. pomonella*, overuse of one material invites the potential for increased tolerance to insecticides among non-target pests of apple, such as leafrollers and codling moth. Finally, the negative effects of insecticides on beneficial insects, which probably are exacerbated by frequent insecticide use, are well documented (e.g., Williams et al. 2003).

In this study, the objectives were to determine the effects of aging new or newer spinosyn insecticides, one incorporated into a bait mix, on the mortality of *R. pomonella*. Effects of aging insecticides on damage to apples caused by the flies were also determined. Tests focused on spinetoram, a new semi-synthetic spinosyn insecticide developed by Dow AgroSciences (Indianapolis, IN) that was accepted for expedited review under the United States Environmental Protection Agency's Reduced Risk Pesticide Program. Spinetoram is derived from fermentation products of the soil bacterium *Saccharopolyspora spinosa* Mertz and Yao, has a high safety profile, may have relatively long residual effects, and has never been tested against *R. pomonella*. Tests were also conducted with GF-120 Fruit Fly Bait (Dow AgroSciences, Indianapolis, IN), which contains spinosad, an insecticide that also has a high safety profile (Dow AgroSciences 2002). Results are discussed with respect to residual toxicities of these materials and their potential use in the management of *R. pomonella* in central Washington.

Materials and Methods

Effects of Field-Aging Spinetoram and Other Insecticides on Fly Mortality and Apple Fruit Damage

Experiment 1 compared a control and spinetoram and 2 other insecticides at various rates and ages: (1) untreated control, (2) a 100-g a.i./L suspension concentrate (SC) formulation of spinetoram at 172 mL/935 L water/ha (100 g a.i/ha), aged 0 d, (3) spinetoram at 100 g a.i./ha, aged 7 d, (4) spinetoram at 100 g a.i./ha, aged 14 d, (5) spinetoram at 127 mL/935 L water/ha (75 g a.i./ha), aged 7 d, (6) spinosad (Entrust® 80 WP, Dow AgroSciences, Indianapolis, IN) at 50.6 g/935 L water/ha (100 g a.i./ha), aged 7 d, (7) azinphos-methyl (Guthion® 50 WP, Gowan Company, Yuma, AZ) at 908 g/935 L water/ha (1,121 g a.i./ha), aged 7 d, and (8) azinphos-methyl at 1,121 g a.i./ha, aged 14 d. Spinosad and azinphos-methyl rates fell within recommended field rates. Materials were used within 1 year of receipt from the manufacturer.

'Fuji' apple trees at the United States Department of Agriculture, Agricultural Research Service (USDA, ARS) experimental orchard in Moxee, WA (46°33.23'N, 120°23.50'W) were sprayed with the various insecticide treatments in Jul and Aug 2004 at 7 or 14 d before exposure to flies. Control apples were from 1 tree and 0-d

treatment apples were from another tree. One treatment was sprayed on apples on each of 7 other trees with 1.18 liter RL Flo-Master® pressurized sprayers (Root-Lowell Manufacturing Co., Lowell, MI) until thorough coverage was achieved visually. Three different sets of trees were used or sprayed for 3 tests: test 1A, insecticides aged 13-27 Jul; test 1B, aged 3-17 Aug; and test 1C, aged 31 Aug to 14 Sep. Mean high and low temperatures and precipitation for tests 1A, 1B, and 1C were (1) 33.5°C and 12.4°C and 8 mm, (2) 31.5°C and 13.0°C and 9 mm (over 2 days), and (3) 25.7°C and 8.9°C and 2 mm (on 1 day), respectively. In tests 1A and 1B, rain occurred before 7-d sprays were made and in test 1C, it occurred after 7-d sprays were made. Most days were sunny, with the mean low humidity being ~30% and the daily mean being ~50%.

Three to 6 apples, each from a different branch, were removed from each tree ~5-6 h before testing. Apples were inserted through the calyxes into single upright nails on a board to keep them from rolling and contacting other surfaces and then transported to the Washington State University Research and Extension Center (WSUREC) in Puyallup in western Washington for the experiment. Quarantine restrictions precluded the maintenance of *R. pomonella* in central Washington for testing.

Flies were collected from feral and unmanaged apple trees in Puyallup in glass vials. Flies were maintained on dry 20% yeast extract (EZ Mix, Sigma, St. Louis, MO) and 80% sucrose (wt/wt) on paper strips ('food' hereafter) and on water in cotton wicks inside 3.8-liter cylindrical paper containers (17.5 cm high × 17.0 cm diameter) for up to 2 weeks before tests. This amount of time was needed to accumulate enough flies for tests. For testing, 1 control or treated apple was placed calyx end down on a shallow plastic dish inside a 3.8-liter container with food and water. For the 0-d treatment (fresh spinetoram at 100 g a.i./ha), apples were hung from a tree branch, sprayed, and dried for 1 h before being placed inside containers. Ten flies—6 males and 4 females in test 1A and 5 males and 5 females in tests 1B and 1C—were then transferred into a test container. Adult mortality was recorded from 1 to 10 d after exposure. Flies were recorded as dead if they could not walk when prodded or within 30 s of observation. Water vials were refilled every 2 d. Apple damage was measured as numbers of stings on fruit (from probing or oviposition) and larval emergence from fruit (an indication of larval infestation). Numbers of stings were counted under a microscope and apples were weighed and their circumferences measured at d 10. Each apple was then placed in a clear plastic 550-ml capacity container covered with organdy cloth. Numbers of larvae that emerged from the apples over 4 weeks were recorded. There were 3 replicates in test 1A, 6 in test 1B, and 3 in test 1C. Laboratory test conditions were 20-27°C and 40-50% RH under a 16 h L: 8 h D cycle.

Effects of Field-Aging GF-120 on Fly Mortality

Experiment 2 tested GF-120 Fruit Fly Bait, which is composed of 0.02% spinosad (wt/vol) (Dow AgroSciences 2002) mixed in a bait of Solulys corn protein, sugar, ammonium acetate, propylene glycol, and other ingredients (Thomas & Mangan 2005). An (1) untreated control and four age treatments of 40% GF-120 (vol/vol) (recommended rate, Dow AgroSciences 2002) were compared: (2) 0-d old, (3) 3-d old, (4) 7-d old, and (5) 14-d old. Trees used were 'Fuji' apple trees at the USDA, ARS orchard in Moxee. One tree provided control leaves and another tree provided 0-d treatment leaves. One age treatment was sprayed on each of 3 other trees. Leaves on 60-90 cm lengths of 5 branches of each tree were sprayed with ~10 mL of GF-120 in Jul and Aug 2004 with RL Flo-Master® pressurized sprayers. Two different sets of trees were used or sprayed for 2 tests: test 2A used GF-120 aged from 12-26 Jul and test 2B used GF-120 aged from 2-16 Aug. Mean high and low temperatures and precipitation during the 14 d of aging for tests 2A and 2B were (1) 29.8°C and 14.1°C and 8 mm (1 d), and (2) 31.9°C and 13.1°C and 9 mm (over 2 d), respectively. Most days were sunny, and humidity values were similar to those during aging for experiment 1. Three or 5 leaves, each from a different branch, were removed from each tree and placed inside plastic bags ~5-6 h before testing at the WSUREC in Puyallup.

Flies were held and tested inside 473-ml paper cartons (7 cm high × 5 cm diameter) (Neptune Paper Products, Newark, NJ) covered with organdy cloth and provided with food and water in the laboratory. Flies used in test 2A were reared from hawthorn fruit collected in 2003. Pupae had been chilled for 6 months at 4°C and then transferred to 20-27°C for adult emergence. Adults were held for 2-4 weeks before testing. Flies used in test 2B were collected from apple trees in Puyallup and held for 2-3 weeks before testing. For testing, a single control or treated leaf was placed inside a carton. The 0-d old treatment was a leaf sprayed with 1 mL of GF-120 1 h before testing. Ten flies (5 of each sex) were then introduced. Mortality was recorded daily, except for d 5 and 6 for both tests (weekends), up until d 10. There were 3 replicates of the control and treatments in test 2A and 5 replicates of each in test 2B. Laboratory test conditions were 25-27°C and ~40-50% RH under a 16 h L: 8 h D cycle.

Data Analyses and Statistics

Repeated-measures analysis of variance (ANOVA) was conducted on mortality data. One-

way ANOVA also was conducted on mortality data within days and on apple injury data. Data from d 1-4, 7, and 10 and from d 1-4 and 7-10 in experiments 1 and 2, respectively, are presented and analyzed. Because there were few replicates in tests 1A and 1B and because weather conditions during insecticide aging in these tests were similar, data from these were pooled for analyses. Test 1C was kept separate because of weather differences. Tests 2A and 2B also were pooled because there were few replicates per test and because weather conditions during GF-120 aging were similar. Percentages were square-root and arcsine-transformed before analyses. Sting and larval counts + 1 were subjected to square-root transformation. Means in one-way ANOVA were separated by using the Tukey test (SAS Institute 2001). This conservative test was chosen because of the high numbers of pairwise comparisons made.

RESULTS

Effects of Field-Aging Spinetoram and Other Insecticides on Fly Mortality and Apple Fruit Damage

Repeated-measures ANOVA indicated there were treatment and day differences, but there were also significant treatment × day interactions (tests 1A and 1B, $F = 16.5$, $df = 7, 408$, $P < 0.0001$; test 1C, $F = 17.0$, $df = 7, 126$, $P < 0.0001$), indicating patterns of mortality among the treatments differed on various days, even though mortality increased over time across all treatments. In tests 1A and 1B (Table 1), one-way ANOVA of data within days showed mortality caused by spinetoram (100) aged 7 d was significantly lower than that caused by fresh spinetoram (100), up until d 7. There were no differences in mortalities caused by spinetoram (100) aged 7 or 14 d until d 4-10, when 7-d old residues caused greater mortality. Spinetoram (75) aged 7 d was similar to spinetoram (100) aged 7 d but caused greater mortality than spinetoram (100) aged 14 d at d 4-10. Spinosad (100) aged 7 d caused greater mortality than spinetoram (100 and 75) aged 7 d at d 2 and 3, but caused similar mortality on other days. Fresh spinetoram usually was not different from aged azinphos-methyl within days. Mortality in tests 1A and 1B and in test 1C differed on 2 of the 6 d, and test number × treatment interactions occurred on 3 of the days (two-way ANOVA, $P < 0.05$). In test 1C (Table 2), similar to tests 1A and 1B, mortality caused by spinetoram (100) aged 7 d was significantly lower than that caused by fresh spinetoram, up until d 4-10. Also similar, in test 1C there were significantly greater mortalities caused by spinetoram (100) aged 7 than 14 d, this time on all 6 d of exposure, and spinetoram (75) aged 7 d caused lower mortality than spinetoram (100) aged 7 d on all days except d 10. Similar to tests 1A and 1B, spinetoram (75) aged 7 d caused greater mortality than spinetoram (100) aged 14 d at d 3 and 10. Unlike in tests 1A and 1B, however, in test 1C, spinosad (100) aged 7 d caused lower mortality than spinetoram (100) aged 7 d at d 1 and not other days, and greater mortality than spinetoram (75) aged 7 d at d 3-7, but not on other days. Fresh spinetoram usually was not different from aged azinphos-methyl within days.

Apples were smaller in tests 1A and 1B than in test 1C, but similar differences were seen in each analysis with respect to numbers of stings in ap-

TABLE 1. TESTS 1A AND 1B: MEAN CUMULATIVE PERCENT MORTALITY ± SE OF *RHAGOLETIS POMONELLA* EXPOSED CONTINUOUSLY TO 0-, 7- OR 14-D OLD INSECTICIDES ON APPLE FRUIT AT 1-10 D AFTER EXPOSURE IN THE LABORATORY.

Insecticides field-aged 13-27 Jul and 3-7 Aug 2004

Treatment		D 1	D 2	D 3	D 4	D 7	D 10
Control		0.0 ± 0.0 c	0.0 ± 0.0 d	0.0 ± 0.0 d	0.0 ± 0.0 d	2.2 ± 2.2 d	6.7 ± 5.5 c
Spinetoram (100) 0 d		36.7 ± 7.8 b	96.7 ± 1.7 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Spinetoram (100) 7 d		6.7 ± 2.4 c	14.4 ± 3.4 c	36.7 ± 8.7 c	47.8 ± 10.8 c	73.3 ± 11.9 ab	85.6 ± 9.3 a
Spinetoram (100) 14 d		5.6 ± 2.4 c	5.6 ± 2.4 cd	7.8 ± 4.3 cd	10.0 ± 4.4 d	24.4 ± 5.8 c	36.7 ± 7.1 b
Spinetoram (75) 7 d		5.6 ± 1.8 c	14.4 ± 5.0 c	28.9 ± 9.2 c	42.2 ± 9.8 c	67.8 ± 10.9 b	82.2 ± 6.8 a
Spinosad (100) 7 d		11.1 ± 4.8 c	43.3 ± 8.7 b	67.8 ± 11.5 b	72.2 ± 11.5 bc	91.1 ± 7.7 ab	95.6 ± 4.4 a
Azinphos-methyl (1,121) 7 d		76.7 ± 2.4 a	92.2 ± 1.5 a	93.3 ± 1.7 ab	95.6 ± 1.8 ab	98.9 ± 1.1 a	100.0 ± 0.0 a
Azinphos-methyl (1,121) 14 d		73.3 ± 3.7 a	94.4 ± 2.4a	95.6 ± 2.4a	98.9 ± 1.1a	100.0 ± 0.0 a	100.0 ± 0.0 a
One-Way ANOVA	*F*	47.1	77.6	41.1	39.0	34.1	44.9
df = 7, 64	*P*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Tests pooled, 9 replicates of 10 flies each (test 1A: 6 males, 4 females, 3 replicates; test 1B: 5 of each sex, 6 replicates); 100 = 100 g a.i./ha; 75 = 75 g a.i./ha; 1,121 = 1,121 g a.i./ha.

Means followed by the same letter within days (columns) are not significantly different (Tukey test, $P > 0.05$).

TABLE 2. TEST 1C: MEAN CUMULATIVE PERCENT MORTALITY ± SE OF *RHAGOLETIS POMONELLA* EXPOSED CONTINUOUSLY TO 0-, 7- OR 14-D OLD INSECTICIDES ON APPLE FRUIT AT 1-10 D AFTER EXPOSURE IN THE LABORATORY.

Insecticides field-aged 31 Aug-14 Sep 2004

Treatment		D 1	D 2	D 3	D 4	D 7	D 10
Control		0.0 ± 0.0 c	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 c	0.0 ± 0.0 c	3.3 ± 3.3 c
Spinetoram (100) 0 d		66.7 ± 8.8 a	96.7 ± 3.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Spinetoram (100) 7 d		20.0 ± 10.0 b	53.3 ± 8.8 bc	86.7 ± 3.3 b	90.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Spinetoram (100) 14 d		0.0 ± 0.0 c	0.0 ± 0.0 e	10.0 ± 0.0 d	33.3 ± 8.8 b	46.7 ± 13.3 b	53.3 ± 17.6 b
Spinetoram (75) 7 d		0.0 ± 0.0 c	16.7 ± 12.0 de	33.3 ± 3.3 c	56.7 ± 12.0 b	73.3 ± 12.0 b	90.0 ± 10.0 a
Spinosad (100) 7 d		0.0 ± 0.0 c	43.3 ± 3.3 cd	90.0 ± 0.0 b	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Azinphos-methyl (1,121) 7 d		80.0 ± 10.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a
Azinphos-methyl (1,121) 14 d		56.7 ± 6.7 a	86.7 ± 3.3 ab	93.3 ± 3.3 b	93.3 ± 3.3 a	100.0 ± 0.0 a	100.0 ± 0.0 a
One-way ANOVA	*F*	45.1	49.5	190.0	70.6	69.7	27.3
df = 7, 16	*P*	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Three replicates of 10 flies each (5 of each sex); 100 = 100 g a.i./ha; 75 = 75 g a.i./ha; 1,121 = 1,121 g a.i./ha.
Means followed by the same letter within days (columns) are not significantly different (Tukey test, $P > 0.05$).

ples and numbers of larvae that emerged from apples (Tables 3 and 4). In tests 1A and 1B (Table 3), spinetoram and other insecticides significantly reduced numbers of stings and numbers of larvae per apple by 90 to 100%. No larvae emerged from the spinetoram (100) treatments, including the 14-d old treatment, even though it killed only 36.7% of flies by d 10, with some females alive at that time. One larva emerged from an apple treated with spinetoram (75) and a total of 3 larvae emerged from 2 apples treated with spinosad. In test 1C (Table 4), there was a tendency toward reduced numbers of stings in all treatments (although not significant, according to the Tukey test) and all treatments prevented larval emergence from apples, including again from the spinetoram (100) aged 14 d treatment, even though it killed only 53.3% of flies.

Effects of Field-Aging GF-120 on Fly Mortality

Mortalities caused by 0-7 d old GF-120 were high, especially as days after exposure to flies increased (Table 5). Repeated-measures ANOVA resulted in a significant treatment × day interaction ($F = 22.0$, $df = 4, 303$, $P < 0.0001$), indicating the

TABLE 3. TESTS 1A AND 1B: MEAN APPLE SIZE AND APPLE DAMAGE ± SE CAUSED BY *RHAGOLETIS POMONELLA* EXPOSED CONTINUOUSLY TO 0-, 7- OR 14-D OLD INSECTICIDES ON APPLE FRUIT IN THE LABORATORY.

Insecticides field-aged 13-27 Jul and 3-7 Aug 2004

		Apple size		Apple damage	
Treatment		Weight (g)	Circ. (cm)	No. stings/apple	No. larvae/apple
Control		70.6 ± 5.8	16.9 ± 0.4	18.3 ± 6.0 a	3.9 ± 2.4 a
Spinetoram (100) 0 d		78.4 ± 8.7	17.3 ± 0.7	0.3 ± 0.2 b	0.0 ± 0.0 b
Spinetoram (100) 7 d		74.2 ± 7.5	17.1 ± 0.6	1.0 ± 0.5 b	0.0 ± 0.0 b
Spinetoram (100) 14 d		77.8 ± 6.8	17.5 ± 0.6	1.9 ± 0.3 b	0.0 ± 0.0 b
Spinetoram (75) 7 d		61.6 ± 5.8	16.2 ± 0.5	0.4 ± 0.2 b	0.1 ± 0.1 b
Spinosad (100) 7 d		67.3 ± 5.0	16.8 ± 0.5	0.9 ± 0.7 b	0.3 ± 0.2 ab
Azinphos-methyl (1,121) 7 d		82.5 ± 4.7	17.9 ± 0.4	0.1 ± 0.1 b	0.0 ± 0.0 b
Azinphos-methyl (1,121) 14 d		72.4 ± 4.4	17.2 ± 0.3	0.0 ± 0.0 b	0.0 ± 0.0 b
One-way ANOVA	*F*	—	—	14.8	2.7
df = 7, 64	*P*	—	—	<0.0001	0.0159

Tests pooled, 9 replicates of 10 flies each (test 1A: 6 males, 4 females, 3 replicates; test 1B: 5 of each sex, 6 replicates); 100 = 100 g a.i./ha; 75 = 75 g a.i./ha; 1,121 = 1,121 g a.i./ha. Circ., circumference.
Means followed by the same letter within apple damage measures (columns) are not significantly different (Tukey test, $P > 0.05$).

TABLE 4. TEST 1C: MEAN APPLE SIZE AND APPLE DAMAGE ± SE CAUSED BY *RHAGOLETIS POMONELLA* EXPOSED CONTINUOUSLY TO 0-, 7- OR 14-D OLD INSECTICIDES ON APPLE FRUIT IN THE LABORATORY.

Insecticides field-aged 31 Aug to 14 Sep 2004

Treatment		Apple size		Apple damage	
		Weight (g)	Circ. (cm)	No. stings/apple	No. larvae/apple
Control		112.8 ± 5.8	20.4 ± 0.1	24.3 ± 19.8	8.3 ± 6.3 a
Spinetoram (100) 0 d		111.7 ± 3.0	19.9 ± 0.2	0.0 ± 0.0	0.0 ± 0.0 b
Spinetoram (100) 7 d		113.5 ± 9.1	20.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.0 b
Spinetoram (100) 14 d		119.1 ± 7.0	20.4 ± 0.4	0.0 ± 0.0	0.0 ± 0.0 b
Spinetoram (75) 7 d		111.5 ± 4.7	19.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0 b
Spinosad (100) 7 d		101.5 ± 5.7	19.4 ± 0.5	1.0 ± 0.6	0.0 ± 0.0 b
Azinphos-methyl (1,121) 7 d		123.0 ± 3.2	20.5 ± 0.3	0.0 ± 0.0	0.0 ± 0.0 b
Azinphos-methyl (1,121) 14 d		127.3 ± 6.2	20.8 ± 0.3	0.0 ± 0.0	0.0 ± 0.0 b
One-Way ANOVA	*F*	—	—	2.8	3.0
df = 7, 16	*P*	—	—	0.0412	0.0312

Three replicates of 10 flies each (5 of each sex); 100 = 100 g a.i./ha; 75 = 75 g a.i./ha; 1,121 = 1,121 g a.i./ha. Circ., circumference. Means followed by the same letter within apple damage measures (columns) are not significantly different (Tukey test, $P > 0.05$).

pattern of mortality among treatments differed at various days after exposure. There was almost no control mortality, large increases in mortality in 0-7 d old treatments, and small increases in mortality in the 14-d old treatment over time. One-way ANOVA indicated that the 14-d old GF-120 was not any more effective than the control until d 7 after exposure, and was never as effective as the 0-3 d old GF-120 during the 10 d (Table 5).

DISCUSSION

In experiment 1, we showed that the new insecticide spinetoram is highly toxic to *R. pomonella* when fresh, on a similar level to that of azinphos-methyl aged 7 to 14 d, but that its toxicity decreases rapidly after 7 d of aging in the field under the sunny, hot, and dry conditions typical of central Washington in Jul and Aug. By 14 d, spinetoram was no longer toxic to *R. pomonella*, except after 3 to 7 d of continuous exposure. Thus, spinetoram at 100 g a.i./ha would probably need to be applied at < 7-d intervals to be effective. The relatively short period of activity (compared with azinphos-methyl) suggests that spinetoram breaks down quickly in central Washington conditions and that ultraviolet light blocking or other agents need to be improved to prolong its toxicity. Also, it is possible that spinetoram was absorbed into plant tissue which occurs with spinosad (Dow

TABLE 5. TESTS 2A AND 2B: MEAN CUMULATIVE PERCENT MORTALITY ± SE OF *RHAGOLETIS POMONELLA* EXPOSED CONTINUOUSLY TO 0-, 3-, 7- OR 14-D OLD 40% GF-120 ON APPLE LEAVES AT 1-10 D AFTER EXPOSURE IN THE LABORATORY.

GF-120 Field-Aged 12-26 Jul and 2-16 Aug 2004

Days after Exposure	Age of GF-120 on apple leaves					One-way ANOVA	
	Control	0 D	3 D	7 D	14 D	*F* (*df* = 4, 35)	*P*
1	0.0 ± 0.0 b	47.5 ± 5.9 a	27.5 ± 4.9 a	25.0 ± 3.3 a	8.8 ± 4.0 b	23.3	<0.0001
2	0.0 ± 0.0 b	77.5 ± 8.2 a	55.0 ± 13.2 a	50.0 ± 9.6 a	12.5 ± 5.3 b	15.6	<0.0001
3	0.0 ± 0.0 b	86.3 ± 5.0 a	70.0 ± 9.1 a	75.0 ± 6.3 a	16.3 ± 6.3 b	30.6	<0.0001
4	0.0 ± 0.0 b	88.8 ± 4.8 a	78.8 ± 6.7 a	83.8 ± 5.6 a	17.5 ± 5.9 b	43.2	<0.0001
7	0.0 ± 0.0 c	100.0 ± 0.0 a	93.8 ± 3.2 a	96.3 ± 1.8 a	38.8 ± 12.0 b	73.1	<0.0001
8	0.0 ± 0.0 c	100.0 ± 0.0 a	98.8 ± 1.3 a	97.5 ± 1.6 a	42.5 ± 11.6 b	114.3	<0.0001
9	0.0 ± 0.0 c	100.0 ± 0.0 a	100.0 ± 0.0 a	97.5 ± 1.6 a	50.0 ± 14.4 b	68.3	<0.0001
10	1.3 ± 1.3 c	100.0 ± 0.0 a	100.0 ± 0.0 a	100.0 ± 0.0 a	53.8 ± 15.8 b	47.6	<0.0001

Tests pooled, 8 replicates of 10 flies each (5 of each sex).
Means followed by the same letter within days after exposure (rows) are not significantly different (Tukey test, $P > 0.05$).

AgroSciences 2004), making it unavailable to flies over time. If so, ingredients that prevent rapid absorption into leaves may help prolong its effectiveness. Spinosad in other studies shows decreased residual activity at 3-7 d after application (Williams et al. 2003), apparently similar to spinetoram. This suggests the different formulations of these 2 spinosyn insecticides do not affect the durations of their toxicity. Spinetoram used at rates described in this study might also be more effective if incorporated into a bait mix, similar to spinosad in GF-120, so that flies ingest more of it. Also, if a fly ingests degraded toxin, it may lead to quicker mortality than a fly that repeatedly contacts the degraded toxin.

The increases in mortality in spinetoram and other insecticide treatments over the 10 d of the tests suggest flies repeatedly contacted the apples as days progressed or that there was a delayed effect from 1 or a few initial contacts with the insecticide. Repeated contacts inside a cage may result in an overestimate of expected mortality under field conditions (Barry & Polavarapu 2005). Repeated contacts or a delayed effect may explain why fresh spinetoram was more toxic than spinetoram aged 7 d at d 1-4 and not 7 and 10 (tests 1A and 1B) or d 1-3 and not d 4-10 (test 1C).

Other results of tests 1A and 1B and of 1C were similar, with one key difference being the relative effectiveness of spinetoram versus spinosad. Overall results suggest spinetoram at 75 g a.i./ha is less effective than at 100 g a.i./ha, perhaps because the smaller amount is broken down more quickly than the larger amount, and that 75 g a.i./ha aged 7 d is more effective than 100 g a.i./ha aged 14 d, so aging may be more critical to effectiveness than the amount. In the one key difference, in tests 1A and 1B, spinosad at 100 g a.i./ha aged 7 d was more effective at d 2 and 3 than spinetoram at 100 g a.i./ha aged 7 d, but in test 1C, spinetoram was more effective than spinosad at d 1 and similar on all other days. The difference could be a result of warmer temperatures or ultraviolet light having a greater negative impact on spinetoram than spinosad over 7 d (5.8-7.8 ºC warmer in tests 1A and 1B than in test 1C). Another conclusion is that 100 g a.i./ha of spinosad, like this amount of spinetoram, is more effective than 75 a.i./ha spinetoram. Azinphos-methyl seemed less affected by warm temperatures or ultraviolet light than spinetoram and spinosad, based on its high effectiveness even after 14 d of aging.

The insecticides in this study did not kill *R. pomonella* adults quickly enough to prevent them from stinging apples. This included azinphos-methyl, albeit only one sting was detected in azinphos-methyl-treated fruit in all 3 tests combined. The inability of insecticides to prevent stinging or oviposition by *R. pomonella* agrees with earlier work with azinphos-methyl (Reissig et al. 1983) and indicates that insecticides must be applied before females are reproductively mature. Protection of apples from stings will therefore be problematic if treatment trees are surrounded by infested trees. However, overall results show that, despite the inability of spinetoram (100 g a.i./ha) to prevent stings, mortality and apple injury data did not lead to the same conclusions concerning effectiveness. Even though 14-d old spinetoram killed only 36.7 and 53.3% of flies after 10 d of exposure, the numbers of stings on the 14-d old spinetoram-treated apples were reduced 90 and 100% compared with controls, and no larvae emerged from any of these apples. This suggests there were non-lethal effects that reduced oviposition (some females were still alive at 10 d), including a repellent effect, or that flies stung the apples but did not oviposit. Eggs or larvae in fruit also may have been affected by spinetoram if it was absorbed into fruit tissue. Thus, mortality of adults is not the only variable to consider when evaluating the ability of spinetoram and the other insecticides to control *R. pomonella*. We did not cut apples and examine them for larvae. This could have resulted in the detection of internal damage in apples that had stings. It is possible some larvae died inside the fruit and did not emerge. If so, our data underestimated larval infestation rates.

In experiment 2 with GF-120, spinosad, similar to spinetoram, was highly toxic when fresh, but appeared to break down or was absorbed into leaves between 7-14 d under sunny, hot, and dry conditions. Whether bait components of GF-120 can be modified to protect spinosad against ultraviolet rays or to prevent rapid absorption into leaves under these conditions needs study. Results suggest that GF-120 in its present form needs to be applied every 7 d to be effective. Against the walnut husk fly, *Rhagoletis completa* Cresson, 20% GF-120 aged for only 3 d in hot weather in California lost toxicity (Van Steenwyk et al. 2003). The inconsistency of GF-120 in controlling *R. pomonella* in the eastern U.S.—ineffective in New York (Reissig 2003) and effective in 1 of 2 years in Michigan (Pelz et al. 2005)—may be caused in part by its short residual activity or by the high precipitation in the regions where it was tested. Residual toxicity of GF-120 in the eastern U.S. may more likely be reduced by rainfall than by dry conditions and high temperatures.

Our overall results indicate spinetoram and GF-120 when fresh are highly toxic to *R. pomonella* but that their residual toxicities need to be prolonged to optimize their performance against flies in central Washington. Direct comparisons of spinetoram and GF-120 are needed to determine whether one holds more promise than the other for fly control. Tests with both at higher rates, in different bait formulations, and in different spray volumes are needed to determine if they can be used in a management program for *R. pomonella*. Aged spinetoram is less toxic against adult flies than

azinphos-methyl, but it and GF-120 are more benign to the environment. Also, results suggest that adult fly mortality caused by spinetoram is not an accurate predictor of larval emergence from apples, and that possible non-lethal effects caused by spinetoram need to be examined.

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REFERENCES CITED

BARRY, J. D., AND S. POLAVARAPU. 2005. Feeding and survivorship of blueberry maggot flies (Diptera: Tephritidae) on protein baits incorporated with insecticides. Florida Entomol. 88: 268-277.

DOW AGROSCIENCES. 2002. Specimen Label, revised 11-27-02. GF-120 Naturalyte Fruit Fly Bait. Indianapolis, IN.

DOW AGROSCIENCES. 2004. Spinosad Technical Bulletin. Indianapolis, Indiana. 7 pp.

FOOD QUALITY & PROTECTION ACT. 1996. U.S. Congressional Record 142: 1489-1538.

GARIBAY, R. 2005. Washington's 2004 apple crop approaches record level. Washington agricultural statistics service. USDA, Olympia, Washington. www.nass.usda.gov/wa/apples.pdf. accessed 25 January 2005.

MOHAMMAD, A. B., AND M. T. ALINIAZEE. 1989. Malathion bait sprays for control of apple maggot (Diptera: Tephritidae). J. Econ. Entomol. 82: 1716-1721.

NEILSON, W. T. A., AND C. W. MAXWELL. 1964. Field tests with a malathion bait spray for control of the apple maggot, *Rhagoletis pomonella*. J. Econ. Entomol. 57: 192-194.

NEILSON, W. T. A., AND K. H. SANFORD. 1974. Apple maggot control with baited and unbaited sprays of azinphos-methyl. J. Econ. Entomol. 67: 556-557.

PELZ, K. S., R. ISAACS, J. C. WISE, AND L. J. GUT. 2005. Protection of fruit against infestation by apple maggot and blueberry maggot (Diptera: Tephritidae) using compounds containing spinosad. J. Econ. Entomol. 98: 432-437.

REISSIG, W. H., B. H. STANLEY, M. E. VALLA, R. C. STEEN, AND J. B. BOURKE. 1983. Effects of surface residues of azinphosmethyl on apple maggot behavior, oviposition, and mortality. Environ. Entomol. 12: 815-822.

REISSIG, W. H. 2003. Field and laboratory tests of new insecticides against the apple maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). J. Econ. Entomol. 96: 1463-1472.

SAS INSTITUTE. 2001. SAS/STAT user's guide, version 8. Cary, NC.

THOMAS, D. B., AND R. L. MANGAN. 2005. Nontarget impact of spinosad GF-120 Bait sprays for control of the Mexican fruit fly (Diptera: Tephritidae) in Texas citrus. J. Econ. Entomol. 98: 1950-1956.

VAN STEENWYK, R. A., S. K. ZOLLBROD, AND R. M. NOMOTO. 2003. Walnut husk fly control with reduced risk insecticides, *In* B. Beers [ed.], Proceedings of the 77th Annual Western Orchard Pest & Disease Management Conference, Portland, Oregon. Washington State University, Pullman. 5 pp. http://entomology.tfrec.wsu.edu/wopdmc/proceedings2003.html. accessed 19 October 2006.

WASHINGTON STATE DEPARTMENT OF AGRICULTURE. 2001. Washington Administrative Code 16-470-108. Distribution of infested or damaged fruit is prohibited. http://agr.wa.gov/ accessed 15 August 2005.

WASHINGTON STATE DEPARTMENT OF AGRICULTURE. 2005. Washington Administrative Code 16-470-105. Area under order for apple maggot—Pest free area—Quarantine areas. http://agr.wa.gov/ accessed 15 August 2005.

WILLIAMS, T., J. VALLE, AND E. VIÑUELA. 2003. Is the naturally derived insecticide spinosad® compatible with insect natural enemies? Biocontrol Sci. Technol. 13: 459-475.

LOW-DOSE IRRADIATION PHYTOSANITARY TREATMENT AGAINST MEDITERRANEAN FRUIT FLY (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the most important quarantine pests in the world. Host commodities shipped from infested parts of the world to non-infested parts that might be susceptible to infestation should undergo a phytosanitary measure to render negligible the risk of shipping viable flies. Ionizing irradiation is a promising phytosanitary treatment that is tolerated by the great majority of hosts of the Mediterranean fruit fly. The current dose in the US is 150 Gy. This research conducted with cage-infested 'Haden' mangoes in Peru showed that 100 Gy is sufficient to provide a high level of quarantine security against this important pest. That dose did not affect pupation when applied to late 3rd instars, but it did prevent any from emerging as adults. A dose of 100 Gy might allow for irradiation of avocados, one of the few fruits that does not tolerate more than 100-200 Gy.

Key Words: *Ceratitis capitata*, ionizing irradiation, quarantine treatment, disinfestation

RESUMEN

La mosca mediterránea de la fruta, *Ceratitis capitata* (Wiedemann), es una de las plagas cuarentenarias más importantes en el mundo. Las materias primas hospederos embarcadas desde regiones del mundo infestadas con destino a zonas no infestadas que podrían ser susceptibles de infestación deberían ser sometidas a una medida fitosanitaria que permita eliminar el riesgo de estos embarques. La radiación ionizante es un tratamiento fitosanitario prometedor y tolerable por la gran mayoría de hospederos de la mosca mediterránea de la fruta. La dosis corriente en los Estados Unidos es 150 Gy. La presente investigación conducida con mangos infestados en jaulas en Perú mostró que 100 Gy son suficientes para proporcionar un nivel alto de seguridad cuarentenaria contra esta importante plaga. La mencionada dosis no afectó el proceso de empupar cuando se aplicó en larvas del tercer instar, pero si evitó la emergencia a adultos. Una dosis de 100 Gy podría adecuarse para la irradiación de aguacates, una de las pocas frutas que no tolera más de 100-200 Gy.

Translation provided by the authors.

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), may be considered the premier quarantine pest in the world. It occurs in much of Africa and southern Europe, most of Latin America from southern Mexico to Argentina, parts of Australia and the Middle East, and Hawaii. Non-infested countries from the tropics into temperate regions place quarantines on a broad range of fresh fruit hosts from infested regions. The host list includes most commercial sweet tree-fruits, avocados, tomatoes, peppers, cotton bolls, walnut fruits, and coffee berries. Over 250 hosts have been listed. Even poor hosts are quarantined because they may carry enough Mediterranean fruit fly individuals for the pest to reproduce and become established.

Much effort and money are spent preventing, managing, and eradicating the Mediterranean fruit fly throughout the world. Millions of sterile males per week are released in Florida, California, and other areas as a preventative measure. Thousands of survey traps are maintained in many countries. The pest has been found and eradicated 7 times in Florida and individuals are found almost every year in California. The United States (US), Mexico, and Guatemala collaborate in trying to eradicate the fly from Mexico and parts of Guatemala. The Mediterranean fruit fly was officially eradicated from Chile in 1995 and cooperation with Peru aims to prevent its reintroduction into Chile as well as achieve eradication in southern Peru.

Phytosanitary treatments may be required to export hosts from Mediterranean fruit fly-infested areas to non-infested areas that could support establishment of the pest. Several treatment options are available. Holding at 1.1-2.2°C for 14-18 d is used to disinfest tangerines shipped from Spain to the US. Methyl bromide fumigation is used for various fruits. Immersion of mangoes in water at 46.1°C for 65-110 min (depending on

shape, mass, and origin of the mangoes) is used to facilitate shipment of mangoes from Latin America to several countries. Heated air is used to facilitate shipment of papayas from Hawaii to Japan and the mainland US. Ionizing irradiation is used for shipment of several fruits from Hawaii to the mainland US.

From the standpoint of fruit quality, irradiation is the most broadly applicable commercial treatment at the doses for tephritid fruit flies (Hallman 2007). It also has advantages over other treatments; e.g., very few external variables affect treatment efficacy and it can be applied after packing and palletizing.

The chief disadvantage of irradiation is that, unlike all other commercially-applied treatments, it does not cause acute mortality but renders insects unable to complete development and/or reproduce. Although preventing development or reproduction is sufficient to prevent the establishment of invasive species, it does not provide inspectors with a simple and reliable independent verification of treatment efficacy, i.e., dead insects. Correct and complete conduction of the research supporting the treatment, robust certification that treatments are done adequately, and careful protection of the treated lots from re-infestation are necessary to ensure commercial viability of phytosanitary irradiation treatments. Irradiation has been used commercially since 1995 for interstate disinfestation of perishable commodities of several pests including tephritids within the US and since 2004 to disinfest mangoes shipped from Australia to New Zealand of tephritids without insurmountable incident.

In the US a minimum absorbed dose of 150 Gy is allowed for any fruit against any tephritid fruit fly (APHIS 2006). Very few fruits do not tolerate that dose applied on a commercial scale, which could be up to 2.5 times the minimum absorbed dose (Hallman & Loaharanu 2002). The reason commercial doses may be up to 2.5 times the minimum prescribed dose is that commercial irradiation facilities may treat pallet-loads and irradiation diminishes as the distance from the source increases. In order to get the minimum absorbed dose required to the farthest fruit in a pallet-load, the nearest fruit may receive a much higher dose.

Because it is such a significant quarantine pest Mediterranean fruit fly has received much phytosanitary attention. For example in the book, Invasive Arthropods in Agriculture: Problems and Solutions (Hallman & Schwalbe 2002), Mediterranean fruit fly is mentioned more than any other arthropod. It is also the most studied pest regarding irradiation phytosanitary treatments. Thirteen studies provide sufficient data to estimate doses required for quarantine security with varying levels of security (Table 1), the most for any quarantine pest. Reasoning for many of the doses listed in Table 1 is given in Hallman (1999).

TABLE 1 MINIMUM IONIZING RADIATION DOSE TO PREVENT ADULT EMERGENCE FROM MEDITERRANEAN FRUIT FLY THIRD INSTARS IN FRUIT ACCORDING TO VARIOUS STUDIES LISTED IN CHRONOLOGICAL ORDER

Dose (Gy)	Fruit	Reference
225	Papaya	Seo et al. (1973)
>200	Orange	Fésüs et al. (1981)
~80	Mango	Potenza et al. (l989)
~80	Mango	Raga (1990)
~80	Peach	Arthur et al. (1993a,b)
~70	Grapefruit	Raga (1996)
~200	Orange	Adamo et al. (1996)
40[1]	Peach, orange	Mansour & Franz (1996)
150	Mango	Bustos et al. (2004)
100[1]	Papaya	Follett & Armstrong (2004)

[1]Fruit infestation involved rearing larvae in diet and inserting them into fruit 24-30 h before treatment.

The literature suggests 2 conflicting peak doses for providing quarantine security against Mediterranean fruit fly, one at 70-100 Gy and the other at 200-225 Gy (Table 1). Hallman & Loaharanu (2002) argue that the upper peak is not well supported and could be dismissed. The currently accepted dose for this pest in the US is 150 Gy (APHIS 2006), but this may not be the minimum absorbed dose that could prevent adult emergence of 3rd instar Mediterranean fruit fly in fruit as evidenced by several studies in Table 1.

Two large-scale studies used Mediterranean fruit fly 3rd instars reared in diet and then inserted in fruit 24-30 h before treatment. Mansour & Franz (1996) obtained no adult emergence when >100,000 3rd instars were reared in diet and then placed in peaches and oranges 30 h prior to irradiation with 40 Gy. Follett and Armstrong (2004) found no adult emergence when 31,920 3rd instars were reared in diet and placed in papayas 24 h before irradiation with 100 Gy. Follett and Armstrong obtained 0.47% emergence of normal-looking adults when 3rd instars were irradiated with 40 Gy and 0.07% at 50 Gy, doses that provided complete prevention of adult emergence in the similar study by Mansour & Franz (1996).

For any phytosanitary treatment, infestation that differs significantly from the natural situation should be tested for relative tolerance to the natural situation. If the semi-artificial technique results in increases in pest tolerance, it would not be of phytosanitary concern, although the treatment may be harsher on the commodity than it need be. But if the semi-artificial infestation increases susceptibility, phytosanitary security will be at risk. Hypoxia reduces radiosusceptibility of organisms (Hallman & Hellmich 2007), and tephritid immatures inside the hypoxic atmosphere of fruit seem to show increased tolerance (Hall-

man & Worley 1999). Lack of hypoxic protection may explain why 40 Gy prevented Mediterranean fruit fly adult emergence in >100,000 third instars reared in diet and placed in peaches and oranges 30 h before irradiation (Mansour & Franz 1996). A higher dose was required using the same techniques with papayas (Follett & Armstrong 2004). Perhaps a hypoxic atmosphere was easier to achieve and maintain in papayas after artificial infestation compared with peaches and oranges making work with papayas more akin to natural conditions, at least in this case.

The most rigorous standard used for confirming the efficacy of a phytosanitary treatment is "probit 9" at the 95% confidence level (Hallman & Loaharanu 2002). Probit 9 represents the effective dose (ED) to achieve a result at the 99.9968 percentile ($ED_{99.9968}$). This entails treating 93,600 individuals with no survivors when done to a confidence level of 95% (Couey & Chew 1986). Efficacy of an irradiation phytosanitary treatment against tephritids is measured by the prevention of the emergence of adults capable of flight when irradiated as 3rd instars inside fruit (Hallman & Loaharanu 2002).

Although Follett & Armstrong (2004) found that 100 Gy would probably control Mediterranean fruit fly in the system that they studied (diet-reared larvae placed in papaya a day before irradiation), they did not do a "probit 9"-level study because their goal was to find a single dose that would control all quarantined tephritid fruit flies in Hawaii. They did not try to make the dose for all fruit flies in Hawaii lower than 150 Gy because their studies indicated that one tephritid, melon fly, *Bactrocera cucurbitae* (Coquillet), would not be controlled to the "probit 9" level with <150 Gy.

To save resources and reduce the potential negative effect of a phytosanitary treatment on commodities the effective dose for any treatment should be made as low as possible. In the case of ionizing irradiation and the Mediterranean fruit fly Hallman & Loaharanu (2002) observed that doses to control all *Anastrepha* fruit flies studied were similar and about 70 Gy achieved a high level of control. They argued that enough phytosanitary research had been done with the neotropical genus to permit a dose of 70 Gy for all *Anastrepha*. Four studies that included both *Anastrepha* spp. and Mediterranean fruit fly showed the latter to require about 1.4× the dose to achieve the same effect against *Anastrepha*. Therefore, if 70 Gy is sufficient for *Anastrepha* then 70 Gy × 1.4 or ~100 Gy should provide a high level of control of Mediterranean fruit fly.

The objective of the research was to determine the dose at the 95% confidence level, beginning at 100 Gy that would prevent the emergence of adults capable of flight when irradiated as 3rd instar Mediterranean fruit flies inside fruit.

Materials and Methods

The flies used in this research were from the colony in the sterile insect release program in La Molina, Peru. About 16,500 Mediterranean fruit fly adults (sex ratio about 1:1) were maintained in each of 3 cages (0.6 × 2 × 0.35 m). They were fed a mixture of sugar, protein, and water. About 60-80 mature green, freshly picked 'Haden' mangoes (0.3-0.5 kg) were placed in each cage for 24 h at about 27°C with constant lighting. The resulting mean infestation rate was about 45 larvae/fruit. Upon removal from the infestation cages mangoes were kept at 27°C until the flies developed to late 3rd instars (9-10 days); mangoes were periodically opened to observe fly development. The test was repeated until at least 93,600 third instars were irradiated at 100 Gy, which would satisfy "probit 9" at the 95% confidence level (Couey & Chew 1986).

When most Mediterranean fruit flies had developed to the 3rd instars 90% of the infested mangoes were irradiated with a cobalt-60 source (model Gammabeam 127, Nordion, Kanata, Canada) that was delivering a dose rate of 2.8 Gy/minute. Dosimetry was done with the Fricke system (ASTM 2006). Timing of irradiation was set so that the maximum dose measured did not exceed the target dose. At that timing the minimum absorbed dose was about 87 Gy when the maximum was 100 Gy. The 10% non-irradiated mangoes were held as controls. After irradiation the mangoes were held at 25°C on a bed of moist sawdust to absorb fluids leaking from the fruit and serve as a burrowing and pupariation medium for emerging larvae. After 5-6 d the mangoes were examined for remaining larvae, both dead and alive. All larvae and puparia were collected, counted, and held at 25°C until after adults emerged. Tests continued until a minimum of 96,400 third instars were irradiated with no adults emerging. If adults capable of flight (fully extended wings) were found the dose would be raised depending on the failure rate and the testing begun anew.

It is expected that Mediterranean fruit fly 3rd instars will largely pupariate at 100 Gy, although adult emergence should be extremely low. Statistical significance of pupariation between irradiated and control 3rd instars was tested via a two-tailed, paired *t*-test (Prism 4, GraphPad Software, San Diego, CA).

Results and Discussion

After 9 tests consisting of 7-12 thousand irradiated 3rd instars each, a total of 99,562 Mediterranean fruit fly 3rd instars were irradiated in mangoes with no adults emerging; 88.5% of these 3rd instars pupariated. Pupariation rate of the control was 90.7%, but it was not significantly different from the irradiated 3rd instars ($t = 0.98$, df

= 8, P = 0.36) showing that 100 Gy did not affect the ability of Mediterranean fruit fly late 3rd instars to pupariate. Adult emergence from control puparia was 86.9%.

This research shows that phytosanitary irradiation at an absorbed minimum dose of 100 Gy provides quarantine security against Mediterranean fruit fly to the highest degree demanded of a commercial phytosanitary treatment, $ED_{99.9968}$ at the 95% confidence level. Almost all hosts of the pest would tolerate this treatment applied on a commercial scale. Even avocado, which has low tolerance to any phytosanitary treatment, such as those based on fumigation, heat, or cold (McDonald & Miller 1994), might have a viable treatment against Mediterranean fruit fly with 100 Gy. Avocado tolerates about 100-200 Gy (Thomas 2001), and the dose uniformity ratio (maximum absorbed dose divided by minimum absorbed dose) expected in commercial irradiation facilities can vary anywhere from 1.2-2.5, depending on the source and arrangement of irradiated product.

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REFERENCES CITED

ADAMO, M., V. D'LLIO, F. GIONFRIDDO, P. NOBILI, A. PASQUALI, E. POSTORINO, G. ROSSI, AND F. ZARBO. 1996. Le technologie di ionizzazione per frutti di arancio infestati da *Ceratitis capitata*. L'Informatore Agrario 52: 73-75.

APHIS. 2006. Treatments for fruits and vegetables. Federal Register 71: 4451-4464.

ARTHUR, V. C. CACERES, F. M. WIENDL, AND J. A. WIENDL. 1993a. Controle da infestação natural de *Ceratitis capitata* (Wied., 1824) (Diptera, Tephritidae) em pêssegos (*Prunus persica*) através das radiações gama. Sci. Agric., Piracicaba 50: 329-332.

ARTHUR, V., F. M. WIENDL, AND J. A. WIENDL. 1993b. Controle de *Ceratitis capitata* (Wied., 1824) (Diptera, Tephritidae) em pêssegos (*Prunus persica*) infestados artificialmente através da radiação gama do cobalto-60. Rev. de Agricultura, Piracicaba 68: 323-330.

ASTM. 2006. Standard practice for using the Fricke reference-standard dosimetry system. E1026-04e1. American Soc. for Testing and Materials, Conshohocken, PA.

BUSTOS, M. E., W. ENKERLIN, J. REYES, AND J. TOLEDO. 2004. Irradiation of mangoes as a postharvest quarantine treatment for fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 97: 286-292.

COUEY, H. M., AND V. CHEW. 1986. Confidence limits and sample size in quarantine research. J. Econ. Entomol. 79: 887-890.

FÉSÜS, I., L. KÁDAS, AND B. KÁLMÁN. (1981) Protection of oranges by gamma radiation against *Ceratitis capitata* Wied. Acta Alimentaria 10: 293-299.

FOLLETT, P. A., AND J. W. ARMSTRONG. 2004. Revised irradiation doses to control melon fly, Mediterranean fruit fly, and oriental fruit fly (Diptera: Tephritidae) and a generic dose for tephritid fruit flies. J. Econ. Entomol. 97: 1254-1262.

HALLMAN, G. J. 1999. Ionizing radiation quarantine treatments against tephritid fruit flies. Postharvest Biol. Technol. 16: 93-106.

HALLMAN, G. J. 2007. Phytosanitary measures to prevent the introduction of invasive species, pp. 367-384 *In* W. Nentwig [ed.], Biological Invasions. Springer, Berlin.

HALLMAN, G. J., AND R. L. HELLMICH. 2007. Modified atmosphere storage may reduce efficacy of irradiation phytosanitary treatments. Acta Horticulturae (in press).

HALLMAN, G. J., AND P. LOAHARANU. 2002. Generic ionizing radiation quarantine treatments against fruit flies (Diptera: Tephritidae) proposed. J. Econ. Entomol. 95: 893-901.

HALLMAN, G. J., AND C. SCHWALBE (eds.). 2002. Invasive Arthropods in Agriculture: Problems and Solutions. Science Publishers, Enfield, NH.

HALLMAN, G. J., AND J. W. WORLEY. 1999. Gamma radiation doses to prevent adult emergence from immatures of Mexican and West Indian fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 92: 967-973.

MANSOUR, M., AND G. FRANZ. 1996. Gamma radiation as a quarantine treatment for the Mediterranean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 89: 1175-1180.

POTENZA, M. R., S. T. YASUO-KA, R. B. P. GIORDANO, AND A RAGA. 1989. Irradiação de frutos de laranja infestados com larvas da mosca das frutas *Ceratitis capitata* (Weid., 1824) (abstract). Congresso Brasileiro de Entomologia, 1989, Belo Horizonte.

RAGA, A. 1990. Uso da Radiação Gama na Desinfestação de Mangas Destinadas À Exportação em Relação À *Ceratitis capitata* (Wied., 1824), *Anastrepha fraterculus* (Weid., 1830) e *Anastrepha obliqua* (Macquart, 1835) (Diptera, Tephritidae). MSc Thesis. Univ. São Paulo.

RAGA, A. 1996. Incidência de Mosca-das-Frutas em Café e Citros e Tratamento Quarentenário de Frutos Cítricos com Radiação Gama. Ph.D. Dissertation. Univ. São Paulo.

SEO, S.T., R. M. KOBAYASHI, D. L. CHAMBERS, D. M. DOLLAR, AND M. HANAOKA. 1973. Hawaiian fruit flies in papaya, bell pepper, and eggplant: quarantine treatment with gamma irradiation. J. Econ. Entomol. 66: 937-939.

THOMAS, P. 2001. Irradiation of fruits and vegetables, pp. 213-240 *In* R. Molins [ed.] Food Irradiation Principles and Applications. Wiley Interscience, New York.

THE THERMAL ENVIRONMENT OF IMMATURE CARIBBEAN FRUIT FLIES, *ANASTREPHA SUSPENSA* DIPTERA: TEPHRITIDAE)

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ABSTRACT

Because many plants regulate their internal temperatures, there is no *a priori* reason to believe air temperature accurately reflects the temperatures faced by tephritid larvae inhabiting fruit interiors. Larvae also move across and burrow into soil to pupate, and immature flies at this point are also likely to encounter temperatures that might be less than or exceed air temperature. Using thermocouples and a computerized data logger we measured a range of temperatures in the 4 major hosts of *Anastrepha suspensa* (Loew), the Caribbean fruit fly: (Surinam cherry, *Eugenia uniflora* L., Cattley guava, *Psidium cattleianum* Sabine, guava, *Psidium guajava* L., and loquat, *Eriobotrya japonica* (Thunb.)), and in grapefruit, *Citrus paradisi* Macf., an economically important secondary host. Generally, temperatures were higher in the southwestern portions of tree canopies relative to those in the northeastern interiors. Fruit on the ground was warmer than in the tree, but there was no significant pattern of maximum fruit core temperatures being warmer than subcutaneous pulp. Soil temperatures were also higher than fruit-in-tree temperatures, and decreased and displayed less variance with increasing depth. Fruit in trees seldom reached temperatures ±0.05 of air temperatures, but fruit on the ground could be more than 0.25 the adjacent air temperature. There were positive relationships between the ratio of mean and minimum fruit temperature/adjacent air temperature and fruit diameter. Information on the temperatures confronted by immature fruit flies can be used to model population dynamics, and to design temperature sensitive strains through conditional gene expression for mass-rearing and release.

Key Words: larvae, pupae, heat, cold, conditional gene expression

RESUMEN

Debido a que muchas plantas regulan su temperatura interna no hay una razón *a priori* para creer que la temperatura ambiental refleja es precisamente la temperatura enfrentada por las moscas tefrítidos que habitan el interior de las frutas. Las larvas a su vez cruzan y escavan en el suelo para empupar, y las moscas inmaduras en este punto también son mas propicias para encontrar temperaturas que pueden ser menos o más alta que la temperatura ambiental. Usando un termoeléctrico y una grabadora de datos computerizados, nosotros medimos el rango de temperaturas en 4 de los hospederos mas importantes de *Anastrepha suspensa* (Loew): (*Eugenia uniflora* L., *Psidium cattleianum* Sabine, *Psidium guajava* L., y *Eriobotrya japonica* (Thunb.)), y en toronja, *Citrus paradisi* Macf. que es un hospedero segundario de importancia económica. En general las temperaturas más altas fueron en las áreas suroeste de las copas de los árboles en relación con las de la parte interior de los árboles en el noreste. Las frutas en el suelo estaban más calidas que las frutas en el árbol, pero no hubo un patrón significativo en la temperatura máxima del interior de la fruta siendo mas caliente que la pulpa subcutánea. Las temperaturas del suelo también fueron más altas que las temperaturas del fruto en el árbol, y diminuyeron y mostraron menos variación con el aumento de la profundidad. Las frutas en el árbol raramente alcanzaron temperaturas de ±0.05 de temperatura ambiental, pero la temperatura de la fruta en el suelo pudo ser más alta de 0.25 que la temperatura ambiental adyacente. Hubo una relación positiva entre la razón del promedio y la temperatura mínima de la fruta/la temperatura ambiental adyacente y el diámetro de la fruta. Información sobre las temperaturas enfrentadas por los estados inmaduros de las moscas de la fruta puede ser usada para hacer un modelo de la dinámica de la población, y para diseñar razas sensibles a la temperatura por medio de la expresión genética condicional para la cría y liberación masiva.

Plants are relatively sessile and exposed to whatever light falls upon them. Characteristics of their parts, such as shapes that minimize surface areas to volumes and high moisture contents,

might evolve for a number of reasons, but can also result in internal temperatures quite different from the surrounding air. This includes fruit, particularly larger species such as apples, which in full sunlight, can be 14°C warmer than an ambient temperature of 27°C (Thorpe 1974). Nor are plants necessarily passive in terms of heat and cold, but rely on several non-behavioral mechanisms to regulate their temperatures. These commonly include (1) emission of infra-red radiation, (2) heat conduction and convection, and (3) evaporative cooling (e.g., Jones 1992; Nobel 1999; Roth-Neblesick 2001). The thermal consequences of various adaptations can be substantial. For example, the alpine cushion plant, *Silene acaulis* spp. *excapa* [All.] J. Braun, and its relatives exploit, among other things, a small, prostrate growth form to avoid heat-loss into the atmosphere and reach temperatures 15-25°C above ambient (Neuner et al. 2000). Flowers of the sacred lotus, *Nelumbo nucifera* (L.) Druce, can maintain temperatures up to 10°C below effective ambient through evaporative cooling (Seymor & Shultze-Motel 1998), as can the leaves of the perennial *Phragmites communis* (Cav.) Trin. (Percy et al. 1972). Fig fruit, *Ficus* spp., in sunlight have temperatures no more than 2-3°C above ambient, but reach temperatures 3-8°C above ambient when an experimentally applied oil coating prevents their evaporative cooling through transpiration (Patiño et al. 1994).

Organisms that inhabit the interior tissues of plants, such as the eggs and larvae of frugivorous tephritid fruit flies, are also relatively limited in their ability to move to different environments to regulate their body temperatures. To a substantial degree they must tolerate the temperature they encounter within the confines of the fruit they infest. However, given the capacity of some plants to maintain temperatures different from the ambient and the variety of lighting that exists within most tree canopies (Aluja & Birke 1993; Aluja et al. 2000; Sivinski et al. 2004), the range of thermal environments encountered by fruit fly larvae may be considerable and is largely undescribed.

The difficulty in simply estimating the temperature faced by immature fruit flies through extrapolation from air temperature is further complicated by the pupation behavior of the larvae that typically exit fallen fruit to pupate in the soil at depths of near-surface to more than 5 cm (e.g., Hodgson et al. 1998). Soil temperatures are known to vary with depth (e.g., Hillel 1982), season and microhabitat (Thomas 1993, 1995).

A better description of tephritid thermal environments would yield several benefits. Temperature is a critical component in modeling population dynamics (e.g., Meats 1981). In addition, proposed new autocidal techniques for tephritid control and eradication rely on temperature sensitivity in offspring (Handler 2002, 2004). Mass-reared and released males would carry genes that, when expressed in immature offspring, result in death after a certain temperature is reached. Such a scheme would avoid the sterilizing radiation believed to diminish male sexual success and which may compromise the Sterile Insect Technique (=SIT) (Lux et al. 2003). The success of the Conditional Gene Expression Technique (=CGE) could be optimized by predicting the minimum and maximum temperatures eggs and larvae are likely to encounter in different locations within the canopies of different hosts fruiting at different times of the year.

The model tephritid we considered was the Caribbean fruit fly, *Anastrepha suspensa* (Loew). Originally from the Greater Antilles, it was accidentally introduced into southern Florida during the mid-1960s and subsequently spread over ~2/3 of the state's peninsular region (Baranowski et al. 1993). Larvae develop in over 90 species of fruit (Norrbom & Kim 1988), but a smaller number of roughly sequentially-fruiting hosts are characteristically the most highly infested. These include: Surinam cherry, *Eugenia uniflora* L. (typically late spring-early summer), Cattley guava, *Psidium cattleianum* Sabine (typically mid-late summer), guava, *Psidium guajava* L. (typically late summer-early autumn), and loquat, *Eriobotrya japonica* (Thunb.) (typically late winter-early spring) (Sivinski et al. 1999). In addition, a number of citrus species are attacked, including grapefruit, *Citrus paradisi* Macf. (Simpson 1993). The temporal distribution and size differences among these fruit suggest that larvae confront considerable within-year variance in temperature (Sivinski et al. 2004).

The present study documented the temperatures near the surfaces and at the cores of the primary hosts (+ grapefruit) in and under tree canopies as they occurred in several geographical locations within the range of the fly. In addition, the temperatures of mature and fallen fruit were measured in the field, as were soil temperatures at several likely pupation depths. Particular attention was given to the minimum and maximum temperatures since these may be important in the distribution/abundance of the fly and its parasitoids (Eitam et al. 2004) and in the design of CGE systems. Finally the relationship of fruit temperatures to air temperature was determined so that the temperature of larval habitats might be estimated by making relatively simple air temperature measurements.

Materials and Methods

Sampling Procedure

Four sets of fruit and 4 sets of soil temperatures were obtained from each host tree, this number determined by the capacity of the measuring and data logging device. Ripe intact fruit on the tree were chosen from what would typi-

cally be those portions of the canopy most and least exposed to sunlight, one on the southwestern exterior and one in the northeastern interior, respectively. Intact, fallen fruit were placed on the soil under the southwest portion of the canopy along an imaginary line extending down from the canopy margin in order to maximize exposure to sunlight. Intact, fallen fruit under the northeast portion of the canopy were placed <0.5 the distance between the canopy margin and the trunk in order to minimize exposure to sunlight.

Thermocouple devices to measure temperature were placed in 2 locations in each piece of fruit, 1 directly under the skin/rind and another as close as possible to the center. For fruit in trees, thermocouple wires were supported by 1 or more twists of wire attached to branches. In some cases, relatively large seeds prevented absolute-center measurements, but regardless, the range of locations potentially occupied by larvae within the fruit pulp was taken into account. A drop of cyanoacrylate gel glue was used to hold thermocouples in the fruit, and cover the wound. Soil temperature measurements were taken within 10 cm of the fruit at 5 depths: on the surface directly under the fruit, at 5 mm, 15 mm, 25 mm, and 50 mm. Air temperature was obtained from within 2 cm above each piece of fruit examined. Tabular data describing actual temperatures in various microhabitats consist of first 24 h of data alone when fruit condition presumably most closely resembled the undisturbed state.

To compare the temperature of larval fruit-microhabitats to local air temperatures, ratios of fruit temperature over air temperature were calculated as follows. Minimum, maximum, and average fruit temperatures from a particular microhabitat (subcutaneous or core, tree canopy or ground, southwest canopy, or northeast canopy) were divided by the minimum, maximum, or average air temperatures recorded directly above the fruit for the same period of time. Because fruit size might influence the thermal dynamics of fruit, these ratios are presented graphically in relation to the log of fruit diameter. In order to balance the needs of maximizing the data set while at the same time minimizing deterioration of the fruit, only the first 3 days of data were considered for comparisons with air temperature regardless of how long the thermocouples were in place.

Temperature Measuring Device

Temperatures were measured by 32 Type T thermocouples 18.29 m in length which were inserted into fruit and soil at the depths described above. The thermocouple consist of shielded thermocouple wire with factory manufactured measuring junctions 1 mm in diameter and covered in Omega Bond (OB-101), a high thermally conductive epoxy to prevent corrosion due to fruit acids. All the thermocouples measuring air temperature were shielded from the effect of thermal radiation by a small sheet of highly reflective aluminum foil.

The thermocouples were connected to a Campbell Scientific CR-10 Datalogger through a Campbell Scientific AM 416 Relay Multiplexer. A thermocouple reference thermistor was wired to the CR-10 datalogger to provide temperature compensation and power was provided by a 12-V car battery. A fifth-order polynomial, resident in the datalogger, converts the EMF to temperature in Celsius. Although calibrated by the manufacturer with an accuracy of ±0.5°C, the 32 thermocouples connected to the Multiplexer and datalogger were left to acclimate in the lab and their readings compared the reference thermistor and the internal datalogger temperature. All the readings were within the accuracy provided by the manufacturer.

The datalogger and multiplexer were housed in UV protected-plastic box (45 cm × 30 cm) to protect them from the elements. Temperature data were obtained every min, and averaged and stored every 30 min.

Fruit Tree Locations

Trees were chosen on the basis of being as isolated as possible so that sunlight on the canopy was unimpeded by neighboring plants. The additional necessity of being secure enough to leave unattended computer equipment resulted in the use of different numbers of trees of the various species. In several cases, as noted in the individual descriptions of the sites, different fruit were later sampled on the same tree to obtain a second data set. All sites were within the perennially-occurring range of *A. suspensa* (Baranowski et al. 1993).

Citrus paradisi. (Two trees, 2 sets of temperature measurements/tree for a total of 4 sets of measurements, dates started: 30-Oct-03, 6-Nov-03, 20-Nov-03, 2-Dec-03): Near Dundee, Florida, Polk County, Florida (28°17'1"N, 81°62'2"W; soil in the area is described as Candler-Tavares-Apopka: excessively drained, moderately drained and well drained, sandy soils underlain by loamy or clayey material; USDA 1990a).

Eriobotrya japonica. (Two trees, 2 sets of temperature measurements on one tree and 1 set on the other for a total of 3 sets of measurements, dates started: 2-Mar-04, 11-Mar-04, 26-Mar-04): Ft. Pierce, Florida, St. Lucie County, (27°44'6"N, 80°32'5"W; soil is described as Waveland-Lawnwood: poorly drained soil, sandy throughout with dark subsoil weakly cemented; USDA 1980).

Eugenia uniflora. (Two trees with 2 sets of temperature measurements on each tree for a total of 4 sets of measurements, dates started: 9-Apr-04, 16-Apr-04, 21-Apr-04, 5-May-04): LaBelle, Florida, Hendry County (26°44'6"N, 80°32'5"W; soil is described as Holopaw-Basinger association: poorly drained or very poorly drained, sandy,

loamy and organic soils that have a loamy subsoil; USDA 1990b).

Psidium cattleianum. (Three trees, 1 set of temperature measurements/tree for a total of 3 sets of measurements, starting dates: 17-July-03, 2-Aug-03, 22-Aug-03): LaBelle, Florida, Hendry County (soil described as Oldsmar-Wabasso association: poorly drained, sandy soils that have a sandy and loamy subsoil with organic staining in the sandy layers; USDA 1990b) and Clewiston, Florida, Hendry County (26°45'12"N, 80°56'1"W; soil is described as Margate association: poorly drained, sandy soils that are underlain by limestone; USDA 1980).

Psidium guajava. (One tree, 1 set of temperature measurements, starting date: 29-Aug-03): LaBelle, Florida, Hendry County (see *P. cattlieanum* above).

Statistical Analyses

Mean, minimum, and maximum temperatures were initially and individually compared on the basis of species, location (within the canopy and on the ground), and the interaction of these 2 variables with SAS (proc GLM) (SAS Inst., Inc., Raleigh, NC). Where applicable, means were compared through analysis of variance followed by the Waller separation of means test (proc ANOVA). Paired comparisons of temperatures fruit in and under particular portions of tree canopies were made by the nonparametric Wilcoxon paired-sample test (Zar 1974). Regressions of fruit diameter to minimum, maximum and mean temperatures were performed with SAS (proc GLM) (SAS Inst., Inc., Raleigh, NC).

Results

As suspected, our "thermal snapshots" demonstrated that immature *A. suspensa* within fruit and in the soil confront a range of temperatures over both a seasonal and spatial scale (Tables 1 and 2). The following are some noteworthy points about this thermal diversity.

Effect of Location In and Under the Canopy on Temperature Maxima and Minima

The maximum fruit temperatures were significantly higher in and under the southwest portion of the canopies. Fruit in the southwest portions of the canopies reached significantly higher temperatures than those in the northeast (Table 3) as did fallen fruit along the southwest margins of the canopy. However, there were no significant differences in the mean and minimum temperatures of fruit in or under the southwestern and northeastern portions of the canopies. This was probably due to maxima occurring during daylight hours with more light striking fruit on the margins of the southwest canopy, while minima occurring during the night when location was relatively unimportant.

Tree species consistently and significantly influenced mean, minimum, and maximum fruit temperatures, but any interspecific differences in fruit and canopy morphology co-occurred with seasonal variation in temperature. However, because there were no significant interactions between tree species and the sites of the fruit within their canopies it is reasonable to assume that tree morphologies were homogeneous relative to seasonal temperature differences.

Within Fruit Differences in Temperature

There was relatively little difference in mean, minimum, or maximum temperatures measured under the surface of fruit and at their cores and no significant pattern in those temperature differences that did occur (Tables 1, 2 and 3). Concentrating on the southwest portion of the trees where temperatures were consistently more extreme, neither fruit still in the tree or on the ground had warmer subcutaneous than core temperatures.

Effect of Remaining on the Tree and Falling on the Ground on Maximum and Minimum Temperatures

In the southwest portion of the canopy, maximum core fruit temperatures were higher in fallen fruit on the ground than, in some case as much as 15°C hotter (Tables 1; $T = 0$, $P < 0.001$). This was also the case in the northeast ($T = 16.5$, $P = 0.05$), although the mean temperature differences between fruit cores in the tree and on the ground was much less in the northeast (6.8°C [southwest] vs. 2.8°C [northeast]; $T = 12$, $P < 0.01$). There are at least 2 reasons for the warmer temperature of the fallen fruit: (1) less effective evaporative cooling after leaving the parent plant, and (2) the higher temperature of the ground surface relative to the air. The later is particularly plausible given the relative insignificance of a ground-effect in the more shaded areas in and under the canopy.

Relationship of Fruit to Air Temperature

As might be expected from the above, the relationship of fruit temperature to the air temperature immediately above the fruit differed in regards to fruit on the tree and on the ground (Figs. 1 and 2). The temperatures of tree-fruits were seldom ±0.05 of the air temperature. However, ground fruit were sometimes ±0.25 of the air temperature. There were no relationships between fruit size and the maximum temperatures fruits reach relative to air temperature. However, there was a consistent pattern of fruit size being positively correlated to minimum and mean temperatures and this pattern held regardless of location

TABLE 1. THE MEAN (SD), MINIMUM, AND MAXIMUM TEMPERATURES OF VARIOUS *ANASTREPHA SUSPENSA* HOST FRUITS (SUBCUTANEOUS TEMPERATURES AND AS NEAR THE CORE AS SEEDS ALLOWED) IN THE TREE CANOPIES AND ON THE GROUND UNDER CANOPIES. FRUIT WERE MEASURED IN AND UNDER THOSE PORTIONS OF THE CANOPY MOST AND LEAST EXPOSED TO SUNLIGHT: THE SOUTHWESTERN MARGIN OF THE CANOPY AND THE NORTHEASTERN INTERIOR OF THE CANOPY. TEMPERATURES WERE RECORDED FOR THE FIRST 24 H AFTER THERMOCOUPLE INSERTION, WHEN THE FRUIT WAS LEAST DECAYED.

Fruit	SW ts	SW tc	NE ts	NE tc	SW gs	SW gc	NE gs	NE gc
Cattley Guava 1	28.4 ± 4.4 (23.7-39.2)	28.5 ± 4.8 (23.4-40.5)	27.7 ± 3.1 (23.8-33.5)	27.7 ± 3.2 (23.9-33.8)	31.0 ± 7.6 (24.4-48.0)	31.0 ± 7.7 (24.2-48.1)	28.8 ± 4.6 (23.7-41.1)	28.9 ± 4.5 (23.7-41.1)
Cattley Guava 2	28.5 ± 3.7 (24.2-36.4)	24.3 ± 3.6 (23.7-35.5)	28.6 ± 3.9 (23.6-36.5)	28.0 ± 3.3 (23.7-34.8)	31.5 ± 8.1 (24.6-48.6)	31.1 ± 6.7 (25.2-44.5)	29.1 ± 4.6 (24.0-40.7)	28.8 ± 3.9 (24.4-37.8)
Cattley Guava 3	27.5 ± 4.7 (22.2-37.6)	27.8 ± 5.3 (22.0-39.8)	27.0 ± 4.3 (22.1-35.8)	27.1 ± 4.4 (22.2-37.2)	29.3 ± 6.1 (23.4-47.5)	29.0 ± 5.9 (23.0-45.9)	29.1 ± 5.7 (23.0-41.5)	29.1 ± 5.3 (23.4-40.0)
Guava	26.4 ± 3.5 (22.6-33.8)	26.6 ± 3.6 (22.6-34.5)	26.0 ± 2.9 (22.6-31.5)	26.0 ± 2.8 (22.6-31.3)	27.8 ± 4.7 (23.0-38.1)	27.6 ± 4.5 (22.9-37.4)	26.3 ± 2.6 (23.3-31.0)	26.2 ± 2.2 (23.5-30.3)
Surinam Cherry 1	19.9 ± 5.8 (12.9-28.6)	20.0 ± 5.9 (12.9-28.7)	18.8 ± 4.6 (12.8-25.2)	18.9 ± 4.7 (12.9-25.2)	22.1 ± 9.1 (13.0-40.4)	22.6 ± 9.1 (13.7-40.8)	18.6 ± 3.9 (13.4-24.5)	18.7 ± 3.7 (13.8-24.5)
Surinam Cherry 2	24.9 ± 3.3 (21.3-32.0)	25.0 ± 3.4 (21.3-32.4)	24.5 ± 2.8 (21.3-29.9)	24.5 ± 2.7 (21.4-29.8)	27.5 ± 7.4 (20.8-46.0)	27.8 ± 7.7 (20.9-47.6)	24.5 ± 2.4 (21.6-29.0)	24.4 ± 2.3 (21.6-28.9)
Surinam Cherry 3	—	22.2 ± 5.7 (14.1-30.6)	20.0 ± 5.4 (14.0-29.9)	22.0 ± 5.3 (14.0-29.5)	24.8 ± 9.5 (14.6-49.1)	24.5 ± 8.7 (15.0-46.0)	21.9 ± 4.4 (15.3-29.5)	21.9 ± 4.4 (15.5-30.8)
Surinam Cherry 4	22.2 ± 7.1 (12.9-33.3)	22.1 ± 7.0 (12.9-33.2)	21.4 ± 6.0 (13.1-29.2)	21.4 ± 5.9 (13.1-29.2)	23.7 ± 10.6 (13.0-45.4)	22.8 ± 9.1 (12.7-39.1)	21.3 ± 5.8 (13.7-31.9)	21.3 ± 6.2 (13.1-32.4)
Loquat 1	23.4 ± 5.3 (17.8-36.7)	23.3 ± 5.1 (17.8-35.5)	22.6 ± 4.2 (17.6-30.2)	22.6 ± 4.5 (17.4-31.2)	23.8 ± 7.8 (16.8-45.8)	23.8 ± 7.8 (16.8-44.8)	22.4 ± 5.4 (16.6-32.5)	22.5 ± 5.7 (16.4-33.6)
Loquat 2	16.7 ± 8.1 (7.6-29.5)	16.9 ± 8.3 (7.6-29.8)	16.2 ± 7.5 (7.4-28.1)	17.1 ± 9.3 (7.2-34.4)	18.2 ± 10.2 (8.6-42.6)	18.6 ± 10.8 (8.5-43.4)	16.4 ± 8.9 (7.2-34.8)	16.6 ± 9.1 (7.3-34.9)
Loquat 3	22.5 ± 3.5 (17.1-28.8)	22.6 ± 3.8 (16.8-29.6)	21.6 ± 2.2 (17.2-25.3)	21.5 ± 2.2 (17.3-25.6)	24.8 ± 7.5 (17.5-41.6)	24.4 ± 6.7 (17.7-39.4)	21.4 ± 2.6 (18.2-41.3)	21.7 ± 3.3 (17.7-36.6)
Grapefruit 1	23.9 ± 6.7 (16.8-37.7)	24.2 ± 7.5 (17.0-37.9)	21.8 ± 3.7 (17.6-28.7)	21.9 ± 3.7 (17.8-28.7)	24.2 ± 6.4 (17.4-37.1)	25.5 ± 7.8 (17.5-40.5)	22.0 ± 3.4 (18.1-28.4)	22.0 ± 3.4 (18.1-28.2)
Grapefruit 2	23.1 ± 2.7 (21.3-33.3)	22.8 ± 1.7 (21.4-27.5)	22.5 ± 1.3 (21.2-26.3)	22.5 ± 1.3 (21.3-26.1)	23.7 ± 3.0 (21.7-33.8)	23.7 ± 2.2 (22.0-30.6)	22.7 ± 1.3 (21.6-26.7)	22.9 ± 1.2 (21.8-26.3)
Grapefruit 3	20.5 ± 8.1 (11.4-34.9)	20.3 ± 7.9 (11.4-33.8)	16.3 ± 3.5 (12.1-25.0)	16.3 ± 3.4 (12.1-23.2)	20.0 ± 10.2 (9.8-37.9)	20.2 ± 8.8 (10.9-36.6)	16.7 ± 3.3 (12.7-26.7)	16.6 ± 3.3 (12.7-28.0)
Grapefruit 4	18.3 ± 8.1 (9.1-32.0)	18.1 ± 7.5 (9.4-29.3)	15.3 ± 4.0 (10.1-21.8)	15.4 ± 4.0 (10.2-21.9)	18.5 ± 9.5 (7.9-35.9)	19.8 ± 9.5 (9.3-36.5)	15.4 ± 4.0 (10.3-21.9)	15.5 ± 3.4 (11.0-21.0)

(tree NE mean int.= 1.0 b = 0.002; tree NE minimum int. = 0.99 b = 0.006; tree SW mean int. = 0.99 b = 0.005; tree SW minimum int. = 1.0 b = 0.001; ground NE mean int. = 0.99 b = 0.002; ground NE minimum int. = 1.007 b = 0.005; ground SW mean int.= 1.01 b = 0.007; ground SW minimum int. = 1.009 b = 0.009). There was considerable variance in many of relative temperature relationships (see r^2 values in Figs. 1 and 2), due presumably to a complex set of factors that differed under individual circumstances (Tables 1 and 2; Figs. 1 and 2). All other things being equal, larger fruits should retain greater amounts of heat derived from sunlight. However, it should be noted that while fruit size and temperatures were sometimes correlated, the different sized fruit also had a variety of morphologies, and that it is possible that it was these morphological differences that were related to temperature. If so, the size relationship was coincidental, and particular attention might be focused on the thermodynamics of grapefruit, the largest fruit measured.

Relationship of Soil Depth to Temperature

As in fruit, maximum soil temperatures were higher under the southwestern margin of the canopy than under the northeastern interior (Table 2; surface temperature: $T = 0$, $P < 0.001$). Maximum temperatures declined with depth on the southwestern canopy margin, but there was no relationship in northeast soils (Table 3).

DISCUSSION

In general, fruit temperatures were higher in the southwestern portions of tree canopies relative to those in the northeastern interiors. Fruit

TABLE 2. THE MEAN (SD), MAXIMUM, AND MINIMUM TEMPERATURES OF VARIOUS *ANASTREPHA SUSPENSA* HOST FRUITS (SUBCUTANEOUS TEMPERATURES AND AS NEAR THE CORE AS SEEDS ALLOWED) ON THE GROUND BENEATH THE CANOPIES. TEMPERATURES AT GROUND LEVEL BENEATH FALLEN FRUIT AND AT DEPTHS OF 5, 25, AND 50 MM WOULD BE ENCOUNTERED AS LARVAE LEFT FALLEN FRUIT AND BURROWED INTO THE SOIL TO PUPATE. FRUIT WERE MEASURED IN THOSE POSITIONS UNDER THE CANOPIES MOST AND LEAST EXPOSED TO SUNLIGHT: BELOW THE SOUTHWESTERN MARGIN OF THE CANOPY AND BELOW THE NORTHEASTERN INTERIOR OF THE CANOPY. TEMPERATURES WERE RECORDED FOR THE FIRST 24 H FOLLOWING THERMOCOUPLE INSERTION, WHEN THE FRUIT WAS LEAST DECAYED.

Fruit	SW s	SW 5	SW 25	SW 50	NE s	NE 5	NE 25	NE 50
Cattley Guava 1	30.9 ± 6.3 (25.3-45.5)	30.7 ± 4.5 (26.4-42.4)	30.4 ± 3.4 (26.7-38.5)	30.2 ± 2.6 (27.2-35.8)	28.7 ± 3.1 (25.5-37.7)	29.0 ± 3.4 (25.5-38.9)	28.6 ± 2.3 (26.0-33.8)	28.6 ± 2.0 (26.2-32.8)
Cattley Guava 2	31.0 ± 5.9 (25.6-44.7)	30.2 ± 4.2 (26.2-39.2)	30.1 ± 3.2 (26.6-36.6)	29.9 ± 2.7 (26.9-35.8)	28.5 ± 2.3 (25.8-33.7)	28.5 ± 2.4 (25.7-34.7)	28.4 ± 2.0 (25.9-33.3)	28.3 ± 1.7 (26.2-31.6)
Cattley Guava 3	30.2 ± 6.0 (24.6-48.2)	29.2 ± 2.7 (26.2-35.8)	29.3 ± 2.3 (26.6-34.8)	29.3 ± 1.7 (27.1-32.5)	29.8 ± 4.1 (25.0-37.7)	29.8 ± 4.0 (25.1-36.8)	29.5 ± 2.8 (25.9-34.4)	29.4 ± 2.3 (26.3-33.2)
Guava	28.3 ± 4.9 (23.6-41.7)	27.8 ± 3.9 (23.7-37.4)	27.7 ± 3.2 (24.1-34.7)	27.6 ± 2.7 (24.5-33.4)	26.0 ± 1.2 (24.5-28.5)	26.0 ± 1.3 (24.3-28.5)	26.0 ± 1.1 (24.6-28.2)	26.1 ± 0.8 (24.8-27.6)
Surinam Cherry 1	22.6 ± 7.2 (15.3-37.9)	22.8 ± 4.5 (17.8-31.7)	23.0 ± 4.2 (18.5-31.3)	23.2 ± 4.0 (18.6-31.2)	18.1 ± 2.3 (14.9-21.2)	18.1 ± 1.8 (15.4-20.6)	17.9 ± 1.2 (15.9-19.6)	17.8 ± 1.1 (16.1-19.3)
Surinam Cherry 2	26.7 ± 6.3 (20.6-42.7)	28.2 ± 5.9 (22.7-43.3)	27.7 ± 4.7 (23.2-39.5)	27.5 ± 3.1 (24.3-34.3)	24.4 ± 2.1 (21.9-28.3)	24.3 ± 1.9 (22.0-28.1)	24.0 ± 1.4 (22.2-26.5)	23.7 ± 1.2 (22.2-25.9)
Surinam Cherry 3	24.4 ± 6.0 (17.3-39.7)	24.8 ± 4.7 (19.2-35.8)	24.7 ± 3.6 (20.3-32.3)	24.8 ± 3.5 (20.4-32.0)	21.0 ± 2.1 (17.5-24.1)	21.1 ± 2.1 (17.8-24.1)	20.8 ± 1.5 (18.2-22.9)	20.6 ± 1.2 (18.6-22.3)
Surinam Cherry 4	23.9 ± 10.7 (13.4-46.1)	24.2 ± 4.6 (19.2-33.7)	24.0 ± 3.1 (20.3-30.2)	24.0 ± 2.8 (20.7-29.3)	21.0 ± 4.4 (14.9-27.2)	21.4 ± 4.1 (14.1-28.3)	20.9 ± 3.1 (16.6-27.8)	21.4 ± 1.6 (19.0-26.0)
Loquat 1	23.6 ± 6.2 (18.0-40.9)	22.5 ± 3.0 (19.3-30.3)	22.3 ± 2.4 (19.7-28.3)	22.1 ± 1.8 (20.0-26.2)	22.4 ± 5.4 (16.7-33.1)	22.0 ± 3.5 (18.1-28.4)	22.0 ± 2.9 (18.8-28.1)	21.6 ± 1.7 (19.6-25.0)
Loquat 2	19.1 ± 10.2 (9.7-45.6)	19.9 ± 6.2 (13.7-34.9)	19.9 ± 4.5 (15.1-30.0)	20.0 ± 2.8 (16.6-26.0)	17.3 ± 8.3 (8.8-33.9)	17.8 ± 6.3 (11.0-29.4)	18.8 ± 5.0 (13.2-28.8)	19.3 ± 3.1 (15.5-24.7)
Loquat 3	25.6 ± 8.0 (18.1-45.0)	25.2 ± 6.7 (18.4-41.3)	24.8 ± 5.8 (18.4-36.6)	24.7 ± 4.5 (19.6-33.9)	21.6 ± 3.4 (17.9-33.0)	21.4 ± 2.7 (18.3-29.5)	21.0 ± 1.6 (18.8-24.0)	21.1 ± 1.6 (18.9-23.6)
Grapefruit 1	25.8 ± 4.9 (20.4-35.5)	26.0 ± 4.9 (20.1-36.1)	25.0 ± 4.4 (20.5-34.7)	25.9 ± 3.4 (21.8-32.6)	21.8 ± 2.1 (19.2-25.5)	21.6 ± 1.9 (19.3-25.3)	21.6 ± 1.4 (19.8-24.1)	21.6 ± 1.1 (20.2-23.5)
Grapefruit 2	24.4 ± 2.3 (22.7-30.3)	24.2 ± 1.9 (22.4-31.9	24.0 ± 1.7 (22.8-29.8)	24.4 ± 1.5 (23.1-28.7)	22.5 ± 1.2 (21.2-26.4)	23.0 ± 0.7 (22.3-25.0)	23.1 ± 0.7 (22.4-25.0)	23.3 ± 0.5 (22.7-24.7)
Grapefruit 3	21.9 ± 7.8 (13.4-36.6)	21.9 ± 7.3 (13.3-35.1)	22.3 ± 7.0 (14.1-35.0)	20.2 ± 6.2 (14.7-30.1)	16.7 ± 2.7 (14.0-30.5)	16.9 ± 1.9 (14.4-23.8)	17.1 ± 2.0 (15.1-27.9)	18.1 ± 1.7 (16.7-28.3)
Grapefruit 4	20.8 ± 7.7 (12.1-35.1)	20.6 ± 7.5 (11.5-34.9)	20.9 ± 6.5 (12.1-34.9)	19.1 ± 6.2 (13.1-31.1)	15.5 ± 2.1 (12.1-19.7)	15.3 ± 1.8 (12.8-17.8)	15.5 ± 1.5 (13.3-17.7)	16.2 ± 1.0 (14.7-17.5)

on the ground were warmer than those in the tree, but there was no significant pattern of maximum fruit core temperatures being warmer than subcutaneous pulp. Soil temperatures were also higher than fruit-in-tree temperatures, and decreased and displayed less variance with increasing depth. Fruit in trees seldom reached temperatures ±0.05 of adjacent air temperatures, but fruit on the ground could be more than 0.25 the adjacent air temperature. There were significant relationships between the ratio of minimum and mean fruit temperatures/adjacent air temperature and fruit diameter. Typically, air temperature in various portions of the canopy are unlikely to grossly underestimate the minimum temperatures faced by the local immature tephritids, but maximum temperatures encountered by larvae in fallen fruit can be substantially higher than suggested by air temperatures. Thus, air temperature could generally be a useful tool in estimating many fruit fly thermal environments.

Fruit Temperature Relative to Ambient and the Function of Cooling

Moist spherical objects in sunlight, sheltered from winds that increase heat flux, will retain solar energy and reach temperatures well above ambient (Thorpe 1974). However, certain fruit, e.g., *Ficus* spp. evaporatively cool by transpiring water through stomata on their surface (Patiño et al. 1994). Fruit in the canopy examined in the present study, even the more exposed southwest portion, tended to show little deviation from sur-

TABLE 3. RESULTS OF ANOVA WITH MEANS COMPARED BY WALLER TEST. THOSE MEANS SHARING A LETTER ARE NOT SIGNIFICANTLY DIFFERENT. SW REFERS TO SOUTHWESTERN PORTION OF THE OUTER MARGIN OF THE CANOPY. NE REFERS TO THE NORTHEASTERN PORTION OF THE INNER MARGIN OF THE CANOPY.

Canopy site	Mean	Minimum	Maximum
SW fruit surface	a 23.3 (0.98)	a 17.2 (1.5)	a 33.8 (0.91)
SW fruit core	a 22.8 (0.94)	a 17.0 (1.4)	a 33.2 (1.0)
NE fruit surface	a 22.0 (1.1)	a 17.1 (1.4)	b 29.1 (1.1)
NE fruit core	a 22.2 (1.0)	a 17.1 (1.4)	b 29.5 (1.2)
Ground Site	**Mean**	**Minimum**	**Maximum**
SW fruit surface	a 24.7 (1.1)	a 17.0 (1.5)	a 42.5 (1.3)
SW fruit core	a 24.8 (1.0)	a 17.4 (1.4)	a 41.8 (1.3)
NE fruit surface	a 22.0 (1.2)	a 17.5 (1.4)	b 32.1 (1.7)
NE fruit core	a 22.5 (1.5)	a 17.6 (1.4)	b 31.6 (1.5)
SW soil depth	**Mean**	**Minimum**	**Maximum**
surface	a 25.3 (0.93)	a 18.7 (1.3)	a 41.0 (1.3)
5 mm	a 25.2 (0.88)	a 20.0 (1.2)	b 36.3(1.0)
25 mm	a 25.0 (0.85)	a 20.7 (1.9)	bc 33.8 (0.87)
50 mm	a 25.9 (0.92)	a 21.2 (1.1)	c 31.5 (0.79)
NE soil depth	**Mean**	**Minimum**	**Maximum**
surface	a 22.4 (1.2)	a 18.7 (1.4)	a 29.9 (1.3)
5 mm	a 22.4 (1.1)	a 19.1 (1.3)	a 27.9 (1.5)
25 mm	a 22.3 (1.1)	a 19.8 (1.2)	a 27.5 (1.4)
50 mm	a 22.5 (1.1)	a 20.5 (1.0)	a 25.7 (1.2)

rounding maximum air temperature. This suggests the possibility of adaptive cooling. Patiño et al. (1994) argued that cooling in *Ficus* spp. was required to protect mutualist pollinators, since figs prevented from transpiration reached temperatures fatal to the agaonid wasps harbored inside the fruit. It is difficult to propose such a hypothesis in the present case since most of the insects located inside the fruit are frugivores, or parasitoids of frugivores that would disperse and be unlikely to protect the subsequent fruit of any particular individual fruit tree (see discussion of larval behavior below). Perhaps such high temperatures damage seeds as well, and fruits are sometimes designed and located to cool and protect plant genetic material.

Temperature and Population Dynamics

The distributions of *Anastrepha* spp. and other tephritids are believed to be influenced by abiotic environmental factors (e.g., Messenger & Flitters 1954; Meats 1981; Drew & Hooper 1983; Sivinski et al. 2000), and temperature is also a principal factor in the distribution of *Anastrepha* spp. parasitoids. For example, the relative abundance of 2 introduced braconid parasitoids of *A. suspensa* in Florida is related to temperature and the effects of temperature on host fruit diversity and availability (Eitam et al. 2004).

On a smaller spatial scale, Aluja & Birke (1993) found fewer *Anastrepha obliqua* (MacQuart) ovipositing in exposed as opposed to shaded host trees. While females might avoid the warmer and drier microenvironment of the exposed trees for their own wellbeing, they could also be seeking more suitable larval habitats in the shade. As to the distribution of subtropical and tropical fruit fly and parasitoid larvae within tree canopies, several studies have yielded somewhat mixed results with the emergence of relatively weak patterns (Sivinski et al. 1997, 1999, 2004). Perhaps the multitudinous combinations of microhabitat-abiotic effects, local natural enemies and competitors make it difficult to generalize about the role of any particular variable. Thomas (1993) found similarly weak correlations between temperature and moisture extremes and the survival of *A. ludens* pupae in the field, and argued that the effects of weather variables were probably masked by predation.

As previously noted, the present work suggests that larval-environment temperatures vary with microhabitat, but are relatively similar to the air temperatures in the same vicinities. However, there are significant relationships between

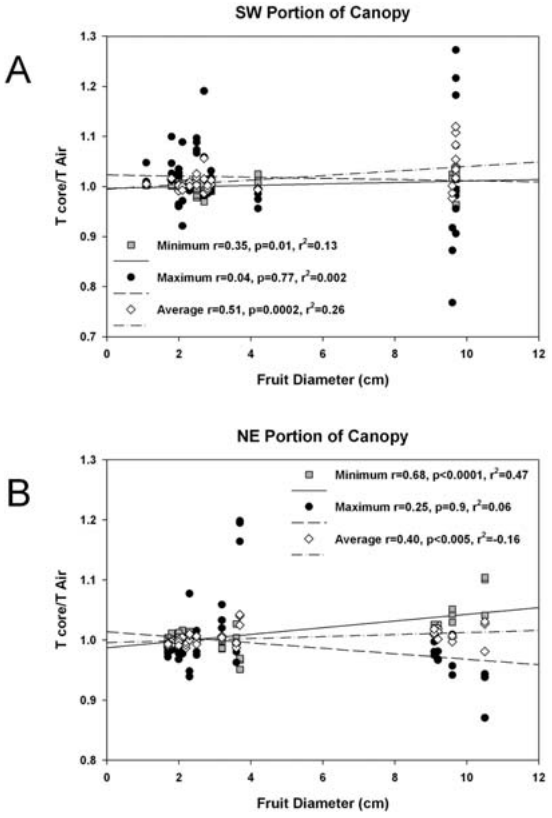

Fig. 1. (A) The ratio of fruit temperature to air temperature in the south west portions of the canopies for each of the first 3 d of monitoring in relation to the diameter of the fruit. The minimum, maximum, and mean ratios of fruit on the southwest margins of the canopies are considered. Because there were no significant differences between subcutaneous and core temperatures, only core temperatures are considered. (B) As above in the north east portions of the canopies.

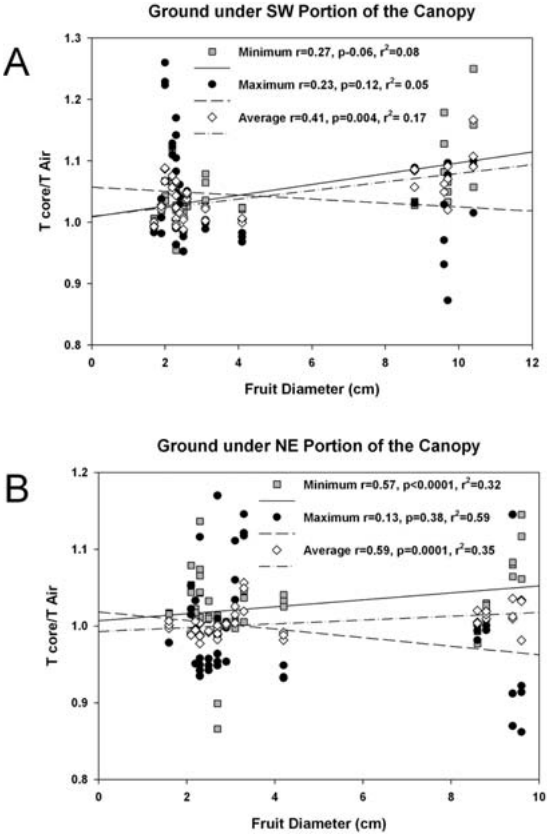

Fig. 2. (A) The ratio of fruit temperature / air temperature for fruit on the ground at the south west margin of the canopies for each of the first 3 d of monitoring in relation to the diameter of the fruit. The minimum, maximum, and mean ratios of fallen fruit on the southwest margins of the canopies are considered. Because there were no significant differences between subcutaneous and core temperatures, only core temperatures are considered. (B) As above, but under the north east-interior portions of the canopies.

minimum and mean temperatures relative to air temperature and fruit size.

Temperature and Larval Behavior

Heating through "forced air" or in water baths has long been used to disinfest fruit destined for export (e.g., Hawkins 1932). In general, but with some variance depending on species, temperatures in excess of 45°C will quickly kill fruit fly eggs and larvae (e.g., Armstrong 1992). At 43°C, the exposure time required for 95% of 3rd instar *A. suspensa* larvae to perish depends on both the medium in which the insects were reared and that in which they are heated (Hall 1996). The adult "L(ethal)T(ime)$_{95}$" of larvae reared in grapefruit and exposed in grapefruit juice, the most natural of the tested regimens, was 24 min. Using temperature probes inserted into olive (*Olea europaea* L.) drupes, Pucci et al. (1981) correlated mortality in immature olive fruit flies *Bactrocera oleae* (Gmelin) to temperature. Eighty-five percent of eggs and first-instar larvae and 95% of mature larvae died when daily maximum temperatures reach just 36°C for a period of a week. Given that fruit in the present study, particularly fallen fruit on the southwest margin of tree canopies, often reached temperatures in excess of 43°C and sometimes temperatures that approached 50°C, it would seem that larvae could frequently find themselves in danger of overheating.

Once on the soil surface, a fruit-exiting larva could still face lethal temperatures. Even 5 mm below the surface temperatures sometimes reached 43°C, and it was only at depths of 25 mm that no temperatures >40°C were recorded. At a

site inhabited by fruit flies in northern Mexico, Thomas (1993) measured temperatures as high as 38°C at depths of 30-40 mm, and noted that exposed soils were 6-7° warmer at than those under shade. In addition to harmful temperatures just beneath the surface, ant predators and pupal parasitoids tend to be more efficient at lesser pupation depths (Hogdson et al. 1998; Baeza et al. 2002; Guillén et al. 2002). Not surprisingly, in one Mexican field survey of *Anastrepha* spp. pupations depths no pupae were found on the surface, 56% were uncovered at depths up to 20 mm and most of the rest at depths of 20-50 mm. Only one occurred deeper than 50 mm (Hogdson et al. 1998).

Perception of soil surface temperature appears to influence the speed with which *Anastrepha* larvae begin to burrow. Under warm condition in Mexico larvae quickly burrow directly beneath, or close to, the fruit they developed within (Aluja et al. 2006). However, under cooler conditions, Thomas (1995) describes *A. ludens* wandering on the surface to find suitable pupations sites.

Temperature and Conditional-effect-lethal Strains

Sterilization through irradiation often harms the performance of released insects and, as a consequence, SIT sometimes fails to reach its theoretical potential (e.g., Proshold 1993; Barry et al. 2003b). Autocidal strains that result in offspring death or sterility and also avoid radiation may be more effective (Alphey 2002). Such strains, based on the conditional regulation of genes that encode lethal products, might be most easily produced through genetic transformation (Robinson & Franz 2000; Handler 2002; Handler & Atkinson 2006). A variety of mutant and normal genes affecting cell viability can be used, including mutant lethal genes affecting vital processes, normal genes involved in programmed cell death (White et al. 1994), and genes for toxin subunit molecules (Kalb et al. 1993). A critical component to the use of these genes is the ability to regulate their expression in terms of developmental stage, tissue, and sex-specificity for the desired phenotype so that breeding populations can be maintained. This can be achieved by conditional regulation where lethal gene expression is determined by manipulation of temperature, chemical treatment, or by interbreeding 2 independent strains. Model systems have already been tested in *Drosophila* spp. with temperature-sensitive lethal alleles and by creating female lethals and steriles by tetracycline-dependent transcriptional repression (Heinrich & Scott 2000; Horn & Wimmer 2003).

Among the temperature regulated lethal systems developed in *Drosophila* is the inclusion of a cold-sensitive allele that kills both heterozygous and homozygous individuals when the temperature falls below 18°C (Fryxell & Miller 1995). Thus the offspring of homozygous individuals reared and released at higher temperatures would die as temperatures fell. In the *A. suspensa* habitats examined minimum temperatures were frequently well above 18°C (e.g., *Psidium* spp.) and this particular scheme, if transferable to *A. suspensa*, would require an upward temperature adjustment to have an immediate effect. However, the proportion of individuals carrying such a gene could be increased by repeated releases during warm seasons of the year and the population would then crash with the onset of winter. Alternatively, conditional systems under consideration/development would release fruit flies reared at relatively low temperatures whose offspring would perish after encountering warmer temperatures in the field (Handler & Atkinson 2006).

In summary, immature Caribbean fruit flies faced a variety of temperatures, but with the exception of fallen fruit exposed to strong sun light, these temperatures are similar to ambient air temperatures. While fruit size was correlated to the mean and minimum temperatures reached, it did so to a relatively minor extent. If other host fruit of other tephritid species have similar thermal properties, then air temperature should be a useful tool to estimate the thermal environments of immature fruit flies outside of Florida. It should be kept in mind that not all subtropical pest tephritids face temperatures identical to those recorded in the present study. For example, *A. ludens* in northern Mexico sometimes encounter and survive below freezing temperatures (Thomas 1993).

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References Cited

Alphey, L. 2002. Re-Engineering the Sterile Insect Technique. Insect Bioch. Mol. Biol. 32: 1243-1247.

Aluja, M., and A. Birke. 1993. Habitat use by adults of *Anastrepha obliqua* (Diptera: Tephritidae) in a mixed mango and tropical plum orchard. Ann. Entomol. Soc. America 86: 799-812.

Aluja, M., J. Piñero, I. Jácome, F. Díaz-Fleischer, and J. Sivinski. 2000. Behavior of flies in the genus *Anastrepha* (Tryptinae; Toxotrypanini), pp. 375-406 *In* M. Aluja and A. Norrbom [eds.], Fruit flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL.

Aluja, M., J. Sivinski, J. Rull, and P. Hodgson. 2006. Behavior and predation of fruit fly larvae (*Anastrepha* spp.) (Diptera: Tephritidae) after exiting fruit. Bull. Entomol. Res. (in press).

Armstrong, J. 1992. Fruit fly disinfestations strategies beyond methyl bromide. New Zealand J. Crop and Hort. Sci. 20: 181-193.

BAEZA-LARIOS, G., J. SIVINSKI, T. HOLLER, AND M. ALUJA. 2002. The ability of *Coptera haywardi* (Ogloblin) (Hymenoptera: Diapriidae) to locate and attack the pupae of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), under seminatural conditions. Biol. Control. 23: 213-218.

BARANOWSKI, R., H. GLENN, AND J. SIVINSKI. 1993. Biological control of the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomol. 76: 245-251.

BARRY, J. D., D. O. MCINNIS, D. GATES, AND J. G. MORSE. 2003. Effects of irradiation on Mediterranean fruit flies (Diptera: Tephritidae): emergence, survivorship, lure attraction, and mating competition. J. Econ. Entomol. 96: 615-622.

DREW, R., AND G. HOOPER. 1983. Population studies of fruit flies (Diptera: Tephritidae) in south-east Queensland. Oecologia 56: 153-159.

EITAM, A., J. SIVINSKI, T. HOLLER, AND M. ALUJA. 2004. Biogeography of braconid parasitoids of the Caribbean fruit fly (Diptera: Tephritdae) in Florida. Ann. Entomol. Soc. Am. 97: 928-939.

FRYXELL, K., AND T. MILLER. 1995. Autocidal biological control: a general strategy for biological control based on genetic transformation with a highly conserved gene. Biol. Microbiol. Control. 88: 1221-1232.

GUILLÉN., L., M. ALUJA, M. EQUIHUA, AND J. SIVINSKI. 2002. Performance of two fruit fly (Diptera: Tephritidae) parasitoids (*Coptera haywardi* [Hymenoptera: Diapriidae] and *Pachycrepoideus vindemiae* [Hymenoptera: Pteromalidae] under different environmental soil conditions. Biol. Control. 23: 219-227.

HANDLER, A. M. 2002. Prospects for using genetic transformation for improved SIT and new biocontrol methods. Genetica. 116: 137-149.

HANDLER, A. M. 2004. Understanding and improving transgene stability and expression in insects for SIT and conditional lethal release programs. Insect Bioch. Mol. Biol. 34: 121-130.

HANDLER, A. M., AND P. ATKINSON. 2006. Insect transgenesis: mechanisms, applications and ecological safety. Biotech. Genet. Eng. Rev. (in press).

HALL, G. 1996. Mortality of third instar Caribbean fruit fly (Diptera: Tephritdae) reared in diet or grapefruits and immersed in heated water or grapefruit juice. Florida Entomol. 79: 168-172.

HAWKINS, L. 1932. Sterilization of citrus fruit by heat. Citriculture. 9: 21-22.

HEINRICH, J. C., AND M. J. SCOTT. 2000. A repressible female-specific lethal genetic system for making transgenic insect strains suitable for a sterile-release program. Proc. Nat. Acad. Sci. 97: 8229-8232.

HILLEL, D. 1982. Introduction to Soil Physics. Academic Press, San Diego, CA.

HODGSON, P., J. SIVINSKI, G. QUINTERO, AND M. ALUJA. 1998. Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. Environ. Entomol. 27: 1310-1314.

HORN, C., AND E. A. WIMMER. 2003. A transgene-based, embryo-specific lethality system for insect pest management. Nature Biotech. 21: 64-70.

KALB, J. M., A. J. DIBENEDETTO, AND M. F. WOLFNER. 1993. Probing the Function of *Drosophila melanogaster* Accessory Glands by Directed Cell Ablation. Proc. Natl Acad Sci. USA 90: 8093-8097.

LUX. S., J. VILARDI, P. LIEDO, K. GAGGI, G. CALCAGNO, F. MUNYIRI, M. VERA, AND F. MANSO. 2002. Effects of irradiation on the courtship behavior of medfly (Diptera: Tephritidae) mass reared for the sterile insect technique. Florida Entomol. 85: 102-112.

MEATS, A. 1981. The bioclimatic potential of the Queensland fruit fly, *Dacus tryoni*, in Australia. Proc. Ecol. Soc. Australia. 11: 151-161.

MESSENGER, P., AND N. FLITTERS. 1954. Bioclimatic studies of three species of fruit flies in Hawaii. J. Econ. Entomol. 47: 756-765.

NEUNER, C., O., BUCHNER AND V. BRAUN. 2000. Short-term changes in heat tolerance in the alpine cushion plant *Silene acaulis* spp. [All.] J. Braun at different altitudes. Plant Biol. 2: 677-683.

NOBEL, P. 1999. Physiochemical and Environmental Plant Physiology. 2nd ed. Academic Press, New York, NY.

NORRBOM, A., AND K. KIM. 1988. A list of the reported host plants of the species of *Anastrepha* (Diptera: Tephritdae). USDA-APHIS Tech. Bull. 1322.

PATIÑO, S., E. HERRE, AND M. TYREE. 1994. Physiological determinants of *Ficus* fruit temperature and implications for survival of pollinator wasp species: comparative physiology through an energy budget approach. Oecologia 100: 13-20.

PEARCY, R., J. BERRY, AND B. BARTHOLOMEW. 1972. Field measurements of the gas exchange capacities of *Phragmites comunis* under summer conditions in Death Valley. Carnegie Institution Year Book 71: 161-164.

PROSHOLD, F. I., V. C. MASTRO, AND G. L. BERNON. 1993. Sperm transfer by gypsy moths (Lepidoptera, Lymantriidae) from irradiated males—implication for control by inherited sterility. J. Econ. Entomol. 86: 1104-1108.

PUCCI, C., A. PORCINA, AND D. SALMISTRARO. 1981. Effects of temperature on the death rate of larvae, pupation and activities of parasites for *Dacus oleae* (Gmel.). Frustula Entomol. 16: 143-155.

ROBINSON, A. S., AND G. FRANZ. 2000. The application of transgenic insect technology in the sterile insect technique, pp. 307-319 *In* A. M. Handler and A. A. James [eds.], Insect Transgenesis: Methods and Applications. CRC Press, Boca Raton, FL.

ROTH-NEBELSICK, A. 2001. Heat transfer in rhyniophytic plant axes. Rev. Plant Paleobot. Palynol. 116: 109-122.

SEYMOUR, R. S., AND P. SCHULTZE-MOTEL. 1998. Physiological temperature regulation by flowers of the sacred lotus. Phil. Trans. R. Soc. London B 353: 935–943.

SIMPSON, S. 1993. Caribbean fruit fly-free zone certification protocol in Florida (Diptera: Tephritidae). Florida Entomol. 76: 228-233.

SIVINSKI, J., M. ALUJA, AND M. LOPEZ. 1997. Spatial and temporal distributions of parasitoids of Mexican species of *Ananstrepha* (Diptera: Tephritidae) within the canopies of fruit trees. Ann. Entomol. Soc. America. 90: 604-618.

SIVINSKI, J., M. ALUJA, T. HOLLER, AND A. EITAM. 1999. Phenological comparison of two braconid parasitoids of the Caribbean fruit fly (Diptera: Tephritidae). Environ. Entomol. 27: 360-365.

SIVINSKI, J., J. PIÑERO, AND M. ALUJA. 2000. The distributions of parasitoids (Hymenoptera) of *Anastrepha* fruit flies (Diptera: Tephritidae) along an altitudinal gradient in Veracruz, Mexico. Biol. Control. 18: 72-81.

SIVINSKI, J., M. ALUJA, J. PIÑERO, AND M. OJEDA. 2004. Novel analysis of spatial and temporal patterns of resource use in a group of tephritid flies of the genus *Anastrepha*. Ann. Entomol. Soc. Am. 97: 504-512.

THOMAS, D. 1993. Survivorship of the pupal stages of the Mexican fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae) in an agricultural and nonagricultural situation. J. Econ. Entomol. 28: 350-362.

THOMAS, D. 1995. Predation on the soil inhabiting stages of the Mexican fruit fly. Southwest. Entomol. 20: 61-71.

THORPE, M. 1974. Radiant heating of apples. The Journal of Applied Ecology 11: 755-760.

(USDA) U.S. DEPARTMENT OF AGRICULTURE. 1980. Soil Survey of St. Lucie County Area, FL.

(USDA) U.S. DEPARTMENT OF AGRICULTURE. 1990a. Soil Survey of Polk, County, FL.

(USDA) U.S. DEPARTMENT OF AGRICULTURE. 1990b. Soil Survey of Hendry County, FL.

WHITE, K., M. E. GRETHER, J. M. ABRAMS, L. YOUNG, K. FARRELL, AND H. STELLER. 1994. Genetic-control of programmed cell-death in *Drosophila*. Science 264: 677-683.

ZAR, J. 1974. Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ.

CITRUS LEAFMINER, *PHYLLOCNISTIS CITRELLA* (LEPIDOPTERA: GRACILLARIIDAE), AND NATURAL ENEMY DYNAMICS IN CENTRAL FLORIDA DURING 2005

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ABSTRACT

After the citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), invaded Florida in 1993, the endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was introduced in 1994 in a classical biological control program. Subsequent to its establishment, only limited information has been obtained regarding the seasonal abundance of *A. citricola* and its host in central Florida citrus groves. During 2005, we monitored replicated plots treated with oil or imidacloprid once on 23 Jun 2005, along with untreated control trees, in a Polk County commercial Valencia orange grove on a weekly basis when tender new growth (= flush) was available. As expected, CLM abundance in the early spring flush was nearly undetectable due to the lack of suitable flush during winter when CLM populations decline nearly to zero. Also as expected, *A. citricola* was not found during this time. During the second flush (Jun through Jul) CLM populations increased and *A. citricola* appeared, parasitizing up to 39% of the pupae in the untreated controls and up to 33% in the blocks treated with oil. Imidacloprid did not significantly reduce the number of CLM larvae but did reduce Asian citrus psyllid, *Diaphorina citri* Kuwayama, nymphal densities. Peak abundance of the CLM occurred during the third flush cycle on 5 Oct from trees treated once with oil, with a mean (SD) of 1.3 (0.8) CLM mines per leaf. Parasitism by *A. citricola* increased through the season, peaking at 56% of the CLM that had pupated prior to the 16 and 23 Nov samples in the untreated control trees and at 37% in the oil-treated trees; *A. citricola* was not found in imidacloprid-treated trees on those dates. During the growing season, a high proportion (up to 100% in some samples) of the CLM mines were empty, presumably due to predation. The data confirmed, for the first time, that *A. citricola* is an important natural enemy of those CLM larvae that escaped predation in this citrus-growing area in Florida. Nymphs of the Asian citrus psyllid were significantly reduced for 3 weeks after the imidacloprid treatment. However, shoots on trees treated with imidacloprid were significantly shorter than shoots on untreated trees and the number of shoots produced in imidacloprid-treated trees was reduced, raising concerns that imidacloprid might affect growth of citrus flush. Brown citrus aphids were nearly absent throughout the growing season.

Key Words: citrus leafminer, *Ageniaspis citricola*, population dynamics, imidacloprid, oil, phytotoxicity

RESUMEN

Después de que el minador de la hoja de los cítricos (MHC), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), invadió el estado de la Florida en 1993, el endoparasitoide *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) fue introducido durante 1994 para un programa de control biológico clásico. Subsiguiente a su establecimiento, información obtenida en cuanto a la abundancia estacional de *A. citricola* y sus hospederos en huertos de cítricos en Florida central fue muy limitada. Durante el año del 2005, nosotros realizamos un monitoreo en parcelas replicadas tratadas con aceite o imidacloprid por una vez en el 23 de junio de 2005, incluidos con árboles no tratados para control, en un huerto comercial de naranjas "Valencia" en el condado de Polk todo ello revisado semanalmente cuando los brotes de nuevas hojas fueron disponibles. Como fue esperado, la abundancia de MHC en los brotes de nuevas hojas en el principio de la primavera fue casi no detectable debido a la falta de brotes apropiados durante el invierno cuando la población de MHC bajó a casi cero. A su vez, como era de esperar, *A. citricola* no fue encontrado durante este tiempo. Durante el segundo brote de hojas (junio a julio) la población de MHC aumentó y *A. citricola* apareció, parasitizando hasta 39% de la pupas en los bloques no tratados de control y hasta 33% de los bloques tratados con aceite. Imidacloprid no redujó significativamente el número de larvas de MHC pero si redujó la densidad de las ninfas del psila de cítrico Asiático, *Diaphorina citri* Kuwayama. El pico de la abundancia de MHC ocurrió durante el ciclo del tercer

brote de las hojas nuevas en el 5 de octubre en árboles tratados una vez con aceite, con un promedio (DS) de 1.3 (0.8) minas de MHC por hoja. El parasitismo por *A. citricola* aumentó a travez de la estación, llegando a un climax de 56% de los MHC que han empupado antes de las muestras de 16 y 23 de noviembre en árboles no tratados y 37% en árboles tratados con aceite; *A. citricola* no fue encontrado en árboles tratados con imidacloprid en estas fechas. Durante la estación de crecimiento, una alta proporción (hasta 100% en algunas muestras) de las minas de MHC fueron vacias, propuestamente debido a la depredación. Los datos confirmaron, por primera vez, que *A. citricola* es un enemigo natural importante de las larvas de de MHC que escapan a los depredadores en esta área en Florida donde se siembra cítricos. Las ninfas del psila de cítrico Asiático fueron reducidas significativamente por 3 semanas después del tratamiento con imidaloprid. Sin embargo, los brotes en los árboles tratados con imidacloprid fueron significativamente mas cortos que en los árboles no tratados y el número de los brotes producidos en árboles tratados con imidacloprid fue reducido, aumentando la preocupación de que el imidacloprid posiblemente puede estar afectando el crecimiento de los brotes de los cítricos. El áfido pardo de los cítricos [*Toxoptera citricida*] durante la estación de crecimiento estuvo casi ausente.

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), was discovered in Florida in May of 1993 and quickly spread through >800,000 acres of citrus, attacking tender new growth (= flush) and, occasionally, fruits and stems when densities were particularly high (Heppner 1993). Shortly after the invasion, native parasitoids (primarily eulophids) attacked this pest, as well as generalist predators (Browning & Peña 1995; Peña et al. 1996; Evans 1999; Amalin et al. 1996), but growers still considered CLM densities too high and treated both mature and young groves multiple times per season (Heppner 1995; Knapp et al. 1996). The host-specific endoparasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was imported from Australia (Neale et al. 1995) and first released in Florida in May 1994 in a classical biological control program (Hoy & Nguyen 1994a; 1997; Smith & Hoy 1995). A subsequent importation of *A. citricola* from Taiwan also was released (Hoy et al. 2000). Although the Australian (which was originally from Thailand) and Taiwan populations appeared morphologically identical, subsequent molecular studies indicated that they were, in fact, cryptic species (Hoy et al. 2000; Alvarez & Hoy 2002). Both populations were released in Florida, but a subsequent analysis failed to show that the Taiwan population had established (Alvarez 2000).

Establishment and spread of *A. citricola*, presumably the Australian population, in Florida was rapid and high rates of parasitism of CLM pupae were observed (Hoy & Nguyen 1994a; 1994b; 1997; Hoy et al. 1995; 1997; Pomerinke & Stansly 1998; Amalin et al. 1996; 2002). However, after establishment and dispersal were documented, funding for monitoring the phenology and dynamics of the CLM and *A. citricola* was unavailable because the CLM 'problem' appeared to have been solved, at least temporarily. As a result, information about the abundance and phenology of *A. citricola* in Florida's citrus groves remained anecdotal. Concerns about CLM population densities in Florida resurfaced during the citrus canker eradication program, because mines produced by CLM larvae allow the canker bacteria access to ideal growing conditions (Sohi & Sandhu 1968; Chagas et al. 2001; Gottwald et al. 2001; Graham et al. 1996; Christiano et al. 2007). In addition, *A. citricola* appeared less effective during 2000-2002 than in previous years because Florida was undergoing a drought and this parasitoid performs poorly when relative humidity is low (Yoder & Hoy 1998). Despite the fact that natural enemies cannot eliminate all CLM in a grove and even a single CLM can cause damage to foliage that increases the susceptibility of a tree to canker infection, consideration was given to importing additional parasitoids of the CLM, with the goal of further reducing CLM densities and, hopefully, canker incidence. *Semielacher petiolatus* Girault (Hymenoptera: Eulophidae) was imported and evaluated in quarantine, but not released because the potential risk of disrupting biological control by *A. citricola* was considered higher than the potential benefit of establishing *S. petiolatus* in Florida (Lim & Hoy 2005: Lim et al. 2006).

During 2005, we monitored a commercial citrus block in central Florida (Polk County) near Haines City each week during the major flush cycles to evaluate the phenology and relative abundance of the CLM and *A. citricola* in Valencia oranges that were untreated or treated with oil or with imidacloprid. We also evaluated additional mortality factors of the CLM. Relative abundances of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, and the brown citrus aphid, *Toxoptera citricida* Kirkaldy, on the flush also were recorded.

Materials and Methods

Plots were established in a Valencia orange grove near Haines City, Florida (GPS coordinates: N 28°03.656, W 081°34.937). The trees were 4-5 years old and spaced 7.3 m apart between the rows and 3 m within the rows. During 2004, the

year prior to this study, the only pesticide applied in this grove was petroleum oil (470 weight oil, (Petro-Canada, Calgary, Alberta), which was applied 3 times (May, Jul, and Sep) at a rate of 7 gal/acre (26.5 L/ 0.4 ha), which should not have had significant negative effects on *A. citricola* in the grove because oil has little residual toxicity (Villanueva-Jimenez & Hoy 1998). Trees were drip irrigated as needed and fertilized in 2004 with Nutri 5 at 1 qt/acre (0.95 L/0.4 ha) and with 3 Key-Plex foliar sprays at a rate of 2 qt/acre (1.89 L/0.4 ha) each. During 2005, a foliar application of potassium nitrate (N:P:K at a rate of 13.75-0-46) was added on 18 Oct 2005 and an organic amendment from poultry houses was added at a rate of 1000 lbs/acre (453.6 kg/0.4 ha) on 1 Sep 2005. During 2005 no sprays, other than those required by the experiment, were applied.

Randomized complete blocks with 4 treatments and 3 replicates of each treatment were set up in Mar, with each replicate consisting of 3 adjacent rows of 10 trees each with 3 buffer rows between each of the treatments. Trees were left untreated between replicates to reduce any effect of spray drift. Out of 30 trees in an experimental unit, 6 central, uniform and healthy trees were labeled and 4 young shoots per tree were collected, when present, at weekly intervals throughout the 2005 growing season from Mar until the end of Nov. Each week the percentage of terminals having new flush was estimated to determine the flushing patterns.

The 4 planned treatments consisted of (A) untreated control, (B) 3 sprays at 6-week intervals starting in Jun when the flush was about 3 cm in length with petroleum oil 455 (Petro-Canada, Calgary, Alberta) at 2% (20 mL/L of water), (C) 1 application of imidacloprid (Provado 1.6 F, Bayer CropScience, North Carolina) at the lowest recommended foliar application rate of 10 oz/acre (295 mL/0.4 ha) when the flush was 3 cm long, and (D) 2 sprays of petroleum oil 455 in weeks 1 and 3 of the Jun flush cycle at 2%. Treatment B was planned because many growers were using this spray schedule and Treatment D was planned to determine whether 2 treatments during the Jun flush cycle (second cycle) would allow *A. citricola* to 'catch up' with the citrus leafminer population and eliminate the need for additional sprays. Because populations of the citrus leafminer and its host-specific parasitoid *A. citricola* decline to very low levels during the winter in Florida when very little tender new growth is available to support reproduction of the leafminer (Lim & Hoy 2006), populations of both species may be nearly undetectable during the first flush cycle in Feb or Mar (Villanueva-Jimenez et al. 2000). Treatment C was considered the standard to which the 2 oil treatments would be compared. However, only 1 application of oil was applied on 23 Jun to treatments B, C, and D because so few CLM were present during Jun through Nov that additional sprays could not be justified.

When flush was present, 4 shoots longer than 0.5 cm were collected from each of 6 trees in each replicate and placed in a labeled plastic bag containing a paper towel to soak up any moisture that could cause the leaves to begin to rot prior to scoring. Plastic bags were placed in an ice chest with ice packs and shipped to the University of Florida, Department of Entomology and Nematology in Gainesville by FedEx overnight delivery.

Samples were scored with the aid of a dissecting microscope. Shoot length and numbers of leaves per shoot were recorded, as well as the number of CLM mines (>0.5 cm) per leaf, the number of CLM larvae in mines that were alive, parasitized, absent, or dead. Larvae missing from the mines were assumed to be dead from predation if no pupal chambers were associated with the mine. Pupal chambers were opened and the number of CLM pupae that were alive, parasitized by *A. citricola* (including number of *A. citricola* pupae) or by other parasitoids, or dead due to unknown causes was recorded. Relative abundance per shoot of Asian citrus psyllids was reported as 0 = none, 1 = 1-20, 2 = 21-50, 3 = 51-80, and 4 = >80 and the relative abundance of brown citrus aphids was reported as 0 = none, 1 = <10, 2 = 10-50, and 3 = >50. Data were analyzed by ANOVA and means separated by Fisher's least significant difference (LSD) test, based on 5% level of significance (SAS Institute, Cary, NC, USA).

Weather data were obtained from the nearest weather station at the Citrus Research and Education Center, Lake Alfred (http://fawn.ifas.ufl.edu/scripts/reportrequest.asp), and averaged each week over the experiment; the weather station is approximately 12.8 km from the experimental site.

Results and Discussion

During 2005, the study trees produced flush suitable for CLM, Asian citrus psyllid, and brown citrus aphid populations 3 times (Fig. 1). The first flush cycle began by 22 Feb and ended by 18 Apr; the second cycle began around 13 Jun and ended around 2 Aug, and the third began 27 Sep and ended by 30 Nov 2005. Tender new growth suitable for oviposition by CLM females was present during the first week of each flush cycle and the flush continued to grow and harden off during the subsequent 5 or 6 weeks. The proportion of tree branches that were flushing during the first 2 flush cycles was not different, but there were fewer ($F = 3.26$, $df = 3$, $P = 0.025$) flushes in the imidacloprid-treated trees during the third flush cycle. The average weekly temperature (°C), relative humidity (% RH), and rainfall (cm) during the trial are shown in Fig. 2.

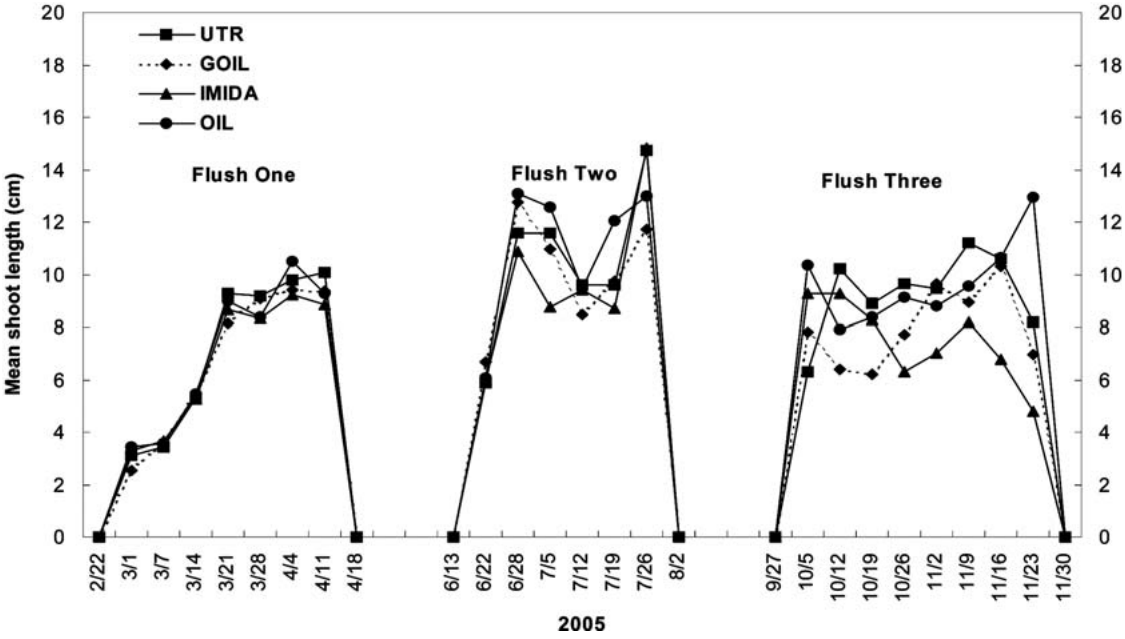

Fig. 1. Mean shoot length (cm) of flushes from a Valencia orange grove near Haines City, Florida in 2005.

First Flush

During the 7 weeks of the first flush cycle there were no differences among the treatments in timing of flush (Fig. 1). Likewise, there were no significant differences in shoot length among the treatments when the data were analyzed weekly, or over the entire flush cycle (Fig. 1) ($F = 0.11$, $df = 3$, $P = 0.95$). No CLM mines were observed except for 1 sample date (1 Mar), when an average of 0.01 (SD ± 0.12) mines/leaf were observed in the untreated control trees (Table 1). No *A. citricola* or other mortality factors were observed during this flush.

Densities of Asian citrus psyllid nymphs during the first flush were in categories 0 and 1, with category 0 indicating no psyllids and category 1 indicating 1-20 psyllids/shoot were present (Table 2). During weeks 1 through 5, there were no significant differences in psyllid densities among the 4 treatments when data were analyzed on a weekly basis ($P = 0.20$ to 0.95). When psyllid densities were analyzed over the entire flush cycle, no significant differences occurred among treatments ($F = 0.03$, $df = 3$, $P = 0.99$). No brown citrus aphids were observed during this flush.

Second Flush

On 22 Jun, prior to the application of sprays, very low numbers of CLM mines (0.04 to 0.07 CLM mines/leaf) were present (Table 1). The proportion of living CLM larvae in the mines on 22 Jun ranged from 79% (in the block to be treated with imidacloprid) to 100% (all other blocks) (Fig. 3). The mortality observed in the imidacloprid-treated trees was probably due to predation, because the mines were empty and no pupal chambers were present (Fig. 4).

After the sprays were applied on 23 Jun, the number of mines remained low throughout the subsequent 5 weeks, with mean densities during week 6 ranging from 0.10 to 0.15 CLM mines/leaf (Table 1). After the trees in treatments B, C, and D were sprayed with oil or imidacloprid, the number of living CLM larvae dropped to 12% in the 28 Jun sample and to zero in the 5 Jul sample in the imidacloprid-treated trees while 28, 36, and 16% of the larvae in the control and 2 oil treatments remained alive, respectively (Fig. 3). However, the number of live CLM larvae in the 4 treatments was not significantly different over the entire second flush when densities were combined over the 6 weeks ($F = 1.25$, $df = 3$, $P = 0.37$). This indicates that neither oil nor imidacloprid significantly reduced CLM larval feeding damage during this flush cycle. The maximum densities of CLM mines in this flush occurred on 28 Jun and ranged from 0.05 (0.03) CLM mines per leaf to 0.29 (0.12) mines per leaf (Table 1). Thus, densities of CLM larvae remained low during the entire second flush.

The proportion of CLM mines that were empty, presumably due to predation, during the second flush ranged from 21 to 97% (Fig. 4). There were no differences ($F = 1.58$, $df = 3$, $P = 0.29$) in the proportion of empty mines among the 4 treatments over the flush cycle, suggesting that the

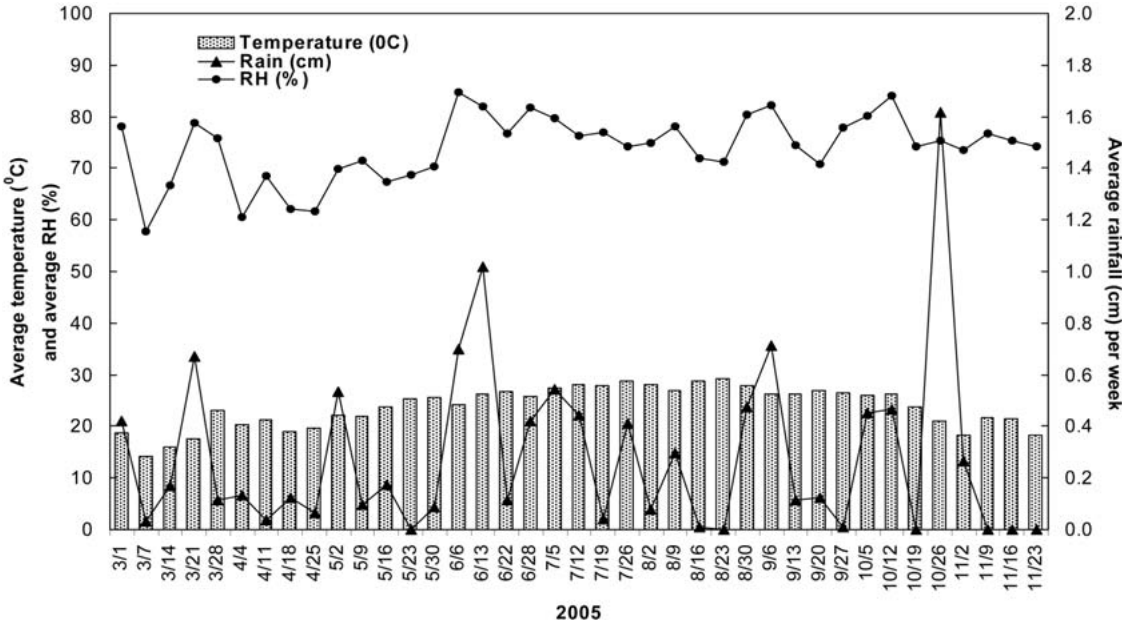

Fig. 2. Average weekly temperature (°C), relative humidity (%), and rainfall (cm) in a Valencia orange grove near Haines City, Florida in 2005.

pesticides applied had no impact on predators, perhaps because oil has a very short residual and imidacloprid is a systemic.

Parasitism by *A. citricola*, as determined by evaluating the pupal chambers of those CLM larvae that survived to the pupal stage, ranged from 0 to 39% over this flush (Fig. 5). There were no differences in parasitism among the 4 treatments ($F = 0.74$, $df = 3$, $P = 0.53$), indicating that oil and imidacloprid did not have a negative effect on *A. citricola* densities in this trial.

Psyllid density categories during the second flush varied by treatment (Table 2). Prior to treatment on 23 Jun, there were no differences in psyllid densities, but after treatment with imidacloprid, a significant reduction in psyllid densities was seen for 3 weeks (28 Jun, 5 and 12 Jul) compared to the untreated control ($F = 25.66$, $df = 3$, $P = 0.001$) (Table 2). By contrast, the growers' oil treatment (treatment B) reduced psyllid densities for only 1 week (7 Jul) compared to the untreated control trees, and the other oil-treated trees (treatment D) did not show a significant difference from the untreated control trees (A). By 19 Jul, there were no differences in psyllid densities among the treatments and again, no differences were observed in densities during the last sample on 26 Jul. No brown citrus aphids were observed in these trees during the second flush.

Third Flush

During the third flush cycle, CLM densities remained relatively low, ranging from 0.05 to 1.27 mines per leaf (Table 1). The number of CLM larvae in the 4 treatments was not different over the entire third flush cycle when densities were combined over the 8 weeks ($F = 0.32$, $df = 3$, $P = 0.80$).

Parasitism by *A. citricola* increased compared to the second flush, ranging from 56% in the untreated control trees to 33% in the oil-treated trees and 22% in the imidacloprid-treated trees (Fig. 5), but these rates were not different among the treatments over this flush cycle ($F = 2.36$, $df = 3$, $P = 0.08$).

Parasitism of the CLM by eulophid parasitoids was not observed during flush cycles 1 or 2, but during flush cycle 3 some pupal chambers contained an unidentified parasitoid. For example, during week 1 of this flush cycle, a total of 7, 34, and 4 pupal chambers were produced in the untreated, imidacloprid- and oil-treated trees, respectively. Of these pupal chambers, 100% of 7 pupal chambers in the untreated control trees, 59% of 34 pupal chambers in the imidacloprid-treated trees, and 25% of 4 pupal chambers in the oil-treated trees contained this unidentified parasitoid; none were found in the growers' oil treatment. During week 2, trees in 2 treatments (untreated and imidacloprid-treated), had 8% (of 12) of the pupal chambers and 67% (of 3) of the pupal chambers, respectively, with an unidentified parasitoid. During weeks 3 and 4, no parasitoids other than *A. citricola* were observed. During week 5, 28% of 18 pupal chambers in the growers' oil treatment contained the unidentified parasitoid. No parasitoids other than *A. citricola* or this

TABLE 1. CITRUS LEAFMINER MINES PER LEAF DURING 3 FLUSH CYCLES IN A VALENCIA ORANGE GROVE NEAR HAINES CITY, FLORIDA IN 2005.

	Mean (± SD) no. of CLM mines per leaf during sample dates							
First flush	1 Mar	7 Mar	14 Mar	21 Mar	28 Mar	4 Apr	11 Apr	
A) Untreated control	0.01 (0.12)	0	0	0	0	0	0	—
B) Grower's oil	0	0	0	0	0	0	0	—
C) Imidacloprid	0	0	0	0	0	0	0	—
D) Oil	0	0	0	0	0	0	0	—
Second flush	22 Jun	28 Jun	7 Jul	12 Jul	19 Jul	26 Jul		
A) Untreated control	0.04 (0.01)	0.13 a (0.08)	0.04 (0.03)	0.05 (0.05)	0.09 (0.02)	0.10 (0.02)	—	—
B) Grower's oil	0.07 (0.03)	0.05 a (0.03)	0.04 (0.03)	0.06 (0.03)	0.09 (0.05)	0.13 (0.08)	—	—
C) Imidacloprid	0.06 (0.04)	0.10 a (0.01)	0.02 (0.03)	0.12 (0.04)	0.06 (0.03)	0.10 (0.02)	—	—
D) Oil	0.04 (0.02)	0.29 b (0.12)	0.13 (0.13)	0.03 (0.03)	0.06 (0.02)	0.15 (0.04)	—	—
P value	0.41	0.001	0.14	0.08	0.45	0.43		
Third flush	5 Oct	12 Oct	19 Oct	26 Oct	2 Nov	9 Nov	16 Nov	23 Nov
A) Untreated control	0.25 (0.25)	1.17 (0.17)	0.55 (0.28)	0.50 (0.30)	0.39 (0.07)	0.32 (0.15)	0.38 (0.17)	0.54 (0.10)
B) Grower's oil	0.05 (0.04)	0.52 (0.49)	0.48 (0.37)	0.33 (0.10)	0.46 (0.14)	0.32 (0.12)	0.36 (0.08)	0.59 (0.30)
C) Imidacloprid	0.29 (0.17)	0.60 (0.22)	0.49 (0.15)	0.33 (0.16)	0.30 (0.06)	0.30 (0.16)	0.40 (0.06)	0.44 (0.11)
D) Oil	1.27 (0.84)	0.65 (0.08)	0.42 (0.16)	0.31 (0.07)	0.28 (0.03)	0.19 (0.10)	0.29 (0.14)	0.44 (0.18)
P value	0.26	0.15	0.93	0.60	0.12	0.71	0.70	0.63

Means were analyzed weekly in each flush cycle by ANOVA and means separated by Fisher's LSD, with $P < 0.05$. Means within a flush cycle within a column with the same letters are not significantly different.

unidentified parasitoid were observed in any samples.

During the third flush cycle, psyllid densities were not different among the 4 treatments during the entire flush cycle when the data were analyzed on a weekly basis (Table 2) or over the entire flush cycle (F =0.59, df = 3, P = 0.62).

Shoot Length and Shoot Numbers

The number of shoots that could be sampled each week was not different among the treatments during flush cycles 1 (F = 1.23, df = 3, P = 0.31, data not shown) and 2 (F = 0.77, df = 3, P = 0.52). However, during flush cycle 3, there were fewer shoots in the imidacloprid-treated trees over the entire flush cycle (F = 3.26, df = 3, P = 0.03).

The length of each shoot sampled was measured weekly throughout the growing season to document when flush cycles began and ended and to determine whether there were differences in growth rates among the treatments (Fig. 1). During the first flush cycle, there were no differences in shoot lengths among the treatments. During the second flush cycle, there were no significant differences in shoot lengths among the treatments, except that treatment B had significantly longer shoots (P = 0.03) on 22 Jun, prior to application of the spray. After that, there were no differences among the treatments, although there was a trend for the trees treated with imidacloprid to have shorter shoots. During flush cycle 3, there were significant differences in shoot lengths on 2 dates when the data were analyzed weekly. On 26 Oct, the imidacloprid-treated trees had shorter shoots (mean = 6.3 cm) compared to the untreated trees (9.6 cm) (P = 0.008). Furthermore, when the combined shoot lengths were compared for flush 2 and 3, (post spray), differences were found (F = 6.29, df = 3, P = 0.03), with the imidacloprid-treated shoots significantly

TABLE 2. ASIAN CITRUS PSYLLID NYMPHAL DENSITIES IN A VALENCIA ORANGE GROVE NEAR HAINES CITY, FLORIDA DURING 2005.

	Mean (± SD) score (range 0-4)* each sample date							
First flush	1 Mar	7 Mar	14 Mar	21 Mar	28 Mar	4 Apr	11 Apr	
A) Untreated	0.90 (0.13)	0.98 (0.02)	1.04 (0.04)	1.04 (0.08)	0.89 (0.11)	0.26 (0.13)	0.09 (0.10)	—
B) Grower's oil	0.86 (0.10)	1.00 (0.04)	1.11 (0.07)	1.02 (0.05)	0.79 (0.11)	0.48 (0.08)	0.12 (0.13)	—
C) Imidacloprid	1.01 (0.02)	0.98 (0.02)	1.07 (0.12)	1.01 (0.02)	0.75 (0.11)	0.23 (0.09)	0.11 (0.09)	—
D) Oil	0.96 (0.08)	0.98 (0.05)	0.96 (0.04)	1.02 (0.05)	0.86 (0.09)	0.33 (0.09)	0.14 (0.07)	—
P value	0.20	0.94	0.26	0.94	0.15	0.11	0.95	
Second flush	22 Jun	28 Jun	7 Jul	12 Jul	19 Jul	26 Jul		
A) Untreated	1.08 (0.08)	0.72 a (0.16)	0.82 a (0.10)	0.96 a (0.04)	0.81 (0.17)	0.44 (0.28)	—	—
B) Grower's oil	1.05 (0.01)	0.47 a (0.13)	0.43 b (0.16)	0.97 a (0.05)	0.73 (0.17)	0.38 (0.19)	—	—
C) Imidacloprid	1.28 (0.35)	0.09 b (0.12)	0 c	0.25 b (0.11)	0.68 (0.17)	0.46 (0.17)	—	—
D) Oil	1.17 (0.12)	0.77 a (0.18)	0.78 a (0.21)	1.07 a (0.12)	0.75 (0.05)	0.51 (0.10)	—	—
P value	0.52	0.006	0.0008	0.001	0.79	0.86		
Third flush	5 Oct	12 Oct	19 Oct	26 Oct	2 Nov	9 Nov	16 Nov	23 Nov
A) Untreated	0	0.79 (0.40)	0.92 (0.14)	0.88 (0.12)	0.82 (0.05)	0.96 (0.03)	0.49 (0.15)	0.74 (0.24)
B) Grower's oil	0	0.97 (0.21)	0.73 (0.23)	0.93 (0.07)	0.89 (0.10)	0.91 (0.15)	0.58 (0.23)	0.45 (0.21)
C) Imidacloprid	0.03 (0.05)	0.50 (0.50)	0.88 (0.03)	0.57 (0.25)	0.89 (0.12)	0.77 (0.18)	0.18 (0.17)	0.67 (0.58)
D) Oil	0	0.81 (0.23)	0.68 (0.26)	0.95 (0.05)	0.89 (0.15)	0.97 (0.05)	0.62 (0.29)	0.27 (0.24)
P value	0.45	0.67	0.28	0.08	0.66	0.35	0.09	0.28

*Psyllid densities were scored as: 0 = none, 1 = 1-20, 2 = 21-50, 3 = 51-80 and 4 = >80. Data were analyzed weekly in each flush cycle by ANOVA and means separated by Fisher's LSD, with $P < 0.05$. Means within a flush cycle within a column with the same letters are not significantly different.

shorter. Interpretation of these results is difficult, because there were no differences over the season in CLM densities among the treatments and there were fewer psyllids in the imidacloprid-treated trees during flush cycles 2 and 3 after treatment. The higher psyllid densities in the untreated or oil-treated trees could have caused reductions in growth. Thus, the data suggest that imidacloprid might have detrimental effects on shoot growth and the number of shoots.

Others have found that imidacloprid may have detrimental effects on growth or yield of crops when there are no pest pressures. Obviously, imidacloprid can result in increased crop growth and yield when pest populations exceed the economic injury level and Oosterhuis & Brown (2003) suggested that imidacloprid might promote plant health, stress recovery, and yield increases in cotton. However, McGuire (2005) evaluated imidacloprid for 2 years and failed to find evidence that imidacloprid enhances growth and/or yield in cotton. By contrast, Wu et al. (2004) and Qiu et al. (2004) found that imidacloprid reduced the size of rice grains in treated plants. Hurley & Patel (2003) found that imidacloprid reduced the growth of *Eucalyptus nitens* Deane & Maiden (Maiden) tree seedlings after a root drench at 2 concentrations by 13 and 8%, respectively. Wallace et al. (2000) found that imidacloprid was phytotoxic to cucumbers in the greenhouse and Ebel et al. (2000) found it was toxic to tomatoes and cucumbers in the greenhouse. Dewar et al. (1997) found that

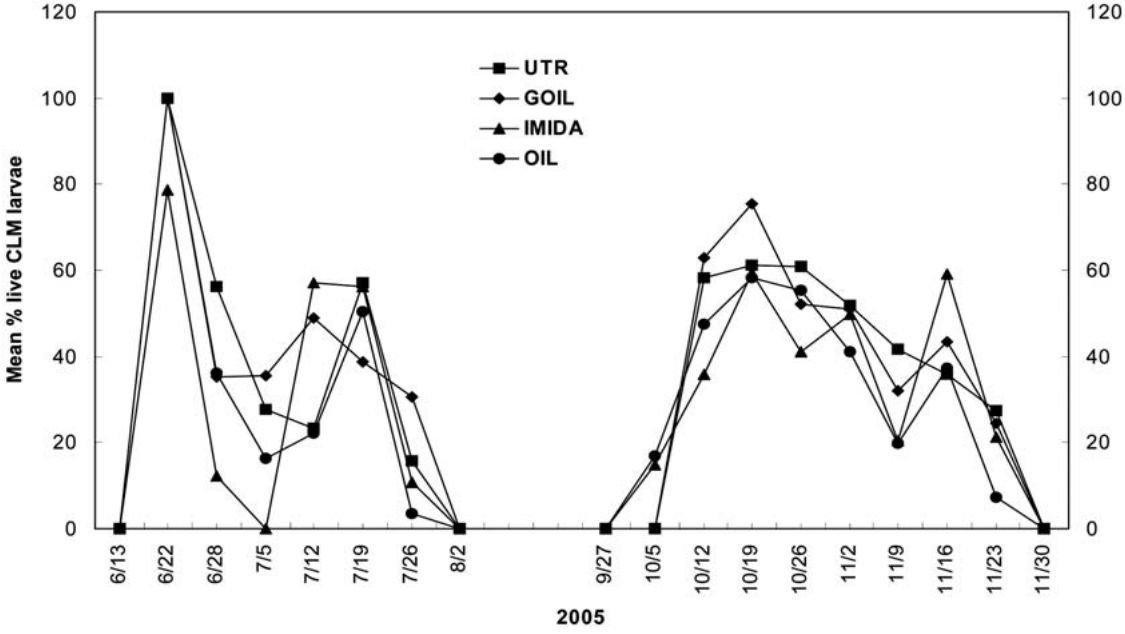

Fig. 3. Mean percentage living citrus leafminer larvae in mines in a Valencia orange grove near Haines City, Florida in 2005.

sugar beet seeds in pellets containing higher rates of imidacloprid had a slower germination rate, and the total number of seedlings emerging was reduced, but different cultivars affected the degree of these effects by imidacloprid. Bhagwat & Lane (2003) found that imidacloprid caused chlorosis of *in vitro* shoot cultures of apples at the end of the 6-week treatment. By contrast, Thielert (2006) reported that imidacloprid protects crops against abiotic stresses such as drought. Our data suggest that imidacloprid could be reducing shoot length in Valencia oranges over the season after a single treatment, but these experiments were not designed to evaluate these effects and there is a possibility that the differences observed are by chance alone. Thus, additional research is needed to confirm any negative effects on growth by imidacloprid in Florida's citrus cultivars. Such research is relevant to developing an IPM program for managing citrus leafminers and Asian citrus psyllids in Florida as a means of reducing the spread of citrus canker and citrus greening disease, respectively, because increased use of imidacloprid to control these disease vectors could have growth or yield costs, as well as benefits.

General Discussion

There were essentially no brown citrus aphids and relatively low densities of psyllid nymphs and CLM larvae in this grove in Polk County, Florida throughout the growing season during 2005. The density of psyllids was estimated by an abundance score, with only nymphs being estimated, because previous experience in monitoring psyllid populations in a grapefruit grove in the Ft. Pierce area during 2004 had found that high rates of predation occurred on eggs and newly hatched nymphs (Hoy et al., unpubl.).

Prior to this study, no information was available on the phenology of *A. citricola* and CLM in this citrus-growing area ('the Ridge') of Florida. These results indicate that *A. citricola* is an important natural enemy of the CLM, as shown by the proportion of those CLM larvae that survived to the pupal stage during 2005 in both treated and control trees. As expected, *A. citricola* populations lagged behind their CLM host during flush cycle 2.

A large number of empty mines were observed in all 4 treatments. Empty mines are often due to predation by ants (Amalin et al. 2002; Zappala et al., 2007). Some dead larvae appeared to have been fed on by lacewing larvae or spiders. Previous work by Browning & Peña (1995) and Amalin et al. (1996, 2002) found that green lacewing larvae (*Chrysoperla rufilabris* (Burmeister)), ants (especially the red imported fire ant, *Solenopsis invicta* Buren), thrips, hunting spiders (*Chiracanthium inclusum* (Hentz), *Hibana velox* (Becker) and *Trachelas volutes* (Gertsch)), and mirid bugs are predators of CLM larvae in lime orchards in south Florida, causing approximately 34 to 39% of the mortality observed. Villanueva-Jimenez et al. (2000) found that total morality of the CLM in a Gainesville, FL grapefruit grove var-

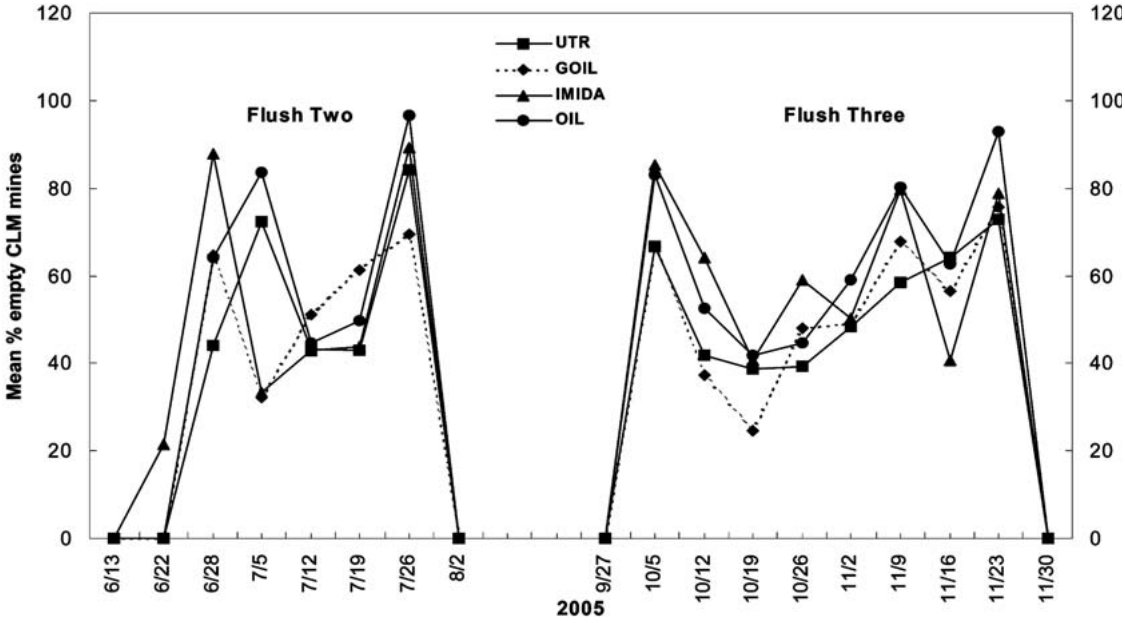

Fig. 4. Mean percentage empty citrus leafminer mines in a Valencia orange grove near Haines City, Florida in 2005.

ied throughout the season, but was greater than 70% after the first flush cycle, with "unexplained larval mortality" that was as high as 70.4% during the fourth flush during 1997. In that study, 32 to 80% of the mortality was caused by *A. citricola* on those CLM that managed to reach the pupal stage (Villanueva-Jimenez et al. 2000), but empty mines also were observed and could have been due to predation by red imported fire ants, which were abundant in the grove. Zappalá et al. (2007) found that red imported fire ants removed CLM larvae that had been parasitized by *A. citricola* from mines in laboratory and field trials.

From about Jul through Nov in this Valencia orange grove in central Florida, a substantial proportion of the few CLM larvae that survived to the pupal stage were parasitized by *A. citricola*; this mortality factor would help to reduce the number of adults entering the winter. However, the proportion of CLM pupae that were parasitized by *A. citricola* was lower than expected, for unknown reasons. Previous samples during Aug through Oct in multiple sites in Florida had found up to 99% of the pupae parasitized by *A. citricola* (Hoy et al. 1995; Hoy & Nguyen 1997; Pomerinke & Stansly 1998; Villanueva-Jimenez et al. 2000). The reason(s) for the relatively lower parasitism rates by *A. citricola* in this citrus grove near Haines City is unknown.

We are unable to conclude that the combined action of natural enemies and pesticide applications suppressed CLM densities below the economic threshold, because it is not known what an economic injury level is in Valencia oranges grown in the 'Ridge' area of Florida, especially now that the canker eradication program has ended (in 2006) and canker is considered established, although there was no canker in the grove at the time of this study. When the CLM attacks trees in nurseries and young trees in groves, direct damage by the CLM can delay growth and alter canopies as well as open the mines to infection by the canker bacterium. The economic impact of CLM on mature orchards in areas where citrus canker is now endemic in Florida is not yet known, and depends on canker bacterial density, weather conditions, tree age, and timing of the damage. It is unclear if there is a surplus of leaf area in citrus grown in central Florida, although Knapp et al. (1995) suggested that a 10% leaf area loss due to CLM mines did not affect yield (prior to canker establishment). In Florida, CLM densities typically are very low during winter and during the first spring flush, but increase during the growing season to peak in the fall, and this pattern was followed in the Valencia grove studied. In China, Huang & Li (1989) found that leaf area loss of less than 20% did not affect yield, and suggested that a loss of 15% of leaf area, or about 0.74 CLM larvae per leaf, was the economic threshold. Garcia-Marí et al. (2002) evaluated the economic injury level of CLM in the Valencia area of eastern Spain from 1996 to 1999 and found that 5-15% of the annual new leaf area of mature trees could be damaged without affecting yield, primarily because the production of new shoots was concentrated

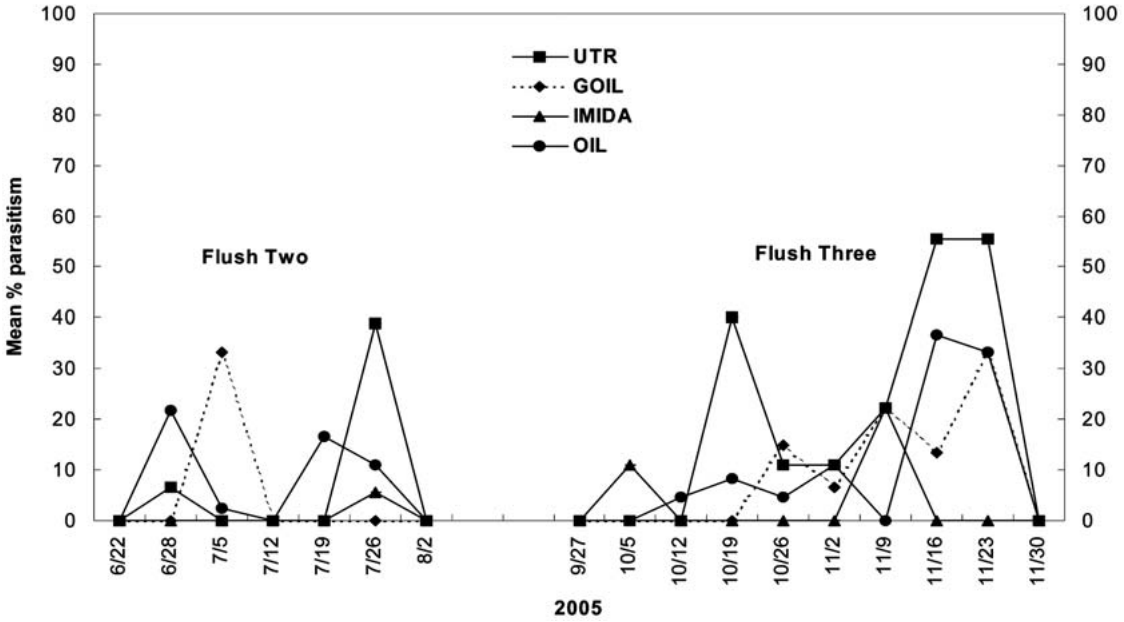

Fig. 5. Mean percentage citrus leafminer pupae parasitized by *Ageniaspis citricola* near Haines City, Florida in 2005.

early in the spring when CLM densities were very low but high CLM populations occurred in summer and fall so the citrus leafminer's effect on biomass, yield and fruit quality was minimal. If this damage level is used to assess potential growth or yield loss for the Valencia grove in this study, then none of the trees reached this level of infestation during either the first or second flush. During the second flush, the maximum density of CLM mines/leaf averaged 0.29 (SD ± 0.12) in the trees treated with oil, while the maximum number of CLM mines/leaf was 0.13 (0.08) in the untreated control. During the third flush, CLM densities peaked in the untreated control and in one set of oil-treated trees at 1.17 (0.22) and 1.27 (0.80) mines/leaf, respectively, but these densities were found only during 1 week. Thus, there is no evidence that the treatments (oil or imidacloprid) significantly reduced CLM densities. The imidacloprid treatment did reduce psyllid densities for 3 weeks, but may have reduced shoot length. The possibility of a detrimental effect by imidacloprid on shoot growth and shoot number should be investigated, particularly if multiple applications of imidacloprid are applied to suppress psyllid populations in an effort to reduce transmission of greening disease in Florida.

Acknowledgments

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Literature Cited

Alvarez, J. M. 2000. Use of Molecular Tools for Discriminating between two Populations of the Citrus Leafminer Parasitoid *Ageniaspis* (Hymenoptera: Encyrtidae). Ph.D. Dissertation, Department of Entomology and Nematology, University of Florida, Gainesville.

Alvarez, J. M., and M. A. Hoy. 2002. Evaluation of the ribosomal ITS2 DNA sequences in separating closely related populations of the parasitoid *Ageniaspis* (Hymenoptera: Encyrtidae). Ann. Entomol. Soc. Am. 95: 250-256.

Amalin, D. M., J. E. Peña, and R. McSorley. 1996. Abundance of spiders in lime groves and their potential role in suppressing the citrus leafminer population, p. 72 *In* M. Hoy [ed.], Proc. Intern. Conf. Managing the Citrus Leafminer, April 23-25. University of Florida, Gainesville.

Amalin, D. M., J. E. Peña, R. E. Duncan, H. W. Browning, and R. McSorley. 2002. Natural mortality factors acting on citrus leafminer, *Phyllocnistis citrella*, in lime orchards in South Florida. BioControl 47: 327-347.

Bhagwat, B., and D. W. Lane. 2003. Eliminating thrips from *in vitro* shoot cultures of apple with insecticides. Hortscience 38: 97-100.

Browning, H. W., and J. E. Peña. 1995. Biological control of the citrus leafminer by its native parasitoids and predators. Citrus Industry 76(4): 46-48.

Chagas, M. C. M., J. R. P. Parra, T. Namekata, J. S. Hartung, and P. T. Yamamoto. 2001. *Phyllocnistis*

citrella Stainton (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* in Brazil. Neotropical Entomol. 30: 55-59.

CHRISTIANO, R. S. C., M. DALLA PRIA, W. C. JESUS JUNIOR, J. R. P. PARRA, L. AMORIM, AND A. BERGAMIN FILHO. 2007. Effect of the citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. Crop Protect. 26: 59-65.

DEWAR, A. M., F. WESTWOOD, K.M. BEAN, L. A. HAYLOCK, AND R. OSBORNE. 1997. The relationship between pellet size and the quantity of imidacloprid applied to sugar beet pellets and the consequences for seedling emergence. Crop Protect. 16: 187-192.

EBEL, R. C., B. WALLACE, AND C. ELKINS. 2000. Phytotoxicity of the systemic insecticide imidacloprid on tomato and cucumber in the greenhouse. HortTechnol. 10: 144-147.

EVANS, G. A. 1999. A new species of *Cirrospilus* (Hymenoptera: Eulophidae) and two new synonymies of parasitoids reared from the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Florida Entomol. 82: 448-453.

GARCIA-MARÍ, F., C. GRANDA, S. ZARAGOZA, AND M. AGUSTI. 2002. Impact of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) on leaf area development and yield of mature citrus trees in the Mediterranean area. J. Econ. Entomol. 95: 966-974.

GOTTWALD, T. R., G. HUGHES, J. H. GRAMAN, X. SUN, AND T. RILEY. 2001. The citrus canker epidemic in Florida: the scientific basis of regulatory eradication policy for an invasive species. Phytopathol. 91: 30-34.

GRAHAM, J. H., T. R. GOTTWALD, H. W. BROWNING, AND D. S. ACHOR. 1996. Citrus leafminer exacerbated the outbreak of Asiatic citrus canker in South Florida, p. 83 *In* M. A. Hoy [ed.], Proceedings, International Meeting: Managing the Citrus Leafminer, 22-25 April 1996, Orlando, Florida. University of Florida, Gainesville.

HEPPNER, J. B. 1993. Citrus leafminer, *Phyllocnistis citrella*, in Florida (Lepidoptera: Gracillariidae: Phyllocnistinae). Trop. Lepidoptera 4: 49-64.

HEPPNER, J. B. 1995. Citrus leafminer (Lepidoptera: Gracillariidae) on fruit in Florida. Florida Entomol. 78: 183-186.

HOY, M. A., AND R. NGUYEN. 1994a. Classical biological control of the citrus leafminer in Florida: a progress report. Citrus Industry 75(6): 61-62.

HOY, M. A., AND R. NGUYEN. 1994b. Current status of *Ageniaspis citricola*, a parasite of the citrus leaf miner, in Florida. Citrus Industry 75 (12): 30-32.

HOY, M. A., AND R. NGUYEN. 1997. Classical biological control of the citrus leafminer *Phyllocnistis citrella* Stainton: Theory, practice, art and science. Trop. Lepidoptera 8 (Suppl. 1): 1-19.

HOY, M. A., R. NGUYEN, D. HALL, R. BULLOCK, M. POMERINKE, J. PEÑA, H. BROWNING, AND P. STANSLY. 1995. Establishment of citrus leafminer parasitoid, *Ageniaspis citricola* in Florida. Citrus Industry 76(12): 12-17.

HOY, M. A., R. NGUYEN, M. POMERINKE, R. BULLOCK, D. HALL, J. KNAPP, J. PEÑA, H. BROWNING, AND P. STANSLY. 1997. Distribution and abundance of *Ageniaspis citricola*, a parasite of the citrus leafminer in Florida. Citrus Industry 78(5): 51-52.

HOY, M. A., A. JEYAPRAKASH, R. MORAKOTE, P. K. C. LO, AND R. NGUYEN. 2000. Genomic analyses of two populations of *Ageniaspis citricola* (Hymenoptera: Encyrtidae) suggest that a cryptic species may exist. BioControl 17: 1-10.

HUANG, M., AND S. LI. 1989. The damage and economic threshold of citrus leafminer, *Phyllocnistis citrella* Stainton, to citrus. Studies on the Integrated Management of Citrus Insect Pests, Academic, New York.

HURLEY, M., AND V. PATEL. 2003. Effect of imidacloprid on the growth of *Eucalyptus nitens* seedlings. Austral. Forest. 66: 100-101.

KNAPP, J. L., L. G. ALBRIGO. H. W. BROWNING, R. C. BULLOCK, J. B. HEPPNER, D. G. HALL, M. A. HOY, R. NGUYEN, J. E. PEÑA, AND P. A. STANSLY. 1995. Citrus leafminer, *Phyllocnistis citrella* Stainton: current status in Florida, 1995. Florida Coop. Ext. Serv., Gainesville, FL.

KNAPP, J. L., H. W. BROWNING, L. G. ALBRIGO, J. E. PEÑA, P. A. STANSLY, AND R. C. BULLOCK. 1996. Management of the citrus leafminer: chemical options. Citrus Industry, Mar 1996: 48-49.

LIM, U. T., AND M. A. HOY. 2005. Biological assessment in quarantine of *Semielacher petiolatus* (Hymenoptera: Eulophidae) as a potential classical biological control agent of citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), in Florida. Biol. Control 33: 87-95.

LIM, U. T., AND M. A. HOY. 2006. Overwintering of the citrus leafminer, *Phyllocnistis citrella* (Lepidoptera: Gracillariidae), without diapause in Florida. Florida Entomol. 89: 361-366.

LIM, U. T., L. ZAPPALÁ, AND M. A. HOY. 2006. Pre-release evaluation of *Semielacher petiolatus* (Hymenoptera: Eulophidae) in quarantine for the control of citrus leafminer: Host discrimination, relative humidity tolerance and alternative hosts. Biol. Control 36: 65-73.

MCGUIRE, C. C. 2005. Evaluation of PGR Properties of Trimax in Cotton. M. S. Thesis. Texas A & M Univ., 63 pp. (http://handle.tamu.edu/1969.1/397).

NEALE, C., D. SMITH, D., G. A. C. BEATTIE, AND M. MILES. 1995. Importation, host specificity testing, rearing and release of three parasitoids of *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) in eastern Australia. J. Aust. Entomol. Soc. 34: 343-348.

OOSTERHUIS, D. M., AND R. S. BROWN. 2003. Effects of Trimax on the physiology, growth and yield of cotton, *In* Proc. Beltwide Cotton Conf. Nashville, TN 1: 1881-1884.

PEÑA, J. E., R. DUNCAN, AND H. BROWNING. 1996. Seasonal abundance of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids in south Florida citrus. Environ. Entomol. 25: 698-702.

PEÑA, J. E., A. HUNSBERGER, AND B. SCHAFFER. 2000. Citrus leafminer (Lepidoptera: Gracillariidae) density: effect on yield of 'Tahiti' lime. J. Econ. Entomol. 93: 374-379.

POMERINKE, M. A., AND P. A. STANSLY. 1998. Establishment of *Ageniaspis citricola* (Hymenoptera: Encyrtidae) for biological control of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) in Florida. Florida Entomol. 81: 361-372.

QIU, H. M., J. C. WU, G. Q. YANG, B. DONG, AND D. H. LI. 2004. Changes in the uptake function of the rice root to nitrogen, phosphorus and potassium under brown plant hopper, *Nilaparvata lugens* (Stal) (Homoptera: Delphacidae) and pesticide stresses, and effect of pesticides on rice-grain filling in field. Crop Protection 23: 1041-1048.

SAS INSTITUTE, INC. 1999. SAS version 9.1. SAS Publ., Cary, NC.

SMITH, J. M., AND M. A. HOY. 1995. Rearing methods for *Ageniaspis citricola* (Hymenoptera: Encyrtidae) and *Cirrospilus quadristriatus* (Hymenoptera: Eulophidae) released in a classical biological control program for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). Florida Entomol. 78: 600-608.

SOHI, G. S., AND M. S. SANDHU. 1968. Relationship between citrus leaf-miner (*Phyllocnistis citrella* Stainton) injury and citrus canker [*Xanthomonas citri* (hasse) Dowson] incidence on citrus leaves. J. Res. Punjab Agric. Univ. 5: 66-69.

THIELERT, W. 2006. A unique product: the story of the imidacloprid stress shield. Pflanzenschutz Nachrichten Bayer 59: 73-86.

VILLANUEVA-JIMENEZ, J. A., AND M. A. HOY. 1998. Toxicity of pesticides to the citrus leafminer (Lepidoptera: Gracillariidae) and its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae) evaluated to assess their suitability for an IPM program in citrus nurseries. BioControl 43: 357-388.

VILLANUEVA-JIMENEZ, J. A., M. A. HOY, AND F. S. DAVIES. 2000. Field evaluation of integrated pest management-compatible pesticides for the citrus leafminer *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae). J. Econ. Entomol. 93: 357-367.

WALLACE, B., R. C. EBEL, AND J. KEMBLE. 2000. Imidacloprid effects on root growth, photosynthesis, and water use of cucumber in the greenhouse. Hortscience 35: 827-832.

WU, J. C., B. DONG, D. H. LI, H. M. QIU, AND G. Q. YANG. 2004. Effects of four pesticides on grain growth parameters of rice. Scientia Agricult. Sinica 37 (3): 376-381.

YODER, J. A., AND M. A. HOY. 1998. Differences in water relations among the citrus leafminer and two different populations of its parasitoid inhabiting the same apparent microhabitat. Entomol. Exp. Appl. 89: 169-173.

ZAPPALÁ, L., M. A. HOY, AND R. D. CAVE. 2007. Interactions between the red imported fire ant, the citrus leafminer, and its parasitoid *Ageniaspis citricola* (Hymenoptera: Encyrtidae): laboratory and field evaluations. Biocontrol Sci. Technol. 17: 353-363.

CONSUMPTION OF BAIT SOLUTIONS BY *ANASTREPHA SUSPENSA*

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ABSTRACT

Nu-Lure and other protein solutions were presented to *Anastrepha suspensa* in J-tubes and consumption was quantified spectrophotometrically. In choice comparisons, flies consumed more or equal water compared to Nu-Lure and more Nu-Lure compared to Bragg's Liquid Aminos, corn steep liquor, NZ case, pepticase, Solulys, soy hydrolysate, Torula yeast, whey, and yeast enzymatic hydrolysate. Consumption of protein solutions was one-half or less than 0.2 M sucrose, the positive control. The addition of 0.2 M sucrose or 0.2 M fructose to Nu-Lure did not increase the consumption of Nu-Lure compared to the consumption of sucrose alone, suggesting that Nu-Lure negates the phagostimulant properties of sucrose and possibly fructose for *A. suspensa*. If higher consumption rates of a bait/toxicant mixture is a goal, 0.2 M sucrose would be a better choice than the protein solutions tested, including Nu-Lure.

RESUMEN

Se suministró Nu-Lure así como otras soluciones protéicas a individuos de *Anastrepha suspensa* en tubos "J" y se cuantificó por via fotométrica el consumo de estas proteinas durante un intervalo adecuado de tiempo. En las comparaciones seleccionadas se observó que el consumo de agua se mantuvo igual o superior al de Nu-Lure; asimismo, el consume de esta proteina fue superior al observado para Amino Líquido de Bragg, licor de maiz, NZ case, pepticase, Solulys, hidrosilato de soya, levadura de Torula y suero e hidrosilato enzimático de levadura. En general, el consumo de soluciones protéicas se mantuvo por debajo de la mitad del correspondiente al control positivo de sacarosa 0.2 M. La adición de sacarosa o fructosa (ambos a la concentración de 0.2 M), a Nu-Lure no incrementó el consumo de dicha proteina en comparación con el consume de azúcar, lo que sugiere que Nu-Lure podría eliminar las propiedades fagoestimulantes de la sacarosa y posiblemente tambien de la fructosa en *A. suspensa*. En aquellos casos en que se desea alcanzar velocidades de consumo mas elevadas de agentes tóxicos mezclados con el sebo correspondiente por parte de *A. suspensa*, la solución de sacarosa 0.2 M podría constituir una mejor alternativa que las soluciones protéicas prepadas, incluído el Nu-Lure.

Translation provided by the authors.

Nu-Lure7, a commercially available, corn protein hydrolysate (Miller Chemical and Fertilizer Corp., P.O. Box 333, Hanover, PA 17331) is combined with malathion for the management of *Anastrepha suspensa* (Loew) in Florida (Nigg et al. 2004a). The 20% malathion/80% Nu-Lure mixture is described as a bait/pesticide and may be applied by air or by ground equipment (Nigg et al. 2004a). We attempted to attract and kill approximately 20,000 *A. suspensa* in the greenhouse with this mixture without success (H. N. Nigg & S. E. Simpson, personal observation).

Others have studied the attractiveness of Nu-Lure to fruit flies under various conditions in trapping studies (Epsky et al. 1993, 1999; Heath et al. 1994; Katsoyannos et al. 1999; Fabre et al. 2003). Although consumption was not determined, Nu-Lure appeared to be an attractant to *A. suspensa* and other tephritidae in those studies.

In *A. suspensa* management programs, Nu-Lure/malathion is applied as a droplet to surfaces. There is an assumption by scientists, growers, and the public that these pesticide-laden bait droplets are consumed by the fly with resultant mortality. Our greenhouse observation appears to be the sole contrary observation to this supposition.

If we could increase the consumption of Nu-Lure, the amount of pesticide added to NuLure could be reduced on a 1:1 basis. That is, if consumption were doubled, pesticide concentration could be halved. Our initial efforts on bait improvement were feeding requirements (Nigg et al. 2004c), development of an individual fly consumption method (Nigg et al. 2004b), and determination of sugar consumption (Nigg et al. 2006). With our development of an accurate method for monitoring individual *A. suspensa* consumption

(Nigg et al. 2004b), the premise that Nu-Lure was consumed by *A. suspensa* could be evaluated.

The purpose of this study was to quantify the consumption of Nu-Lure and other protein solutions by adult *A. suspensa*.

Materials and Methods

Insects

Anastrepha suspensa pupae were shipped overnight from the Florida Department of Agriculture and Consumer Services (Division of Plant Industry, Gainesville, FL) fly-rearing facility. The ziplock bags in which they were shipped were opened, the pupae were gently manipulated by hand, and the bags were resealed and placed in a refrigerator at 4°C. This procedure allowed for gas exchange and resulted in better adult emergence. Flies destined to be tested at 24 h were held in the refrigerator as pupae for 48 h before being placed in emergence cages. Flies destined to be tested at 6 d of age were held in the refrigerator as pupae for 24 h. This procedure allowed coordination of fly emergence so experiments could be conducted Monday through Friday. Flies were allowed to emerge into cages that were 30 × 30 × 30 cm (Bioquip, Inc., Gardena, CA) and were tested as immature (24-h) and sexually mature (6-d) flies. Flies were fed yeast, sugar, and water according to Nigg et al. (1994, 1995) in their emergence cages. Once adult emergence began, the pupae were removed to an empty cage, emergence was allowed to continue for 12 h, and all remaining pupae were discarded. This procedure resulted in flies 1-2 and 6-7 d old on the day of an experiment. Twenty-four h prior to an experiment, flies were selected directly from their emergence cage. Only active flies with normal wings were transferred by grasping one wing and placing the fly into a 950 mL translucent plastic container. Flies were provided only on agar patty for water for 16 h prior to an experiment.

The consumption of solutions by flies was studied in cages by allowing flies to feed for 45 min (Nigg et al. 2004a). Each cage contained 5 males and 5 females and was treated as a replicate. Five positive control cages, presented with 0.2 M sucrose plus 0.1% cresol red in a J-tube, were included in each trial (Nigg et al. 2006). If the flies in the positive control did not average 2.5 µL or greater consumption over 45 min, the entire data set for that week was discarded. This procedure eliminated 1 data set during these experiments.

Nu-Lure was obtained from Miller Chemical and Fertilizer Corp. (P.O. Box 333, Hanover, PA 17331); whey protein (W-1500) from bovine milk, pepticase (P1192), N-Z-Case M (C7585), and soy protein acid hydrolysate (S-1674) were from Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178); sodium caseinate (spray dried) and hydrolyzed casein (HCA411) from American Casein Company (Burlington, NJ 08016-4123); yeast hydrolysate enzymatic (103304), corn gluten meal (960015), and Torula yeast (903085) from MP Biomedicals, LLC (1263 South Chillicothe Road, Aurora, OH 44202); soy protein (Prolisse) from Cargill Health & Food Technologies (15407 McGinty Road W., Wayzata, MN 55391); and Bragg Liquid Aminos (Live Food Products, Inc., Box 7, Santa Barbara, CA 93102) from a local supermarket. Solulys was from Roquette America, Inc. (1417 Exchange St., P.O. Box 6647, Keokuk, IA 52632-6647).

Consumption Quantification

Flies were allowed to feed for 45 min as this is the time for maximum initial consumption (Nigg et al. 2004b). Quantification of consumption was according to Nigg et al. (2004b). Briefly, flies were presented with protein solutions containing 0.1% fluorescein or 0.1% cresol red in 5-mL J-tubes. Solutions containing 0.1% cresol red or 0.1% fluorescein are consumed equally by these flies (Nigg et al. 2006). Different dyes allowed the direct comparison of two solutions in the same fly (Nigg et al. 2004a). Consumption was measured by extracting each fly in 0.1 M NaOH and quantifying the dye spectrophotometrically, cresol red at 573 nm and fluorescein at 491 nm (Nigg et al. 2004b).

Experiment One

This experiment was designed to directly compare the consumption of NuLure with other protein solutions. Two J-tubes with different solutions were presented in each treatment cage for 45 min. Consumption of 10% Nu-Lure was compared to distilled water and to 10% solutions of the proteins listed above except for Solulys which was tested as packaged. There were 5 replicates of each treatment. All flies were included in the statistical analysis of this experiment whether they had fed or not. To calculate the mean for each replicate, the sum of each solution by sex and cage (replicate) was divided by the number of that sex in the cage.

Experiment Two

Our previous work showed that *A. suspensa* readily consumed 0.2 M sucrose so we compared its consumption to consumption of NuLure (Nigg et al. 2006). This experiment indirectly compared the consumption of NuLure, water, and 0.2 M sucrose. A single J-tube was presented in each cage for 45 min. Treatments were 10% Nu-Lure plus 0.1% cresol red or glass-distilled deionized water plus 0.1% fluorescein or 0.2 M sucrose plus 0.1% fluorescein, or 0.2 M sucrose in 10% Nu-Lure plus 0.1% fluorescein. After 45 min, flies were pro-

cessed and consumption was quantified as described above. There were 5 replicates of each treatment.

Experiment Three

Sugars are phagostimulants for many insects (Hagen & Finney 1950; Peacock & Fisk 1970; Sutherland 1971; Ma & Kubo 1977; Friend 1981; Cobbinah et al. 1982; Doss & Shanks, Jr. 1984; Mochizuki et al. 1985; Shanks & Doss 1987; Ladd 1988; Schmidt & Friend 1991; Allsop 1992; Sharma 1994; Soetens & Pasteels 1994; Shields & Mitchell 1995; Yazawa 1997; Saran & Rust 2005), including *A. suspensa* (Nigg et al. 2006). This experiment examined the influence on the consumption of NuLure by the addition of sucrose, fructose, valine, or sodium tetraborate to 10% Nu-Lure. Two J-tubes containing different solutions were presented in each treatment cage for 45 min. The choice comparisons for experiment 3 were as follows: (1) 10% Nu-Lure plus 0.1% cresol red vs. distilled water plus 0.1% fluorescein; (2) 10% Nu-Lure plus 0.1% cresol red vs. 0.2 M sucrose plus 0.1% fluorescein; (3) 10% Nu-Lure plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.1% fluorescein; (4) 0.2 M sucrose plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.1% fluorescein; (5) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure in 0.2 M fructose plus 0.1% cresol red; (6) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure plus 0.05 M valine plus 0.1% cresol red; (7) 10% Nu-Lure in 0.2 M sucrose plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.05 M valine plus 0.1% fluorescein; and (8) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure in 5% sodium tetraborate plus 0.1% cresol red. There were 5 replicates of each comparison except there were 10 replications for 10% Nu-Lure vs. distilled water and for 10% Nu-Lure vs. 10% Nu-Lure in 0.2 M sucrose. We compared statistically the percent of flies that did not feed, flies that fed only on one of the solutions, and flies that fed on both solutions. We examined in detail the consumption of flies that fed on both solutions.

Statistics

A replicate for all experiments is the mean of a cage by sex. For example, a five-replicate experiment is 5 cages. The means of the 5 cages by sex are the basis for the means and variation of each treatment. Standard deviation is used throughout. Means in Table 2 were compared with paired *t*-tests $\alpha = 0.05$, 0.01, or 0.001 (Microsoft Office Excel 2003). Means in Tables 3, 4, and 5 were statistically compared by analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at $\alpha = 0.05$ (SAS Institute 2001).

Results and Discussion

The means and standard deviations of the consumption of the sucrose positive controls by males were 2.50 ± 0.31 µL (range 2.07-3.08 µL) and by females 3.27 ± 0.74 µL (range 2.15-4.43 µL). There were no statistical differences week to week in the consumption of sucrose by the sucrose control flies except for one week with less than 2.5 µL/fly; that data set was discarded.

The pH of the protein solutions ranged from a low of 3.79 (Nu-Lure in 0.2 M sucrose + 0.05 M valine) to 7.12 (EZ Case M), a factor that may affect attractiveness (Flath et al. 1989; Heath et al. 1994), and ranged from completely soluble to insoluble (Table 1). The 10% Nu-Lure sugar and Nu-Lure valine solutions pHs ranged from 3.80 to 3.78. The pH of 10% Nu-Lure + 5% sodium tetraborate was 8.01. Materials that were insoluble and unsuitable for a liquid bait were Torula yeast, Prolisse, and sodium caseinate (Table 1).

Experiment One

No fly consumed Nu-Lure only. The percentage of flies feeding ranged from 36-100% compared to sucrose controls at 98-100%. Male and female flies consumed about 5× more water compared to Nu-Lure, although 24-h fly consumption was low (Table 2). There was no difference in the consumption of Braggs liquid amino acids vs. Nu-Lure for 6-d flies (Table 2); more NuLure was consumed by 24-h flies. Six-day flies preferred Nu-Lure compared to corn steep liquor; there were no differences for 24-h flies (Table 2). Nu-Lure was preferred to NZ Case and pepticase by 24-h and 6-d flies (Table 2). Nu-Lure was preferred over Solulys by 6-d males only (Table 2). Nu-Lure was preferred over soy protein hydrolysate only by 24-h females (Table 2). Nu-Lure was preferred over Torula yeast except by 6-d males (Table 2). Whey protein was consumed less than Nu-Lure by 24-h males and 6-d females (Table 2). Nu-Lure was preferred over yeast hydrolysate by 24-h flies, but not by 6-d flies (Table 2). The important point about Table 2 data is the less than 2.0 µL average consumption of protein solutions, actually most below 1.0 µL, compared to an average sucrose control consumption of 2.50 µL for males and 3.27 µL for females.

Experiment Two

With the discovery in Experiment 1 that the consumption of protein solutions was low compared to the sucrose controls, we designed Experiment 2 to examine a no-choice comparison of Nu-Lure, sucrose, and water. Experiment 2 no-choice consumption data are presented in Table 3. For males, the percent feeding was not different across solutions (Table 3). For 6-d females, the

TABLE 1. SOLUBILITY AND PH OF PROTEIN SOLUTIONS

10% type	Solubility	pH
Corn steep liquor	Slight sediment	3.98
EZ Case M	Soluble	7.12
Hydrolyzed casein	Stable suspension	5.02
Liquid amino acids	Soluble	5.58
Nu-Lure	Soluble	3.82
Nu-Lure in 0.2 M fructose	Soluble	3.82
Nu-Lure + 5% sodium tetraborate	Soluble	8.01
Nu-Lure in 0.2 M sucrose	Soluble	3.80
Nu-Lure in 0.2 M sucrose + 0.05 M valine	Soluble	3.79
Nu-Lure plus 0.05 M valine	Soluble	3.78
Pepticase	Soluble	6.96
Prolisse	Thick suspension	6.96
Sodium caseinate	Not soluble	6.22
Solulys	Slight sediment	3.98
Soy protein acid hydrolysate	Some sediment	5.70
Torula yeast	Not soluble	6.31
Whey	Some sediment	5.38
Yeast hydrolysate enzymatic	Some sediment	5.60

percent feeding on water was lower than the other solutions, but the amount of water consumed was not different than Nu-Lure or Nu-Lure plus sucrose. For males and females, the amount of 0.2 M sucrose consumed was 2× to 5× greater than water, Nu-Lure, or Nu-Lure plus sucrose (Table 3). The addition of sucrose to Nu-Lure did not enhance its consumption compared to Nu-Lure alone (Table 3).

Experiment Three

Experiment 3 examined the choices flies made in their consumption of the solutions in Experiment 2 (Table 2) and the possible improvement of Nu-Lure consumption. The percentage of flies that fed ranged from 36 to 100% (data not presented). For most experiments, the percent feeding was 70% or more (data not presented). Only the flies that fed on both solutions were included in these analyses.

When comparing the quantities consumed, there was no difference between Nu-Lure and water (line 1, Tables 4 and 5). This is the same result as in Table 3, that is, no difference between the consumption of Nu-Lure and the consumption of water.

Flies fed more on sucrose than on 10% Nu-Lure; this reached statistical significance with 6-d females (line 2, Tables 4 and 5). The addition of sucrose to Nu-Lure led to more consumption of sucrose/Nu-Lure compared to Nu-Lure alone for 24-h females only (line 3, Tables 4 and 5).

Valine improved Nu-Lure and Nu-Lure in 0.2 M sucrose consumption by 6-d, but not 24-h males and females (line 6, Tables 4 and 5). Although more NuLure plus 0.2 M sucrose was consumed when valine was added, this reached statistical significance only with 6-d females (line 7, Tables 4 and 5). The addition of 5% borax to 10% Nu-Lure did not improve its consumption (line 8, Tables 4 and 5),a combination known to increase Nu-Lure attractiveness to *Anastrepha* spp. (Heath et al. 1994). Our interpretation of these data is that the addition of NuLure to 0.2 M sucrose decreased the consumption of sucrose and the inclusion of NuLure in a comparison decreased the consumption of solutions in general. If we total the consumption of flies in Tables 4 and 5, we can compare these totals to the consumption of sucrose controls. Overall, male sucrose controls averaged 2.5 µL/fly; females 3.27 µL/fly. By comparison, 24-h males consumed 3.41 µL/fly; females 5.81 µL for the Nu-Lure/sucrose comparison (line 2, Table 4). This is the only set of totals for Table 4 that meet or exceed the control average. Sucrose control consumption was exceeded by males in Table 5 (line 2); females consumed 3.28 µL/fly (line 2, Table 5). In some cases, sucrose may overcome a deterrent effect of a substance (Shields & Mitchell 1995), but apparently not with Nu-Lure and *A. suspensa*.

Ninety-eight to 100% of the sucrose positive controls fed over the 10 weeks of these experiments (data not presented). There were no differences in the consumption of water and 10% Nu-Lure by 24-h and 6-d males and females (Table 3). In the water-Nu-Lure comparison, an average of 92% of males and 96% of females in the sucrose checks fed with a mean consumption of 3.24 ± 0.21 µL (males) and 3.89 ± 0.33 µL (females). The consumption of the protein solutions was generally less than one-half of the consumption of the 0.2 M sucrose controls. The addition of Nu-Lure to

TABLE 2. *ANASTREPHA SUSPENSA* CONSUMPTION OF PROTEIN SOLUTIONS (µL, EXPERIMENT 1).

	Comparison	24-h				6-d			
		% feeding	Male	% feeding	Female	% feeding	Male	% feeding	Female
1.	Nu-Lure Water	87 ± 12	0.14 ± 0.12*** 0.59 ± 0.19	53 ± 23	0.04 ± 0.04* 0.16 ± 0.12	56 ± 26	0.17 ± 0.07* 1.10 ± 0.56	92 ± 11	0.37 ± 0.11* 1.75 ± 0.84
2.	Nu-Lure Braggs Liquid Aminos	52 ± 34	0.33 ± 0.21* 0.05 ± 0.04	88 ± 18	0.67 ± 0.26** 0.28 ± 0.16	100 ± 0	0.45 ± 0.28 NS 0.24 ± 0.20	96 ± 9	0.42 ± 0.33 NS 0.49 ± 0.24
3.	Nu-Lure Corn Steep Liquor	64 ± 17	0.10 ± 0.11 NS 0.11 ± 0.03	72 ± 17	0.27 ± 0.24 NS 0.10 ± 0.03	100 ± 0	0.65 ± 0.32* 0.06 ± 0.06	100 ± 0	0.63 ± 0.24** 0.04 ± 0.03
4.	Nu-Lure NZ Case	56 ± 9	0.23 ± 0.14 NS 0.03 ± 0.04	60 ± 32	0.62 ± 0.50* 0.03 ± 0.05	91 ± 12	0.99 ± 0.22*** 0.02 ± 0.04	100 ± 0	1.76 ± 0.45*** 0.33 ± 0.15
5.	Nu-Lure Pepticase	100 ± 0	0.40 ± 0.13*** 0.01 ± 0.03	96 ± 9	0.83 ± 0.30** 0.12 ± 0.05	84 ± 26	0.61 ± 0.29** 0.11 ± 0.07	100 ± 0	1.34 ± 0.41** 0.26 ± 0.17
6.	Nu-Lure Solulys	56 ± 26	0.11 ± 0.10 NS 0.10 ± 0.10	100 ± 0	0.49 ± 0.41 NS 0.46 ± 0.10	100 ± 0	0.72 ± 0.18*** 0.03 ± 0.03	96 ± 9	0.52 ± 0.34 NS 0.20 ± 0.21
7.	Nu-Lure Soy Protein Hydrolysate	48 ± 11	0.38 ± 0.32 NS 0.09 ± 0.07	76 ± 17	1.39 ± 0.90* 0.09 ± 0.09	64 ± 9	0.38 ± 0.20 NS 0.13 ± 0.09	76 ± 17	0.84 ± 0.47 NS 0.28 ± 0.31
8.	Nu-Lure Torula Yeast	100 ± 0	0.88 ± 0.21** 0.04 ± 0.09	100 ± 0	1.07 ± 0.47* 0.33 ± 0.11	36 ± 22	0.28 ± 0.18 NS 0.07 ± 0.08	88 ± 18	1.91 ± 0.31*** 0.20 ± 0.09
9.	Nu-Lure Whey	36 ± 22	0.21 ± 0.18 NS 0.03 ± 0.02	48 ± 27	0.95 ± 0.69* 0.05 ± 0.03	93 ± 12	0.91 ± 0.36** 0.07 ± 0.11	80 ± 20	0.86 ± 0.82 NS 0.09 ± 0.07
10.	Nu-Lure Yeast Hydrolysate	56 ± 33	0.14 ± 0.09* 0.01 ± 0.01	100 ± 0	0.84 ± 0.23*** 0.004 ± 0.01	88 ± 18	0.34 ± 0.15 NS 0.37 ± 0.12	88 ± 18	0.50 ± 0.24 NS 0.59 ± 0.48

Mean ± standard deviation, n = 5; means are different at *0.05, **0.01, and ***0.001 by paired t-tests (Microsoft Office Excel 2003) or NS = not significantly different.

TABLE 3. NO-CHOICE CONSUMPTION OF WATER, 10% NU-LURE PLUS 0.2 M SUCROSE, 10% NU-LURE AND 0.2 M SUCROSE BY *ANASTREPHA SUSPENSA* (EXPERIMENT 2).

	% feeding	Mean ± SD µL per fly consumed	% feeding	Mean ± SD µL per fly consumed
	24-h Male		24-h Female	
DDI water	66 ± 24 a*	0.51 ± 0.44 b	48 ± 39 b	0.70 ± 0.52 b
10% Nu-Lure plus 0.2 M sucrose	80 ± 20 a	0.45 ± 0.12 b	74 ± 19 ab	0.44 ± 0.20 b
10% Nu-Lure	68 ± 30 a	0.78 ± 0.23 b	96 ± 9 a	1.11 ± 0.34 b
0.2 M sucrose	76 ± 17 a	2.10 ± 0.33 a	84 ± 17 ab	2.68 ± 0.77 a
	6-d Male		6-d Female	
DDI water	52 ± 33 a	0.69 ± 0.36 b	20 ± 0 b	0.13 ± 0.07 b
10% Nu-Lure plus 0.2 M sucrose	96 ± 9 a	0.78 ± 0.29 b	100 ± 0 a	0.80 ± 0.13 b
10% Nu-Lure	65 ± 25 a	1.61 ± 0.37 b	82 ± 10 a	0.84 ± 0.59 b
0.2 M sucrose	76 ± 33 a	2.57 ± 0.70 a	84 ± 17 a	2.79 ± 1.43 a

*Means by age and sex followed by the same letter are not statistically different by ANOVA followed by Tukey's HSD test, ± = 0.05, n = 5. SD = standard deviation.

a consumption comparison appears to decrease the total consumption of both solutions (Tables 4 and 5). One possibility for our data is that *A. suspensa* self-selected an optimal diet (Hagen & Finney 1950; Waldbauer & Friedman 1991). *Anastrepha suspensa* seems to prefer sugar as an immature fly and protein when sexually mature (Nigg et al. 1995). Our previous data suggested that 6-d-old females would have preferentially consumed protein (Nigg et al. 1995). However, in the present study, both sexually mature and immature flies preferentially consumed 0.2 M sucrose (Table 3). This said, the goal here was an increase in consumption so that pesticide quantity might be reduced. The mechanism of the increase might be studied in the future.

TABLE 4. CHOICE COMPARISON OF NU-LURE CONSUMPTION BY 24-H *ANASTREPHA SUSPENSA* (EXPERIMENT 3).

Feeding category	Mean ± SD µL per fly consumed	Mean ± SD µL per fly 6-d
	24-h male	24-h female
1. 10% Nu-Lure	0.46 ± 0.21 NS	0.82 ± 0.13 NS
Water	0.59 ± 0.29	0.67 ± 0.13
2. 10% Nu-Lure	1.19 ± 0.34 NS	1.93 ± 0.33 NS
0.2 M sucrose	2.22 ± 2.82	3.88 ± 1.92
3. 10% Nu-Lure	0.36 ± 0.13 NS	0.48 ± 0.15*
10% Nu-Lure in 0.2 M sucrose	1.39 ± 0.85	1.40 ± 0.52
4. 0.2 M sucrose	1.13 ± 0.95 NS	0.67 ± 0.29 NS
10% Nu-Lure in 0.2 M sucrose	0.14 ± 0.05	0.24 ± 0.16
5. 10% Nu-Lure	0.36 ± 0.16 NS	0.48 ± 0.26 NS
10% Nu-Lure in 0.2 M fructose	1.37 ± 1.03	2.19 ± 1.62
6. 10% Nu-Lure	0.17 ± 0.06 NS	0.44 ± 0.20 NS
10% Nu-Lure in 0.05 M valine	1.16 ± 1.14	1.30 ± 0.76
7. 10% Nu-Lure in 0.2 M sucrose	0.35 ± 0.16 NS	0.66 ± 0.28 NS
10% Nu-Lure in 0.2 M sucrose + 0.05 M valine	1.79 ± 0.88	2.32 ± 1.92
8. 10% Nu-Lure	0.31 ± 0.12**	0.52 ± 0.38 NS
10% Nu-Lure in 5% borax	0.54 ± 0.18	0.77 ± 0.43

Means are significantly different at *0.05, **0.01, ***0.001. Rows with the same number were compared statistically. SD = standard deviation.

TABLE 5. CHOICE COMPARISON OF NU-LURE CONSUMPTION BY 6-D *ANASTREPHA SUSPENSA* (EXPERIMENT 3).

Feeding category	Mean ± SD µL per fly consumed	Mean ± SD µL per fly 6-d
	6-d Male	24-h Female
1. 10% Nu-Lure	0.75 ± 0.22 NS	1.03 ± 0.27 NS
Water	1.39 ± 0.73	1.41 ± 0.46
2. 10% Nu-Lure	0.80 ± 0.49 NS	1.21 ± 0.51*
0.2 M sucrose	2.20 ± 0.76	2.07 ± 0.57
3. 10% Nu-Lure	0.70 ± 0.55 NS	1.15 ± 0.73 NS
10% Nu-Lure in 0.2 M sucrose	1.32 ± 0.95	0.89 ± 0.78
4. 0.2 M sucrose	0.30 ± 0.47 NS	0.88 ± 1.09 NS
10% Nu-Lure in 0.2 M sucrose	0.37 ± 0.07	0.80 ± 0.30
5. 10% Nu-Lure	0.30 ± 0.20 NS	0.51 ± 0.14 NS
10% Nu-Lure in 0.2 M fructose	1.13 ± 0.61	1.96 ± 1.21
6. 10% Nu-Lure	0.20 ± 0.12**	0.56 ± 0.15**
10% Nu-Lure in 0.05 M valine	2.09 ± 0.55	3.71 ± 0.71
7. 10% Nu-Lure in 0.2 M sucrose	0.31 ± 0.17 NS	0.61 ± 0.36*
10% Nu-Lure in 0.2 M sucrose + 0.05 M valine	0.50 ± 0.47	2.34 ± 0.95
8. 10% Nu-Lure	0.65 ± 0.16 NS	0.84 ± 0.48 NS
10% Nu-Lure in 5% borax	1.00 ± 0.25	0.62 ± 0.20

Means are significantly different at *0.05, **0.01, ***0.001. Rows with the same number were compared statistically. SD = standard deviation.

A bait must be both attractive and readily consumed. Maximum consumption is desirable in order to reduce pesticide while maintaining effectiveness. For consumption, our data suggest that Nu-Lure and other tested protein solutions are inappropriate as *consumed* baits for *A. suspensa* and could be replaced by 0.2 M sucrose.

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REFERENCES CITED

ALLSOP, P. G. 1992. Sugars, amino acids, and ascorbic acid as phagostimulants for larvae of *Antitrogus parvulus* and *Lepidiota negatoria* (Coleoptera: Scarabaeidae). J. Econ. Entomol. 85: 106-111.

COBBINAH, J. R., F. DAVID MORGAN, AND T. J. DOUGLAS. 1982. Feeding responses of the gum leaf skeletoniser *Uraba lugens* Walker to sugars, amino acids, lipids, sterols, salts, vitamins, and certain extracts of eucalypt leaves. J. Australian Entomol. Soc. 21: 225-236.

DOSS, R. P. AND C. H. SHANKS, JR. 1984. Black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), phagostimulants from 'Alpine' strawberry. Environ. Entomol. 13: 691-695.

EPSKY, N. D., R. R. HEATH, J. M. SIVINSKI, C. O. CALKINS, R. M. BARANOWSKI, AND A. H. FRITZ. 1993. Evaluation of protein bait formulations for the Caribbean fruit fly (Diptera: Tephritidae). Florida Entomol. 76: 626-635.

EPSKY, N. D., J. HENDRICHS, B. I. KATSOYANNOS, L. A. VÁSQUEZ, J. P. ROS, A. ZÜMREOGLU, R. PEREIRA, A. BAKRI, S. I. SEEWOORUTHUN, AND R. R. HEATH. 1999. Field evaluation of female-targeted trapping systems for *Ceratitis capitata* (Diptera: Tephritidae) in seven countries. J. Econ. Entomol. 92: 156-164.

FABRE, F., P. RYCKEWAERT, P. F. DUYCK, F. CHIROLEU, AND S. QUILICI. 2003. Comparison of the efficacy of different food attractants and their concentration for melon fly (Diptera: Tephritidae). J. Econ. Entomol. 96: 231-238.

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

FRIEND, W. G. 1981. Diet destination in *Culiseta inornata* (Williston): Effect of feeding conditions on the response to ATP and sucrose. Ann. Entomol. Soc. Amer. 74: 151-154.

HAGEN, K. S., AND G. L. FINNEY. 1950. A food supplement for effectively increasing the fecundity of certain tephritid species. J. Econ. Entomol. 43: 735.

HEATH, R. R., N. D. EPSKY, S. BLOEM, K. BLOEM, F. ACAJABON, A. GUZMAN, AND D. CHAMBERS. 1994. pH effect on the attractiveness of a corn hydrolysate to the Mediterranean fruit fly and several *Anastrepha* species (Diptera: Tephritidae). J. Econ. Entomol. 87: 1008-1013.

KATSOYANNOS, B. I., R. R. HEATH, N. T. PAPADOPOULOS, N. D. EPSKY, AND J. HENDRICHS. 1999. Field evaluation of Mediterranean fruit fly (Diptera: Tephritidae) female selective attractants for use in monitoring programs. J. Econ. Entomol. 92: 583-589.

LADD, T. L., JR. Japanese beetle (Coleoptera: Scarabaeidae): influence of sugars on feeding response of larvae. J. Econ. Entomol. 81: 1390-1393.

MA, W.-C., AND ISAO KUBO. 1977. Phagostimulants for *Spodoptera exempta*: identification of adenosine from *Zea mays*. Entomol. Exp. 22(2): 107-112.

MICROSOFT OFFICE EXCEL. 2003. Microsoft Office Excel 2003. Microsoft Corporation, One Microsoft Way, Redmond, WA 98052-6399.

MOCHIZUKI, A., Y. ISHIKAWA, AND Y. MATSUMOTO. 1985. Sugars as phagostimulants for larvae of the onion fly, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae). Appl. Entomol. Zool. 20(4): 465-469.

NIGG, H. N., L. L. MALLORY, S. FRASER, S. E. SIMPSON, J. L. ROBERTSON, J. A. ATTAWAY, S. B. CALLAHAM, AND R. E. BROWN. 1994. Test protocols and toxicity of organophosphate insecticides to Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 87: 589-595.

NIGG, H. N., S. E. SIMPSON, J. A. ATTAWAY, S. FRASER, E. BURNS, AND R. C. LITTELL. 1995. Age-related response of *Anastrepha suspensa* (Diptera: Tephritidae) to protein hydrolysate and sucrose. J. Econ. Entomol. 88: 669-677.

NIGG, H. N., S. E. SIMPSON, AND J. L. KNAPP. 2004a. The Caribbean fruit fly-free zone programme in Florida, U.S.A. pp. 179-182 *In* Proc. 6th Intl. Fruit Fly Symp.

NIGG, H. N., R. A. SCHUMANN, J. J. YANG, L. K. YANG, S. E. SIMPSON, E. ETXEBERRIA, R. E. BURNS, D. L. HARRIS, AND S. FRASER. 2004b. Quantifying individual fruit fly consumption with *Anastrepha suspensa* (Diptera: Tephritidae). J. Econ. Entomol. 97: 1850-1860.

NIGG, H. N., S. E. SIMPSON, R. A. SCHUMANN, E. ETXEBERRIA, AND E. B. JANG. 2004C. Kairomones for the management of *Anastrepha* spp. fruit flies, pp. 335-347 *In* Proc. 6th Intl. Fruit Fly Symp.

NIGG, H. N., R. A. SCHUMANN, R. J. STUART, E. ETXEBERRIA, J. J. YANG, AND S. FRASER. 2006. Consumption of sugars by *Anastrepha suspensa* Loew (Diptera: Tephritidae). Ann. Entomol. Soc. America (in press).

PEACOCK, J. W., AND F. W. FISK. 1970. Phagostimulants for larvae of the Mimosa webworm, *Homadaula anisocentra*. Ann. Entomol. Soc. Amer. 63: 1755-1762.

SARAN, R. K., AND M. K. RUST. 2005. Feeding, uptake, and utilization of carbohydrates by western subterranean termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 98: 1284-1293.

SAS INSTITUTE. 2001. SAS version 8.2. SAS Institute, Cary, NC.

SCHMIDT, J. M., AND W. G. FRIEND. 1991. Ingestion and diet destination in the mosquito *Culiseta inornata*: effects of carbohydrate configuration. J. Insect Physiol. 37: 817-828.

SHANKS, C. H., JR. AND R. P. DOSS. 1987. Feeding responses by adults of five species of weevils (Coleoptera: Curculionidae) to sucrose and sterols. Ann. Entomol. Soc. Amer. 80: 41-46.

SHARMA, H. C. 1994. Phagostimulant activity of sucrose, sterols, and soybean leaf extractables to the cabbage looper *Trichoplusia ni* (Lepidoptera: Noctuidae). Insect Sci. App. 15: 281-286.

SHIELDS, V. D. C., AND B. K. MITCHELL. 1995. The effect of phagostimulant mixtures on deterrent receptor(s) in two crucifer-feeding lepidopterous species. 347: 459-464.

SOETENS, PH., AND J. M. PASTEELS. 1994. Synergistic effect of secondary compounds and nutrients in the host plant choice of a salicaceous-feeding leaf beetle: *Phrator vitellinae* (Coleoptera: Chrysomelidae). Med. Fac. Landbouww. Univ. Gent. 59(2b): 695-689.

SUTHERLAND, O. R. W. 1971. Feeding behaviour of the grass grub *Costelytra zealandica* (White) (Coleoptera: Melolonthinae) -1 The influence of carbohydrates. New Zealand J. Sci. 14: 18-24.

WALDBAUER, G. P., AND S. FRIEDMAN. 1991. Self-selection of optimal diets by insects. Annu. Rev. Entomol. 36: 43-63.

YAZAWA, M. 1997. Characteristics of sucrose formation, and the influence of UV irradiation on the feeding by the silkworm, in leaves of the mulberry. Natl. Inst. Seric. Entomol. Sci. 18: 1-77.

TWO NEW SPECIES OF THE GENUS *XENYLLA* TULLBERG, 1869 FROM CHINA (COLLEMBOLA: HYPOGASTRURIDAE)

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ABSTRACT

Two new species of *Xenylla* from Jilin Province, Northeast China are described and illustrated. *Xenylla changlingensis*, **new species** clearly differs from the closely related species *X. piceeta* Stebaeva & Potapov, 1994 in the presence of dorsal *la*2 in the thoracic segments II and III, the absence of ventral seta *p*2 on abdominal segment II, 1 median ventral seta above the retinaculum on abdominal segment III, and lack of teeth on the mucro. *Xenylla changchunensis*, **new species** is similar to the species *X. osetica* Stebaeva & Potapov, 1994. However, it is separable from the latter by the presence of a furca, a tenaculum, and the ventral chaetotaxy on abdominal segment III.

Key words: Collembola, Hypogastruridae, *Xenylla*, new species, China

RESUMEN

Se describe e ilustran dos especies del género *Xenylla* de la Provincia de Jilin, en el noreste de China. *Xenylla changlingensis* **nueva especie** claramente se distingue de su especie cercana *X. piceeta* Stebaeva & Potapov, 1994 por la presencia de *la*2 dorsal de los segmentos torácicos II y III, la ausencia de la seta ventral *p*2 en el segmento abdominal II, una seta mediana ventral arriba del retinaculum en el segmento abdominal III, y la falta de dientes sobre el mucro. *Xenylla changchunensis* **nueva especie** es parecida a *X. osetica* Stebaeva & Potapov, 1994. Sin embargo, se distingue de la especie posterior por la presencia de una furca, el tenaculum y la chaetotaxia ventral del segmento abdominal III.

The genus *Xenylla* was established by Tullberg for *X. maritima* Tullberg, 1869 as type species. It is one of the largest and most widespread genera in the family of Hypogastruridae. According to Thibaud et al. (2004), species in the genus *Xenylla* are mainly characterized by (1) 5+5, rarely 4+4 ommatidia, (2) postantennal organ absent, (3) mandible short with a well developed molar plate, maxillary head with normal lamellae, (4) furca rarely absent, showing a diverse morphology, if mucro separated from the dens, which normally bears 2 setae; mucro, however, fused with the dens or mucro absent, the dens has 1 or 2 setae, (5) empodium absent, and (6) abdominal segment V tergite with *p*3 as sensilla.

So far, about 126 species of the genus *Xenylla* have been described worldwide (Christiansen 2006). However, only one, *X. boerneri* Axelson, 1905, has been reported from East China (Zhao et al. 1997). The taxonomy of the fauna of many Chinese habitats is poorly known, especially those of soil. In the present paper, two new species of the genus *Xenylla* that were found from Northeast China are described.

Abbreviations

*a*1, 2, … — setae 1, 2, … of the anterior row, counted from the "middle line", *m*1, 2, … — setae 1, 2, … of the middle row, counted from the "middle line", *p*1, 2, … — setae 1, 2, … of the posterior row, counted from the "middle line", *c*1, 2, … — cervical setae 1, 2, … of area occipitalis, counted from the "middle line", L*a*1, 2, …— setae 1, 2, … of the lateral anterior row in thoracic segments, L1, 2, …— lateral setae 1, 2, …. in head (Yosii 1960; Gama 1988).

Xenylla changlingensis, **new species**

(Figs. 1-10)

Type Materials

Holotype: Female, from the grassland of *Leymus chinensis*, 44°35'N, 123°30'E, 141 m altitude, Changling county, Jilin Province, Northeast China, 6-5-2005, collected by Dr. Donghui Wu. Paratypes: Two females, 3 males, same data as holotype. Holotype and paratypes deposited in Shanghai Institute of Plant Physiology & Ecology.

Description

Body length up to 0.95 mm. Body color in alcohol dark blue-violet. With 5+5 ommatidia in head (Fig. 1). Antennal segment I with 7 setae, antennal segment II with 12 setae. Sensory organ of antennal segment III consists of 2 microsensilla which are embedded in a tegumentary fold and

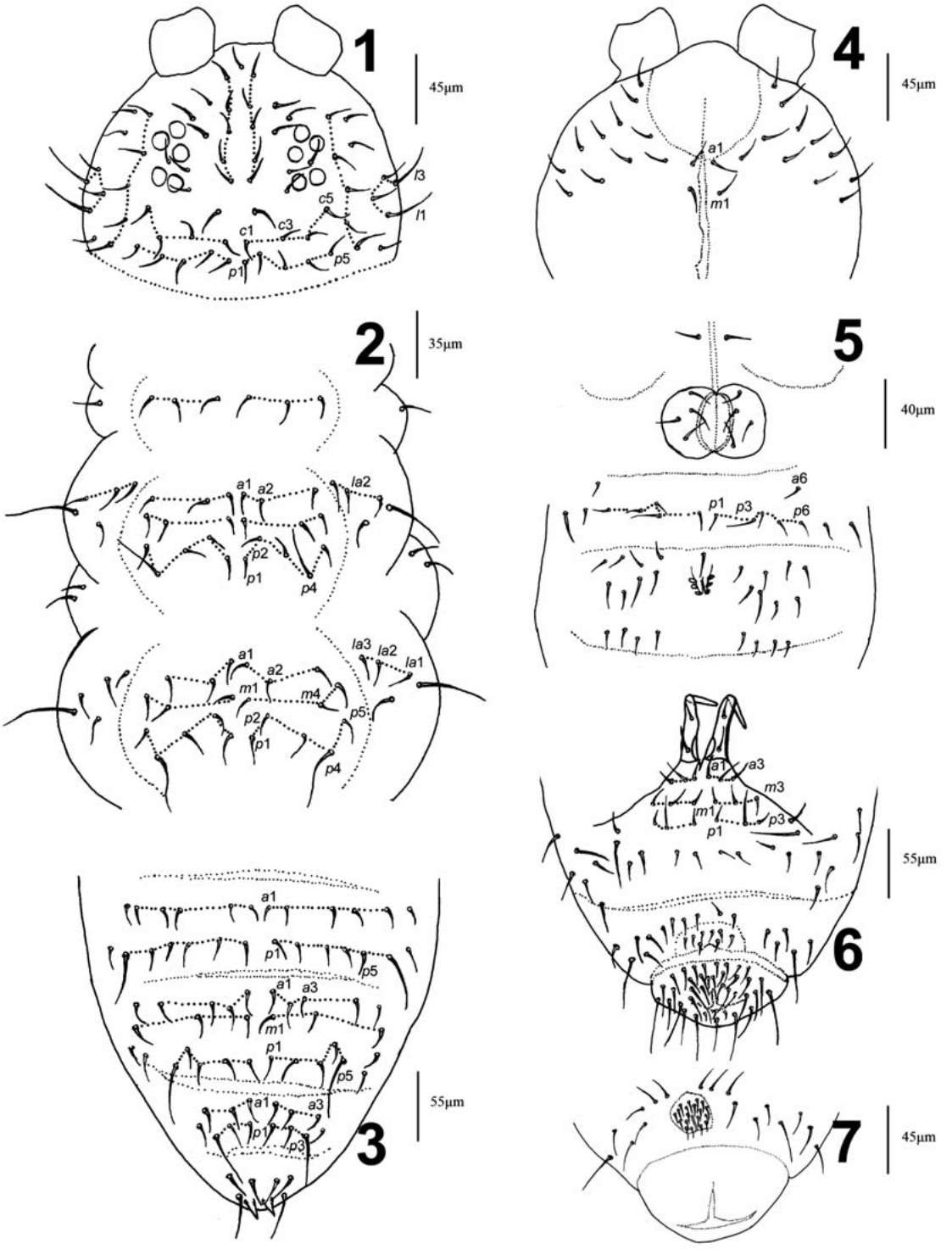

Fig. 1-7. *Xenylla changlingensis*, **new species**. 1. Dorsal chaetotaxy of the head. 2. Dorsal chaetotaxy of Th. I - III. 3. Dorsal chaetotaxy of Abd. III - VI. 4. Ventral chaetotaxy of the head. 5. Ventral chaetotaxy of Th. III, Abd. II and III, ventral tube, and retinaculum. 6. Ventral chaetotaxy of Abd. IV and V female genital plate, and anal plate. 7. Male genital plate.

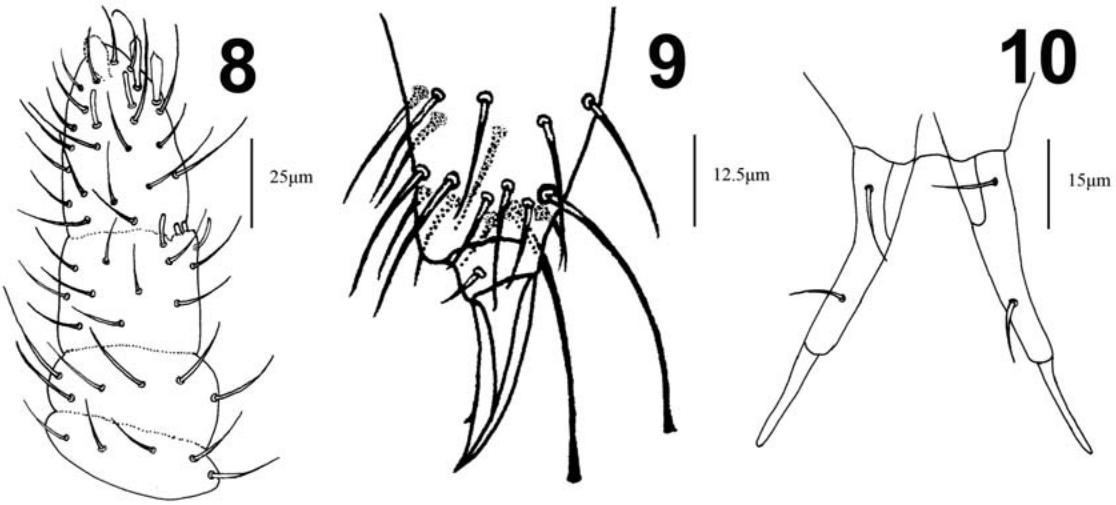

Fig. 8-10. *Xenylla changlingensis*, **new species**. 8. Antenna, dorsal view. 9. Tibiotarsi III with claw. 10. Furca, posterior view.

flanked by 2 longer guard sensillum. Antennae IV with a simple apical bulb and 4 weakly thickened sensillum, of which 3 are dorso-external and 1 dorso-internal, and 2 inernal sensillum, which are thinner and shorter than the others (Fig. 8). External maxillary lobe with 2 sublobal hairs.

Tibiotarsi each with 2 capitate, dorsal tenent hairs, which are longer than the inner edge of the claws. Claws with a small, distal internal tooth, 2/3 as long as tibiotarsal hairs (Fig. 9). Mucro well separated from the dens with 2 posterior setae, 1/2 as long as the dens but dens and mucro particularly slender, width of dens at distal seta about an eighth its length, mucro straight and without teeth (Fig. 10). Ventral tube with 4+4 setae. Retinaculum with 3+3 teeth (Fig. 5). Female genital (Fig. 6) and male genital plate (Fig. 7) normal. Anal spines small, on weakly developed papillae separated at the base, 1/4 as long as claws (Fig. 3).

Chaetotaxy, consisting of short setae and longer and fine sensorial setae. Dorsally head without seta *c*2 (*a2 a*/*c* to Babenko), cephalic setae *l*1 and *l*3 subequal (Fig. 1), thoracic segments II-III with central setae in 3 rows, on thoracic segments II-III *p*2 displaced apically relative to *p*1, and on thoracic segment III *a*2 displaced distally compared with *a*1 (Fig. 2), on abdominal segments I-III *p*5 present, abdominal segments IV with *a*3. Abd. V with *a*2 (Fig.3). Ventrally head without seta *p*1 (Fig. 4), thoracic segments II and III with a pair of medial setae, abdominal segments II without *p*2 and *a*5, abdominal segments III with 1 median seta above the retinaculum (Fig. 5).

Comment

The new species is distinguished from all the known species of the genus *Xenylla* by the absence of mucronal teeth, dorsal side of head without *c*2 seta, seta *p*2 on tergite of thoracic segments II-III set in front of *p*1 seta, head without ventral seta *p*1, thoracic segments II and III with a pair of ventral medial setae, abdominal segment II without ventral setae *p*2 and *a*5, abdominal segment III with 1 median ventral seta above the retinaculum, abdominal segment IV with ventral seta *m*1, unguis with 1 internal tooth.

Etymology

This species is named after the type locality.

Taxonomic Remarks

This species keys out to *X. piceeta* Stebaeva & Potapov, 1994 (Babenko et al. 1994), from Far East, southern maritime province, Russia, which was collected in litter of a fir forest (Babenko et al. 1994), but the new species clearly differs from *X. piceeta* by the presence of dorsal *la*2 of thoracic segments II and III, which is stable on the tergites, and the absence of ventral seta *p*2 on abdominal segment II. On abdominal segment III, *X. changlingensis* has only 1 median ventral seta above the retinaculum, while *X. piceeta* has a pair of medial setae. In addition, the mucro of *X. changlingensis* is straight and thin, but lacking teeth.

Xenylla changchunensis, **new species**
(Figs. 11-19)

Type Materials

Holotype: Female, from the deciduous-coniferous mixed forest of Jingyuetan Park, 43°45'N, 125°27'E, 242 m altitude, Changchun city, Jilin

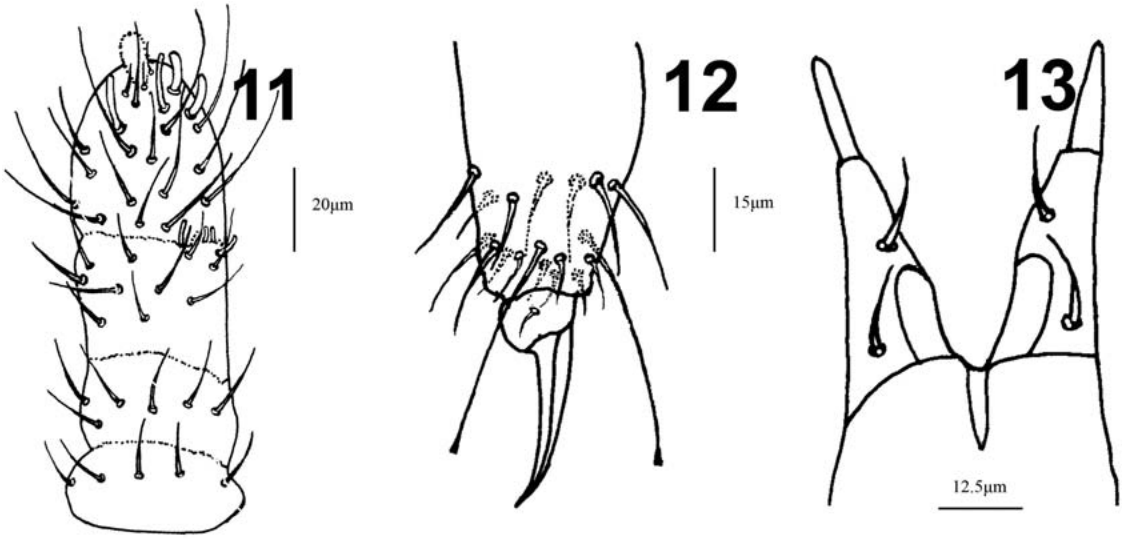

Fig. 11-13. *Xenylla changchunensis*, **new species**. 11. Antenna, dorsal view. 12. Tibiotarsi III with claw. 13. Furca, posterior view.

Province, Northeast China, 3-9-2003, collected by Dr. Donghui Wu. Paratypes: Two females, same data as holotype. Holotype and paratypes deposited in Shanghai Institute of Plant Physiology & Ecology.

Description

Body length up to 0.81 mm. Body color in alcohol red-brown. Antennal segment I with 7 setae, antennal segment II with 12 setae. Sensory organ of antennal segment III consists of 2 microsensilla, embedded in a tegumentary fold and flanked by 2 longer guard sensillum. Antennae IV with a simple apical bulb and 4 weakly thickened sensillum, of which 3 dorso-external and 1 dorsointernal, and 2 internal sensillum, thinner and longer than the others (Fig. 11). External maxillary lobe with 3 sublobal hairs.

Tibiotarsi each with 2 capitate, dorsal tenent hairs, longer than the inner edge of the claws. Claws toothless, 11/15 as long as tibiotarsal hairs (Fig. 12). Dens with 2 posterior setae. Mucro well separated from the dens, straight and fine without teeth, dens broad, at level of distal setae length of dens about 3 times breadth**,** ratio mucro: dens = 1/2 (Fig. 13). There are 4+4 setae on ventral tube. Retinaculum with 3+3 teeth (Fig. 18). Female genital plate (Fig. 19). Anal spines short, inserted on poorly developed papillae, 1/4 as long as claws (Fig. 16).

Chaetotaxy, consisting of short setae and longer and fine sensorial setae. Dorsally head without seta $c1$ with both $p1$ and $p2$, $l1$ longer than $l3$ (Fig. 14), thoracic segments II-III with medial setae in 3 rows, seta $p2$ on tergite of thoracic segments II-III set in front of $p1$, on thoracic segments III $a2$ displaced distally relative to $a1$ (Fig. 15), on abdominal segments I-III, $p5$ present, abdominal segments IV without $a3$, abdominal segments V without $a2$ (Fig. 16). Ventrally head with seta $p1$ (Fig. 17), thoracic segments II and III with a pair of medial setae, abdominal segment II without $p2$ and $a5$, abdominal segment III with 1 median seta above the retinaculum (Fig. 18), abdominal segment IV without $m1$ (Fig. 19).

Comment

The new species is distinguished from other species of *Xenylla* by possessing a mucro without teeth, dorsal side of head with $c1$ ($p1\ a/c$ Babenko et al. 1994) seta absent, seta $p2$ on tergite of thoracic segments II-III set in front of $p1$ seta, thoracic segments II and III with a pair of ventral medial setae, abdominal segment II without ventral setae $p2$ and $a5$, abdominal segment III with 1 median ventral seta above the retinaculum, abdominal segment IV without ventral seta $m1$, unguis lacking teeth.

Etymology

Named *changchunensis* alluding to Changchun, the city where the species was found.

Taxonomic Remarks

The new species resembles *X. osetica* Stebaeva & Potapov, 1994 in general shape, antenna, tibiotarsi, and claws, especially in dorsal chaetotaxy,

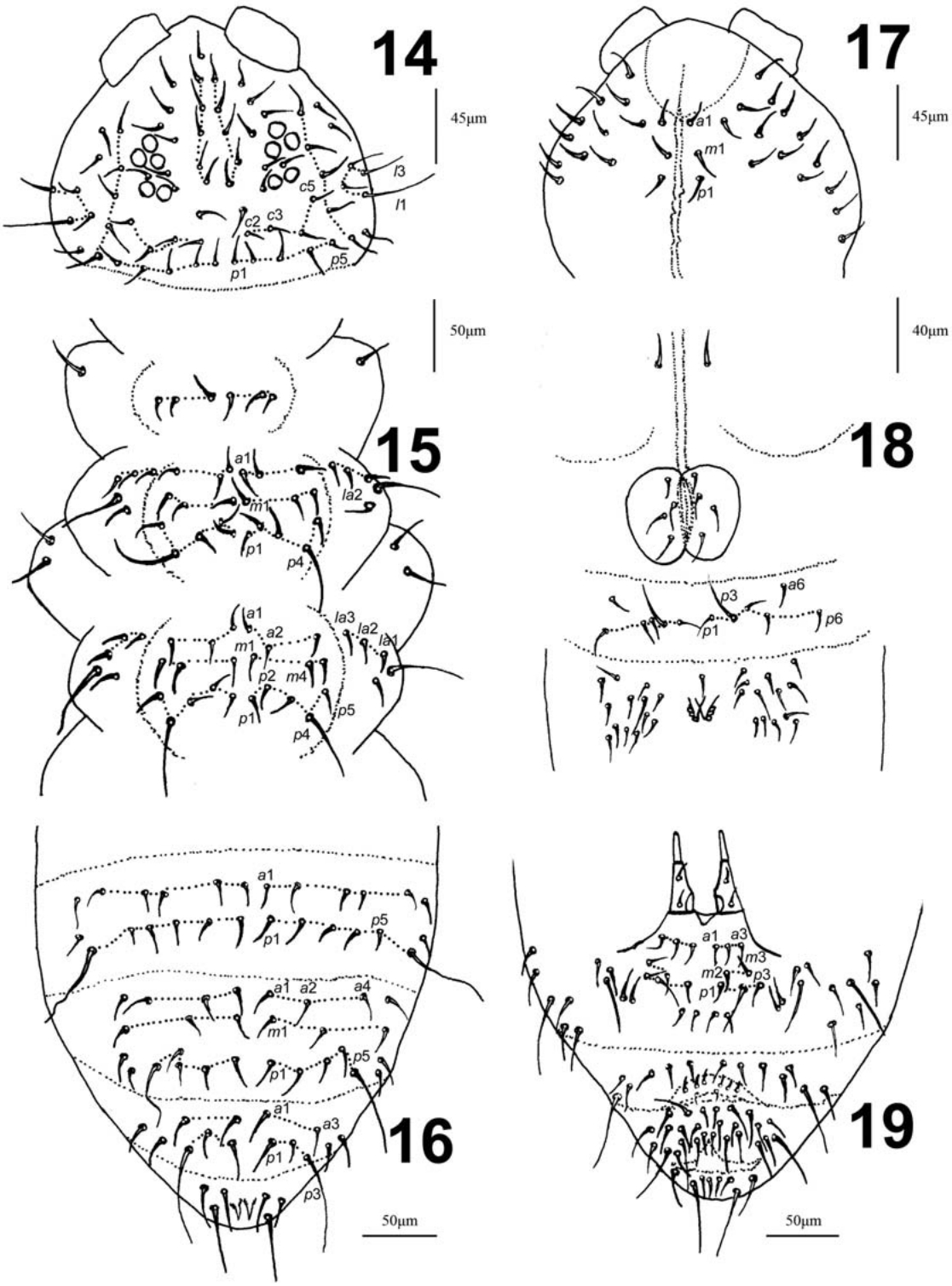

Fig. 14-19. *Xenylla changchunensis*, **new species**. 14. Dorsal chaetotaxy of the head. 15. Dorsal chaetotaxy of Th. I-III. 16. Dorsal chaetotaxy of Abd. III-VI. 17. Ventral chaetotaxy of the head. 18. Ventral chaetotaxy of Th. III, Abd. II and III, ventral tube, and retinaculum. 19. Ventral chaetotaxy of Abd. IV and V, female genital plate, and anal plate.

but distinctly differs from *X. osetica* in the following characters: (1) furca and tenaculum present, and (2) ventral chaetotaxy on abdominal segment III.

KEY TO SPECIES OF THE CHINESE *XENYLLA* TULLBERG, 1869

1. Furca without mucro, retinaculum with 2+2 teeth . . . . . *X. boerneri* Axelson, 1905
—Mucro separated from dens that has 2 setae, retinaculum with 3+3 teeth 2

2. Dorsal side of head without c2 seta, abdominal segment IV with ventral seta *m*1, unguis with 1 internal tooth . . . . . *X. Changlingensis* sp. nov
Dorsal side of head with *c*1 seta absent, abdominal segment IV without ventral seta *m*1, unguis lacking teeth . . . . . *X. Changchunensis* sp. nov

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REFERENCES CITED

BABENKO, A. B., N. M. CHERNOVA, M. B. POTAPOV, AND S. K. STEBAEVA. 1994. Family Hypogastruridae, pp. 259-305, 329 *In* N. M. Chernova [ed.], Collembola of Russia and Adjacent Countries.

CHRISTIANSEN, K. A. 2006. Http://www.collembola.org.

GAMA, M. M. DA. 1988. Filogenia des espécies de *Xenylla* à escala mundial (Insecta, Collembola). Evolución boilógia 2: 139-147.

THIBAUD, J. M., H. J. SCHULZ, AND M. M. G. ASSALINO. 2004. Hypogastruridae, *In* W. Dunger [ed.], Synopses on Palaearctic Collembola 4: 7-10, 217-250.

YOSII, R. 1960. Studies on the Collembolan genus *Hypogastrura*. The American Midland Naturalist 64: 257-281.

ZHAO, L., A. H. TAMUR, AND X. KE. 1997. Tentative Checklist of Collembolan Species from China (Insect). Publications of the Itako Hydrobiological Station 9: 15-40.

HOST STATUS OF MAMEY SAPOTE, *POUTERIA SAPOTA* (SAPOTACEAE), TO THE WEST INDIAN FRUIT FLY, *ANASTREPHA OBLIQUA* (DIPTERA: TEPHRITIDAE) IN PUERTO RICO

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ABSTRACT

The authors evaluated the host status of mamey sapote, *Pouteria sapota* (Sapotaceae) to *Anastrepha obliqua* by collecting mature fruits and monitoring them for the emergence of larval Tephritidae. Fruits were also scarred and placed in cages with female *A. obliqua* and monitored for the emergence of larvae and adults. Multi-lure traps baited with putrescine and ammonium acetate were used to compare the number of flies in orchards of mamey sapote to the number of flies in nearby orchards of carambola (*Averrhoa carambola*: Oxalidaceae). There are a number of references citing mamey sapote as a host of *A. obliqua* in different countries. However, we only found two unidentified tephritid larva from 1,160 mamey sapote fruits collected in the field and these fly larvae did not survive to adulthood. We were not able to rear adult *A. obliqua* on scarred, mature fruit of mamey sapote, whereas we were able to do so on mango under identical conditions. Abundance in orchards based on trapping indicates that flies are very rarely encountered in orchards of mamey sapote compared with orchards of carambola. We conclude that in Puerto Rico mamey sapote has a very low (undetectable) rate of infestation by fruit flies in the family Tephritidae.

Key Words: Mamey sapote, *Pouteria sapota*, *Anastrepha*, hosts

RESUMEN

Se evaluó si el mamey sapote, *Pouteria sapota* (Sapotaceae) puede ser hospedero de la mosca de las frutas *Anastrepha obliqua*. Con este fin se colectaron frutas maduras las cuales fueron monitoreadas para detectar la presencia de larvas Tephritidae. Las frutas fueron también rasgadas y colocadas en jaulas conteniendo moscas hembras de *A. obliqua* e inspeccionadas regularmente para determinar si larvas y adultos emergían de las frutas. Trampas con cebo de putrescina y acetato de amonio fueron colocadas en los predios de mamey sapote y huertos cercanos de carambola (*Averrhoa carambola*: Oxalidaceae) para comparar la población de moscas de las frutas en estos huertos. Aunque varios escritos citan el mamey sapote como un hospedero de *A. obliqua*, los autores solo pudimos encontrar dos larvas en varios centenares de frutas y estas larvas no se desarrollaron a su estado adulto. Tampoco se pudo inducir oviposicion de moscas fruteras en frutas de mamey sapote con la superficie rasgada. Los datos obtenidos de las trampas indicaron una población insignificante de moscas de las frutas en huertos de mamey sapote en comparación con aquellos de carambola. Concluimos que en Puerto Rico el mamey sapote tiene un nivel de infestacion extremadamente bajo (indetectable) para las moscas de las frutas de la familia Tephritidae.

Translation provided by the authors.

Mamey sapote, *Pouteria sapota* (Jacq.) H.E. Moore & Stearn (Sapotaceae), is native to Central America (Morton 1987) and its fruits are prized throughout Central America and the Caribbean for their sweetness. It is currently cultivated and sold in Puerto Rico, but some growers would like to expand their market to include Latin American populations in the continental US. However, the possible introduction of new insect pests, including fruit flies in the genus *Anastrepha*, precludes importation of this fruit crop into the continental US. Gould & Hallman (2001) concluded that mamey sapote presents no discernible risk of transporting *Anastrepha suspensa* (Loew). However, a second species of economic importance, *A. obliqua* (Marquart), is present in Puerto Rico and the host-status of mamey sapote with respect to this fly is unclear. Cowley et al. (1992) defined a host as a fruit or vegetable that fruit flies oviposit in under field conditions and that these eggs subsequently develop into larvae, pupae and adults.

Anastrepha obliqua is not thought to occur in Florida (Steck 2001) so the importation of any fruit that may serve as a host for this tephritid poses a serious risk for agriculture in Florida and, potentially, elsewhere in the subtropical mainland. At least 13 reports indicate that the West Indian fruit fly, *A. obliqua*, does indeed use mamey sapote as a host (Emmart 1933; Stone 1942; Aczél 1950; Oakley 1950; Gonzalez Mendoza 1952;

Blanchard 1961; Korytkowski & Ojeda Pena 1970; Weems 1970; Wasbauer 1972; Kandybina 1977; Norrbom & Kim 1988; White & Elson-Harris 1992; Fernández et al. 1998; Norrbom 2004). However, these reports are based on unreliable identifications of host and insect species (confusion remains about the plant or insect species names used in these reports), or are citations of unreliable literature.

Our objective was to estimate the likelihood of infestation of mamey sapote by *A. obliqua* by surveying the incidence of infestation in fruits collected from the field, assessing the incidence of infestation when *A. obliqua* females have no other host options, and by monitoring fruit fly populations in orchards of mamey sapote with baited traps. We used the principles outlined in Cowley et al. (1992) as guidelines for our investigation.

Materials and Methods

From Jun 2005 to Jun 2006 mature mamey sapote fruits of cultivars Magaña, Mayapan, Pantin (Key West), Tazumal, Pace, and Copan from orchards in Isabela and Corozal, PR, were scarred by removing approximately 20 cm^2 of skin from each fruit. Fruit were scarred in order to give access to the pulp, in case the fruit flies could only oviposit in damaged fruit. Mature fruits reveal a deep orange color when a thin layer of the coarse skin of the mamey sapote is removed. At this stage fruits are typically very hard and yield sticky latex when cut. Harvested mature fruits will ripen and soften over the next 3-6 days and no longer yield sticky latex. We decided to harvest fruit in this stage because that is the prevailing practice in PR: fruit left on the tree typically do not abscise until they have mummified, so we could not collect dropped fruit, as is often done with other species of fruits when surveying for fruit fly infestations. Different varieties of mameys were harvested as they were available.

The Isabela location is on the north coast on the west side of the island (18°28'18.97"N; 67°02'49.66"W) and is 15.24 meters above sea level. The mean rainfall for 2005 was 14.58 cm with a range per month of 0-21.59 cm. The mean rainfall for 2006 was 10.68 cm with a range per month of 0-23.42 cm. The mean temperatures for 2005 and 2006 were 24.58°C and 24.78°C, respectively. The Corozal location is on the north-central portion of the island (18°19'39.05" N; 66°21'38.04" W) and is 212.14 meters above sea level. The mean rainfall for 2005 was 20.6 cm with a range per month of 1.12-42.82 cm. The mean rainfall for 2006 was 13.69 cm with a range per month of 2.34-29.36 cm. The mean temperatures for 2005 and 2006 were 24.84°C and 25.29°C, respectively.

One week after scarring, all scarred fruits were harvested, weighed, and placed on a wire mesh over vermiculite. Harvested fruits were stored in a room at 25-27°C and approximately 60% RH (never less than 50% RH). The vermiculite was monitored weekly for the presence of fruit fly larvae or pupae. Any recovered larvae or pupae were collected and placed in a plastic Petri-dish with a small amount of moistened vermiculite and stored at 25°C and 85% RH in an environmental chamber (12:12 D:L) (White & Elson-Harris 1992). Petri-dishes containing pupae were monitored daily for the emergence of adults.

Between 1 and 4 mature mamey sapote fruits of each variety were scarred as described above and placed in collapsible mesh cages ($60 \times 60 \times 60$ cm) (Bioquip, Rancho Dominguez, CA) with 20 female and 20 male *Anastrepha obliqua* flies, 12 d post emergence. Fruit was exposed to flies for 48 h, removed and stored as described above to collect emerging larval tephrititids. Concomitantly, mature naturalized Mayaguezano variety mangoes that had been covered with brown paper bags when they were green (preventing infestation by fruit flies) were exposed to male and female *A. obliqua* under conditions identical to those described for the mamey sapote fruit. The exposure described was conducted for all 6 varieties of mamey and replicated 3 times for each variety. To ensure that the mangoes used in the cage-trials were not infested prior to the trials, mature mangoes that had been bagged were stored over vermiculite as described above and monitored for the emergence of larvae and pupae.

To demonstrate that fruit flies occurred at the experimental sites, 5 plastic multilure traps (A Better World, Inc.) baited with ammonium acetate and putrescine were placed in each mamey sapote orchard and monitored weekly. Five traps were also placed in nearby (120 m) carambola orchards (*Averrhoa carambola*: Oxalidaceae) and monitored weekly. All adult flies obtained from traps or from fruit were identified by the author (D.J.) and voucher specimens were deposited in the Entomological Laboratory of the Tropical Agriculture Research Station, Mayaguez, PR.

Results

A total of 1160 mamey sapote fruits weighing 777 kg were collected from orchards in Corozal and Isabela, PR (Table 1). Of the fruit collected, only 1 fruit of the Tazumal variety, harvested on 21 Nov 2005, yielded 2 larval tephritids and these did not become adults. None of the 15 mamey sapote fruits exposed to colonies of *A. obliqua* yielded larvae, while mango fruit similarly exposed yielded an average of 5.8 ± 0.7 (mean ± SE) larvae (Table 2). Control mangoes that had been bagged when green and not exposed to *A. obliqua* in cage-trials did not produce any larvae or pupae. The number of adult *Anastrepha obliqua* observed per trap per week in mamey sapote orchards was always 2 or less (Fig. 1). Traps in

TABLE 1. COLLECTIONS OF MAMEY SAPOTE BY DATE AND VARIETY.

Variety	Dates collected	Number of fruit	Kg of fruit	Total fruit per variety	Total kg per variety
Magania	18-Aug-05	14	17.5	48	56.8
	23-Aug-05	12	15.0		
	13-Sep-05	22	24.3		
Mayapan	24-Jun-05	15	12.3	369	259.6
	15-Jul-05	15	11.6		
	16-Aug-05	15	13.6		
	13-Apr-06	242	155.5		
	5-Jun-06	82	66.6		
Tazumal	28-Jul-05	8	3.9	238	102.7
	30-Aug-05	2	1.5		
	6-Sep-05	2	0.9		
	18-Oct-05	80	29.5		
	21-Nov-05	146	66.9		
Pantin	16-Aug-05	5	4.2	97	83.7
	13-Sep-05	10	5.6		
	20-Sep-05	2	1.8		
	2-Jun-06	80	72.1		
Pace	24-Jun-05	15	10.2	156	103.6
	15-Jul-05	15	10.4		
	30-Aug-05	4	3.1		
	20-Sep-05	24	14.9		
	22-Feb-06	98	65.0		
Copan	3-Jun-05	6	3.4	252	170.2
	28-Jul-05	10	6.1		
	29-Sep-05	6	3.7		
	21-Nov-05	42	61.2		
	13-Dec-05	64	31.0		
	19-Jan-06	124	64.8		
Total		1160	776.6		

nearby carambola orchards indicated that adult *A. obliqua* were present and, at times, abundant (1 trap in Corozal had 103 flies in it one week) in the area being surveyed (Fig. 1). Traps in the carambola orchards also caught *A. suspensa* adults in numbers similar to those reported for *A. obliqua*, but these data will be published in a future manuscript.

TABLE 2. FLY PUPAE RECOVERED FROM FRUIT EXPOSED TO 20 MALE AND 20 FEMALE *ANASTREPHA OBLIQUA* (12 D POST EMERGENCE) FOR 48 H.

			Pupae recovered			
Date		Fruit/rep	Rep 1	Rep 2	Rep 3	Mean + SE
13-Apr-06	Mango	5	5	7	3	5.0 + 1.2
	Mayapan	2	0	0	0	0.0 + 0.0
3-May-06	Mango	5	8	2	4	4.7 + 1.8
	Tazumal	3	0	0	0	0.0 + 0.0
8-May-06	Mango	5	3	3	7	4.3 + 1.4
	Magania	1	0	0	0	0.0 + 0.0
15-May-06	Mango	5	13	5	8	8.7 + 2.4
	Pace	4	0	0	0	0.0 + 0.0
2-Jun-06	Mango	5	5	6	9	6.7 + 1.2
	Pantin	3	0	0	0	0.0 + 0.0
5-Jun-06	Mango	5	9	4	3	5.3 + 1.9
	Copan	2	0	0	0	0.0 + 0.0

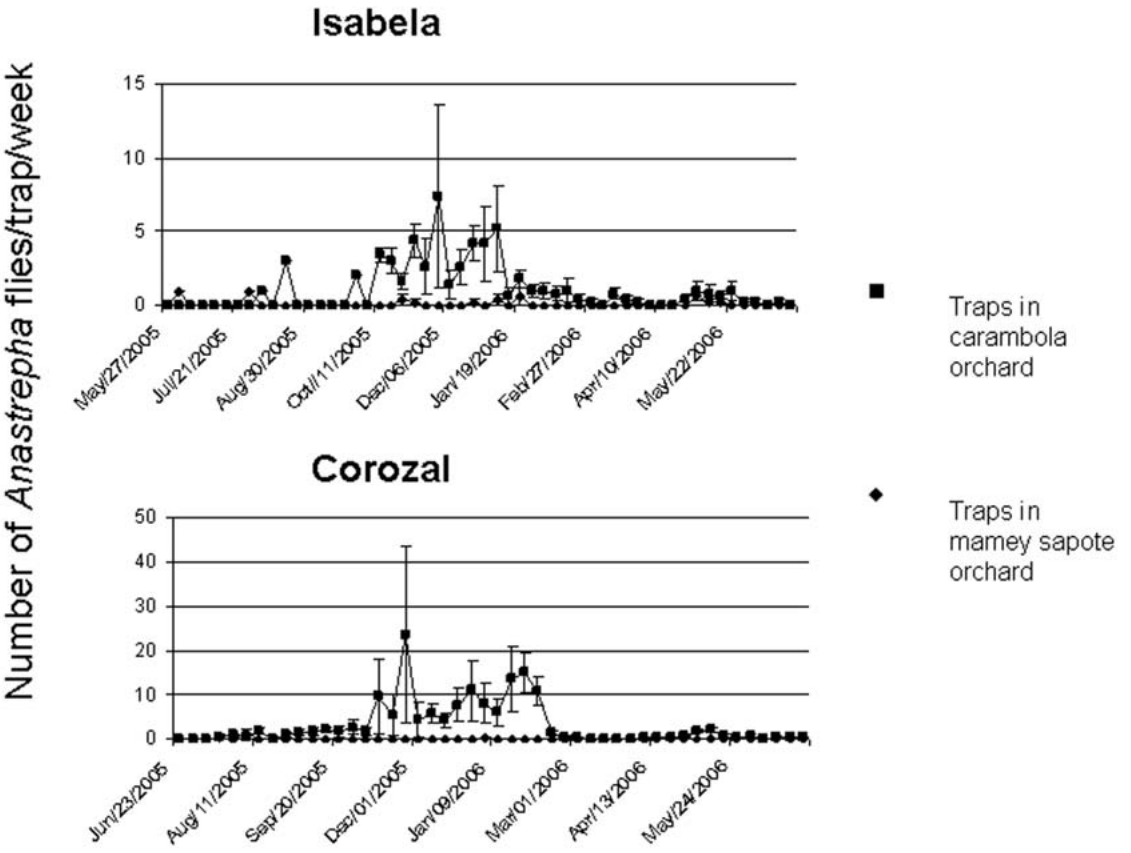

Fig. 1. Mean number of *Anastrepha obliqua* per trap per week (± SEM) in orchards of mamey sapote and carambola at Isabela and Corozal, Puerto Rico (n = 5 traps per orchard).

Discussion

Our data show that the likelihood of infestation of mamey sapote by *A. obliqua* is extremely small. Similar methods have been used to demonstrate the non-host status of litchi and longan (*Litchi chinensis* Sonn. and *Euphoria longana* (Lour.), respectively: Sapindaceae) and mamey sapote to *A. suspensa* (Gould et al. 1999; Gould & Hallman 2001). We collected more than 1000 mature mamey sapote fruits and reared only 2 larvae from these. These larvae did not survive to adulthood. Fruit exposed to fecund female fruit flies did not yield any larvae or pupae. This indicates that either *A. obliqua* females refuse to oviposit in mamey sapote fruit or that eggs put in mamey sapote are unlikely to develop.

We noted that the process of scarring mamey sapotes yields a sticky latex, in common with many sapotaceaous plants (Morton 1987). This latex persists for up to 24 h. After this time the scar is healed, resulting in a rough, corky tissue. We suspect that the latex and the corky tissue may preclude oviposition by *A. obliqua*. *Anastrepha serpentina* is known to oviposit in sapotaceous hosts that release sticky latex upon being punctured and presumably have adapted their oviposition behavior to deal with this defense system (Aluja et al. 2000). *Anastrepha obliqua* often infest mangoes and other anacardiaceous fruits (White & Elson-Harris 1992) that exude sticky polyphenolic resins (Morton 1987; Zomlefer 1994). Females may oviposit when the fruit is ripe and the resin levels in the peel are reduced. There is also reason to believe that mango cultivars vary in their susceptibility to *A. obliqua* and that the level of infestation is correlated with the density of resin canals in the fruit peel (Alex Segarra, unpubl.).

Mamey sapote has the potential to become an important tropical fruit crop in Southern Florida and Puerto Rico. We have compiled evidence that mamey sapote is an extremely unlikely host of *A. obliqua* in Puerto Rico and that the threat of transporting larval fruit flies of this species in fruit of mamey sapote is not likely.

Acknowledgments

Mention of trade names or commercial products in this article is solely for the purpose of providing specific

information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. We thank Yadzaida Garcia and Elkin Vargas for excellent field work. We also thank Drs. Guy Hallman and Jorge Peña and two anonymous reviewers for critiquing an earlier version of this manuscript.

References Cited

ACZÉL, M. L. 1950. Catalogo de la familia 'Trypetidae' (Dipt. Acalypt.) de la region neotropical. Acta Zool. Lilloana (1949) 7: 177-328.

ALUJA, M., J. PIÑERO, I. JÁCOME, F. DÍAZ-FLEISCHER, AND J. SIVINSKI. 2000. Behavior of flies in the genus *Anastrepha* (Trypetinae: Toxotrypanini), p. 384 *In* M. Aluja and A. L. Norrbom [eds.], Fruit Flies (Tephritidae): Phylogeny and Evolution of Behavior. CRC Press, Boca Raton, FL. 963 pp.

BLANCHARD, E. E. 1961. Especies argentinas del género *Anastrepha* Schiner (sens. lat.) (Diptera, Trypetidae). Rev. Invest. Agric. 15 (2): 281-342.

COWLEY, J. M., R. T. BAKER, AND D. S. HARTE. 1992. Definition and determination of host status for multivoltine fruit fly (Diptera: Tephritidae) species. J. Econ. Entomol. 85: 312-317.

EMMART, E. W. 1933. The eggs of four species of fruit flies of the genus Anastrepha. Proc. Entomol. Soc. Wash. 35: 184-191.

FERNÁNDEZ, A. M., D. RODRÍGUEZ, AND V. HERNÁNDEZ-ORTIZ. 1998. Notas sobre el genero *Anastrepha* Schiner en Cuba con descripcion de una nueva especie (Diptera: Tephritidae). Folia Entomol. Mex. (1997) No. 99: 29-36.

GONZALEZ MENDOZA, R. 1952. Contribucion al estudio de las moscas *Anastrephas* en Colombia. Rev. Fac. Nac. Agron. Medellin 12: 423-549.

GOULD, W. P., M. K. HENNESSEY, J. PEÑA, A. CASTINEIRAS, R. NGUYEN, AND J. CRANE. 1999. Nonhost status of lychess and longans to Caribbean fruit fly (Diptera: Tephritidae). J. Econ. Entomol. 92: 1212-1216.

GOULD, W. P., AND G. HALLMAN. 2001. Host status of mamey sapote to Caribbean fruit fly (Diptera: Tephritidae). Florida Entomol. 84: 730-375.

KANDYBINA, M. N. 1977. Lichinki plodovykh mukh-pestrokrylok (Diptera, Tephritidae). [Larvae of fruit-infesting fruit flies (Diptera, Tephritidae)]. Opred. Faune SSSR No. 114: 1-210.

KORYTKOWSKI G., C. A., AND D. OJEDA PEÑA. 1970. Especies del genero *Anastrepha* Schiner 1868 en el noroeste peruano. Rev. Peru. Entomol. (1968) 11: 32-70.

MORTON, J. F. 1987. Fruits of Warm Climates. Media Incorporated, Greensboro, NC. 506 pp.

NORRBOM, A. L., AND K. C. KIM. 1988. A list of the reported host plants of the species of *Anastrepha* (Diptera: Tephritidae). U.S. Dep. Agric. Animal Plant Health Insp. Serv. APHIS 81-52: 114 p.

NORRBOM, A. L. 2004. Host plant database for *Anastrepha* and *Toxotrypana* (Diptera: Tephritidae: Toxotrypanini). Diptera Data Dissemination Disk (CD-ROM) Volume 2, Systematic Entomology Laboratory, USDA.

OAKLEY, R. G. 1950. Part III Fruit Flies (Tephritidae), pp. 169-246 *In* Manual of Foreign Plant Pests. United States Department of Agriculture, Agricultural Research Administration, Bureau of Entomology and Plant Quarantine, Division of Foreign Plant Quarantines.

STECK, G. J. 2001. Concerning the occurrence of *Anastrepha obliqua* (Diptera: Tephritidae), in Florida. Florida Entomol. 84: 320-321.

STONE, A. 1942. The Fruitflies of the Genus *Anastrepha*. U.S. Dep. Agric. Misc. Publ. 439: 112 p.

WASBAUER, M. S. 1972. An Annotated Host Catalog of the Fruit Flies of America North of Mexico (Diptera: Tephritidae). Occas. Pap. Calif. Dep. Agric. Bur. Entomol. 19: [i] + 172 p.

WEEMS, H. V., JR. 1970. West Indian fruit fly *Anastrepha mombinpraeoptans* Seín (Diptera: Tephritidae). Florida Dept. Agric. Consum. Serv., Div. Plant Ind. Entomol. Circ. 101: 2 p.

WHITE, I. M., AND M. M. ELSON-HARRIS. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International, Wallingford. xii + 601 p.

ZOMLEFER, W. B. 1994. Guide to Flowering Plant Families. University of North Carolina Press, Chapel Hill & London. 430 pp.

BIOLOGY AND NATURAL HISTORY OF *ANASTREPHA INTERRUPTA* (DIPTERA: TEPHRITIDAE)

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Florida's best known member of the genus *Anastrepha* is an introduced pest, the Caribbean fruit fly, *A. suspensa* (Loew). However, Florida is also home to other innocuous species, including *Anastrepha interrupta* Stone. The latter was first reported as an undescribed "species E" collected during an early fruit fly survey program in south Florida (Brown 1937) and eventually described by Stone (1942). It is also known from the Bahamas, Virgin Islands, and Dominica (Steyskal 1977), Cuba (Fernandez et al. 1997), and Puerto Rico (A. L. Norrbom, personal communication). The larvae infest fruits of *Schoepfia schreberi* J. F. Gmel. ("gulf greytwig", family Olacaceae; previously known as *Schoepfia chrysophylloides* (A. Rich.) Planch. (McClahanan & Merrill 1951); because this is its only known host, the fly has been called the "Schoepfia fruit fly" (Heppner 1990). In Florida, *A. interrupta* has been trapped since 1934, and many museum specimens exist up to the early 1960s, but far fewer were collected during later years. The literature contains only a few bits of information concerning this species: Brown (1937)—detection; Stone (1942), Shaw (1962), Weems (1967)—adult identification and taxonomy; McClanahan & Merrill (1951)—host plant; Marsh (1970), Wharton & Marsh (1978)—parasitoids; Steck & Wharton (1988), Steck et al. (1990), Heppner (1990)—larval identification.

In early 2003, a population of *A. interrupta* was discovered through adult trapping and fruit collections in Miami-Dade County near Homestead, Florida at Camp Owaissa Bauer. This population offered an unusual opportunity to make additional observations on *A. interupta*. At the same time, an effort was made to detect other populations of the fly where the hosts were abundant.

Field work was conducted at the Deering Estate at Cutler (Miami) and Camp Owaissa Bauer, both Miami-Dade County Parks. *Schoepfia* fruiting phenology was followed in both parks, and two distinct patterns were observed. There was one fruiting season (Mar-Apr) at Deering Estate at Cutler, and two fruiting seasons at Camp Owaissa Bauer (Mar-Apr and Oct-Nov). Even though they are near each other (32 km apart), Camp Owaissa Bauer is in the interior (11 km from the Atlantic coast), unlike Deering Estate at Cutler, which is directly on the Atlantic coast. It has been observed that populations of *Schoepfia* in the Florida Keys flower and fruit at different times than the mainland populations (R. Hammer, personal communication). Phenological differences may be due to climatic differences among locations, or, alternatively, differences in soil composition and nutrients or plant variety may result in different capacities to produce fruit or different adaptive fruiting schedules.

Adult trapping was conducted during Mar and Apr, 2004 at Deering Estate at Cutler in 3 locations within the park where *S. schreberi* occurred. Five multi-lure traps (Better World, Fresno. CA) with torula yeast pellets dissolved in water were deployed and serviced every 2 weeks. Only one adult female specimen was trapped in Apr. At Camp Owaissa Bauer we did not conduct any trapping activities for fear of reducing the resident fly population.

Two hundred fruit were collected at the Deering Estate at Cutler in Mar and Apr, 2004, and 150 fruits in Mar, 2005, but no larvae were obtained. At Camp Owaissa Bauer, fruits were collected in Oct 2003, Mar, Oct, and Nov 2004, and Mar 2005 (about 100 fruits on each occasion). The fruits ranged in fresh weight from 61.1 mg to 316.0 mg and averaged 146.9 ± 54.9 mg (n = 25). More than 50% of the collected fruit was infested.

Anastrepha interrupta apparently oviposits into the fruit of *S. schreberi* while they are still green and not yet full size. We observed only 1 larva per fruit based on field observation of about 100 infested fruits, in which the seed had been entirely consumed leaving most of the space inside the fruit occupied by the third instar. Normal fruits became purple as they matured, but infested fruits did not appear to fully mature and change color. The oviposition puncture was clearly evident to the naked eye, and the larval exit hole was relatively large, obvious, and circular.

The samples of collected fruit were maintained in the laboratory (temperature of 25 ± 1°C and relative humidity of 55 ± 5%), in a container with vermiculite until larvae emerged and pupation occurred. The mean weight of *A. interrupta* pupae was 13.6 ± 4.8 mg (n = 25), with a range of 4.9 to 22.7 mg. *Anastrepha interrupta*'s closest relative, *Anastrepha spatulata* Stone (Foote et al. 1993), infests the same host plant in Mexico, where the fruit are slightly larger (mean = 185.0 mg), but the pupae are slightly smaller (12.0 mg) (Aluja et al. 2000).

The adult flies emerged 20-24 d after pupation at 25 ± 1°C and RH of 55 ± 5%. Under the same conditions, adults took about 20 d to mature sexually; i.e., the start of male pheromone production, sexual signaling, and mating. Maximum adult longevity in the laboratory was 177 d (female). This is similar to the lifespan reported for other *Anastrepha* species such as *A. suspensa* (Sivinski 1994). No diapause in the pupal stage was observed; all pupae that developed from the fall collections and the spring collections eclosed as adults within a few weeks of pupation.

We searched museum collections and records of the Florida State Collection of Arthropods (FSCA) and the U.S. National Museum of Natural History (NMHN) (Norrbom 2006) for information on flight time of *A. interrupta* (Fig. 1). Almost all records are based on specimens captured in fruit fly detection traps (McPhail traps). We excluded those few records based on adults reared from fruits. As previously noted (Weems 1967), adults have been collected in every month of the year. Both the number of collection records and the number of flies collected clearly indicate elevated population levels in the months from Dec to May, which overlaps with the primary fruiting seasons of its host. During the winter months of 2004/2005, *A. interrupta* clearly produced at least 2 generations at Camp Owaissa Bauer. Herbarium records at the Fairchild Tropical Botanic Garden (http://www.virtualherbarium.org/, accessed 20 Oct 2006) show that *Schoepfia schreberi* fruits at least sporadically during other months than observed here, e.g. Jan and Feb; and fruiting has also been observed during late Jul, Aug and Sep (R. Hammer, personal communication). The opportunistic availability of fruit throughout the year probably allows additional generations to be produced. An adult life span of several months as observed in the laboratory would allow this specialist species to persist as adults from one fruiting period to another as occurs at Camp Owaissa Bauer. A population may not be sustainable at sites with only a single fruiting season, such as Deering Estate at Cutler, and such sites might be repopulated only by immigration.

After emergence the flies were separated by sex and maintained with food (3 parts sugar and 1 part yeast) and water *ad libitum* in separate cages. At 20 d of adult age, flies from both sexes were placed in the same cage and their sexual behaviors observed. During the mid-afternoon males performed the following peculiar behavior: after approaching a female, the male faced her and then moved laterally, back and forth, in a half circle pattern. It is not known if this behavior is confined to interactions between males and females. Male pheromone calling, recognized by the extrusion of anal membranes and pleural glands, as seen in other tephritids (Aluja 1994), took place only in complete darkness, followed by mating. Male pheromone components were identified and will be de-

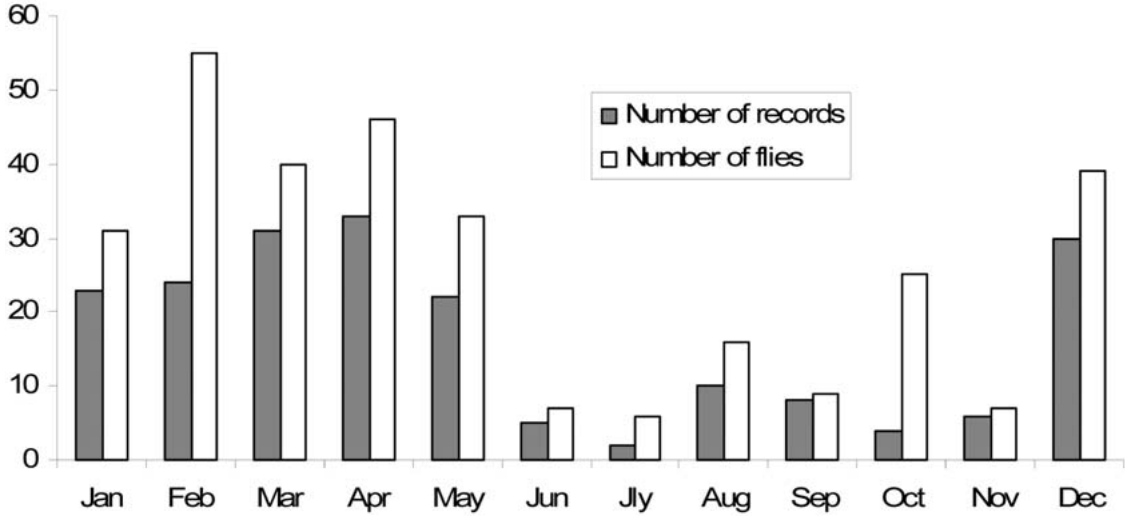

Fig. 1. Phenology of *Anastrepha interrupta* based on Florida State Collection of Arthropods and the U.S. National Museum of Natural History specimens and records, 1934-2003.

scribed elsewhere (B. D. Dueben, personal communication). Male-male interaction was not observed during the behavioral observations in laboratory.

Two females of the parasitoid *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) were reared from fly pupae derived from fruit sampled in Mar 2005. This is the first record of this parasitoid attacking *A. interrupta*. The only other parasitoid previously reported was another braconid, *Doryctobracon anastrephilus* (Marsh) (Marsh 1970; Wharton & Marsh 1978). *Utetes anastrephae* is widespread in South Florida, where it also parasitizes *Anastrepha suspensa* (Loew) (Eitam et al. 2004).

This study was conducted under permit #061 from Miami-Dade County Park and Recreation Department. We offer special thanks to Alice Warren-Bradley (Manager of Deering Estate Park) and Pam Rose (Manager of Camp Owaissa Bauer) for their cooperation and Robert A. Wharton (Texas A&M University, College Station, TX) for identification of the parasitoids. Roger Hammer (Miami-Dade Parks Department) provided comments on phenology of *Schoepfia schreberi* in south Florida. We thank Allen L. Norrbom (Systematic Entomology Laboratory, USDA-ARS, Washington DC) and Robert A. Wharton for critical reviews of an earlier version of this manuscript. Financial support was provided to RP by the Centro de Ciência e Tecnologia da Madeira through the BD I/ 2002-004 grant. This is Entomology Contribution No. 1049, Bureau of Entomology, Nematology, and Plant Pathology, FDACS-DPI.

Summary

Observations on the phenology, larval host feeding, adult longevity, behavior, and parasites of *Anastrepha interrupta* Stone (Diptera: Tephritidae) are presented. A population of this fly was bivoltine in Homestead, Florida where its host plant fruited twice per year. *Anastrepha interrupta* larvae were found singly in fruits of *Schoepfia schreberi* (Olacaceae) in which they consumed the seed. Adult flies lived up to 177 d, and there was no evidence of diapause in the immature stages. Male calling behavior begins after dusk and continues in darkness. *Utetes anastrephae* (Viereck) (Hymenoptera: Braconidae) was reared from pupae, the first observation of this parasitoid on *A. interrupta*.

References Cited

Aluja, M. 1994. Bionomics and management of *Anastrepha*. Annu. Rev. Entomol. 39: 155-178.

Aluja, M., E. Herrera, M. Lopéz, and J. Sivinski. 2000. First host plant and parasitoid record for *Anastrepha spatulata* Stone (Diptera: Tephritidae). Proc. Entomol. Soc. Washington 102: 1072-1073.

Brown, A. C. 1937. Report of the grove inspection department. State Plant Board of Florida, Report for the period July 1, 1934-June 30, 1936 (Eleventh Biennial Report). 37 pp.

Eitam, A., J. Sivinski, T. Holler, and M. Aluja. 2004. Biogeography of braconid parasitoids of the Caribbean fruit fly (Diptera: Tephritidae) in Florida. Ann. Entomol. Soc. America 97: 928-939.

Fernandez, A. M., D. Rodriguez, and V. Hernandez-Ortiz. 1997. Notas sobre el genero *Anastrepha* Schiner en Cuba con description de una nueva especie (Diptera: Tephritidae). Folia Entomol. Mexicana 99: 29-36.

Foote, R. H., F. L. Blanc, and A. L. Norrbom. 1993. Handbook of the Fruit Flies (Diptera: Tephritidae) of America North of Mexico. Comstock Publishing Associates, Ithaca, NY.

Heppner, J. B. 1990. Larvae of Fruit Flies. 6. *Anastrepha interrupta* (Schoepfia fruit fly) (Diptera: Tephritidae). Florida Dept. Agric., Div. Plant Ind. Entomol. Circ. 327.

Marsh, P. M. 1970. A new species of fruit fly parasite from Florida (Hymenoptera: Braconidae). Florida Entomol. 53: 31-32.

McClanahan, H. S., and G. B. Merrill. 1951. Reports of the Grove Inspection and Entomological Departments, Florida State Plant Board Biennial Report 18: 41-46.

Norrbom, A. L. (ed.). F. Louie Blanc New World Fruit Fly Specimen Database (http://www.sel.barc.usda.gov:591/diptera/Tephritidae/TephIntro.html, accessed on 16 Oct. 2006).

Shaw, J. G. 1962. Species of the *spatulata* group of *Anastrepha*. J. Kansas Entomol. Soc. 35: 408-414.

Sivinski, J. 1994. Longevity in the Caribbean fruit fly; effects of sex, strain and sexual experience. Florida Entomol. 76: 635-644.

Steck, G. J., and R. A. Wharton. 1988. Description of immature stages of *Anastrepha interrupta, A. limae, and A. grandis* (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 81: 994-1003

Steck, G. J., L. E. Carrol, H. Celedonio-Hurtado, and J. Guillen-Aguilar. 1990. Methods for identification of *Anastrepha* larvae (Diptera: Tephritidae), and key to 13 species. Proc. Entomol. Soc. Washington 92: 333-346.

Steyskal, G. C. 1977. Pictorial Key to Species of the Genus *Anastrepha* (Diptera: Tephritidae). Special Publ. Entomol. Soc. Washington. 35 pp.

Stone, A. 1942. The Fruit Flies of the Genus *Anastrepha*. U.S. Dep. Agric. Misc. Publ. 439: 1-112.

Wharton, R. A., and P. M. Marsh. 1978. New World Opiinae (Hymenoptera: Braconidae) parasitic on Tephritidae (Diptera). J. Washington Acad. Sci. 68: 147-167.

Weems, H. V. Jr. 1967. *Anastrepha interrupta* Stone (Diptera: Tephritidae). Florida Dept. Agric., Div. Plant Ind. Entomol. Circ. 61.

DUPLACHIONASPIS DIVERGENS (HEMIPTERA: DIASPIDIDAE), A NEW EXOTIC PEST OF SUGARCANE AND OTHER GRASSES IN FLORIDA

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The grasses (Poaceae) are some of the most important agricultural plants in the world with corn, sugarcane and wheat all being widely used as food crops. In addition to agricultural importance, grasses also are used as ornamentals in landscapes and other horticultural situations, as well as being the major plants used in turf for lawns and recreational activities. Because these plants are so widely used in a variety of monocultural plantings, they attract a wide array of pests. One of the most common and taxonomically difficult groups of insects that diagnosticians have to identify are scale insects (Hemiptera: Coccoidea).

Twenty-one families of scale insects are known worldwide with the most commonly encountered pest species found within the following 3 families: (1) Diaspididae (armored scales), (2) Pseudococcidae (mealybugs) and (3) Coccidae (soft scales). The most common scale insects associated with grasses are armored scales and mealybugs, and several species of armored scales are commonly collected on grasses in Florida. The most common of these are *Odonaspis* species, *Aspidiella sacchari* (Cockerell), *Haliaspis* species, in addition to *Frogattiella* and *Kuwanaspsis* species, which are found on bamboo.

More recently, *Duplachionaspis divergens* (Green) has steadily become one of the most commonly encountered grass infesting armored scales in Florida. This armored scale has been reported throughout much of the Eastern Hemisphere including Algeria, Australia, China, Egypt, India, Japan, Sri Lanka, Taiwan, and Thailand, and is the only species of the 35 described species of the genus known to occur in the United States. Lastra and Gomez (1997) reported the first occurrence in the Western Hemisphere from collections on sugarcane in Colombia in 1996. However, specimens collected by Fred Bennett in Venezuela on sugarcane confirmed its presence in the Western Hemisphere as early as 1991. Its occurrence in Florida and the continental United States was first recorded from specimens on a grass in Seminole Co., Florida in 2002. However, a re-examination of specimens collected in Manatee Co., Florida on *Miscanthus* species in 2000 is the earliest record of this species occurring in Florida. Since the initial finds of *D. divergens* in Florida, interceptions have occurred in both Alabama (Charles Ray, Auburn University, pers. comm.) and Texas (Scott Ludwing, Texas A&M University, pers. comm.).

The biology of *D. divergens* was studied by Lastra and Gomez (1997) in Colombia. They reported that adult females lay an average of 130 eggs and that 9 generations/year occurred with an average generation time of 39 days. The scale cover of the adult female (Fig. 1) resembles that of false oleander scale (*Pseudaulacaspis cockerelli* (Cooley) in that they appear as small white "tear drops" that are about 3 mm long. Male covers are much smaller (1 mm) and appear as white tricarinate tubes.

The economic importance of *D. divergens* in Florida is not clearly defined. However, Pruthi & Rao (1942) reported it as a minor pest of sugarcane in India, and Lastra & Gomez (1997) noted it as a pest of sugarcane in Colombia. Therefore, it is a potential pest of sugarcane in Florida where about 450,000 acres are grown annually (Meagher 2003). This species has also been found on St. Augustine grass (*Stenatophrum secundatum* (Walter) Kuntze), a common lawn grass and Bahia grass (*Paspalum notatum* Flugge), an important pasture grass. Sugarcane growers usually implement natural control strategies to control pests and seldom use pesticides. Natural enemies are known for *D. divergens* (Sankaran 1984; Shafee et al. 1975; Lastra & Gomez 1997); however, we have reared a species in the *Aphytis lingnanesis* group and *Encarsia citrina* (Craw) (Hymenoptera: Aphelinidae) from specimens collected in Florida.

Fig. 1. Field specimen, adult female cover of *Duplachionaspis divergens*. Photograph credit: Avas Hamon, FDACS-DPI.

SUMMARY

Duplachionaspis divergens is established in Florida and has been intercepted in both Alabama and Texas, but its overall economic impact is yet unknown. However, due to its potential as a pest of sugarcane and other grasses, it warrants close observation to ensure that this insect does not become a major pest of grasses in the southeastern United States.

REFERENCES CITED

LASTRA, L. A., AND L. A. GOMEZ. 1997. Observaciones del ciclo devida de la escama blanca, *Duplachionaspis divergens* (Green) (Homoptera: Diaspididae) y reconcimiento de enemigos naturals, pp. 41-51 *In* IV Congreso Colombiana de la Asociación de técnios de la can´a de azucar. Cali, Colombia 24-26 de Sept. de 1997, 473 pp.

MEAGHER, R. L. 2003. Sugarcane IPM. http://ipm-world.umn.edu/chapters/meagher.htm

PRUTHI, H. S., AND V. P. RAO. 1942. Coccids attacking sugarcane in India. Indian J. Entomol. 4: 87-88.

SANKARAN, T. 1984. Survey for natural enemies of Diaspine Scale Insects in South India: Final Technical Report for the Period November 5, 1980 to November 4, 1983. Commonwealth Institute of Biological Control, Bangalore India. 87 pp.

SHAFEE, S. A., S. F. ALAM, AND M. M. AGARWAL. 1975. Taxonomic survey of encyrtid parasites (Hymenoptera: Encyrtidae) in India. Publications (Aligarh Muslim University) 10: 123 pp.

LOW INCIDENCE OF *CANDIDATUS* LIBERIBACTER ASIATICUS IN *DIAPHORINA CITRI* (HEMIPTERA: PSYLLIDAE) POPULATIONS BETWEEN NOV 2005 AND JAN 2006: RELEVANCE TO MANAGEMENT OF CITRUS GREENING DISEASE IN FLORIDA

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Key Words: *Diaphorina*, Huanglongbing, Asian citrus psyllid, *Candidatus* Liberibacter asiaticus

Citrus greening disease or Huanglongbing (HLB) is caused by the gram-negative bacterium *Candidatus* Liberibacter asiaticus (*Ca.* L. asiaticus) (Garnier et al. 2000) and was confirmed in southern Florida in 2005 (Halbert 2005; Bouffard 2006). This disease is vectored by *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), which colonized the citrus-growing regions of Florida after it was discovered in 1998 (Knapp et al. 1998; Halbert 1998; Halbert et al. 2000). *Diaphorina citri* acquires the greening bacterium while feeding on infected phloem (Hung et al. 2004). HLB ultimately is fatal to susceptible citrus trees, so early detection and removal of infected trees is important for disease management. Unfortunately, citrus trees often are asymptomatic for years before the common signs of HLB, including yellowing and mottling of leaf veins and misshapen green-colored fruit, are noticeable (da Graça 1991). Current chemical and biological controls reduce *D. citri* populations (Rae et al. 1997; Hoy et al. 1999; Hoy & Nguyen 2000; Michaud 2004; Browning et al. 2006), but may not be sufficient to eliminate all HLB transmission.

It will be important to understand the epidemiology of HLB to control the spread of this disease. The regions of Florida with citrus showing symptoms of HLB currently are being mapped (http://www.doacs.state.fl.us/pi/chrp/greening/maps/cgsit_map.pdf). However, little currently is known about infection rates and transmission frequency of HLB by the psyllid vector. We surveyed the vector, *D. citri*, for the greening bacterium in 11 citrus-growing counties in Florida (Table 1). In most of the counties sampled, citrus trees did not show signs of HLB infection, so we anticipated a low incidence of the greening bacterium (perhaps <1-2%) in these psyllid populations. However, we hypothesized that if citrus trees had acquired HLB recently and did not show disease symptoms, HLB could still be spread in these regions and detected in vector populations by molecular analyses.

Adult psyllids collected in this survey were killed in 95% ethanol in the field and placed on ice during transit to the Department of Entomology and Nematology at the University of Florida, Gainesville FL. Adult and immature *D. citri* were separated, counted, and stored in fresh 95% ethanol or acetone at -80°C (Fukatsu 2005). Tools used to separate insect specimens were washed with bleach, which degrades DNA, to avoid cross-contamination between samples. A maximum of 10 *D. citri* were pooled for DNA isolation by PUREGENE reagents according to the manufacturer's instructions (Gentra Systems, Minneapolis, MN). DNA pellets were re-suspended in 50 µL of sterile water or TE buffer and stored at -80°C. High-fidelity PCR was used to analyze each sample for the 16S rRNA (Subandiyah et al. 2000) and *nus*G-*rpl*K (Villechanoux et al. 1993; Hoy et al. 2001) gene sequences of *Ca.* L. asiaticus, which would yield DNA bands 0.5 kb and 0.6 kb in length, respectively. The samples also were screened for a 0.6-kb portion of the *wsp* gene of *Wolbachia* (Braig et al. 1998), an endosymbiotic bacterium found in *D. citri* (Subandiyah et al. 2000), to control for DNA quality.

A positive control was obtained from Vernon Damsteegt at the USDA-ARS quarantine facility in Beltsville, MD, where adult *D. citri* fed on citrus trees positive for HLB. A total of 3 DNA extractions from these adult *D. citri*, including 2 extractions from single adults and 1 extraction from 10 pooled adults, was conducted by Micki Kuhlmann with the methods described above. The DNA was shipped from Beltsville, MD to the University of Florida and used in a high-fidelity PCR assay. Amplification products were detected in each of the 3 samples with primers for the *nus*G-*rpl*K gene of *Ca.* L. asiaticus and for the *wsp* gene of *Wolbachia*.

To quantify the sensitivity of our high-fidelity PCR assay, a dilution series of plasmid DNA containing the *nus*G-*rpl*K gene of *Ca.* L. asiaticus, mixed with DNA from adult *D. citri* from a laboratory colony that previously had tested negative *Ca.* L. asiaticus, was amplified with high-fidelity PCR (Fig. 1). As little as 1 fg of the target template could be detected 100% of the time, which is approximately equivalent to 100 copies of the *nus*G-*rpl*K gene sequence (Fig. 1), while as few as 10 copies could be detected 50% of the time (Hoy et al. 2001). Control reactions containing no DNA

TABLE 1. COLLECTION DATA FOR *DIAPHORINA CITRI* AND RESULTS OF THE HIGH-FIDELITY PCR ASSAY FOR *CANDIDATUS* L. ASIATICUS AND THE ENDOSYMBIONT *WOLBACHIA* DURING SEP 2005 AND JAN 2006

County	°N	°W	No. adults tested[1]	No. nymphs tested[1]	Host[2]	Grove[3]	PCR: *Ca.* L. asiaticus	PCR: *Wolbachia*
De Soto	27°13.877'	81°53.990'	56	0	G	C	-	+
Glades	27°06.559'	80°56.427'	30	25	O	A	-	+
Glades	27°00.372'	81°03.003'	10	12	G	D	-	+
Hendry	26°44.256'	81°10.490'	5	0	O	C	-	+
Hendry	26°46.322'	81°12.654'	61	0	G	C	-	+
Hendry	26°33.898'	81°26.202'	0	38	G	D	-	+
Hendry	26°44.423'	81°28.029'	90	0	G	C	-	+
Hendry	26°20.307'	80°54.597'	486	0	O	C	-	+
Highlands	27°24.781'	81°24.714'	48	39	O	C	-	+
Highlands	27°09.201'	81°19.877'	22	27	G	C	-	+
Indian River	27°40.953'	80°27.621'	48	0	G	A	-	+
Lake	28°51.627'	81°38.306'	69	0	O	C	-	+
Lake	28°23.967'	81°41.768'	52	0	O	A	-	+
Lee	26°42.707'	81°36.559'	36	38	O	D	-	+
Marion	28°59.204'	81°55.267'	41	0	G	C	-	+
Pasco	28°19.505'	82°11.240'	116	0	O	C	-	+
Polk	28°03.656'	81°34.937'	140	0	O	C	-	+
Polk	28°02.880'	81°37.035'	91	0	O	C	-	+
Polk	27°52.542'	81°34.654'	70	0	O	C	-	+
Polk	27°47.537'	81°32.044'	79	0	O	C	-	+
Polk	28°06.295'	81°42.895'	197	0	G	R	-	+
St. Lucie	27°32.976'	80°25.852'	40	0	G	N	-	+
St. Lucie	27°23.360'	80°28.376'	6	0	O	C	-	+
		Total	1793	179				

[1]A maximum of 10 specimens were pooled for each DNA extraction.
[2]Host: O = Oranges, G = Grapefruit.
[3]Grove: C = Commercial, A = Abandoned, D = Dooryard, R = Research Plot, N = Non-Commercial.

were negative for the 16S rRNA and *nus*G-*rpl*K genes of *Ca.* L. asiaticus and for the *wsp* gene for *Wolbachia*, as expected.

Altogether, 1,793 adult and 179 immature *D. citri* were collected from 23 sites in 11 counties between Sep 2005 and Jan 2006 (Table 1). All field-collected *D. citri* tested negative for *Ca.* L. asiaticus (<1 in 1,972 psyllids surveyed = <0.05% infection frequency). All samples were positive for *Wolbachia* in the PCR assays, which indicates that the DNA extractions and PCR protocols were working for these samples of microbial DNA mixed with *D. citri* genomic DNA (Table 1). The amount of *Ca.* L. asiaticus DNA in our field-collected *D. citri* was either below the sensitivity of the high-fidelity PCR assay (which reliably detects 100 copies and can detect as few as 10 copies), or the psyllids were truly negative for the HLB-causing bacterium. The failure to obtain any positives for *Ca.* L. asiaticus was surprising, particularly for the site in Hendry County (26°20.307'°N: 80°54.597'°W) where 486 adult *D. citri* were collected from flushing citrus trees with symptoms of HLB. However, the psyllids were aggregating on these trees to mate and oviposit on the tender flush, so they may not have acquired *Ca.* L. asiaticus before they were collected or the greening bacterium had not multiplied sufficiently in the host to facilitate detection.

The results from this study can be used as a benchmark for the infection status of *D. citri* in these sites in Florida during 2005-2006. The absence of *Ca*. L. asiaticus in the psyllid populations surveyed could be due to the recent detection of HLB in Florida and because most psyllids were collected from trees that did not appear to have HLB. However, the lack of any PCR positives in the 486 psyllids collected in Hendry county (Table 1) from trees with HLB symptoms was perplexing; with even a 1% infection frequency we would have expected positives in at least 4 psyllids. In Indonesia, where HLB is more prevalent, up to 45.2% of individual adult *D. citri* tested positive for *Ca.* L. asiaticus in a standard PCR assay (Subandiyah et al. 2000), which is approximately 7-fold less sensitive than the high-fidelity PCR method used here (Hoy et al. 2001). However, in 1992 in India fewer than 1% of the *D. citri* tested were positive for *Ca.* L. asiaticus (Bové et al. 1993). The proportion of *D. citri* carrying *Ca.* L.

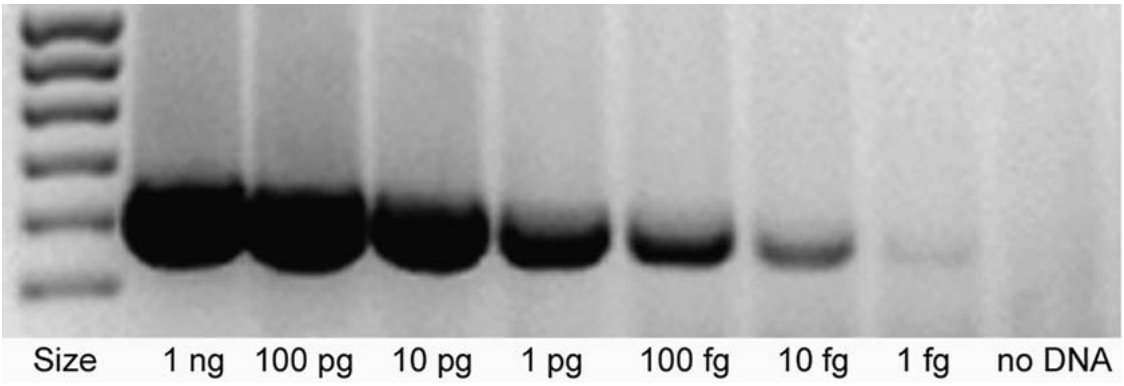

Fig. 1. Sensitivity analysis for high-fidelity PCR-amplification of plasmid DNA containing the *nus*G-*rpl*K gene of *Ca.* L. asiaticus mixed with *D. citri* DNA. PCR-products were obtained with as little as 1 fg (approximately 100 copies) of the 0.6 kb amplification target.

asiaticus in Florida will likely increase if the titer and distribution of the pathogen increases in infected trees and the number of citrus trees with HLB multiplies.

The findings of this study raised a number of important questions concerning the epidemiology and management of HLB in Florida. For instance, how appropriate is it to attempt to kill every psyllid in citrus groves with chemical control if the infection frequency is less than 1%? The cost of widespread chemical control of psyllids and the potential disruption of biological control of other citrus pests needs to be considered. Other questions remain unanswered. How long does it take psyllids to acquire and transmit *Ca.* L. asiaticus when feeding on infected citrus under Florida conditions? How many citrus trees can be infected by a single psyllid hosting *Ca.* L. asiaticus? How does the duration and level of infection in HLB-positive trees affect disease acquisition and transmission by psyllids? Are there seasonal factors that influence HLB transmission? What roles do other host plants, such as *Murraya paniculata* (L.) Jack. play in HLB transmission? Are there other mechanisms that contribute to the spread of HLB, such as mechanical transmission or native vectors? A more extensive effort to survey psyllid populations for *Ca*. L. asiaticus is needed to better understand the epidemiology of HLB. It is thought that destruction of HLB-positive citrus trees, along with suppression of *D. citri* populations in infected citrus groves, will slow the spread of HLB (Stansly & Rogers 2005). However, research is needed to determine which management tactics should have priority in order to minimize the spread of HLB while maintaining existing biological control of other economically-important citrus pests. Possibly, detecting and removing infected trees should be considered a higher priority than attempting to kill all *D. citri* in citrus groves, due to the apparently low proportion of the vector population carrying *Ca*. L asiaticus.

We thank Vernon Damsteegt (USDA Beltsville, MD) for providing infected psyllids and Micki Kuhlmann for extracting their DNA for the positive control experiment. We thank Mike Rogers for assistance and Tim Gast, who allowed us to collect psyllids from citrus trees with HLB symptoms at Southern Gardens. This research was funded in part by the Davies, Fischer and Eckes Endowment in biological control to M. A. Hoy.

Summary

Populations of *D. citri* in Florida citrus were surveyed between Sep 2005 and Jan 2006 for *Ca.* L. asiaticus, the causal agent of HLB. No field-collected adults or immatures of the 1,972 *D. citri* tested were positive for the HLB pathogen in these samples, indicating that the proportion of *D. citri* populations hosting *Ca.* L. asiaticus in the regions sampled was very low (<0.05%) during this survey. More extensive surveys for *Ca.* L. asiaticus in *D. citri* are recommended to learn more about the epidemiology of disease transmission in Florida.

References Cited

Bouffard, K. 2006. Greening found in 10 counties. Citrus Industry 87(1): 5-26.

Bové, J. M., M. Garnier, Y. S. Ahlawat, N. K. Chakraborty, and A. Varma. 1993. Detection of the Asian strains of the greening BLO by DNA-DNA hybridization in Indian orchard trees and Malaysian *Diaphorina citri* psyllids, pp. 258-263 *In* P. Moreno, J. V. da Graça, and L. W. Timmer [eds.], Proc. 12th Conference of the International Organization of Citrus Virologists. University of California, Riverside.

Braig, H. R., W. Zhou, S. L. Dobson, and S. L. O'Neill. 1998. Cloning and characterization of a

gene encoding the major surface protein of the bacterial endosymbiont *Wolbachia pipientis*. J. Bacteriol. 180: 2373-2378.

BROWNING. H. W., C. C. CHILDERS, P. A. STANSLY, J. PEÑA, AND M. E. ROGERS. 2006. Florida citrus pest management guide: soft-bodied insects attacking foliage and fruit: University of Florida IFAS Extension. http://edis.ifas.ufl.edu/BODY_CG004.

FUKATSU, T. 2005. Acetone preservation: a practical technique for molecular analysis. Mol. Ecol. 8: 1935-1945.

GARNIER, M., S. JAGOUEIX-EVEILLARD, P. R. CORNJE, H. F. LE ROUX, AND J. M. BOVÉ. 2000. Genomic characterization of a *Liberibacter* present in an ornamental rutaceous tree, *Calodendrum capense*, in the Western Cape province of South Africa. Proposal of '*Candidatus Liberibacter africanus* subsp. *capensis*'. Int. J. System. Evol. Microbiol. 50: 2119-2125.

DA GRAÇA, J. V. 1991. Citrus greening disease. Annu. Rev. Phytopathol. 19: 109-136.

HALBERT, S. E. 1998. Asian citrus psyllid—A serious potential exotic pest of Florida citrus. http://www.ifas.ufl.edu/~entweb/DCITRI.htm.

HALBERT, S. E. 2005. Pest Alert: Citrus Greening/Huanglongbing. http://www.doacs.state.fl.us/pi/chrp/greening/citrusgreeningalert.html.

HALBERT, S. E., X. SUN, AND W. N. DIXON. 2000. Asian citrus psyllid and citrus greening disease. Citrus Industry 91(5): 22-24.

HOY, M. A., AND R. NGUYEN. 2000. Classical biological control of Asian citrus psylla. Citrus Industry 81(12): 48-50.

HOY, M. A., R. NGUYEN, AND A. JEYAPRAKASH. 1999. Classical biological control of Asian citrus psylla. Citrus Industry 80(9): 20-22.

HOY, M. A., A. JEYAPRAKASH, AND R. NGUYEN. 2001. Long PCR is a sensitive method for detecting *Liberobacter asiaticum* in parasitoids undergoing risk assessment in quarantine. Biol. Control 22: 278-287.

HUNG, T. H., S. C. HUNG, C. N. CHEN, M. H. HSU, AND H. J. SU. 2004. Detection by PCR of Candidatus *Liberibacter asiaticus*, the bacterium causing citrus Huanglongbing in vector psyllids: application to the study of vector-pathogen relationships. Plant Path. 53: 96-102.

KNAPP, J. L., S. HALBERT, R. LEE, M. HOY, R. CLARK, AND M. KESINGER. 1998. The Asian citrus psyllid and citrus greening disease. Citrus Industry 79(10): 28-29.

MICHAUD, J. P. 2004. Natural mortality of Asian citrus psyllid (Homoptera: Psyllidae) in central Florida. Biol. Control 29: 260-269.

RAE, D. J., W. G. LIANG, D. M. WATSON, G. A. C. BEATTIE, AND M. D. HUANG. 1997. Evaluation of petroleum spray oils for control for the Asian citrus psylla, *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), in China. Intern. J. Pest Management. 43: 71-75.

STANSLY, P. A., AND M. E. ROGERS. 2006. Managing Asian citrus psyllid populations. Citrus Industry 87(3): 17-19

SUBANDIYAH, S., N. NIKOH, S. TSUYUMU, S. SOMOWIYARJO, AND T. FUKATSU. 2000. Complex endosymbiotic microbiota of the citrus psyllid *Diaphorina citri* (Homoptera: Psylloidea). Zool. Sci. 17: 983-989.

VILLECHANOUX, S., M. GARNIER, F. LAIGRET, J. RENAUDIN, AND J. M. BOVÉ. 1993. The genome of the noncultured, bacterial-like organism associated with citrus greening disease contains the *nus*G-*rpl*KAJL-*rpo*BC gene cluster and the gene for a bacteriophage type DNA polymerase. Curr. Microbiol. 26(3): 161-166.

OBSERVATIONS OF A SUBSOCIAL TREEHOPPER, *STALOTYPA FAIRMAIRII* FROM CUBA (HEMIPTERA: MEMBRACIDAE)

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The treehopper *Stalotypa fairmairii* (Guérin-Méneville) is endemic to the Caribbean Island of Cuba and has been placed within the membracine tribe Hoplophorionini (McKamey & Deitz 1996). The hoplophorionine treehoppers are mainly Neotropical, and occur mostly at higher elevations, with greatest species diversity near the equator of South America. Hoplophorionines for which the biology is known have highly developed subsocial behavior of guarding eggs and nymphs (Wood 1984; McKamey & Deitz 1991, 1996; Lin 2003, 2006). Unlike other treehoppers, they do not interact with mutualistic ants or other hymenopterans.

Among the Hoplophorionine, detailed data on natural history is available only for the North American species *Platycotis vittata* (Fabricius) and *Umbonia crassicornis* (Amyot & Serville) (Wood 1974, 1976). Natural histories of the majority of tropical hoplophorionines are less known except for a few species in the Central America (Wood 1984; McKamey & Deitz 1991, 1996). No natural history information for *S. fairmairii* is known but it has been presumed to be subsocial (McKamey & Deitz 1996).

Observations of *S. fairmairii* were made in Soroa, Pinar del Rio Province, Cuba between 19 and 26 of Jun 2001. The observation site was approximately 400 m wide on the edge of the road leading to Manto Bonito from Soroa (mileage marker 11, near Campismo La Caridad) (Fig. 1A). The terrain is agricultural land, open-air dairy farms, fruit trees, and secondary forest remnants. The habitat is on a humid lowland hill with tropical thunderstorms occurring almost every afternoon. Additional treehopper populations were located in similar habitats a few kilometers north of Soroa, near the town of San Diego de Nunez.

Aggregations of adults, nymphs, and females on eggs or with nymphal aggregations were tagged individually with tapes on the branches and observed every 3 h from 8 AM to 5 PM for 8 consecutive days. Plant stems with egg masses guarded by females were collected and dissected. Eggs were counted with the aid of a microscope. Voucher specimens of *S. fairmairii* resulting from this study are deposited in the insect collections of the Department of Life Sciences, Tunghai University, Taichung, Taiwan.

All life stages of *S. fairmairii* were found on cultivated guava, *Psidium guajava* L. (Myrtaceae) (Fig. 1B-H). The plants were blossoming and small fruits were developing during the study period. The only other host-plant record for *S. fairmairii* is an introduced Old World *Eucalyptus* (Myrtaceae) (Scaramuzza 1951). In that study, only adult treehoppers were observed to be associated with *Eucalyptus*. Moreover, the absence of egg masses, nymphs, or ovipositioning behavior makes this host-plant record dubious.

A single clutch of eggs is deposited on the underside of stems toward the apical portion of the branches (Fig. 1C, D). Females appear to lay eggs on branches that lack ovipositions from other females (n = 16). Nevertheless, two egg masses were found on a single stem, one deposited about 3 cm below the other. Egg masses were usually located in the woody stem about 3 ± 1.8 cm (n = 18) below the apical green shoot, but 1 mass left in the petiole was below a developing fruit. Eggs were deposited in 4-8 longitudinal slits about 1 cm long and parallel to the bark (Fig. 1C). The average number of eggs per mass is 66 ± 13.9 (n = 12), greater than that of *P. vittata* (32 ± 19.93, Wood 1976), but smaller than that of *U. crassicornis* (97.62 ± 24.34, Wood & Dowell 1984).

Females cover eggs with watery accessory secretion during oviposition, and this degrades in a few days as eggs swell during development. Egg covering of *S. fairmairii* appears to be similar in color and shape to that of other hoplophorionines (Fig. 1C). Egg-guarding females sitting on top of egg masses oriented themselves toward the apical meristem of the branch (n = 18) (Fig. 1C). Brooding females maintain close body contact with egg masses, but when disturbed raise their body above egg masses, perhaps as a defense response.

The average aggregation size of the first instars is 61 ± 13.2 (n = 3). Newly hatched nymphs feed in the slits made by females, which are located below the egg masses (Fig. 1D). Unlike spiral-shaped feeding slits in *Umbonia*, *Platycotis* or *Romosella* (Wood 1984; Lin 2003, 2006), these scattered feeding slits of *Stalotypa* appear to be randomly arranged without a regular pattern, similar to those made by *Metcalfiella* (McKamey & Deitz 1991). Females of *S. fairmairii* sit below the offspring aggregation (n = 10) (Fig. 1E, F). The size of aggregations of 2-3rd and 4-5th instars is 59 ± 4.7 (n = 4) and 23 ± 9.1 (n = 6), respectively. The number of 4-5th instars in aggregations without adult females is 9 ± 5.7 (n = 2), suggesting a decrease in nymphal survival without maternal care. The average size of teneral adults in aggregations is 28 ± 5.6 (n = 3) before dispersal (inferred from the presence of 5th instars and the unsclerotized adult coloration). Unlike other hoplophorionines with female biased sex ratios (*P. vittata*, Wood 1976; *Potnia* sp., McKamey & Deitz 1996; *U. crassicornis* and *U. ataliba*,

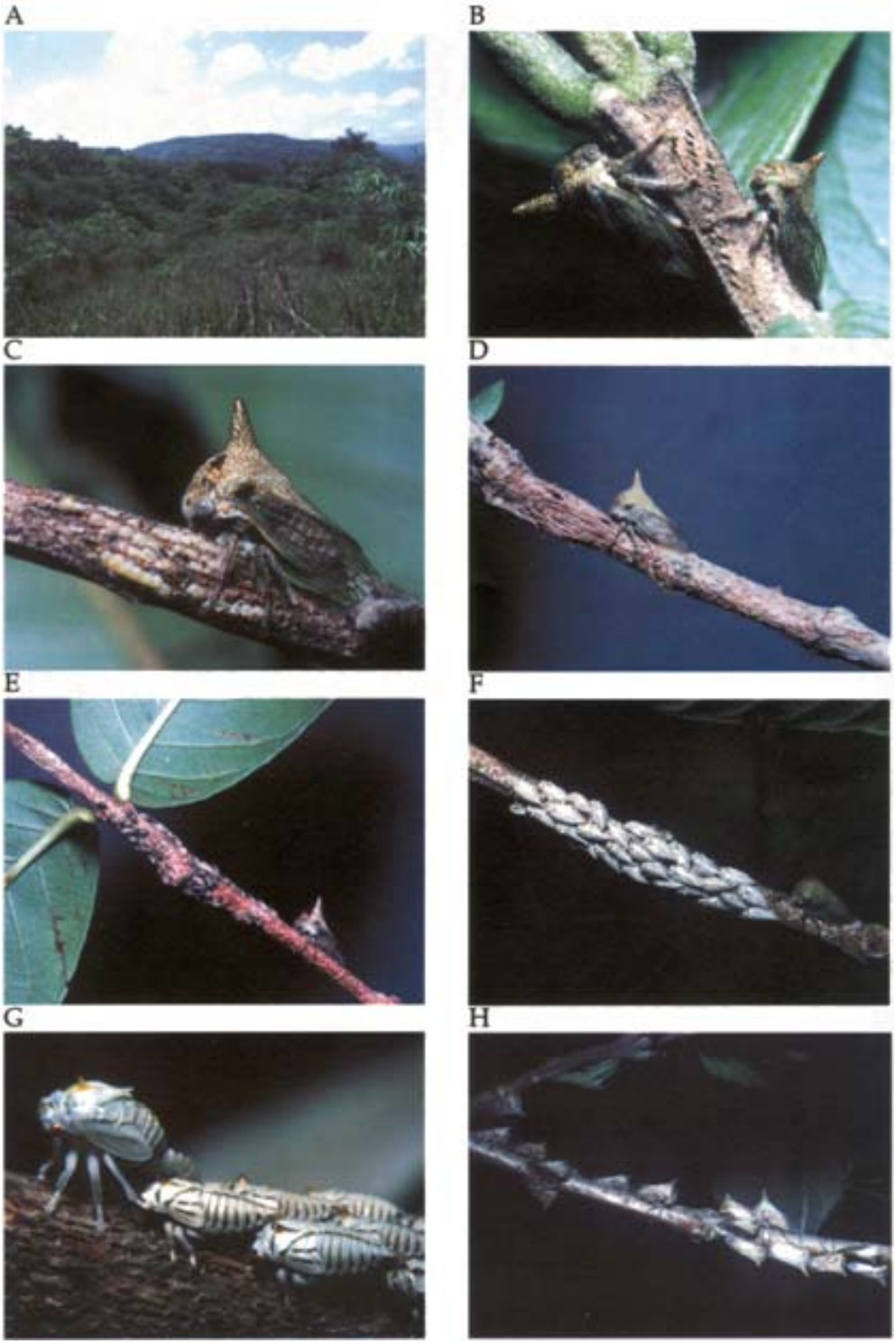

Fig. 1. Life stages of *Stalotypa fairmairii*. A, the study site. B, a female (left) and a male (right), with a female guarding her egg masses which are inserted into the bark. C, a female with egg masses and randomly arranged ovipositional slits. D, the position of a guarding female, egg masses and newly hatched nymphs. E, the position and orientation of a female with newly hatched nymphs. F. a female and an aggregation of 4-5th instars. G, the 4-5th nymphs. H, a teneral adult aggregation. This figure can be accessed on line in color at http://www.fcla.edu/FlaEnt/fe902.htm.

Wood & Dowell 1984; Master 1989), sex ratio of teneral adults in aggregations of *S. fairmairii* appears to be equal (14 ± 2.7 for males and 14 ± 3.5 for females, $n = 3$).

A mating pair was observed copulating around 11 AM for at least 45 min before separating near the apex of the branch. *Stalotypa* are sexually dimorphic. Males are smaller than females and sexes differ in pronotal shapes with short (male) or long (female) humeral horns. Teneral adults and late instars show presumably aposematic coloration while sclerotized adults are dark brown (Fig. 1B, C, G, F).

As in other hoplophorionines, *S. fairmairii* are not ant-tended, although ants were observed tending scale insects on the same trees. An egg-guarding female was observed to kick an approaching ant with her hind legs. Several egg-guarding females also delivered kicks toward approaching conspecific adults. In addition to kicking, females fanned their wings and made audible buzzes upon disturbance. A female maintained its nymphal aggregation by stopping nymphs from moving down the stem by using movement of her front legs. When disturbed, females walked back and forth along the stem above nymphal aggregations. An unidentified spider was observed to prey on an adult female.

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SUMMARY

Based on observations of treehopper *Stalotypa fairmairii* (Guérin-Méneville) in Soroa, Pinar del Rio Province of Cuba, this work presents the first documentation of various aspects of its life history and behavior including host plants, ovipositional sites, egg mass characteristics, nymphal and adult aggregations, and maternal care.

REFERENCES CITED

LIN, C.-P. 2003. Phylogeny and Evolution of Subsocial Behavior and Life History Traits in the Neotropical Treehopper Subfamily Membracinae (Hemiptera: Membracidae). Ph.D. Dissertation. Cornell Univ., Ithaca, NY.

LIN, C.-P. 2006. Social behavior and life history of membracine treehoppers. J. Nat. Hist. 40: 1887-1907.

MASTER, K. L. 1989. The Adaptive Significance of Female-biased Sex Ratios in the Neotropical Treehopper *Umbonia ataliba* (Homoptera: Membracidae). M.S. thesis, Univ. of Florida, Gainesville.

MCKAMEY, S. H., AND L. L. DEITZ. 1991. Revision of the Neotropical Treehopper Genus *Metcalfiella* (Homoptera: Membracidae). North Carolina Agric. Res. Sev. Tec. Bul. 294: 1-89.

MCKAMEY, S. H., AND L. L. DEITZ. 1996. Generic revision of the New World tribe Hoplophorionini (Hemiptera: Membracidae: Membracinae). Syst. Entomol. 21: 295-342.

SCARAMUZZA, L. C. 1951. Insectos observados en *Eucalyptus* spp. Bol. Hist. Nat. Soc. 'Felipe Poey' 2: 86-89.

WOOD, T. K. 1974. Aggregation behavior of *Umbonia crassicornis* (Homoptera: Membracidae). Can. Entomol. 106: 169-173.

WOOD, T. K. 1976. Biology and presocial behavior of *Platycotis vittata* (Homoptera: Membracidae). Ann. Entomol. Soc. America 69: 807-811.

WOOD, T. K. 1984. Life history patterns of tropical membracids (Homoptera: Membracidae). Sociobiology 8: 299-344.

WOOD, T. K., AND R. DOWELL. 1984. Sex ratio in *Umbonia crassicornis* Amyot and Serville (Homoptera: Membracidae). American Mid. Nat. 112: 58-66.

VIRULENCE OF ENTOMOPATHOGENIC NEMATODES AGAINST *DIAPREPES ABBREVIATUS* IN AN OXISOL

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Diaprepes abbreviatus (L.) (Coleoptera: Curculionidae) is an insect whose host range includes more than 270 species of plants, including many economic species (Martorell 1976; Simpson et al. 1996; Wolcott 1936). Damage to roots by larvae can reduce yield and impact the long term health of host plants. There is a need to identify biocontrol options for this pest that are efficacious in Puerto Rico.

Entomopathogenic nematodes in the families Heterorhabditidae and Steinernematidae are lethal parasites of insects (Poinar 1990) and have proven effective against *D. abbreviatus* in Florida (McCoy et al. 2000; Shapiro-Ilan et al. 2002, 2005). However, these assays were conducted in the sandy soils typical of the regions where citrus is cultivated in Florida. For infective juvenile nematodes to successfully infect a host they must be able to move through the soil. Therefore, soil physical properties, such as those typical of sandy soils (porous and aerated), should facilitate nematode infectivity than denser soils, such as clays. Indeed a number of researchers have noted that the clay content of a soil is inversely proportional to the ability of nematodes to disperse in that soil (Georgis & Poinar 1983; Barbercheck & Kaya 1991; Barbercheck 1992). However, recent research has shown that the role of soil physical properties in nematode dispersal and survival is more complex, varying with species of nematode (Portillo-Aguilar et al. 1999; Koppenhöfer & Fuzy 2006). Additionally, research shows that nematode virulence to *D. abbreviatus* can be significantly higher in certain high clay content soils (Shapiro et al. 2000). Greenhouse assays with *Steinernema feltiae* (=*Neoaplectana carpocapsae*) conducted in Puerto Rico against *D. abbreviatus* revealed limited mortality, but results were ambiguous and the soil type assayed was not identified (Román & Figueroa 1985). Another study of the virulence of *S. feltiae* against *D. abbreviatus* in soils from various regions of Puerto Rico indicated that infection rates were higher in soils from regions that had a higher sand content, suggesting that increased infectivity was positively correlated with the porosity of the soil (Román & Beavers 1983).

Oxisols are representative of the ultimate stages of soil development in the tropical and subtropical regions to which they are restricted. They are characterized by extremely weathered, acidic red clay completely lacking in weatherable minerals and bases (Beinroth 1971). Although Oxisols occupy a relatively small percentage of Puerto Rico's surface, tropical fruit crops are commonly grown in this soil type and sustain significant damage from *D. abbreviatus*. The objective of this study was to assay the virulence of a number of nematode species and strains against *D. abbreviatus* larvae in an Oxisol.

Thirty kg of soil were collected from a fruit orchard at the USDA-ARS Experiment Station in Isabela, PR. The soil was oven-dried for 2 d and shipped to the USDA Research Laboratory in Byron, GA. A sample of the soil was analyzed for nutrient content and physical properties at USDA-ARS-TARS in Mayaguez, PR. The soil was identified as belonging to the Cotito series in the Oxisol order. The soil composition, determined by the hydrometer method, was 13.26% sand, 12.86% silt, and 73.87% clay, with a pH of 5.85. The percent of total nitrogen in the soil, determined by the micro-Kjeldahl method, was 0.22. The concentrations of other critical elements in the soil, determined by atomic absorption spectroscopy (K, Ca, Mg, Cu, Fe, Mn, and Zn) or the Bray II Method (P), were as follows: P = 39 µg/g; K = 558 µg/g; Ca = 1,136 µg/g; Mg = 122 µg/g; Cu = 0.98 µg/g; Fe = 9 µg/g; Mn = 301 µg/g; and Zn = 4 µg/g.

Larvae of *D. abbreviatus* were obtained from the Biological Control Mass Rearing Facility of the Florida Division of Plant Industry. Nine species/strains of nematodes were assayed against larvae of *D. abbreviatus*: *Steinernema riobrave* Cabanillas, Poinar & Raulston (strains 355, 7-12, 3-8b, and TP), *S. feltiae* (Filipjev) (SN strain), *S. rarum* (Doucet) (J. Levy strain), *S. diaprepesi* Nguyen & Duncan, *Heterorhabditis indica* Poinar, Karunakar & David (HOM1 strain), and *H. megidis* Poinar, Jackson & Klein (UK211 strain). Before experimentation, nematodes were reared in and transferred from no more than 5 live last-instar greater wax moth larvae, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae). Nematodes were reared at approximately 25°C according to procedures described in Kaya & Stock (1997). After harvesting, nematodes were stored in tap water at 13°C (Kaya & Stock 1997) for up to 2 weeks prior to use. Viability of all nematodes was >95% at the time of application.

Bioassay methods were based on those described by Shapiro & McCoy (2000a, b). Experimental units consisted of plastic pots (10.5 cm diam., 6.5 cm deep). Each pot contained 400 grams

of soil (dry weight), 10 *D. abbreviatus* larvae, and 10 pieces of carrot. The final soil moisture in each pot was 22%, which was determined to be field capacity for this soil (graduated cylinder method). Larvae and carrot pieces were placed 1 cm from the bottom of the pot on a bed of soil and were covered with 5.5 cm of soil. Nematodes were applied in 1 mL of tap water (using a 1 mL pipette) at a rate of 40 infective juveniles/cm^2 24 h after the larvae were placed in the pots. Controls received only water. The pots were tightly covered with plastic lids after application of the nematodes and stored at 25°C. After 14 d, the pots were emptied and the number of surviving larvae was recorded. Each treatment was replicated 3 times and the experiment was repeated the following day.

Analysis of variance (PROC GLM) and Student-Newman-Keuls multiple range test (SAS 2003) were used to analyze the effect of nematode species or strain on the survival of *D. abbreviatus* larvae. Analyses were performed on arcsin transformed data (proportion surviving).

There was not a significant interaction between the treatment and trial effects ($F = 0.98$, $P = 0.4713$, $df = 9, 39$) so the data from the two trials were combined. Nor was there a significant effect attributable to the trial ($F = 2.35$, $P = 0.1332$, $df = 1, 39$). Analysis indicated that all of the nematode treatments had lower mean survival than the control treatments ($F = 5.98$, $P \leq 0.0001$, $df = 1, 39$) (Table 1). Three strains of *S. riobrave* (3-8b, TP, and 7-12) had lower mean survivorship than all other treatments except *H. indica* (HOM1), and *S. riobrave* (355), with zero *D. abbreviatus* larvae surviving in 2 of the treatments (strains TP and 7-12), and all *S. riobrave* strains caused lower *D. abbreviatus* survival than *S. feltiae* or *S. rarum*; no other treatment differences were detected (Table 1).

TABLE 1. PROPORTION OF *DIAPREPES ABBREVIATUS* LARVAE SURVIVING (ANALYSIS PERFORMED ON ARCSINE TRANSFORMED DATA) AFTER 14 D EXPOSURE TO NEMATODES IN AN OXISOL FROM PUERTO RICO. MEANS FOLLOWED BY THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT (SNK, $P \leq 0.05$). THE NUMBER OF ALL TREATMENTS WAS 6, EXCEPT FOR THE CONTROL, WHICH WAS 5.

Treatment	Proportion surviving ± SEM
Control	0.78 ± 0.04 a
Steinernema feltiae (SN)	0.50 ± 0.12 b
S. rarum (J. Levy)	0.45 ± 0.12 b
S. diaprepesi	0.33 ± 0.13 bc
S. riobrave (355)	0.08 ± 0.03 cd
S. riobrave (3-8b)	0.02 ± 0.02 d
S. riobrave (TP)	0.00 ± 0 d
S. riobrave (7-12)	0.00 ± 0 d
Heterorhabditis indica (HOM1)	0.15 ± 0.06 cbd
H. megidis (UK211)	0.25 ± 0.06 bc

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation nor endorsement by the U.S. Department of Agriculture. We thank Kathy Halat, Wanda Evans, and Rebeckah Long. We thank Jorge Lugo, NRCS, Mayaguez, PR, for classifying soil samples, and Ulises Chardon, USDA-ARS-TARS, Mayaguez, PR, for soil analysis, and Drs. Wayne Hunter, David Hall, and two anonymous reviewers for comments on an earlier version of this manuscript.

SUMMARY

We evaluated the virulence of 9 species/strains of entomopathogenic nematode against *D. abbreviatus* in a high clay content soil typical of fruit growing regions of Puerto Rico. All nematode species and nematode strains provided significant mean mortality as compared to a water control. Strains of *Steinernema riobrave* performed particularly well, with some strains resulting in 100% mortality. These laboratory and field tests indicate that some species/strains of entomopathogenic nematodes may be suitable for the control of *D. abbreviatus* in Puerto Rican soils of high clay content.

REFERENCES CITED

BARBERCHECK, M. E. 1992. Effect of soil physical factors on biological control agents of soil insect pests. Florida Entomol. 75: 539-548.

BARBERCHECK, M. E., AND H. K. KAYA. 1991. Effect of host condition and soil texture on host finding by the entomogenous nematodes *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) and *Steinernema carpocapsae* (Rhabditida: Steinernematidae). Environ. Entomol. 20: 582-589.

BEINROTH, F. H. 1971. The general pattern of the soils of Puerto Rico, pp. 225-230 *In* Transcripts of the Fifth Caribbean Geological Conference, Geol. Bull. No. 5. Queens College Press.

GEORGIS, R., AND G. O. POINAR, JR. 1983. Effect of soil temperature on the distribution and infectivity of *Neoaplectana carpocapsae* (Nematoda: Steinernematidae). J. Nematol. 15: 308-311.

KAYA, H. K., AND S. P. STOCK. 1997. Techniques in insect nematology *In* L. A. Lacey [ed.], Manual of Techniques in Insect Pathology. Academic, San Diego, CA.

KOPPENHÖFER, A. M. AND EUGENE M. FUZY. 2006. Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei*, *Steinernema glaseri*, *Heterorhabditis zealandica*, and *Heterorhabditis bacteriophora*. J. Invertebr. Pathol. 92: 11-22.

MARTORELL, L. F. 1976. Annotated food plant catalog of the insects of Puerto Rico. Agric. Exp. Stn., Univ. Puerto Rico, Department of Entomology. 303 pp.

McCoy, C. W., D. I. Shapiro, L. W. Duncan, and K. Nguyen. 2000. Entomopathogenic nematodes and other natural enemies as mortality factors for larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Biol. Control 19: 182-190.

Poinar, G. O. 1990. Biology and Taxonomy of Steinernematidae and Heterorhabditidae, pp 23-62 *In* R. Gaugler and H. K. Kaya [eds.], Entomopathogenic Nematodes in Biological Control. CRC, Boca Raton, FL.

Portillo-Aguilar, C., M. G. Villani, M. J. Tauber, C. A. Tauber, and J. P. Nyrop. 1999. Entomopathogenic nematode (Rhabditida: Heterorhabditidae and Steinernematidae) response to soil texture and bulk density. Environ. Entomol. 28: 1021-1035.

Román, J., and J. B. Beavers. 1983. A survey of Puerto Rican soils for entomogenous nematodes which attack *Diaprepes abbreviatus* (L.) (Coleoptera: Curculionidae). J. Agric. Univ. Puerto Rico 67: 311-316.

Román, J., and W. Figueroa. 1985. Control of the larva of the sugarcane rootstalk borer, *Diaprepes abbreviatus* (L), with the entomogenous nematode *Neoaplectana carpocapsae* Weiser. J. Agric. Univ. Puerto Rico 69: 153-158.

SAS. 2003. Version 9.1. SAS Institute, Cary, NC.

Shapiro, D. I., and C. W. McCoy. 2000a. Susceptibility of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) larvae to different rates of entomopathogenic nematodes in the greenhouse. Florida Entomol. 83: 1-9.

Shapiro, D. I., and C. W. McCoy. 2000b. Virulence of entomopathogenic nematodes to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in the laboratory. J. Econ. Entomol. 93: 1090-1095.

Shapiro, D. I., C. W. McCoy, A. Fares, T. Obreza, and H. Dou. 2000. Effects of soil type on virulence and persistence of entomopathogenic nematodes in relation to control of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). Environ. Entomol. 29: 1083-1087.

Shapiro-Ilan, D. I., D. H., Gouge, and A. M. Koppenhöfer. 2002. Factors affecting commercial success: case studies in cotton, turf and citrus, pp. 333-355 *In* R. Gaugler [ed.], Entomopathogenic Nematology. CABI, New York, NY.

Shapiro-Ilan, D. I., L. W. Duncan, L. A. Lacey, and R. Han. 2005. Orchard crops, pp. 215-230 *In* P. Grewal, R.U. Ehlers, and D. Shapiro-Ilan [eds.], Nematodes as Biological Control Agents. CABI Publishing, New York, NY.

Simpson, S. E., H. N. Nigg, N. C. Coile, and R. A. Adair. 1996. *Diaprepes abbreviatus* (Coleoptera: Curculionidae): Host plant associations. Environ. Entomol. 25: 333-349.

Wolcott, G. N. 1936. The life history of *Diaprepes abbreviatus* at Rio Piedras, Puerto Rico.J. Agr. Univ. Puerto Rico 20: 883-914.

AN EFFECTIVE TRAP AND BAIT COMBINATION FOR MONITORING THE SMALL HIVE BEETLE, *AETHINA TUMIDA* (COLEOPTERA: NITIDULIDAE)

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The small hive beetle (SHB), *Aethina tumida* Murray (Coleoptera: Nitidulidae), is a pest of European honeybees *Apis mellifera* (L.) in the United States. Surveys in the US in 2004 indicated that the beetle had spread to 30 states (Hood 2004). The beetle is weakly attracted to bucket traps baited with a combination of honey, pollen and adult bees, but not to traps baited with honey and pollen, or brood alone (Elzen et al. 1999). The beetle is also attracted to bumblebee colonies, suggesting that these may serve as alternative hosts (Spiewok & Neumann 2006). This paper reports field tests of an effective trap and bait combination for monitoring flying SHB.

The bait consisted of pollen dough (a mixture of pollen and honey) conditioned by allowing male SHB to feed on it for 3 d. Its attractiveness was tested by trapping at 2 beeyards in north-central Florida and 7 in Pennsylvania, all with previous histories of SHB infestation. The traps were 25.5-cm sections of black PVC pipe (7.5 cm ID) with a removable cap at each end. Two openings (8×13 cm) covered with 4-mesh aluminum screen allowed entry of SHB, but not honey bees. An inverted 18-mesh-screen cone (8 cm deep), located just below the windows, funneled beetles into the bottom cap through a small hole at the apex. Three pin holes in the bottom allowed for drainage of rainwater. The bottom cap of each baited trap contained 100 g of pollen dough tied in a cotton stockinette. Two 15-ml plastic vials of water with dental wicks inserted through the caps gradually moistened the dough. The control traps contained only vials of water.

Three groups of 6 baited and 6 control traps were placed in one of the Florida beeyards. In 2 of the groups, the traps were suspended 1 m above the ground on T-shaped metal poles, with a baited trap on 1 arm and a control trap on the other (about 1 m apart). The poles were placed 7.5 m apart in 2 parallel rows, 1 row in full sun in front of the hives and the other in the shade of trees behind the hives. Traps in the third group were placed on the ground and attached to platforms supporting the hives, with 1 baited and 1 control trap on each of 6 platforms. Trapped beetles were removed and counted, and the water vials were topped up every 3 d for 12 d. Comparisons of numbers captured were made between hanging traps in the sun and those in the shade, and between hanging traps in the sun and traps on the ground, which were also in the sun.

The baited traps captured 90 beetles, mostly in the shade. The proportion of beetles captured in the shade (0.90) was significantly greater than 0.5 (binomial test, normal approximation, $z = 7.59, P < 0.01$) (Zar 1999), suggesting a preference for traps in the shade. There was no difference in trap catch between traps on the ground and those hanging in full sunlight; that is, the proportion captured on the ground (0.78) was not significantly greater than 0.5 ($z = 1.67, P = 0.096$).

Seven beeyards in Pennsylvania, each in a different county, were used to compare baited and control traps. One baited and 1 control trap was placed at each location. These were suspended about 30 cm apart from tree limbs at a height of about 1.5 m and at least 90 m from the beeyards. Trapped beetles were counted and the bait replaced weekly for 6 weeks. The total number of SHB captured was 419. Of these, 350 (83%) were caught at 1 location in Bucks County. A second trial done the following year also showed a preponderance of captures in Bucks County, 219 out of 265. These results suggest that SHB infestation in Pennsylvania was highly localized.

A second Florida beeyard was used to study the spatial distribution of trap catch, and to determine the effect of shade and distance from bee colonies on numbers captured. The hives were located in a sparse pine plantation with scattered clumps of oaks that provided the only shade of any significance. Baited traps were scattered over an area of about 9 ha at various distances from the hives (Fig. 1A); some were in shade and others in full sun (Fig. 1B). A total of 46 SHB were captured during an 18-week trapping period, suggesting a low level of infestation. Of this total, 39 were captured by 7 traps shaded by oak canopy, 5 by 2 traps in partial shade, and 2 by 10 traps in full sunlight. The spatial distribution of trap catch is illustrated in Fig. 1B. The proportion of captures in full shade (0.85) was significantly greater than 0.5 ($z = 4.72, P < 0.01$), suggesting a preference for shaded traps, consistent with results for the first Florida location. Most of the beetles (23) were captured in trap 2 (Fig. 1B), which was in deep shade and close to the hives. Overall, however, there was no significant relationship

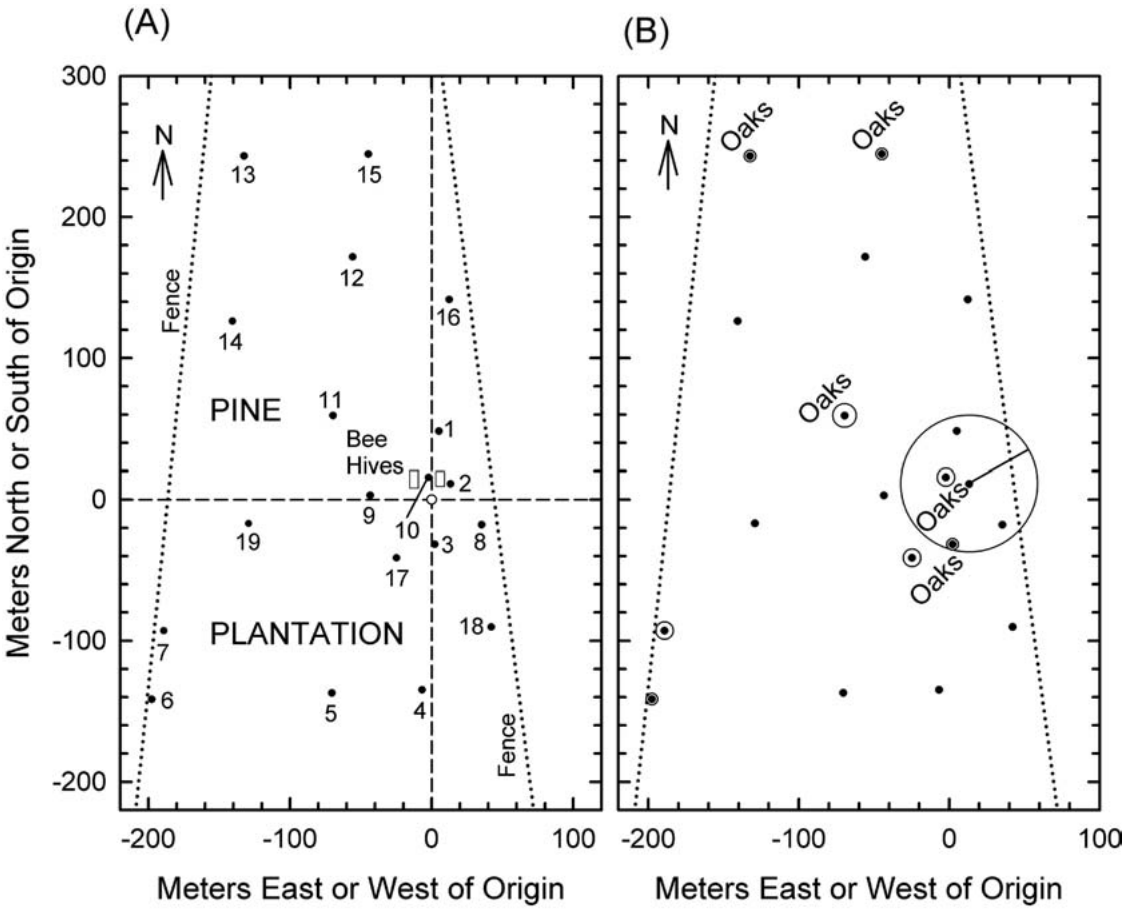

Fig 1. Trapping adult *A. tumida* in a sparse pine plantation with scattered clumps of oak trees, north-central Florida. (A) Dots with numbers 1-19 indicate trap positions. The open circle and dashed lines indicate the origin and axes of the coordinate system used to specify trap positions. The 2 open rectangles indicate groups of bee hives. (B) Spatial distribution of beetles captured in baited flight traps between Jun 15 and Oct 26, 2005. The areas of the circles are proportional to the total trap catch at each trap position.

between trap catch and distance from the hives (Spearman's rank correlation coefficient $r_s = 0.03$, $P = 0.89$). The lack of correlation may reflect the nearly equal distribution of traps between shady and sunny locations, together with the spatial distribution of trees (Fig. 1A) and the preference of the beetles for shade.

No SHB were captured by the control traps in any of the beeyards. The difference between control and baited traps clearly establishes the effectiveness of conditioned pollen dough in attracting the beetle, which contradicts the statement by Spiewok & Neumann (2006) that free-flying SHB cannot be trapped in the field with bee products unless adult bees are present. However, because of the marked preference for shade, baited traps placed in full sunshine captured very few beetles. Future studies are planned to examine the relationship between the intensity of incident solar radiation and numbers of beetles captured in baited traps.

Charlotte Skov and Mary Searle provided technical assistance, and we appreciate their contributions to the success of the research.

Summary

Traps baited with pollen dough conditioned by allowing male SHB to feed on it for 3 d were effective in capturing SHB if the traps were located in shade. Traps placed in full sunshine captured very few.

References Cited

Elzen, P. J., J. R. Baxter, D. Westervelt, C. Randall, K. S. Delaplane, L. Cutts and W. T. Wilson.

1999. Field control and biology studies of a new pest species, *Aethina tumida* Murray (Coleoptera, Nitidulidae), attacking European honey bees in the western hemisphere. Apidologie 31: 361-366.

HOOD, M. 2004. The small hive beetle, *Aethina tumida*: A review. Bee World 85: 51-59.

SPIEWOK, S., AND P. NEUMANN. 2006. Infestation of commercial bumblebee (*Bombus impatiens*) field colonies by small hive beetles (*Aethina tumida*). Ecol. Entomol. 31: 623-628.

ZAR, J. H. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.

NOTES ON THE LONGAN SCALE, *THYSANOFIORINIA NEPHELII* (HEMIPTERA: COCCOIDEA: DIASPIDIDAE) IN FLORIDA[1]

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In Florida, the most common armored scales found on tropical fruits are considered exotics or not native to Florida and 3 examples of introductions in the last 10 years include the white mango scale, *Aulacaspis tubercularis* Newstead, the litchi scale, *Andaspis punicae* (Laing), and the longan scale, *Thysanofiorinia nephelii* (Maskell) (Hodges et al. 2005). The longan scale was first collected in the continental U.S. in Homestead, Florida in 1996. Even though the longan scale is believed to be native to the Orient, it has spread and invaded several regions worldwide prior to this detection including Autralasian, Neotropical, and Palearctic regions. Neotropical introductions detected prior to the Florida report included Brazil, Rio de Janeiro, and Cuba (Ben-Dov et al. 2003). The longan scale is now considered established in southern Florida but has not been catalogued as a significant economic pest in the United States (Miller et al. 2005). Primary hosts of concern in Florida for the longan scale include plants in the Sapindaceae, in particular longan (*Dimocarpus longan*) and lychee (*Litchi chinensis* Sonnerat). Populations may occur on leaves, stems, or fruits. Other potential host plants for the longan scale include Arecaceae (*Kentia* spp.) and Euphorbiaceae (*Euphorbia longena*) (Ben Dov et al. 1993).

Increased sample submissions of pest population reports to the Florida Department of Agriculture, Division of Plant Industry have occurred particularly in Broward, Miami-Dade, and Palm Beach Counties since 2005 (Table 1). Isolated occurrences of detection have also occurred at retail stores of tropical fruit trees, but the established pest distribution will probably be restricted to climate zones suitable for tropical fruit trees. It is unknown if this species will be a significant economic pest for tropical fruits in Florida, but increased report incidences suggest that it may be an emerging pest of concern. Basic life history and control information is unknown for this species. General and taxonomic information are summarized below.

In the field, the covering or "armor" of this scale looks similar to that of a Parlatoria scale but differs both by the color and by the actual formation of the cover. In *Thysanofiorinia*, the cover is light yellow to green and this species is considered pupillarial in that the cover is actually an enlarged shed skin of the second instar. In the *Parlatoria* scales, the covers are gray to dark in color and the covers are not formed as above; waxes are secreted and combined with shed skins. Slide mounted specimens are fairly distinctive with the slightly pyriform body shape and with strongly pronounced anal lobes separated by a considerable distance.

TABLE 1. LONGAN SCALE COLLECTION RECORDS.

Date	Month	County	Host
1996	Dec	Miami-Dade	Longan
1996	Dec	Miami-Dade	Longan
2001	Dec	Miami-Dade	Lychee
2002	Apr	Miami-Dade	Longan
2002	Apr	Miami-Dade	Longan
2002	May	Palm Beach	Lychee
2002	Jul	Miami-Dade	Longan
2004	Dec	Broward	Lychee
2005	Jan	Miami-Dade	Longan
2005	Jan	Miami-Dade	Lychee
2005	Mar	Palm Beach	Longan
2005	Apr	Alachua	Longan
2006	Jan	Collier	Longan
2006	Feb	Polk	Longan
2006	Feb	Collier	Longan
2006	Mar	Miami-Dade	Longan
2006	Mar	Pinellas	Longan
2006	May	Miami-Dade	Longan

Thysanofiorinia nephelii (Maskell)
Fiorinia nephelii Maskell, 1897

Diagnosis. Balchowsky (1954) gave a good description and illustration of *T. nephelii*. A summary of some of the key characters are listed in the following paragraphs. Slide-mounted adult females with median lobes produced out of the broad apical recess of the pygidium, separated from each other by about the width of one anal lobe, divergent, serrate on the inner margin, with

[1]FDACS Contribution No. 1032

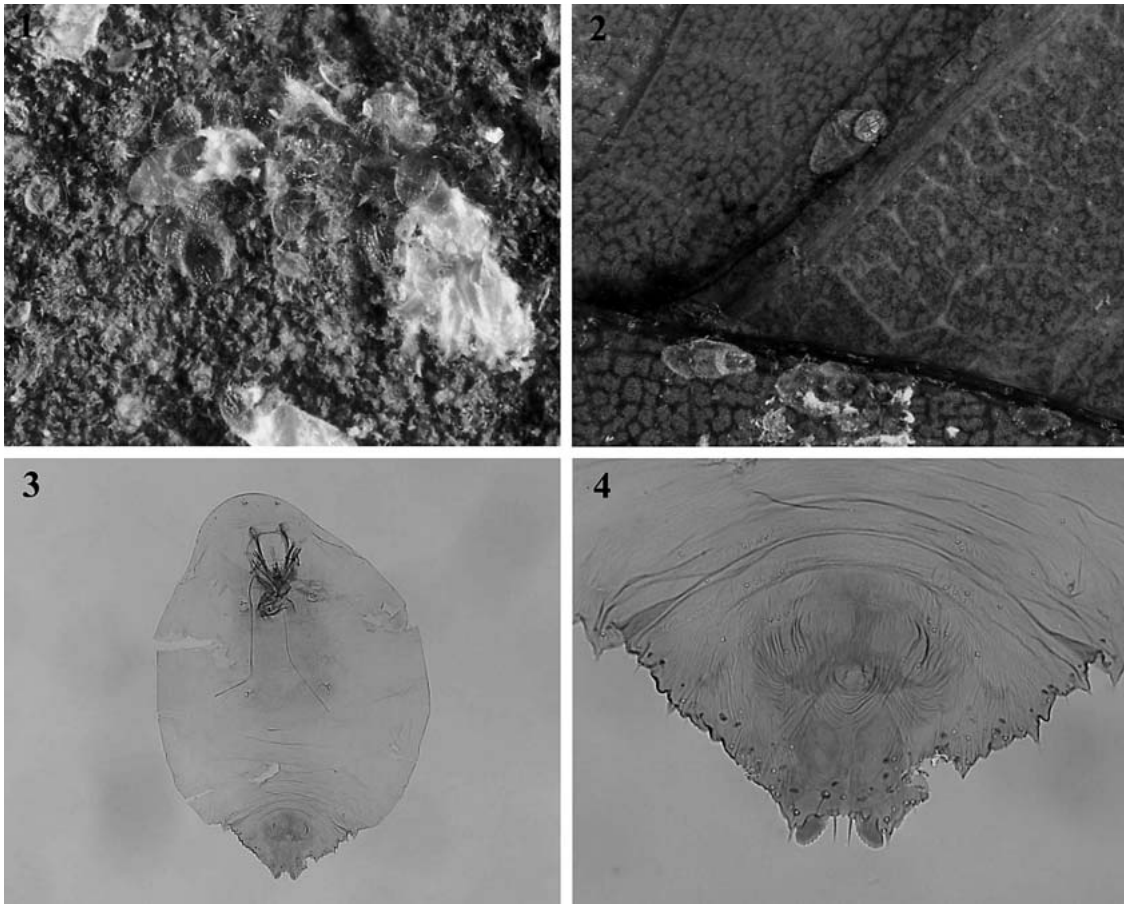

Fig. 1. *Thysanofiorinia nephelii* (Maskell) on the fruit of *Dimocarpus longan* (photo L. Buss, UF 2005).
Figs. 2-4. Adult female; 2: on the leaf of *D. longan*; 3: body; 4: pygidium.

a pair of marginal setae between them, and with the inner margin of the lobe being longer than the outer margin, the apex rounded. Second lobe reduced into a point. One marginal gland spine between the median and 2nd lobes, scarcely extending beyond the apex of the median lobe; one on each of the 3rd and 5th abdominal segments, broadened basally and somewhat conical. Perivulvar pores absent. Dorsal marcroduct distribution as follows: abdominal segment V with 1 on margin, segments VI-VII with 3 on margin, segment VIII with 1 located submarginally. First instar with a marginal series of prominent setae around the body, antennae 6-segmented (Takagi 1970).

Specimens Examined. [United States: Florida] Alachua Co.: Gainesville, on *Dimocarpus longan* (Sapindaceae), 18. IV. 2005 (J. Brambila). Miami-Dade Co.: Homestead, same host plant, 13. VI. 1996, (M. Biondo); same data, except for 23. IX. 1996 (L. Lodyga); same data, except for 5. III. 2005 (J. Brambila); Largo, same host plant, 13. XII. 2001 (D. Mooney); Miami, same host plant, 13. IX. 1996 (M. Biondo).

Summary

The longan scale, *Thysanofiorinia nephelii*, was reported for the first time in Florida in 1996 with 2 independent finds in Miami-Dade County. The scale was not found or reported during the time period of 1996-2000. Longan scale was reported 1 time in 2001, 4 times in 2002, 1 time in 2004, 4 times in 2005 and 6 times in 2006 (as of Jul 6, 2006). This scale is being seen more frequently in the field and field populations are beginning to reach high densities in some areas of Miami-Dade County (personal observation). The overall economic impact of this invasive scale insect is unknown at this time. However, increasing finds, movement on plant material for sale and increasing populations in the field may indicate that the longan scale may be an economic pest in the future for longan and lychee crops in south Florida.

References Cited

BALACHOWSKY, A. S. 1954e. Les cochenilles Paléarctiques de la tribu des Diaspidini (In French). Memmoires Scientifiques de l'Institut Pasteur, Paris. 450 pp.

BEN-DOV, T. D. R. MILLER, AND G. A. C. GIBSON. 2003. scalenet http://www.sel.barc.usda.gov/scalenet/scalenet.htm (1 June 2006).

HODGES, A. C., G. S. HODGES, AND G. C. WISLER. 2005. Exotic scale insects (Hemiptera: Coccoidea) and whiteflies (Hemiptera: Aleyrodidae) in Florida's tropical fruits: An example of the vital role of early detection in pest prevention and management. Proc. Florida State Hort. Soc. 118: 215-217.

MASKELL, W. M. 1897. On a collection of Coccidae, principally from China and Japan. Entomologist's Monthly Magazine 33: 239-244.

MILLER, D. R. AND M. E. GIMPEL. 2005.Diaspididae. Part of ScaleNet. http://www.sel.barc.usda.gov/scalenet/scalenet.htm.

MILLER, D. R., G. L. MILLER, G. S. HODGES, AND J. A. DAVIDSON. 2005. Introduced scale insects (Hemiptera: Coccoidea) of the United States and their impact on U.S. agriculture. Proc. Entomological Soc. Washington 107(1): 123-158.

TAKAGI, S. 1970. Diaspididae of Taiwan based on material collected in connection with the Japan-U.S. cooperative science programme, 1965 (Homoptera: Coccoidea). Insecta Matsumurana 33(1): 1-146.

TANG, F. T. 1986. The Scale Insects of Horticulture & Forest of China, Vol. III. Research publication No. 3. Shanxi Agricultural University Press Taigu, Shanxi, China. 305 pp.

PRESENCE OF *DIACHASMIMORPHA LONGICAUDATA* (HYMENOPTERA: BRACONIDAE) IN A GUILD OF PARASITOIDS ATTACKING *ANASTREPHA FRATERCULUS* (DIPTERA: TEPHRITIDAE) IN NORTHWESTERN ARGENTINA

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The braconid *Diachasmimorpha longicaudata* (Ashmead) is a fruit fly parasitoid native to the Indo-Pacific region, which has been widely disseminated into America via Hawaii (Ovruski et al. 2000). It was used in augmentative release programs against *Anastrepha suspensa* (Loew) in the United States of America (Florida state) (Sivinski et al. 1996), against *Anastrepha ludens* (Loew) and *Anastrepha obliqua* (Macquart) in Mexico (Montoya et al. 2000), and against *Anastrepha fraterculus* (Wiedemann) and *Ceratitis capitata* (Wiedemann) in Brazil (Carvalho 2005). In Mexico, this exotic parasitoid is currently a common parasitoid species of *Anastrepha* larvae, particularly in exotic commercial fruit in the state of Veracruz (Sivinski et al. 2000; Sivinski et al. 2001), and it is also being mass-reared on *A. ludens* larvae in the state of Chiapas (Cancino et al. 2002; Montoya & Cancino 2004). During 1961, *D. longicaudata* and the eulophid *Ceratoneuromyia indica* (Silvestri) were introduced into Argentina from Mexico and released in limited numbers in citrus-growing areas of the northwestern provinces of Jujuy, Salta, and Tucumán, and of the northeastern provinces of Misiones and Entre Rios (Ovruski et al. 1999). Although *D. longicaudata* was recovered immediately after release in Jujuy and Tucumán (Turica 1968), up to this time, there was no evidence of permanent establishment of this parasitoid species in any release sites of the northwestern Argentinean region. However, that *D. longicaudata* is permanently established on *A. fraterculus* has been documented in the northeastern province of Misiones (Schliserman et al. 2003). Similarly, the exotic *A. indica* was recently recorded on *A. fraterculus* in both Misiones and Jujuy provinces (Ovruski et al. 2006). Recent fruit fly parasitoid surveys made in Salta province (El Oculto locality) included specimens of *D. longicaudata*. Thus, *D. longicaudata* was recovered 40 years after its first release in the northwestern Argentinean region.

Between Nov and Dec 2001, 103 (= 4.3 kg, individual weight 37.5 ± 5.3 g) peaches (*Prunus persica* (L.) Batsch, Rosaceae) were collected in patches of disturbed wild vegetation with high diversity of exotic fruits in the locality of "El Oculto" (23°06'S, 64°24'W, 530 m above sea level). The collecting area is located in the northern-most extension of the Argentinean subtropical mountain rainforest (locally known as "Las Yungas forest") (Cabrera 1976). Climate is defined as temperate-hot humid with a summer rainy season (Dec through Mar), winter dry season, and annual rainfall varies from 259 to 1,947 mm. The temperature of the warmest month is >22°C with a mean annual temperature of 18°C.

The fruit samples consisted of fallen ripe fruit (80%) and ripe fruit still on the tree (20%). In the laboratory, all fruits in the sample were weighed and rinsed with a 20% solution of sodium benzoate, and each fruit was placed in a plastic glass (250 cm^3) with damp sand in the bottom as a pupation substrate for fly larvae. Pupae were removed weekly and the *A. fraterculus* and *C. capitata* pupae were separated by external pupal characters (White & Elson-Harris 1992). Then, pupae were placed in plastic vials containing sterilized humid sand until either a fruit fly or a parasitoid emerged. Fruit fly species were identified by L. Oroño based upon Zucchi's (2000) taxonomic key. Parasitoid specimens were identified to species by S. Ovruski with the keys from Wharton & Marsh (1978), Wharton & Gilstrap (1983), and Ovruski (2003) for Opiinae (Braconidae), and the taxonomic description by Wharton et al. (1998) for Eucoilinae (Figitidae). Voucher specimens were placed in the entomological collection of the Fundación Miguel Lillo (FML) (San Miguel de Tucumán, Argentina).

In total, 316 *C. capitata* and 25 *A. fraterculus* pupae were recovered from all infested peach fruits. From *C. capitata* pupae, 151 adult flies (47.8% emergence rate) and 25 *Aganaspis pelleranoi* (Brethes) (Hymenoptera: Figitidae) adult parasitoids (19 females and 6 males) were recovered. From *A. fraterculus* pupae, 8 adult flies (32.0% emergence rate) and 7 adult parasitoids (3 *D. longicaudata* females, 2 *Doryctobracon brasiliensis* (Szépligeti) (Hymenoptera: Braconidae) males, and 2 *A. pelleranoi* females) were obtained. Pupal viabilities (number of emerging adult flies and wasps) were 60.0% and 55.1% in *A. fraterculus* and *C. capitata*, respectively. Parasitism rates were 28.0% and 7.3% in *A. fraterculus* and *C. capitata*, respectively.

All wasp species identified are solitary, koinobiont larval-pupal endoparasitoids belonging to the fruit fly parasitoid guild number "2" defined by Ovruski et al. (2000). *Aganaspis pelleranoi* and

the braconid *Doryctobracon brasiliensis* are native species from the Neotropical region. *Aganaspis pelleranoi* accounted for more than 80% of all parasitoids recovered from *P. persica* we sampled. This eucoiline species and the braconid *Doryctobracon areolatus* (Szépligeti) (Hymenoptera: Braconidae) are the most abundant *A. fraterculus* parasitoid species in wild guava habitats from the northernmost to the southernmost portion of the Yungas forest in Argentina (Ovruski et al. 2004; Ovruski et al. 2005). Furthermore, *A. pelleranoi* would be better adapted to *C. capitata* larvae than any of the native braconid parasitoid common in Latin America (Ovruski et al. 2004). *Doryctobracon brasiliensis* was previously recorded from Las Yungas forest of the northwestern Argentina in association with *A. fraterculus* in several native and exotic host fruit species (Ovruski et al. 2004).

Even though *D. longicaudata* was recovered in smaller numbers, the data presented here and also those published by Schliserman et al. (2003) show the successful establishment of this exotic parasitoid in 2 different Argentinian biogeographical areas: Las Yungas forest in the northwestern region and Paranaense forest in the northeastern region.

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Summary

Specimens of *Diachasmimorpha longicaudata* (Ashmead), native to Indo-Pacific region, *Aganaspis pelleranoi* (Brethes) and *Doryctobracon brasiliensis* (Szépligeti), both native to Neotropical region, were recovered from *Anastrepha fraterculus* (Wiedemann) pupae collected from *Prunus persica* (L.) Batsch in the province of Salta. Thus, the braconid *D. longicaudata* was recovered 40 years after its first release in the northwestern Argentinean region.

References Cited

Cabrera, A. 1976. Regiones fitogeográficas argentinas. Enciclopedia Agricultura y Jardinería. Ediciones ACME, SACI, Buenos Aires, Argentina. 135 pp.

Cancino, J., L. Ruiz, Y. Gomez, and J. Toledo. 2002. Irradiación de larvas de *Anastrepha ludens* (Loew) (Diptera: Tephritidae) para inhibir la emergencia de moscas en la cría del parasitoide *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Folia Entomol. Mex. 41: 195-208.

Carvalho, R. Da S. 2005. Avaliaça˜o das liberações inoculativas do parasitóide exótico *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) em pomar diversificado em Conceição do Almeida, BA. Neotr. Ent. 34: 799-805.

Montoya, P., P. Liedo, B. Benrey, J. Cancino, J. F. Barrera, J. Sivinski, and M. Aluja. 2000. Biological control of *Anastrepha* spp. (Diptera: Tephritidae), in mango orchards through augmentative releases of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). Biol. Control 18: 216-224.

Montoya, P., and J. Cancino. 2004. Control biológico por aumento en moscas de la fruta (Diptera: Tephritidae). Folia Entomol. Mex. 43: 257-270.

Ovruski, S. M. 2003. Nuevos aportes a la taxonomía de las especies de Opiinae (Hymenoptera: Braconidae) parasitoides de *Anastrepha fraterculus* (Wiedemann) (Diptera: Tephritidae) en la provincia de Tucumán. Acta Zool. Lilloana 47: 39-68.

Ovruski, S. M., J. L. Cancino, P. Fidalgo, and P. Liedo. 1999. Nuevas perspectivas para la aplicación del control biológico contra moscas de la fruta (Diptera: Tephritidae) en Argentina. Rev. Manejo Integrado de Plagas 54: 1-12.

Ovruski, S. M., M. Aluja, J. Sivinski, and R. A. Wharton. 2000. Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. Int. Pest Management Rev. 5: 81-107.

Ovruski, S. M., P. Schliserman, and M. Aluja. 2004. Indigenous parasitoids (Hymenoptera) attacking *Anastrepha fraterculus* and *Ceratitis capitata* (Diptera: Tephritidae) in native and exotic host plants in Northwestern Argentina. Biol. Control 29: 43-57.

Ovruski, S. M., R. A. Wharton, P. Schliserman, and M. Aluja. 2005. Abundance of *Anastrepha fraterculus* (Diptera: Tephritidae) and its associated native parasitoids (Hymenoptera) in "feral" guavas growing in the endangered northernmost Yungas forest of Argentina with an update on the taxonomic status of opiine parasitoids previously reported in this country. Environ. Entomol. 34: 807-818.

Ovruski, S. M., P. Schliserman, O. R. Decoll, C. Peñaloza, L. Oroño, and C. Colin. 2006. The establishment of *Aceratoneuromyia indica* (Hymenoptera: Eulophidae) in three biogeographical regions of Argentina. Florida Entomol. 89: 270-273.

Schliserman, P., S. M. Ovruski, and O. R. Decoll. 2003. The recovery and permanent establishment of *Diachasmimorpha longicaudata* (Hymenoptera: Braconidae) in Misiones, northeastern Argentina. Florida Entomol. 86: 491-492.

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

Sivinski, J., J. Piñero, and M. Aluja. 2000. The distributions of parasitoids (Hymenoptera) of *Anastrepha* fruit flies (Diptera: Tephritidae) along an altitudinal gradient in Veracruz, Mexico. Biol. Control 18: 258-269.

Sivinski, J., K. Vulinec, and M. Aluja. 2001. Oviposition length in a guild of parasitoids (Hymenoptera: Braconidae) attacking *Anastrepha* spp. fruit flies (Diptera: Tephritidae) in southern Mexico. Ann. Entomol. Soc. Am. 94: 886-895.

Turica, A. 1968. Lucha biológica como medio de control de las moscas de los frutos. Revista IDIA 241: 29-38.

WHARTON, R. A., AND P. M. MARSH. 1978. New world Opiinae (Hymenoptera: Braconidae) parasitic on Tephritidae (Diptera). J. Wash. Acad. Sci. 68: 147-167.

WHARTON, R. A., AND F. E. GILSTRAP. 1983. Key to and status of Opiinae braconid (Hymenoptera: Braconidae) parasitoids used in Biological Control of *Ceratitis* and *Dacus* s. l. (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 76: 721-741.

WHARTON, R. A., S. M. OVRUSKI, AND F. E. GILSTRAP. 1998. Neotropical Eucoilidae (Cynipoidea) associated with fruit infesting Tephritidae, with new records from Argentina, Bolivia and Costa Rica. J. Hymenoptera. Res. 7: 102-115.

WHITE, I. M., AND M. M. ELSON-HARRIS. 1992. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB international, ACIAR, Redwood Press Ltd., Melksham, UK. 601 pp.

ZUCCHI, R. A. 2000. Taxonomía, pp. 13-24 *In* A. Malavasi and R. A. Zucchi [eds.], Moscas-das-frutas de Importância Econômica no Brasil. Conhecimento Básico e Aplicado. Holos Editora, Riberão Preto, Brasil.

NEW HOST AND EXPANDED GEOGRAPHIC RANGE OF STELLATE SCALE, *VINSONIA STELLIFERA* (HEMIPTERA: COCCIDAE: CEROPLASTINAE)

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Stellate scale, *Vinsonia stellifera* (Westwood), is a polyphagous wax scale with a distribution spanning across the tropics and subtropics of both the northern and southern hemispheres (Williams & Watson 1990). It has been reported to occur as far north as Virginia in the U.S. (Hamon & Williams 1984) and as far south as the Northern Territory of Australia (Qin & Gullan 1994). Jansen (1995) reported the occurrence *V. stellifera* in the Netherlands within a glasshouse environment. This insect feeds on a wide range of plant taxa and can occur in high densities on a single plant. As a result, it was considered a potential threat to several economically important plants in Florida (Hamon & Williams 1984).

We report the occurrence of stellate scale both on a new host, *Melaleuca quinquenervia* (Myrtaceae), and in a new locality, on the island of New Providence of the Bahamas (near the Nassau airport; N25.05827, W-77.45352). U.S. quarantine records document the interception of stellate scale on imports of *Eugenia* (Myrtaceae) from the Bahamas. However, to our knowledge a specific locality in the Bahamas has not been reported in the scientific literature for stellate scale.

The native distribution of the melaleuca tree, *M. quinquenervia*, extends along the coastal region of New South Wales and Queensland in Australia. Over the past century, *M. quinquenervia* has been introduced into the Bahamas and Puerto Rico, as well as into California, Hawaii, Texas, Louisiana and Florida in the U.S. for ornamental, revegetation and agroforestry purposes (Turner et al. 1998; Dray 2003). While *M. quinquenervia* has not become a pest in all areas it was introduced, it has been categorized as an invasive weed in south Florida, the Bahamas and Puerto Rico (Turner et al. 1998; Pratt et al. 2005).

Stellate scale can be easily identified by its star-shaped wax covering (Hamon & Williams 1984) (Fig. 1). We observed the scale while performing regular demographic surveys of *M. quinquenervia* on New Providence. Typical densities of *V. stellifera* observed on *M. quinquenervia* were *ca.* 10-15 nymphs and adults per leaf. However, no evidence of damage to these leaves as a result of the feeding was apparent, and it is doubtful that the scale will have a significant impact as a natural enemy against this exotic tree.

Simberloff & Von Holle (1999) cautioned that commensalistic and mutualistic relationships between invaders may accelerate the rate of invasion of exotic species and may serve to magnify the cumulative impacts of the invaders on native communities. If *V. stellifera* does have the potential to impact other, native or economically important plants when present in sufficient densities, the coupling of *V. stellifera* and *M. quinquenervia* could heighten the risk that scales will achieve the numbers necessary to inflict detrimental impacts on these other plants. Adding to this risk, stellate scale has been observed to utilize both the camphor tree, *Cinnamomum camphora* (Lauraceae) (Jansen 1995), and a congener (*Schefflera arbolicola*) (Qin & Gullan 1994) of the Queensland umbrella tree, *S. actinophylla* (Araliaceae), as hosts. Both the camphor tree and the Queensland umbrella tree are invasive in Florida. Stellate scale has not yet been reported as an ecologically or economically important pest (Qin & Gullan 1994), but it should be monitored closely as the adventive ranges of *M. quinquenervia* and these other invasives expand.

Summary

Stellate scale, *Vinsonia stellifera*, was observed utilizing *Melaleuca quinquenervia* as a host on New Providence of the Bahamas. This expands the known host and geographic ranges of this polyphagous and widespread scale insect.

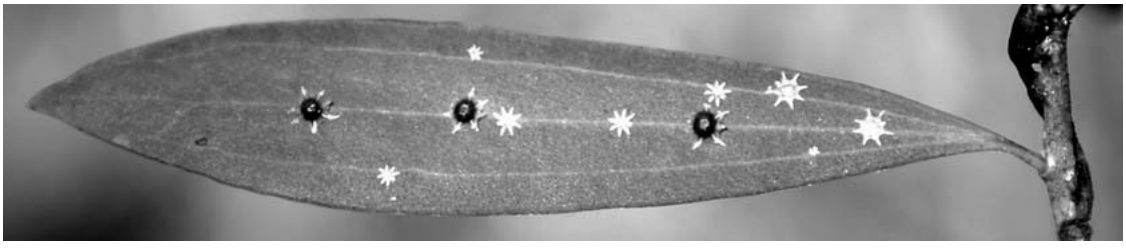

Fig. 1. Photograph of stellate scale, *Vinsonia stellifera*, on *Melaleuca quinquenervia*.

References Cited

Dray, F. A. 2003. Ecological Genetics of *Melaleuca quinquenervia* (Myrtaceae): Population Variation in Florida and its Influence on Performance of the Biological Control Agent *Oxyops vitiosa* (Coleoptera: Curculionidae). Ph.D. Dissertation. Florida International University, Miami, FL. 161 pp.

Hamon, A. B., and M. L. Williams. 1984. The Soft Scale Insects of Florida (Homoptera: Cocoidea: Coccidae). Florida Dept. Agric. Cons. Serv. Div. Plant Ind. 11: 1-94.

Jansen, M. G. M. 1995. Scale insects (Homoptera: Coccinea) from import interceptions and greenhouses in the Netherlands. Israeli J. Entomol. 29: 131-146.

Pratt, P. D., V. Quevedo, L. Bernier, J. Sustache and T. D. Center. 2005. Invasions of Puerto Rican Wetlands by the Australian tree, *Melaleuca quinquenervia*. Caribbean J. Sci. 41: 42-54.

Qin, T. K., and P. J. Gullan. 1994. Taxonomy of the wax scales (Hemiptera: Coccidae: *Cero plastinae*) in Australia. Invert. Taxon. 8: 923-959.

Simberloff, D., and B. Von Holle. 1999. Positive interactions of nonindigenous species: invasional meltdown? Biol. Invasions 1: 21-32

Turner, C. E., T. D. Center, D. W. Burrows, and G. R. Buckingham. 1998. Ecology and management of *Melaleuca quinquenervia*, an invader of wetlands in Florida, U.S.A. Wetlands Ecol. Man. 5: 165-178.

Williams, J. R., and D. J. Watson. 1990. The Scale Insects of the Tropical South Pacific Region. Part 3. The Soft Scales (Coccidae) and Other Families. C.A.B. International, Wallingford. 267 pp.